Impact of molecular markers on treatment selection in advanced colorectal cancer

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Introduction

For decades, 5-fluorouracil (5-FU) ± leucovorin (LV) was the only active agent in the treatment of advanced colorectal cancer with an overall response rate of 10% and a median survival of up to 12 months [1,2]. At the end of the 1990s, the topoisomerase-1 inhibitor irinotecan and the platinum derivative oxaliplatin were introduced as active drugs in the treatment of metastatic colorectal cancer (CRC) [3–7]. More recently, the importance of the epidermal growth factor receptor (EGFR) as a therapeutic target has been demonstrated in many cancers, including colorectal cancer. The EGFR is indeed frequently overexpressed in CRC and the monoclonal antibodies against the EGFR, cetuximab and panitumumab, have been developed and approved. Another approach to inhibit cancer growth is the use of anti-angiogenic agents such as bevacizumab, which is a humanised monoclonal antibody to vascular endothelial growth factor (VEGF). Bevacizumab was the first angiogenesis inhibitor to demonstrate prolonged survival in patients with metastatic CRC [8].

The progress in the management of metastatic CRC over the last 5 to 10 years is unprecedented and has prolonged the median survival to approximately 24 months today. This has also presented clinicians with many questions and challenges such as the choice of best treatment, the best combination, the best sequence and the most optimal selection of patients eligible for the different treatment options. In this regard the use of molecular markers for determining prognosis and also for selecting the most appropriate treatment is crucial for the optimal treatment approach for patients with metastatic CRC.

This review will summarise the available data of molecular markers that are important for treatment selection in advanced CRC.

Which molecular markers are we looking for?

CRC is a heterogeneous disease resulting from the co-occurrence of multiple oncogenic mutations. Molecular classification of CRC is evolving. CRC is not one homogeneous disease but at least three molecular subtypes exist, currently defined by the presence of CIN (chromosome instability), MIN (microsatellite instability) and CIMP (CpG island methylator phenotype) [9]. Studying molecular classification and molecular correlates can provide clues to the pathogenesis and provide surrogate markers in clinical or research studies. Moreover, there is an urgent need to identify markers that will help in predicting response to therapy and in determining prognosis. It is important to make a difference between prognostic and predictive makers.

A predictive marker can be defined as a marker that indicates the sensitivity or resistance of the tumour to a specific treatment. Predictive markers are very important in oncology, since only a proportion of patients will respond to a particular treatment, in order to select the best treatment. Although insight on the potential role of molecular markers is growing rapidly, only a very few predictive markers are validated in CRC in association with one of the available treatment options. An example of a recently validated predictive marker to determine resistance to the anti-EGFR antibodies is the mutated KRAS gene. In the future it will be crucial to further characterise and analyse specific mutations present in the tumour on which our therapeutic decisions can be based for a more individualised treatment. Molecular profiling is also important to test the presence of the target in the tumour and to validate whether the target is inhibited and functionally significant.

Prognostic factors on the other hand do not select responsiveness or resistance to a specific treatment, but provide information on the outcome and the prognosis of the disease itself independent of a specific therapy. There is a large unmet need of prognostic subgroup identification in colorectal cancer. Comprehensive

annotation of known colorectal cancer gene mutations will help us to identify these subgroups.

Some markers can be both prognostic and predictive. For example, in breast cancer the presence of oestrogen receptors are both predictive (predicts response to hormone therapy) and prognostic (correlates with good prognosis).

The techniques to evaluate prognostic and predictive markers are usually measurement of protein expression, gene expression and DNA alterations. Germ-line polymorphisms can explain some of the differences in toxicity and efficacy of a drug used in different patients with the same disease. In the future, molecular markers can become an integrated part of our daily practice; however, rigorous validation is first required.

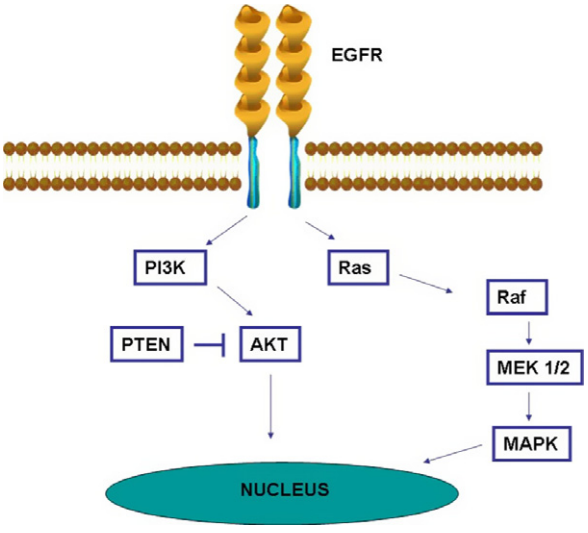

Fig. 1. Schematic overview of the most important downstream pathways of the EGFR.

