The role of personalized medicine in metastatic colorectal cancer: an evolving landscape

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Abstract: Advances in the treatment of metastatic colorectal cancer have led to an improvement in survival from 12 months with fluorouracil monotherapy to approximately 2 years. This is partly as a result of the addition of irinotecan and oxaliplatin, but is also due to the use of monoclonal antibodies against the epidermal growth factor receptor (EGFR) and antiangiogenic drugs such as bevacizumab. However, there are significant molecular differences between tumours which can affect both prognosis and response to treatment. Personalized medicine aims to tailor treatment according to the characteristics of the individual patient and is now a clinical reality as testing for *KRAS* mutations to guide treatment with the anti-EGFR monoclonal antibodies cetuximab and panitumumab is now part of routine clinical practice. However, not all patients who are *KRAS* wild type respond to anti-EGFR therapy and a validated biomarker for antiangiogenic therapy is still lacking. Therefore, other biomarkers are needed to assist with predicting response to both existing drugs as well as to drugs currently under investigation. This review summarizes the molecular biology of colorectal cancer, focusing on the genetic features that are currently most clinically relevant. Current and emerging biomarkers are reviewed along with their roles in selecting patients for targeted treatment with currently licensed therapies and drugs being evaluated in clinical trials. The value of predictive biomarkers of chemosensitivity and potential future treatment strategies are also discussed.

Keywords: biomarker, colorectal cancer, personalized medicine, targeted therapy

Introduction

Advances in the treatment of metastatic colorectal cancer (CRC) have led to an improvement in survival from 12 months with fluorouracil monotherapy to approximately 2 years [Cunningham *et al.* 2010]. Despite these advances, CRC remains the fourth most common cause of cancer death worldwide [International Agency for Research on Cancer, 2010] and therefore more effective treatments are urgently needed. Many new cancer drugs target specific molecular aberrations or cell-signalling pathways, but these drugs are only active in a subset of patients due to molecular differences between tumours. Consequently, a 'one-size-fits-all' approach to treatment is suboptimal and so there has been increasing interest in a more personalized approach to treatment.

Personalized medicine is defined by the US National Cancer Institute as 'a form of medicine that uses information about a person's genes, proteins, and environment to prevent, diagnose, and treat disease'. The potential benefits of this treatment approach include increased response rates and survival, as well as reduced toxicity [Diamandis *et al.* 2010]. In addition, the cost effectiveness of oncology treatment may be improved as expensive drugs can be given to the patients most likely to benefit [Frank and Mittendorf, 2013].

Biomarkers are characteristics that indicate a normal or pathogenic process or a response to a specific therapeutic intervention. Biomarkers may have prognostic and/or predictive value. Prognostic biomarkers provide information on the natural

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history of the patient's disease independent of treatment, whereas predictive biomarkers provide information on the likelihood of response to a particular treatment [Alymani *et al.* 2010].

There are many challenges to overcome in personalizing medicine. These include the cost of developing biomarker-related drugs and biomarker testing, standardization of testing (including specimen type, collection and storage), ethical issues occurring as a result of genetic testing, regulatory hurdles for biomarkers and the need to establish the benefit of targeted drugs over alternative approaches [Diamandis *et al.* 2010; Wistuba *et al.* 2011]. In addition, many targeted drugs are cytostatic rather than cytotoxic and therefore may not be optimally assessed by standard response criteria such as RECIST [Wistuba *et al.* 2011]. Despite these challenges, personalized medicine is increasingly becoming a reality. For example, in the UK, the Cancer Research UK stratified medicine programme is developing large-scale molecular diagnostic testing for National Health Service patients.

In this review we focus on the medical management of CRC. We summarize the molecular biology of CRC and discuss current and emerging biomarkers and their role in personalized medicine, including drugs currently being evaluated in clinical trials. We also review the importance of genomic stability, markers of chemosensitivity and the role of biomarkers in antiangiogenic therapy. Finally, we conclude by examining potential future treatment strategies.

Molecular biology of colorectal cancer

CRC is a complex, heterogeneous disease that involves multiple signalling pathways [Deschoolmeester *et al.* 2010] and tumours that appear histologically identical can have different prognoses and different responses to treatment [Ferte *et al.* 2010; Zlobec and Lugli, 2008]. The morphology of tumours and the pattern of molecular abnormalities vary depending on their anatomical location [Cancer Genome Atlas Network, 2012; Gervaz *et al.* 2004; Yamauchi *et al.* 2012], with a probable gradual change in molecular characteristics between the right and left side of the bowel [Yamauchi *et al.* 2012].

