Targeted treatments in colorectal cancer: state of the art and future perspectives

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ABSTRACT
Targeted treatments have generated a lot of hope and hype in the treatment of gastrointestinal tumours. Indeed, the introduction of targeted treatments particularly for colorectal cancer has resulted in substantial improvements in tumour response and progression-free survival of patients. However, it is not fully understood how these agents act in patients, and the preclinical models are not appropriate to predict clinical efficacy. Here the current state of targeted treatments in colorectal cancer is reviewed, focusing on antiproliferal growth factor receptor (EGFR) and antiangiogenic strategies, describing how these agents fit into the therapeutic algorithm of this disease and discussing current understanding of the mechanism of action, biomarkers as well as future therapeutic strategies targeting multiple signalling pathways in colorectal cancer.

INTRODUCTION
Over the last 30 years multiple signalling networks have been discovered that play a major role in vitro and in vivo for the regulation of growth, metastases and survival of tumours. These data resulted in strategies to inhibit pathways selectively that are critical for tumours and generate novel, ‘targeted’ anticancer drugs. Ideally, these drugs exhibit only minor side effects due to expected tumour specificity.

Protein phosphorylation is a critical requirement of most processes involved in cancer biology. Therefore, it is not surprising that protein kinases are amongst the most popular drug targets in oncology to date. There are >500 protein kinases in the human genome. Currently ~30 kinases have been selectively targeted in clinical trials. Protein kinases initiate and propagate gastrointestinal cancer, for example the V600E mutation in B-Raf has been implicated in the carcinogenesis of a subgroup of colorectal and pancreatic cancers.1 2

Protein kinases such as MEK1/2, FRAP1, ribosomal S6 kinase, cyclin-dependent kinases, Aurora kinases and Polo-like kinases are critical for proliferation or tumour cell survival3 or are involved in tumour maintenance and invasion, such as the vascular endothelial growth factor receptor (VEGFR) and the fibroblast growth factor receptor (FGFR).

MECHANISMS OF TARGETING PROTEIN KINASES
There are several options to target a protein kinase. Receptor tyrosine kinases can be targeted by antibodies that block the receptor and thereby prevent ligand binding or by antibodies that bind to the ligand and prevent its interaction with the receptor. Another approach is the use of a decoy receptor that does not transmit signals and quenches the ligand. This approach is, for example, used as ‘VEGF trap’ to block VEGF signalling. VEGF trap is a fully human decoy receptor protein that consists of a fusion of the second immunoglobulin (Ig) domain of human VEGFR1 and the third Ig domain of human VEGFR2 with the constant region (Fc) of human IgG1.4 5

Small molecules are also used to block the catalytic activity of protein kinases. These inhibitors can be differentiated by their ability to recognise either the active conformation of a protein kinase (type I) or its inactive conformation (type II; eg, imatinib, sorafenib). They mostly compete with ATP for the ATP-binding site in the hinge region of the target kinase by mimicking the hydrogen bonds that are formed by the adenine ring of ATP.6 Other compounds allosterically inhibit the catalytic activity by binding outside the ATP-binding site, or by binding covalently to the active site of the kinase (type IV), respectively.3

TARGETING PROTEIN KINASES IN GASTROINTESTINAL TUMOURS: A ROAD TO SUCCESS?
So far the most striking success using targeted treatments has been achieved in tumours that are characterised by a single genetic defect in one protein kinase that renders the kinase constitutively active. Examples are the inhibition of Bcr-Abl in chronic myelogenous leukaemia and the inhibition of c-Kit in gastrointestinal stroma tumours (GISTs) both by the small molecule imatinib. The striking efficacy of imatinib in these tumours has given the drug the status of a ‘magic bullet’.

Box 1 Receptor tyrosine kinases can be targeted by various mechanisms:

- Antibodies that bind to the receptor and block ligand binding
- Antibodies that bind to the ligand and thereby prevent its binding to the receptor
- Decoy receptors that bind the ligand
- Small molecules that compete with ATP for the ATP-binding site in the hinge region of the target kinase
Unfortunately, most solid tumours exhibit far more than one genetic alteration in a particular protein and their malignant phenotype also results from numerous epigenetic alterations. Furthermore, in many solid tumours protein kinases are not mutated, but act in a cell type-, context- and/or even compartment-specific manner.

Recent evidence shows that an average of 63 genetic alterations can be found, for example, in pancreatic cancers. Many of these genes can be allocated to ~12 major signalling networks. Consequently, it is unlikely that targeting of a single signalling pathway will eradicate such a tumour. In addition, widely used models to evaluate the efficacy of a targeted treatment—tissue culture or rapidly growing xenograft tumours—often do not reflect the situation in man, and results obtained in clinical trials frequently differ from the preclinical data.