Predictive markers of response to EGFR-targeted therapies

Introduction

The EGFR, also referred to as HER-1, is overexpressed in many types of cancers, especially in CRC. EGFR is a 170-kDa transmembrane glycoprotein composed of an intracellular tyrosine-kinase domain, a transmembrane lipophilic segment and an extracellular ligand-binding domain whose autocrine ligands are epidermal growth factor (EGF), transforming growth factor-alpha (TGFα), amphiregulin, heparin-binding EGF-like growth factor (HB-EGF), betacellulin (BTC), epigen (EPI) and epiregulin [10]. After binding its ligand, EGFR is known to homodimerise or heterodimerise with other HER family members and subsequently to phosphorylate several tyrosine kinase domains and thus to initiate intracellular signalling pathways (Fig. 1). The major downstream signalling route of the HER family is via the Ras–Raf–MAPK pathway. Another important target in EGFR signalling is phophatidyl-inositol 3-kinase (PI3K) and the downstream kinase AKT. The activation of these pathways translates in the nucleus into transcription of genes that mediate a variety of cellular responses. Based on the importance of the EGFR pathway in tumourigenesis, expression has been investigated in CRC and was found in up to 80% of CRC [11] and is therefore an important therapeutic target. EGFR signalling can be targeted by either monoclonal antibodies that interfere directly with receptor signalling or tyrosine kinase inhibitors (TKIs) that interfere with the cytoplasmatic domain.

Both the EGFR-targeting monoclonal antibodies cetuximab (chimeric IgG1) as well as panitumumab (fully human IgG2) are approved treatments in patients with metastatic CRC. Cetuximab has been shown to be active as a single agent as well as in combination with irinotecan in patients with chemotherapy-refractory CRC and in combination with chemotherapy in earlier lines of treatment [12]. Panitumumab has been shown to be active as a single agent in chemotherapy-refractory metastatic CRC [13]. The anti-EGFR antibodies were initially investigated in patients with EGFR-expressing metastatic CRC. However, no clear association between EGFR expression and response to EGFR targeted therapy was seen in CRC [14]. The reason why immunohistochemical (IHC) detection of EGFR is a poor indicator of response to EGFR-targeting monoclonal antibodies may include a variety of factors. It has been suggested that the technique to detect EGFR by IHC is not sensitive enough. On the other hand, the EGFR signalling pathway is very complex and the level of expression of the receptor ligands, the level of tyrosine phosphorylation of the receptor and the expression of downstream molecules may be more predictive of treatment response than the total level of the receptor. Moreover, the expression of EGFR by IHC is very heterogeneous within a specific tumour localisation and between different tumour sites in a patient.

EGFR as a molecular prognostic and predictive marker

Several studies have addressed the question about EGFR as a potential prognostic marker. In a review by Nicholson and colleagues, it was stated that increased EGFR expression was associated with reduced overall survival (OS) rates in about 50% of the studies [15].

Table 1
Single-arm studies of treatment of metastatic CRC with anti-EGFR monoclonal antibodies

Study	Treatment arm	Total patients	KRAS WT		KRAS mutated	
			Number	RR%	Number	RR%
Lièvre et al. [21]	C ± CT	89	65	40	24	0
De Roock et al. [22]	C ± CT	113	57	40	46	0
Khambata-Ford et al. [23]	C	80	50	10	30	0
Di Fiore et al. [24]	C + CT	59	43	28	16	0
Benvenuti et al. [25]	P or C ± CT	48	32	31	16	6
Finocchiaro et al. [26]	C ± CT	81	49	26	32	6

Abbreviations: C, cetuximab; CT, chemotherapy; P, panitumumab; WT, wild type; RR, response rate.

Table 2
Randomised studies of treatment of metastatic CRC with anti-EGFR monoclonal antibodies

Study	Treatment arms	Total patients	KRAS WT				KRAS mutated			
			Number		RR (%)		Number		RR (%)	
			A	C	A	C	A	C	A	C
Amado et al. [27]	Panitumumab vs BSC	427	124	119	17	0	84	100	0	0
Van Cutsem et al. [28]	FOLFIRI +/– cetuximab	540	172	176	59	43	105	87	36	40
Bokemeyer et al. [29]	FOLFOX +/– cetuximab	233	61	73	60	37	52	47	33	49
Karapetis et al. [30]	Cetuximab vs BSC	394	117	113	13	0	81	83	1	0

Abbreviations: BSC, best supportive care; A, antibody arm; C, control arm; vs, versus; RR, response rate, WT, wild type.

Because of important methodological issues, however, a prognostic role can not be attributed to EGFR.

Is EGFR then a predictive marker? There is no clear association between EGFR expression and response to EGFR targeted therapy. Moreover, in contrast with non-small lung cancer where EGFR mutations predict response to gefitinib, this is not the case for CRC where EGFR mutations are very rare [16]. Similarly, the type III mutated variant of the human EGFR (EGFR vIII) characterised by a deletion in the extracellular domain is very rare in CRC [17].