There are thought to be at least three main mechanisms by which CRC occurs. The majority of cancers start as adenomas, which then undergo other mutational events such as loss of the adenomatous polyposis coli (*APC*) gene and p53 mutations and result in the chromosome instability (CIN) phenotype [Greystoke and Mullamitha, 2012]. In contrast, patients with Lynch syndrome (hereditary nonpolyposis CRC) have germline loss of DNA mismatch repair genes, most commonly *MLH1* and *MLH2* [Sinicrope and Sargent, 2012]. This results in the accumulation of DNA defects, predominantly in microsatellite areas (areas of the genome where short sequences of nucleotide bases are repeated multiple times) and leads to the microsatellite instability high (MSI-high) phenotype [Grady and Carethers, 2008]. Lynch syndrome is uncommon, accounting for approximately 2–3% of CRC [Kerber *et al.* 2005]. However, approximately 15% of patients with CRC have extensive epigenetic changes resulting in the CpG island methylator phenotype (CIMP), which is relatively similar to the MSI-high hypermutated phenotype [Jenkins *et al.* 2007; Samowitz *et al.* 2005].

CRC is therefore frequently divided into two main groups: hypermutated patients with MSI (these are mainly right-sided tumours and are often associated with CIMP) and nonhypermutated patients with CIN [Cancer Genome Atlas Network, 2012]. There are significant differences in the molecular profile of these two groups. For example, both *TP53* (60% *versus* 20%, $p = 0.0001$) and *APC* (81% versus 51%, P= 0.0023) mutations are more frequently found in nonhypermutated cancers [Cancer Genome Atlas Network, 2012]. Other commonly mutated genes in nonhypermutated cancers include *KRAS*, *PIK3CA* and *NRAS*; whereas *MSH3*, *MSH6* and *BRAF* mutations are more frequently found in hypermutated cancers [Cancer Genome Atlas Network, 2012].

The Cancer Genome Atlas Network has recently published a comprehensive molecular characterization of CRC [Cancer Genome Atlas Network, 2012]. Specific signalling pathways or genes have been found to be commonly affected in CRC (see Figure 1). For example, almost 100% of tumours have changes in MYC transcriptional targets, 93% of all tumours have alterations in the WNT signalling pathway, 55% of tumours have alterations in KRAS, BRAF or NRAS and 33% of tumours have alterations in both the phosphoinositol 3 kinase (PI3K) and RAS pathways [Cancer Genome Atlas Network, 2012]. However, some mutations are mutually exclusive and therefore rarely occur in the same patient. For example,

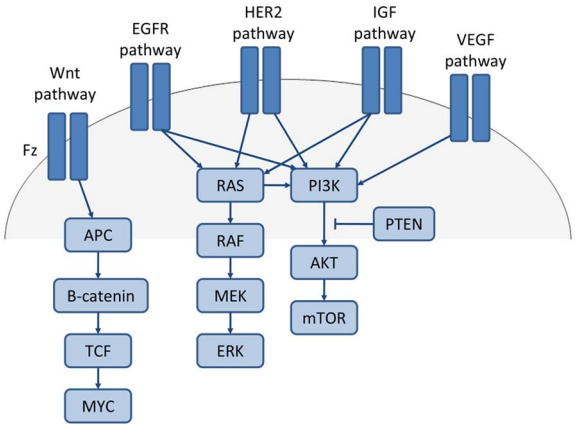

Figure 1. Cell signalling pathways in colorectal cancer. AKT, protein kinase B; APC, adenomatous polyposis coli; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; Fz, Frizzled receptor; HER2, human epidermal growth factor receptor 2; IGF, insulin-like growth factor; mTOR, mammalian target of rapamycin; MEK, mitogen-activated protein kinase kinase; MYC, v-myc myelocytomatosis viral oncogene homolog (avian); PI3K, phosphoinositol 3 kinase; PTEN, phosphatase and tensin homolog; TCF, T cell factor; VEGF, vascular endothelial growth factor.

although mutations in PIK3CA and BRAF or KRAS may coexist, *KRAS* and *BRAF* mutations are mutually exclusive [Tol *et al.* 2009b; Yokota *et al.* 2011] and insulin-like growth factor 2 (IGF2) amplification/overexpression is mutually exclusive with mutations in the PI3K signalling pathway [Cancer Genome Atlas Network, 2012]. Chromosomal changes are also relatively common and include loss of chromosome arms 18p and q (including SMAD4) and loss of 17p and q (including TP53) [Cancer Genome Atlas Network, 2012]. The deletion of other important tumour suppressor genes [including phosphatase and tensin homolog (*PTEN*)] as well as gene amplifications (including *IGF2* and *ERBB2*) have also been reported [Cancer Genome Atlas Network, 2012].

Tumour heterogeneity means that it is challenging to elucidate the roles of individual mutations [Markowitz and Bertagnolli, 2009] and poses significant challenges to personalized medicine [Gerlinger *et al.* 2012; Lee and Swanton, 2012; Oltedal *et al.* 2011]. There may be significant differences not only within the primary tumour, but also between the primary tumour and metastases [Gerlinger *et al.* 2012]. For example, Baldus and colleagues found that *KRAS* mutations were heterogeneous in 20% of tumours with *KRAS* mutations, and the results were particularly discordant when primary tumours were compared with lymph node metastases [Baldus *et al.* 2010]. Furthermore, anticancer treatment can affect tumour heterogeneity due to selection pressures [Gerlinger *et al.* 2012; Lee and Swanton, 2012; Oltedal *et al.* 2011].

This review will now discuss some of the main molecular characteristics that are of current or potential future significance in CRC.

Antiepidermal growth factor receptor therapies and the role of KRAS

One of the major advances in the treatment of CRC has been the development of targeted therapies. Amongst the most well established of these are the monoclonal antibodies cetuximab and panitumumab, which target the EGFR. Cetuximab has been shown to have efficacy both as monotherapy and in combination with chemotherapy for patients with pretreated metastatic CRC [Cunningham *et al.* 2004; Jonker *et al.* 2007; Saltz *et al.* 2004; Sobrero *et al.* 2008; Souglakos *et al.* 2007]. However, the situation is less clear in the first-line setting. A phase II trial showed encouraging results with the addition of cetuximab to capecitabine and oxaliplatin [Borner *et al.* 2008] and the phase III CRYSTAL study showed an improvement in progression-free survival (PFS) with the addition of cetuximab to FOLFIRI [Van Cutsem *et al.* 2009]. However, this was not confirmed by the phase III COIN and NORDIC-VII studies [Maughan *et al.* 2011; Tveit *et al.* 2012]. Whether cetuximab has a role in downsizing liver metastases has also been evaluated, with mixed results. The CELIM trial showed an improvement in the resectability rate with cetuximab compared with historical controls [Folprecht *et al.* 2010] and the CRYSTAL and OPUS trials also showed improvements in the R0 resection rate [Bokemeyer *et al.* 2012; Van Cutsem

et al. 2009]. However, this was not subsequently confirmed by the COIN trial [Maughan *et al.* 2011]. The results of the CALGB C80405 study, which is comparing the addition of cetuximab or bevacizumab to FOLFOX or FOLFIRI in patients with unresectable metastatic disease [ClinicalTrials.gov identifier: NCT00265850], are awaited and should hopefully help to answer this question.

The addition of panitumumab to first-line treatment in the phase III PRIME study resulted in an improvement in PFS of 1.6 months (hazard ratio 0.8, $p = 0.02$) [Douillard *et al.* 2010]. In addition, Peeters and colleagues demonstrated that panitumumab plus FOLFIRI improves PFS in the second-line setting [Peeters *et al.* 2010], and panitumumab monotherapy also results in a modest improvement in PFS compared with best supportive care [Van Cutsem *et al.* 2007]. The efficacy of cetuximab and panitumumab is broadly similar, although cetuximab is the more widely used.

However, not all patients respond to anti-EGFR therapies and a variety of molecular characteristics have been evaluated to see if they have a predictive role. The most established biomarker is the presence or absence of *KRAS* mutations. Mutations in KRAS, PIK3CA or BRAF result in the downstream activation of the RAS-mitogen-activated protein kinase (MAPK) or PI3K pathways irrespective of EGFR activation [Siena *et al.* 2009]. It is therefore logical that *KRAS* mutations could lead to resistance to anti-EGFR therapy and this was subsequently established by analyzing multiple, well designed phase III randomized controlled trials [Amado *et al.* 2008; Bokemeyer *et al.* 2009; Douillard *et al.* 2010; Karapetis *et al.* 2008]. However, the acceptance of the reliability and importance of KRAS status in predicting response to anti-EGFR therapy took several years to evolve [Blanke *et al.* 2011].