An increased *EGFR* copy number as determined by FISH has been described in metastatic CRC as being associated with a better response to anti-EGFR treatment [18,19], but there are still some reproducibility and methodological concerns that prevent its routine use as a predictive marker in clinical practice.

The type of HER hetero- and homodimers expressed by the tumour cells may be indicative of response to EGFR inhibitors, but this remains to be explored.

KRAS, BRAF and PI3K mutations

While more than 80% of CRC express the EGFR, only 23% of patients refractory to oxaliplatin- and irinotecan-containing regimens respond to a treatment with cetuximab plus irinotecan [12]. A treatment with cetuximab or panitumumab in monotherapy is also active, but with only a response rate of approximately 10% [12,13].

Since activation of the EGFR leads to activation of intracellular effectors, such as the G protein K-ras, the protein kinase RAF, mitogen-activated protein kinase (MAPK) and PI3K (Fig. 1), it was hypothesised that a mutation in the KRAS and BRAF coding genes could affect clinical response to EGFR-inhibitors.

A small retrospective analysis by the group of Pierre Laurent-Puig showed, in 30 patients with metastatic CRC treated with cetuximab-based therapy, that *KRAS* mutations were linked to an absence of response to cetuximab [20]. Mutation at key sites within the gene, commonly codons 12, 13 and 61, leads to constitutive activation of *KRAS*-associated signalling downstream of the EGFR. *KRAS* mutations were present in approximately 40% of patients and none of these patients responded to cetuximab. These findings were confirmed in several other studies, as well as single-arm retrospective analyses (Table 1), as randomised clinical trials (Table 2) [21–30]. This was the case for cetuximab and panitumumab used as single agents as well as for cetuximab/chemotherapy combinations. A meta-analysis of seven retrospective studies in 281 patients with chemotherapy-refractory

metastatic CRC, treated with cetuximab/irinotecan, showed a response rate of 42% in KRAS wild type patients, while no responses were found in KRAS mutant patients. The progression-free survival (PFS) and OS were also significantly longer in KRAS wild type patients compared to the mutant patients [31].

In a randomised study of Best Supportive Care (BSC) plus panitumumab versus BSC in chemotherapy-refractory CRC, an analysis of the KRAS status clearly showed that the activity of panitumumab was confined to the KRAS wild type (WT) patients: the response rate in KRAS wild type patients was 17% and the PFS was clearly longer in the panitumumab-treated WT patients compared to KRAS mutant patients. There were no responses in the KRAS mutant population [13,27]. On the basis of these results the European Medicines Agency (EMEA) has approved panitumumab in monotherapy as a third-line treatment only for patients with tumours that are WT KRAS. In a large randomised study of BSC plus cetuximab versus BSC in chemotherapy-refractory metastatic CRC by the Canadian and Australian group, similar findings were published: the activity of cetuximab was confined to the KRAS WT patients and cetuximab led to an important prolongation in survival in KRAS WT patients compared to patients treated with only BSC [30].

The CRYSTAL trial is a randomised phase III trial evaluating cetuximab combined with 5-FU/LV/irinotecan (FOLFIRI) versus FOLFIRI alone in first-line in patients with metastatic CRC. A statistically significant improvement in the overall response rate (response rate 47% versus 39%; $P = 0.0038$) and median PFS (Hazard ratio (HR) for PFS 0.85; $P = 0.048$) in the cetuximab-containing arm was found. Subsequently, the investigators evaluated the KRAS mutational status on a subset of the intent-to-treat population with available tissue for KRAS testing. KRAS mutations were detected in 35% of patients, and the benefit of cetuximab was restricted to patients without mutations in the *KRAS* gene. Moreover, a statistically significant difference in favour of cetuximab was seen in KRAS WT patients for PFS (HR 0.68; $P = 0.017$) and best overall response (59% versus 43%; $P = 0.0025$).

The OPUS study is a randomised phase II trial evaluating 5-FU/LV/oxaliplatin (FOLFOX) ± cetuximab in first-line treatment of metastatic CRC [29]. In this study the KRAS mutational status was also evaluated on a subset of the intent-to-treat population (233/337). KRAS mutations were detected in 42% of evaluable samples and also in this study KRAS WT patients obtained benefit from receiving cetuximab in addition to FOLFOX compared to those receiving FOLFOX alone, both in terms of response rate (61% versus 37%) and PFS (median 7.7 months versus 7.2 months; HR 0.57; $P = 0.011$) [29].

The American Society of Clinical Oncology (ASCO) now recommends, based on a review of the literature, to test all patients with metastatic CRC who are candidates for anti-EGFR monoclonal antibody therapy for their KRAS status in an accredited laboratory [32]. If a mutation is detected, those patients should not receive anti-EGFR therapy.

To address the need for standardised *KRAS* mutation testing, working groups convened an expert panel to develop guideline recommendations which have been published recently [33]. They conclude that although many robust techniques have been developed for *KRAS* genotyping, most of these techniques have not been validated in the clinical setting and therefore there is an urgent need for validated methods and standardised testing procedures [33].