It is now routine clinical practice to test for the presence of *KRAS* mutations (which occur in approximately 45–50% of patients with CRC) [Yamauchi *et al.* 2012] and anti-EGFR treatment is only given to patients who are *KRAS* wild type. This is the first true use of personalized medicine in CRC. Although it has been suggested that not all *KRAS* mutations are equivalent and that patients with *KRAS* G13D mutations may benefit from anti-EGFR treatment [Tejpar *et al.* 2012], this has not subsequently been confirmed by a recent retrospective analysis of three phase III studies [Peeters *et al.* 2013]. Furthermore, not all patients who are *KRAS* wild type respond to anti-EGFR therapy and therefore there has been substantial research into other potential predictive biomarkers.

EGFR mutations are very rare in CRC and are not associated with response to treatment [Barber *et al.* 2004; Moroni *et al.* 2005] and positive EGFR protein expression by immunohistochemistry also does not predict response to treatment [Chung *et al.* 2005; Cunningham *et al.* 2004]. In contrast, increased EGFR copy number has been associated with response to anti-EGFR therapy in small retrospective studies [Laurent-Puig *et al.* 2009; Moroni *et al.* 2005]. However, EGFR copy number is not used in clinical practice to select patients for treatment, partly due to the lack of standardization of fluorescence *in situ* hybridization technology and scoring.

Increased expression of the EGFR ligands amphiregulin and epiregulin may generate an autocrine or paracrine loop that drives tumour growth [Bardelli and Siena, 2010] and have been shown in retrospective studies to be predictive of response to cetuximab [Adams *et al.* 2012; Jacobs *et al.* 2009]. An exploratory four-gene predictive classifier for response to cetuximab has been developed, which includes the genes encoding amphiregulin and epiregulin as well as the genes *DUSP6* (which encodes a dual-specificity phosphatase) and *SLC26A3* (which encodes an intestinal chloride ion transporter) [Baker *et al.* 2011]. Amphiregulin and epiregulin are not routinely measured in clinical practice and further evaluation of their role is required.

The EGFR tyrosine kinase inhibitors erlotinib and gefitinib have also been studied in CRC but results have been generally disappointing, with no objective responses seen with single agent erlotinib [Townsley *et al.* 2006] and no improvement in PFS or overall survival (OS) with the addition of gefitinib to FOLFIRI [Santoro *et al.* 2008]. In lung cancer, erlotinib and gefitinib are effective in patients with activating mutations of EGFR and therefore these results may reflect the rarity of *EGFR* mutations in CRC as these studies involved unselected populations. At the moment, erlotinib and gefitinib are not routinely used in CRC. However, this may change in the future as the phase III GERCOR DREAM trial showed that the addition of erlotinib to bevacizumab maintenance

after induction chemotherapy led to a small, but statistically significant improvement in PFS from 4.6 months to 5.8 months ($p = 0.005$) [Tournigand *et al.* 2012].

BRAF

It has been challenging to determine the prognostic role of BRAF due to its association with other prognostic variables such as MSI [Sclafani *et al.* 2012]. However, patients with *BRAF* mutations have been shown in a number of studies to have a significantly shorter PFS and OS [Bokemeyer *et al.* 2012; Di Nicolantonio *et al.* 2008; Tol *et al.* 2009b; Yokota *et al.* 2011]. For example, Tol and colleagues reported that the median OS for patients with *BRAF* mutation treated with capecitabine, oxaliplatin and bevacizumab was 15.0 months compared with 24.6 months for patients who were *BRAF* wild type.

As a consequence of the prognostic significance of *BRAF* mutations, it has also been difficult to clarify whether BRAF also has value as a predictive biomarker. Initial studies suggested that *BRAF* mutations are associated with resistance to anti-EGFR monoclonal antibody treatment [De Roock *et al.* 2010; Di Nicolantonio *et al.* 2008; Laurent-Puig *et al.* 2009; Loupakis *et al.* 2009b]. For example, in a retrospective analysis performed by De Roock and colleagues 2 of 24 (8.3%) patients with *BRAF* mutation responded to cetuximab compared with 124 of 326 (38%) of patients who were *BRAF* wild type [De Roock *et al.* 2010]. This led to the suggestion that BRAF status should be used in combination with KRAS to select patients who are suitable for treatment with anti-EGFR monoclonal antibodies [De Roock *et al.* 2010; Di Nicolantonio *et al.* 2008; Loupakis *et al.* 2009b]. However, a subsequent pooled analysis of the CRYSTAL and OPUS trials did not confirm BRAF as a predictive biomarker for response to anti-EGFR monoclonal antibodies [Bokemeyer *et al.* 2012].

Vemurafenib is a BRAF inhibitor that targets the *BRAF* V600E mutation, resulting in dramatic responses in patients with melanoma [Chapman *et al.* 2011]. Unfortunately, the effect of targeting BRAF in patients with CRC has, so far, been disappointing. In a phase I trial of single agent vemurafenib in patients with *BRAF* mutant CRC, only 1 of 19 patients had a partial response and 4 patients had minor responses (≥10% shrinkage) [Kopetz *et al.* 2010]. One possible explanation for the resistance to vemurafenib monotherapy in patients with *BRAF*-mutant CRC is that BRAF inhibition causes feedback activation of EGFR [Prahallad *et al.* 2012]. Combining BRAF and EGFR inhibition may therefore be more effective, and indeed this has resulted in a strong synergistic effect in preclinical studies [Higgins *et al.* 2012; Prahallad *et al.* 2012]. The combination of vemurafenib and cetuximab for patients with metastatic CRC is currently being evaluated in a dose-finding, multicentre study (EUDRACT number 2011-004426-10).

The PI3K pathway

The main alterations in the PI3K pathway in CRC are mutations in PIK3CA and loss of PTEN protein expression. These molecular alterations may coexist with *KRAS* and *BRAF* mutations and this makes it more challenging to ascertain their clinical significance [Bardelli and Siena, 2010]. However, *PTEN* loss correlates with advanced and metastatic tumours and has been associated with worse survival outcomes in CRC [Jang *et al.* 2010; Loupakis *et al.* 2009a; Sawai *et al.* 2008].

EGFR stimulates the PI3K pathway and therefore it would seem logical that alterations in the PI3K pathway might affect response to anti-EGFR treatment [Bardelli and Siena, 2010; Razis *et al.* 2008]. A number of studies have shown that *PIK3CA* mutations or *PTEN* loss are associated with a lack of response to anti-EGFR therapies and these alterations therefore appear to have a negative predictive role [Bardelli and Siena, 2010; De Roock *et al.* 2010; Laurent-Puig *et al.* 2009; Razis *et al.* 2008; Sartore-Bianchi *et al.* 2009a, 2009b].

It has been suggested that by combining the results of KRAS, BRAF and PIK3CA mutational analyses, more patients may be identified who are unlikely to respond to cetuximab [Spindler *et al.* 2011]. Patients with mutations in any of these three genes had a high risk of progression, whereas patients who were 'triple-negative' had had a response rate of 41% ($p < 0.001$) and a significantly higher PFS of 7.7 *versus* 2.3 months ($p < 0.000$). In a similar fashion, Sartore-Bianchi and colleagues proposed that up to 70% of patients who are unlikely to respond to panitumumab or cetuximab can be identified by testing patients for *PTEN* loss as well as mutations in these three genes [Sartore-Bianchi *et al.* 2009a, 2009b]. However, the situation may not be quite as

straightforward as this because different mutations can have different effects [Bardelli and Siena, 2010; Zhao and Vogt, 2008]. For example, mutations in exon 20 of *PIK3CA* have been associated with a low response rate to anti-EGFR therapy, whereas mutations in exon 9 do not appear to have this effect [De Roock *et al.* 2010].

Aspirin may block the PI3K pathway and a large study has shown that patients with *PIK3CA* mutant-resected CRC who took regular aspirin had improved cancer-specific and OS compared with patients with wild-type *PIK3CA* [Liao *et al.* 2012], leading to interest in aspirin as an adjuvant treatment. In addition, various drugs targeting the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway have been developed and are currently in clinical trials [Yu and Grady, 2012]. Unfortunately, although a phase II trial of capecitabine plus perifosine (an inhibitor of the PI3K/Akt/mTOR pathway) showed promising activity, the phase III X-PECT trial did not show any improvement with the addition of perifosine to capecitabine [Bendell *et al.* 2012]. However, further biomarker analysis may identify a subgroup of patients who may benefit from PI3K pathway inhibitors, as, for example, single-agent PI3K inhibitors have not been shown to be effective in patients with *KRAS* mutations [Di Nicolantonio *et al.* 2010; Ihle *et al.* 2009].

However, just as combination therapy incorporating a BRAF inhibitor holds greater promise than monotherapy, a combination approach to treatment with PI3K inhibitors may yield the greatest benefits. For example, combining PI3K/AKT inhibitors with a BRAF inhibitor results in a synergistic effect in colorectal cell lines [Mao *et al.* 2012] and the combination of MEK and PI3K/mTOR inhibitors is currently being evaluated in a phase I trial [ClinicalTrials.gov identifier: 01390818]. MEK inhibitors may also have a role in combination with other treatments. Hochster and colleagues recently reported encouraging results from the addition of the MEK inhibitor selumetinib to irinotecan [Hochster *et al.* 2012] and a trial of MEK inhibitors in combination with BRAF and EGFR inhibitors is currently being set up [ClinicalTrials.gov identifier: NCT01750918].