These data reopened the discussion on the prognostic role of KRAS in metastatic CRC. It has been suggested that KRAS has a potential prognostic role. The RASCAL studies evaluated the association between KRAS mutations and patient outcome and reported that KRAS mutations were associated with increased risk of relapse ($P < 0.001$) and death ($P = 0.004$) [34,35]. More specifically, some mutations, such as the codon 12 glycine to valine mutation, seemed more aggressive than others. The prognostic role of KRAS, however, has never been clearly established. In several recent large studies the potential prognostic role of KRAS was also evaluated. In the study of the National Cancer Institute of Canada (NCIC) it was shown that KRAS had no prognostic role at least in the setting of chemotherapy-refractory patients who had a short survival: indeed, patients with KRAS mutant and KRAS WT tumours had a similar median survival (4.6 months versus 4.8 months respectively) [30]. In the Crystal study in previously untreated patients KRAS mutant patients had a shorter survival than KRAS WT patients when treated with chemotherapy alone (5-FU/LV/irinotecan), suggesting some prognostic role of KRAS mutation. This statement has to be interpreted with caution since some WT patients were treated in later lines with cetuximab and this influenced the outcome of these patients [28]. Looking at a large number of patients with stage II and III colon cancer in the PETACC-3 study, there was no clear prognostic role for KRAS [36].

Thus, the presence of a KRAS mutation is the first validated predictive biomarker in metastatic CRC that influences treatment selection for anti-EGFR

antibodies and this opens a new era in biomarker-driven therapy in CRC. While a *KRAS* mutation is responsible for resistance to EGFR inhibitors in about 40% of the cases, a considerable number of KRAS WT tumours also show resistance to a treatment with EGFR-inhibitors, for which mutations in other genes may be responsible. More recently, among 79 colorectal cancers with WT *KRAS* status, Di Nicolantonio and colleagues [37] found 11 B-type Raf kinase (*BRAF*) mutations (14%); none of the 11 tumours with these mutations had a response to cetuximab. In our own experience [36,37], *BRAF* mutations were found in 16 of 136 colorectal cancers with WT *K-ras* genes (12%); 15 of these tumours did not have a response to cetuximab. In addition, we found *N-ras* mutations in five of 108 tumours with WT *K-ras* status (5%); none of these tumours had a response to cetuximab. Since *KRAS, BRAF,* and *NRAS* mutations are mutually exclusive, these *BRAF* and *NRAS* mutations could explain the lack of a response in an additional 17% of patients with WT *KRAS* CRC who were treated with cetuximab [38,39].

Mutations in the PI3K gene have also been correlated with response to EGFR inhibitors. *PIK3CA* mutations occur mainly in the hotspots located in exon 9 and exon 20. Sartore-Bianchi and colleagues evaluated the mutational status of 110 patients treated with anti-EGFR monoclonal antibodies [40]. They reported a frequency of 13.6% of *PIK3CA* mutations and showed a significant association with clinical resistance to panitumumab or cetuximab. Our group evaluated a total of 200 patients with chemotherapy-refractory metastatic CRC and reported a *PIK3CA* mutation in 12% of patients, but did not find a correlation between the presence of a *PIK3CA* mutation and impaired response to cetuximab [41]. Larger patient populations will be necessary to determine whether there is a role of *PIK3CA* mutations as a single marker in determining response to cetuximab.

Finally, the role of loss of PTEN, resulting in overactivation of the AKT pathway, and response to cetuximab is still under investigation. It has been reported in a small retrospective study that PTEN-positive tumours may have a better outcome than PTEN-negative tumours. These data certainly need confirmation and also have the potential drawback of the IHC method that was used [42].

Fc gammaR polymorphism

Modulation of the immune response could be another important mechanism of sensitivity to anti-EGFR monoclonal antibodies. The immunological mechanism mediated through Fc receptors (FcγR) carried by immune cells plays an important role in the antitumour effect of IgG1 antibodies [43]. FcγR are under the influence of a germinal genetic polymorphism, which impacts the binding between the Fc receptor and its Fc target. In metastatic breast cancer and follicular lymphoma, different allelic variations of FcγRs are significantly associated with better response rate and PFS when treated by trastuzumab and rituximab respectively [44,45]. The data are conflicting in chemotherapy-refractory CRC patients treated with anti-EGFR monoclonal antibodies. Zhang and colleagues studied 39 chemotherapy-refractory metastatic CRC patients treated with single agent cetuximab [46] and found that patients with FcγRIIa (CD32) 131 H/H or H/R genotype showed a longer time to tumour progression compared to patients with a homogeneous R/R genotype ($P = 0.037$). Surprisingly, the FcγRIIa-158V/V genotype was associated with a shorter PFS compared to FCγRIIa-158 F carriers. The study of Bibeau and colleagues showed that FcγRIIa-131 H/H and/or FcγRIIIa-158 V/V were favourable alleles and suggests a more effective ADCC (antibody cell-mediated cytotoxicity) antitumour response on cetuximab treatment [47]. A possible explanation of these conflicting data could be due to the low number of patients included in the study by Zhang and colleagues, single agent treatment instead of combination with irinotecan in the other study and the type and level of pre-treatment (second-line versus third-line). Prospective studies are therefore needed to rule out a role of FcγR polymorphisms and response to anti-EGFR monoclonal antibodies.