Human epidermal growth factor receptor 2

In contrast to breast and gastric cancer, human epidermal growth factor receptor 2 (*HER2*) gene amplification and HER2 protein overexpression is relatively rare in CRC. Kavanagh and colleagues reported that of 132 patients who underwent colorectal resections, 11% had HER2 overexpression and 3% had *HER2* amplification [Kavanagh *et al.* 2009]. A number of studies have investigated whether *HER2* gene amplification has a predictive role in the identification of patients who respond to anti-EGFR therapy, but results have been conflicting. Some studies have shown that *HER2* gene amplification was significantly related to resistance to cetuximab or panitumumab and was also associated with a significantly worse PFS and a trend towards a worse OS [Barbara *et al.* 2012; Finocchiaro *et al.* 2007]. However, other studies have not found a predictive or prognostic role for HER2 [Tol *et al.* 2010; Troiani *et al.* 2013].

Anti-HER2 therapy is used successfully in other cancer types, such as breast and gastric cancer. In CRC, preclinical studies have shown that combined HER2 and EGFR inhibition can induce long-lasting tumour regression [Bertotti *et al.* 2011] and that the HER2 dimerization inhibitor pertuzumab has some antitumour effect, particularly in combination with erlotinib [Pohl *et al.* 2009]. In clinical trials of anti-HER2 therapy initial results suggested that trastuzumab may have clinical activity (e.g. five out of seven patients with had a partial response to irinotecan plus trastuzumab), but the trials were limited by the low prevalence of HER2 amplification and closed prematurely due to low accrual [Clark *et al.* 2003; Ramanathan *et al.* 2004].

The insulin-like growth factor system

The IGF system activates a number of signalling pathways, including the Ras/Raf/MAPK pathway and the PI3K/Akt pathway [Scartozzi *et al.* 2010] and is therefore important for the growth of both normal and tumour cells [Vrieling *et al.* 2009]. Consequently, the IGF system is a target for a number of drugs currently in development. For example, dalotuzumab (MK-066) is a monoclonal antibody that binds to IGF-R1 and prevents activation of the receptor. Although dalotuzumab failed proof of concept at an interim analysis of a phase II/III trial in patients with metastatic CRC, biomarker analysis suggested that patients with rectal tumours expressing high IGF-1 levels and low IGF-2 levels may be a subgroup of patients who benefit from treatment and this is being evaluated in a phase IIA trial [ClinicalTrials.gov identifier: NCT01609231] [Watkins *et al.* 2011].

In addition, anti-EGFR therapy can lead to high levels of Akt overexpression and so theoretically the IGF system may be involved in resistance to anti-EGFR therapies [Hu *et al.* 2008]. This theory is supported by limited evidence showing higher response rates to cetuximab in patients who had IGF-1-negative tumours [Scartozzi *et al.* 2010].

Microsatellite instability

MSI is seen in approximately 15% of sporadic CRC and is usually mutually exclusive with CIN [Walther *et al.* 2008]. Patients with MSI have different outcomes following adjuvant chemotherapy [Sargent *et al.* 2010] and have a better prognosis than patients with CIN [Grady and Carethers, 2008]. As a result, MSI testing is increasingly being used in the adjuvant setting to facilitate decision making. However, the significance of MSI in the metastatic setting is not yet established. Nevertheless, patients with MSI may be suitable for targeted therapies that take advantage of the sensitivity of these tumours to drugs that cause specific types of DNA damage [Hewish *et al.* 2010]. For example, due to a synthetic lethal relationship, MSH2-defective tumours are highly sensitive to methotrexate [Martin *et al.* 2009] and this is the focus of a current phase II trial [ClinicalTrials.gov identifier: NCT00952016].

Antiangiogenic therapies

Antiangiogenic drugs have been used in CRC since 2004 and have improved patient outcomes. The first and most common of these is the monoclonal antibody bevacizumab, which has shown efficacy in both previously treated [Giantonio *et al.* 2007] and untreated metastatic CRC [Hurwitz *et al.* 2004; Kabbinavar *et al.* 2005; Saltz *et al.* 2008]. In addition, the continuation of bevacizumab beyond initial disease progression has been shown to improve survival [Grothey *et al.* 2008] and bevacizumab is also used in the neoadjuvant setting for patients with potentially resectable liver metastases [Gruenberger *et al.* 2008]. However, the addition of anti-EGFR therapies to chemotherapy plus bevacizumab was not successful, resulting in increased toxicity and reduced PFS [Hecht *et al.* 2009; Tol *et al.* 2009a]. The antiangiogenic drugs regorafenib (an oral multikinase inhibitor) and aflibercept (a recombinant fusion protein) have also been licensed by the US Food and Drug Administration) based on trials showing modest improvements in OS [Grothey *et al.* 2013; Van Cutsem *et al.* 2012].

In addition, a number of other antiangiogenic drugs that have been evaluated in clinical trials, with mixed results. For example, cediranib (a VEGFR inhibitor) showed comparable clinical efficacy to bevacizumab (although the predefined boundary for PFS noninferiority was not met) but was associated with a worse toxicity profile [Schmoll *et al.* 2012]. Similarly, the dual EGFR and VEGFR inhibitor vandetanib has also not shown efficacy in CRC [Morabito *et al.* 2010]. More encouraging, ramicirumab is an anti-VEGFR-2 monoclonal antibody that is currently being evaluated in a phase III trial [ClinicalTrials.gov identifier: NCT01183780] following promising phase II results [Garcia-Carbonero *et al.* 2012].

However, despite the increasing use of various antiangiogenic drugs and intense research efforts, there is a lack of evidence for validated biomarkers for response to antiangiogenic therapy. An association between the development of arterial hypertension and improvement in PFS was initially reported [Scartozzi *et al.* 2009], but this was not confirmed by other studies, including the BOXER study [Dewdney *et al.* 2012]. Similarly, early studies suggested that high serum levels of VEGF-A and TGF-β1 were associated with a poorer prognosis, but this was not confirmed by subsequent research [Pectasides *et al.* 2012]. Research is ongoing and potential promising biomarkers include baseline plasma osteopontin [Pectasides *et al.* 2012] and VEGF polymorphisms [Koutras *et al.* 2012], but it is not currently possible to personalize treatment with antiangiogenic therapies.

Personalizing chemotherapy

Various studies have investigated whether molecular differences between patients can predict response to standard chemotherapy drugs to facilitate a more personalized approach to chemotherapy. The main chemotherapeutic agents used in metastatic CRC are 5-fluorouracil (5-FU)/capecitabine, irinotecan and oxaliplatin and this section of the review discusses each of these in turn.

The main target of 5-FU is thymidylate synthase (TS). Tumours with low expression of TS are less proliferative and may therefore be associated with a better prognosis [Koopman *et al.* 2009a]. This may partly explain why studies evaluating TS expression and response to 5-FU show conflicting

results [Koopman *et al.* 2009a]. The metabolism of 5-FU is mediated by thymidine phosphorylase and dihydropyrimidine dehydrogenase, but again there are conflicting results regarding their role in response to treatment [Koopman *et al.* 2009a].

In the adjuvant setting, 5-FU-based chemotherapy is not effective in patients with MSI tumours and may even be detrimental [Ribic *et al.* 2003], and patients with loss of 18q (and therefore SMAD4 deletion) also appear to obtain less benefit from adjuvant 5-FU [Boulay *et al.* 2002], but whether this is relevant in the metastatic setting is not yet clear. In contrast, a small retrospective study of 72 patients suggested that MSI tumours are more responsive to irinotecan [Fallik *et al.* 2003], but this does not currently influence routine clinical practice for these patients.

Irinotecan is a topoisomerase-1 inhibitor and Topo1 is overexpressed in 43–51% of CRCs [Koopman *et al.* 2009a]. The large randomized FOCUS trial showed that patients with high levels of Topo-1 expression had improved OS with first-line combination chemotherapy compared with patients with low or moderate Topo1 levels [Braun *et al.* 2008]. Irinotecan is detoxified by the enzyme UGT1A1. However, although a homozygous polymorphism that leads to a reduction in UGT1A1 activity has been associated with increased irinotecan toxicity [Palomaki *et al.* 2009], this was not confirmed by the FOCUS trial [Braun *et al.* 2008] and there is no current evidence for the benefit or harm of modifying irinotecan regimes based on an individual patient's UGT1A1 genotype [Palomaki *et al.* 2009].