EGFR ligands

Recently, EGFR ligands Amphiregulin (AREG) and Epiregulin (EREG) were found by biomarker exploratory analysis using Affimetrix profiling arrays to be amongst the top genes associated with response to cetuximab treatment [23]. The analysis was performed on fresh frozen biopsies from liver metastases in a small group of patients. Our group investigated the role of these ligands in a larger group of 220 patients who were enrolled in clinical trials, used formalin-fixed paraffin-embedded (FFPE) tissue from primary tumours and evaluated the relationship with outcome [48]. We found that it is technically feasible to measure ligand expression in FFPE tissue of primary CRCs and that there is a conservation of ligand expression between primary tumours and metastases. Moreover, we identified a relationship

between ligand expression and outcome after treatment with cetuximab in KRAS WT patients [48].

Predictive markers of response to angiogenesis inhibitors

Angiogenesis refers to the proliferation of blood vessels, which is an important natural process to ensure that all cells are within a small distance of a supply of oxygen. It occurs in the healthy body, e.g. for healing wounds or for restoring blood flow to tissues after an injury. Angiogenesis is regulated by a complex control system with pro-angiogenic and anti-angiogenic factors [49]. Early in the development of cancer, the angiogenic switch induces expression of pro-angiogenic factors and down-regulates anti-angiogenic proteins. In cancer it is one of the most important steps in progression from localised to metastatic disease [50]; therefore, inhibition of tumour vascularisation is an attractive anti-cancer approach. The anti-VEGF humanised monoclonal antibody bevacizumab is the first anti-angiogenic agent that demonstrated substantial activity in CRC [8,51,52]. The number of anti-vascular compounds entering clinical trials is constantly increasing. Therefore, biomarkers that have the potential for response prediction are urgently needed. New imaging techniques are under development trying to predict response to anti-angiogenic agents. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), using gadolinium-based paramagnetic contrast media, and DCE-ultrasonography are able to assess vascular physiology of tumours, by combining good anatomical detail with the ability to quantify vascular parameters. The DCE-MRI and possibly also DCE-ultrasonography are imaging techniques with great potential, but several challenges still remain.

It is still a great challenge to explore predictive molecular biomarkers that may allow us to treat patients who are likely to benefit from bevacizumab and avoid treating patients with an expensive and potentially toxic drug who are unlikely to benefit. To date, no validated molecular marker has been identified that can predict response to anti-angiogenic agents. While research on biomarkers for bevacizumab mainly focused on tumour-derived molecular markers such as KRAS, BRAF, p53 and VEGF [53–55], results have been disappointing, which is not a surprise since angiogenesis is a host-regulated, as well as a tumour-related, process. In these studies, VEGF score and microvessel density did not have a predictive value for bevacizumab. There is substantial inherited genetic variability within *VEGF* and its receptor, including multiple single nucleotide polymorphisms (SNPs). Recently, a study was published that showed an association between *VEGF* genotype and median OS, as well as grade 3 or 4 hypertension in patients with metastatic breast cancer treated with bevacizumab [56]. However, several questions remain, such as e.g. the biological mechanism underlying these findings. It is probable that the interplay between the biology of angiogenesis and the use of VEGF inhibitors is more complex than initially thought. Also, other genes independent of VEGF could play a critical role in the mechanism of resistance to anti-VEGF therapy.

Circulating tumour cells

Since micrometastases are undetectable by the classic imaging and laboratory studies, their identification in early cancer may have a substantial effect on prognosis and individualising treatment strategies for these patients. Epithelial cells have been identified in the bone marrow or in the peripheral blood which we refer to as circulating tumour cells (CTC). Due to the refinement of an immunomagnetic separation technique, CTCs can now more reliably and reproducibly be isolated and studied as a prognostic and predictive marker in epithelial malignancies [57]. In breast cancer, CTCs are an independent predictor of PFS and OS [58].

In CRC, Cohen and colleagues reported on a multicentre study where CTCs were enumerated in the peripheral blood of 430 patients with metastatic CRC at baseline and after the first-, second, or third-line therapy [59]. They showed that CTCs can serve as both a prognostic and predictive factor. The patients were stratified into unfavourable ($\geqslant$3 CTCs/7.5 mL) and favourable prognostic groups (<3 CTCs/7.5 mL). The median PFS and OS for patients with unfavourable CTCs at baseline was 4.5 and 9.4 months, respectively, which was significantly different from those with favourable CTCs at baseline (PFS and OS 7.9 months and 18.5 months, respectively). They also showed that PFS and OS were significantly shorter at all time points for patients with at least three CTCs during therapy compared with those with fewer than three CTCs. The authors suggested several applications for which CTCs can be used in CRC, such as the possibility to inform treatment choices before typical imaging intervals, and to spare patients unnecessary toxicity by suggesting that an early change in therapy is warranted [57]. Currently, however, the low detection rate of CTCs in CRC patients (30% of patients) make it an unlikely candidate for clinical application.