The excision nuclease ERCC1 is involved in the repair of platinum-induced DNA damage and early data suggested that there was an association between low ERCC1 expression and improved OS in patients with metastatic CRC who were treated with oxaliplatin [Shirota *et al.* 2001]. However, this was not confirmed in an analysis of the CAIRO phase III trial [Koopman *et al.* 2009b]. Another enzyme involved in the detoxification is glutathione-S transferase, and again, the relevance of specific polymorphisms is unclear as individual polymorphisms have been associated with both improved and reduced survival [Stoehlmacher *et al.* 2004; Sun *et al.* 2005].

Germline polymorphisms have the potential to be useful in personalizing chemotherapy to individual patients. However, these are not routinely used in clinical practice as many studies have shown conflicting results and most polymorphisms have not yet been thoroughly validated. In addition, other potential biomarkers such as *KRAS* and *BRAF* mutations are not predictive of response to chemotherapy and therefore chemotherapy is not yet tailored to individual patients [Richman *et al.* 2009].

Immunotherapy

There have been major recent advances in immunotherapy for other tumours, particularly with the use of the anti-CTLA-4 antibody ipilimumab in patients with melanoma. There are also encouraging results from monoclonal antibodies that target the programmed death 1 (PD-1) receptor and its ligand (PD-L1). Unfortunately, immunotherapy in CRC has, so far, been unsuccessful. No responses were seen in patients with CRC who were treated in early phase trials with ipilimumab [O'Mahony *et al.* 2007], anti-PD-1 antibodies [Topalian *et al.* 2012] or anti-PD-L1 antibodies [Brahmer *et al.* 2012] and therefore this does not appear to be a promising strategy in the treatment of CRC at this time.

Outcomes of a personalized medicine approach and future directions

The hypothesis underpinning a personalized medicine approach is that this will lead to improvements in clinical outcomes. Apart from the use of anti-EGFR therapies in patients who are *KRAS* wild type, initial results from clinical trials have been mixed. For example, in a nonrandomized phase I trial, 175 patients with one molecular aberration were treated with matched targeted therapy and 116 patients had unmatched therapy. The patients receiving matched therapy had a higher overall response rate (27% *versus* 5%), longer time to treatment failure (median 5.2 *versus* 2.2 months) and longer survival (median 13.4 *versus* 9.0 months) [Kurzrock *et al.* 2012]. However, a phase I trial showed no benefit in patients with advanced CRC in matching treatment to their current molecular profile [Dienstmann *et al.* 2012]. However, this study had important limitations. For example, some of the biomarkers were exploratory (e.g. many patients were treated with PI3K inhibitors based on PTEN expression levels), the targeted agents had different mechanisms of action, archival tumour specimens may not have reflected the patients' current molecular characteristics and because this was a phase I

study patients may have been treated at nonbiologically active doses.

In the future, clinical trials are likely to be smaller, involving a selected group of patients who are thought most likely to benefit from a specific treatment. It has been proposed that patients could act as their own controls, with the PFS on a targeted regime being compared with the PFS of their previous regime (the $N = 1$ concept) but this has inherent biases [Von Hoff *et al.* 2010].

In addition, due to the low frequency of specific molecular characteristics and the increasing number of potential biomarkers, new techniques are required to efficiently screen patients for suitability to molecularly targeted treatments. In the past, genetic sequencing was both expensive and time consuming and therefore was not extensively used to facilitate personalized treatment. The development of next-generation sequencing techniques means that it is now much cheaper and faster to perform genetic sequencing [Liu *et al.* 2012]. Genetic sequencing is therefore now a feasible approach in patients with cancer, but is not currently used outside of clinical trials. In the adjuvant setting, gene expression profiling (e.g. using the Oncotype DX colon cancer test or the ColoPrint assay) is emerging as a tool with potential prognostic potential [Kelley and Venook, 2011] but, as yet, there are no similar assays available for use in patients with advanced disease.

Conclusion

Personalized medicine has made some major advances in CRC, with KRAS testing now part of routine clinical practice. However, KRAS has some limitations as a biomarker and despite extensive research into other biomarkers for antiangiogenic drugs, chemotherapy and other targeted agents, these are not yet established in clinical practice. Therefore, truly personalized medicine in CRC currently remains an aspiration for the future rather than a clinical reality. However, it is likely that a molecular screening approach to treatment will become increasingly used in the future to fully characterize tumours and identify patients who are most likely to benefit from targeted treatments. This holds great promise for the improvement of patient outcomes, but brings its own logistical and financial challenges as well as new complexities, such as how to overcome tumour heterogeneity, how to interpret a patient's molecular profile to select the most appropriate treatment and how to prevent rapid development of treatment resistance.

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Conflict of interest statement

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