Other molecular prognostic and predictive markers

Several other prognostic and predictive markers have also been investigated in metastatic CRC, but none of them are currently being used in clinical practice because of lack of confirmatory and validated data and studies.

Prognostic markers

Allelic loss of the region on the long arm of chromosome 18, which encompasses three domains (DCC, SMAD4 and SMAD2), occurs commonly in CRC. There is evidence that loss of heterozygosity (LOH at 18q) is linked with inferior prognosis [60]. However, the data are inconsistent and some studies failed to show this association. Prospective studies using consistent methodology are needed to determine the role of 18q in patients with CRC.

Mutations in one of several DNA mismatch repair genes are found in hereditary non-polyposis CRC (HNPCC) and in 15% to 20% of sporadic colon cancers [61]. Some studies suggest that tumours with high levels of DNA microsatellite instability (MSI-H) are associated with a longer survival than either MSI-low (MSI-L) or microsatellite-stable (MSS) tumours, both in HNPCC and in sporadic cases [62]. These data still need to be validated.

Predictive markers

There are four basic DNA repair pathways: nucleotide excision repair, base excision repair, mismatch repair and double-strand break repair. Excision repair cross complementing 1 (ERCC1) is an excision nuclease within the nucleotide excision repair pathway. ERCC1 has been reported to play a major role in the response to platinum-based therapies. In contrast to cisplatin, oxaliplatin-induced adducts are not recognised or processed by mismatch repair, being predominantly repaired by the nucleotide excision repair pathway. Viguer and colleagues demonstrated that the *ERCC1* polymorphism at codon 118 affects the response to oxaliplatin in colon cancer [63]. Because DNA adducts formed by cisplatin and oxaliplatin are equally well recognised by nucleotide excision repair, it is not surprising that the effect of ERCC1 activity on the cytotoxicity of cisplatin also applies to oxaliplatin. Prospective trials are still necessary to confirm the role of this molecular marker, which could guide us to avoid random selection of first-line chemotherapy by selecting those who are more likely to benefit from treatment with oxaliplatin.

Finally, topoisomerase-1 (Topo1), which cuts one strand of a DNA double helix, has been suggested as a plausible predictive marker for irinotecan, a Topo1 inhibitor. The randomised FOCUS trial incorporated this predictive biomarker for the treatment of metastatic CRC [64]. They found that in patients with low Topo1, PFS was not improved by the addition of irinotecan. In contrast, patients with moderate/high Topo1 benefited from the addition of irinotecan ($P = 0.001$). These data still need to be validated before clinical application.

Conclusion

The understanding of the role of the prognostic and predictive markers in colon cancer has progressed rapidly over past years, but more information is needed until we can classify CRC according to a molecular profile in different subgroups. Moreover, despite the development of several new targeted therapies, we still do not completely understand the entire mechanisms of action of some of these new drugs and therefore are unable to develop good biomarkers that select exactly which patients are likely to respond to a particular drug. The data of KRAS mutation status as a predictive marker to predict resistance to an anti-EGFR antibody have, however, paved the route for more studies on predictive markers and led us to an era of individualised anti-cancer treatment in patients with metastatic CRC. Although the possibility of individualising cancer treatment by using molecular markers is gaining wide acceptance, we believe this is just the start of a new postgenomic era and many open questions still need to be answered.

Conflict of interest statement

None declared.

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References

1 Thirion P, Michiels S, Pignon JP, et al. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: an updated meta-analysis. *J Clin Oncol* 2004;**22**:3766–75.
2 Buyse M, Thirion P, Carlson RW, Burzykowski T, Molenberghs G, Piedbois P. Relation between tumour response to first-line chemotherapy and survival in advanced colorectal

cancer: a meta-analysis. Meta-analysis Group in Cancer. *Lancet* 2000;**356**:373–8.
3 Douillard JY, Cunningham D, Roth AD, et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet* 2000;**355**:1041–7.
4 Saltz LB, Cox JV, Blanke C, et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. *N Engl J Med* 2000;**343**:905–14.
5 Kohne CH, Van Cutsem E, Wils J, et al. Phase III study of weekly high-dose infusional fluorouracil plus folinic acid with or without irinotecan in patients with metastatic colorectal cancer: European Organisation for Research and Treatment of Cancer Gastrointestinal Group Study 40986. *J Clin Oncol* 2005;**23**: 4856–65.
6 De Gramont A, Figer A, Seymour M, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* 2000;**18**:2938–47.
7 Van Cutsem E, Geboes K. The multidisciplinary management of gastrointestinal cancer. The integration of cytotoxics and biologicals in the treatment of metastatic colorectal cancer. *Best Pract Res Clin Gastrroenterol* 2007;**21**:1089–108.
8 Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;**350**:2335–42.
9 Ogino S, Goel A. Molecular classification and correlates in colorectal cancer. *J Mol Diagn* 2008;**10**(1):13–27.
10 Singh AB, Harris RC. Autocrine, paracrine and juxtacrine signalling by EGFR ligands. *Cellular Signaling* 2005;**17**: 1183–93.
11 Goldstein NS, Armin M. Epidermal growth factor receptor immunohistochemical reactivity in patients with American Joint Committee on Cancer Stage IV colon adenocarcinoma: implications for a standardized scoring system. *Cancer* 2001;**92**:1331–46.
12 Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004;**351**:337–45.
13 Van Cutsem E, Peeters M, Siena S, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemo-refractory metastatic colorectal cancer. *J Clin Oncol* 2007;**25**:1658–64.
14 Van Cutsem E. Challenges in the use of epidermal growth factor receptor inhibitors in colorectal cancer. *Oncologist* 2006;**11**:1010–7.
15 Nicholson RI, Gee JM, Harper ME. EGFR and cancer prognosis. *Eur J Cancer* 2001;**37**(Suppl 4):S9–15.
16 Ogino S, Meyerhardt JA, Cantor M, et al. Molecular alterations in tumors and response to combination chemotherapy with gefitinib for advanced colorectal cancer. *Clin Cancer Res* 2005;**11**:6650–6.
17 Azuma M, Danenberg KD, Iqbal S, et al. Epidermal growth factor receptor and epidermal growth factor receptor variant III gene expression in metastatic colorectal cancer. *Clin Colorectal Cancer* 2006;**6**:214–8.
18 Moroni M, Veronese S, Benvenuti S, et al. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. *Lancet Oncol* 2005;**6**:279–86.
19 Personeni N, Fieuws S, Piessevaux H, et al. Clinical usefulness of EGFR gene copy number as a predictive marker in colorectal cancer patients treated with cetuximab: a fluorescent in situ hybridization study. *Clin Cancer Res* 2008;**14**:5869–76.
20 Lièvre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 2006;**66**:3992–5.
21 Lièvre A, Bachet JB, Boige V, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol* 2008;**26**:374–9.
22 De Roock W, Piessevaux H, De Schutter J, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann Oncol* 2008;**19**:508–15.
23 Khambata-Ford S, Garrett CR, Meropol NJ, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastastic colorectal cancer patients treated with cetuximab. *J Clin Oncol* 2007;**25**:3230–7.
24 Di Fiore F, Blanchard F, Charbonnier F, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. *Br J Cancer* 2007;**96**: 1166–9.
25 Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, et al. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. *Cancer Res* 2007;**67**:2643–8.
26 Finnochiaro G, Capuzzo F, Janne K, et al. EGFR, HER2 and Kras as predictive factors for cetuximab sensitivity in colorectal cancer. *J Clin Oncol* 2007;**25**:168s (suppl;abstr 4021).
27 Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 2008;**26**:1626–34.
28 Van Cutsem E, Köhne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastastic colorectal cancer. *N Engl J Med* 2009;**360**:1408–17.
29 Bokemeyer C, Bondarenko I, Makhson A, et al. Fluorouracil, leucovorin and oxaliplatin with and without cetuximab in the first line treatment of metastatic colorectal cancer. *J Clin Oncol* 2009;**27**:663–71.
30 Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008;**359**:1757–65.
31 Di Fiore F, Van Cutsem E, Laurent-Puig P, et al. Role of *KRAS* mutation in predicting response, progression-free survival, and overall survival in irinotecan-refractory patients treated with cetuximab plus irinotecan for a metastatic colorectal cancer: Analysis of 281 individual data from published series. *J Clin Oncol* 2008;**26**:abstr 4035.
32 Allegra CJ, Jessup JM, Somerfield MR, et al. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. *J Clin Oncol* 2009;**27**(12):2091–6.
33 Van Krieken JHJM, Jung A, Kirchner T, et al. *KRAS* mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for an European quality assurance. *Virchows Arch* 2009;**454**:233–5.
34 Andreyev HJ, Norman AR, Cunningham D, Oates JR, Clarke PA. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. *J Natl Cancer Inst* 1998;**90**: 675–84.

35 Andreyev HJ, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: the "RASCAL II" study. *Br J Cancer* 2001;**85**:692–6.
36 Roth AD, Tejpar S, Yan R, et al. Correlation of molecular markers in colon cancer with stage-specific prognosis: Results of the translational study on the PETACC 3 - EORTC 40993-SAKK 60-00 trial. *Gastrointestinal Cancer Symposium, San Francisco* 2009, www.asco.org.
37 Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008;**26**:5705–12.
38 De Roock W, Lambrechts D, Tejpar S. K-RAS mutations and cetuximab in colorectal cancer. *N Engl J Med* 2009;**360**:834.
39 Lambrechts D, Prenen H, De Schutter J, et al. The role of KRAS, BRAF, NRAS & PIK3CA mutations as markers of resistance to cetuximab in chemorefractory metastatic colorectal cancer. *ASCO 2009 Proc* [in press].
40 Sartore-Bianchi A, Martini M, Molinari F, et al. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. *Cancer Res* 2009;**69**:1851–7.
41 Prenen H, Deschutter J, Jacobs B, et al. PIK3CA mutations are not a major determinant of resistance to the EGFR inhibitor cetuximab in metastatic colorectal cancer. *Clin Cancer Res* 2009 [Epub ahead of print].
42 Loupakis F, Pollina L, Stasi I, et al. Evaluation of PTEN expression in colorectal cancer (CRC) metastases (mets) and in primary tumors as predictors of activity of cetuximab plus irinotecan treatment. *J Clin Oncol* 2008;**26**: abstract No. 4003.
43 Weiner GJ. Monoclonal antibody mechanisms of action in cancer. *Immunol Res* 2007;**39**:271–8.
44 Weng WK, Levy R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *J Clin Oncol* 2003;**21**:3940–7.
45 Musolino A, Naldi N, Bortesi B, et al. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. *J Clin Oncol* 2008;**26**:1789–96.
46 Zhang W, Gordon M, Schultheis AM, et al. FCGR2A and FCGR3A polymorphisms associated with clinical outcome of epidermal growth factor receptor expressing metastatic colorectal cancer patients treated with single-agent cetuximab. *J Clin Oncol* 2007;**25**:3712–8.
47 Bibeau F, Lopez-Crapez E, Di Fiori F, et al. Impact of FcγRIIa-FcγRIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. *J Clin Oncol* 2009;**27**:1122–9.
48 Jacobs B, De Roock W, Piessevaux H, et al. Amphiregulin and Epiregulin mRNA expression in primary tumors predict outcome in metastastic colorectal cancer treated with cetuximab. Submitted for publication.
49 Roskoski R Jr. Vascular endothelial growth factor (VEGF) signaling in tumor progression. *Crit Rev Oncol Hematol* 2007;**62**:179–213.
50 Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971;**285**:1182–6.
51 Giantonio BJ, Catalano PJ, Meropol NJ, et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. *J Clin Oncol* 2007;**25**:1539–44.
52 Saltz LB, Clarke S, Diaz-Rubio E, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. *J Clin Oncol* 2008;**26**:2013–9.
53 Ince WL, Jubb AM, Holden SN, et al. Association of k-ras, b-raf, and p53 status with the treatment effect of bevacizumab. *J Natl Cancer Inst* 2005;**97**:981–9.
54 Jubb AM, Hurwitz HI, Bai W, et al. Impact of vascular endothelial growth factor-A expression, thrombospondin-2 expression, and microvessel density on the treatment effect of Bevacizumab in metastatic colorectal cancer. *J Clin Oncol* 2006;**24**:217–27.
55 Hurwitz HI, Yi J, Ince W, Novotny WF, Rosen O. The clinical benefit of Bevacizumab in metastatic colorectal cancer is independent of K-ras mutation status: analysis of a phase III study of Bevacizumab with chemotherapy in previously untreated metastatic colorectal cancer. *Oncologist* 2009;**14**:22–8.
56 Schneider BP, Wang M, Radovich M, et al. Association of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 genetic polymorphisms with outcome in a trial of paclitaxel compared with paclitaxel plus bevacizumab in advanced breast cancer: ECOG 2100. *J Clin Oncol* 2008;**26**:4672–8.
57 Allard WJ, Matera J, Miller MC, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with non-malignant diseases. *Clin Cancer Res* 2004;**10**:6897–904.
58 Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastastic breast cancer. *N Engl J Med* 2004;**351**:781–91.
59 Cohen ST, Punt CJA, Iannotti N, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J Clin Oncol* 2008;**26**:3213–21.
60 Popat S, Houlston RS. A systematic review and meta-analysis of the relationship between chromosome 18q genotype, DCC status and colorectal cancer prognosis. *Eur J Cancer* 2005;**41**: 2060–70.
61 Shibata D, Peinado MA, Ionov Y, Malkhosyan S, Perucho M. Genomic instability in repeated sequences is an early somatic event in colorectal tumorigenesis that persists after transformation. *Nat Genet* 1994;**6**:273–81.
62 Malesci A, Laghi L, Bianchi P, et al. Reduced likelihood of metastases in patients with microsatellite-unstable colorectal cancer. *Clin Cancer Res* 2007;**13**:3831–9.
63 Viguier J, Boige V, Miquel C, et al. ERCC1 Codon 118 polymorphism is a predictive factor for the tumor response to oxaliplatin/5-fluorouracil combination chemotherapy in patients with advanced colorectal cancer. *Clin Cancer Res* 2005;**11**:6212–7.
64 Braun MS, Richman SD, Quirke P, et al. Predictive biomarkers of chemotherapy efficacy in colorectal cancer: results from the UK MRC FOCUS trial. *J Clin Oncol* 2008;**26**:2690–8.