COLORECTAL CANCER (CRC)
CRC is the second leading cause of cancer death in the Western world in males and females. Over the last years CRC mortality has progressively decreased—which is probably due to the earlier diagnosis through screening, an improvement in surgical procedures and both more efficacious chemotherapy and radiotherapy approaches. The better knowledge of the molecular biology of CRC and the resulting therapeutic strategies increasingly have a positive impact on survival of patients with CRC, but there is a need to optimise and define the best use of these novel approaches.

Currently, two major strategies of targeted treatments are used in CRC in clinical practice: inhibition of the epidermal growth factor receptor (EGFR) by the monoclonal antibodies cetuximab and panitumumab and blocking angiogenesis using bevacizumab, a monoclonal antibody directed against VEGF. Further therapeutic approaches have entered clinical trials in CRC, indicating both the medical need for further improvement and the ongoing role of CRC as a ‘model’ tumour entity for the development of targeted treatments in epithelial malignancies.

INHIBITION OF EGFR SIGNAL TRANSDUCTION IN CRC
Receptor tyrosine kinases are transmembrane glycoproteins that play a crucial role in cellular signalling regulating proliferation, transformation, apoptosis, angiogenesis and migration. The EGFR is a member of the family of transmembrane protein kinase receptors known as the erbB or HER receptor family that comprises EGFR (HER1 or erbB1), erbB2 (HER2), erbB3 (HER3) and erb4 (HER4). Several EGFR ligands have been identified including EGF, heparin-binding EGF-like growth factor, transforming growth factor (TGF), epiregulin, betacellulin and amphiregulin. These ligands induce dimerisation and autophosphorylation of the receptor and therefore initiate intracellular signalling pathways linked to cellular proliferation, control of apoptosis and angiogenesis. About 80% of all CRCs exhibit EGFR expression or even overexpression that correlates with reduced survival and increased metastases. Therefore, various strategies targeting the EGFR were examined in metastatic CRC (mCRC).

EGFR signal transduction can be blocked by all approaches described above. At present, in CRC most clinical data are available from receptor-blocking monoclonal antibodies such as cetuximab and panitumumab.

CETUXIMAB
Cetuximab in refractory mCRC
Cetuximab is a recombinant chimeric monoclonal IgG1 antibody that specifically binds the extracellular domain of the human EGFR and blocks binding of endogenous ligands such as EGF and TGFβ. This results in inhibition of EGFR signalling. In preclinical models, cetuximab exhibited anticancer effects both alone and in combination with chemotherapy. Clinically, cetuximab given as single agent in 568 pretreated patients refractory to chemotherapy, of whom >80% had received at least three preceding treatment lines, has shown efficacy, with partial responses in 8% of patients, whereas disease stabilisation occurred in >30%. In a phase III trial, cetuximab was tested versus best supportive care (BSC) only, and administration of the antibody also resulted in improvement of progression-free survival (PFS) and overall survival (OS) (see table 1).

Furthermore, cetuximab can restore chemotherapeutic sensitivity in irinotecan- or oxaliplatin-refractory tumour xenografts and thereby acts as a chemosensitiser. This chemosensitising effect could at least in part be due to the inhibition of antiapoptotic properties of the EGFR pathway that otherwise lead to chemotherapy resistance of these tumours. A phase II trial based on the preclinical findings in patients with EGFR-expressing, irinotecan-refractory CRC demonstrated an objective tumour response (overall response rate (ORR)) in 10% of the patients receiving cetuximab alone, whereas the ORR was 19% for the patients receiving irinotecan and cetuximab. These results were confirmed in a second trial by Cunningham and colleagues in 329 patients with irinotecan-refractory mCRC: ~40% of the patients in this trial had received three or more previous chemotherapeutic regimens. The objective response rate was 22.9% in patients receiving cetuximab plus irinotecan and 10.8% in patients receiving single agent cetuximab. The median time to tumour progression was 4.1 months in combination treatment and 1.5 months in the monotherapy arm. However, there was no significant survival difference in both arms, most probably due to crossover upon progression from the cetuximab into the combination arm. Age, performance status, gender and number of previous treatments did not affect the outcome.

In a large, multinational phase IV study in a community practice setting these findings were confirmed in 1147 patients who received irinotecan in different schedules (weekly, or every 2 or 3 weeks): the response rate and rate of patients without tumour progression at 12 weeks were 20% and 61%, respectively, whereas the median survival...
was 9.2 months. However, toxicity profiles favoured the fortnightly administration. Therefore, cetuximab plus irinotecan has become a standard treatment in refractory mCRC.

In the phase III EPIC study, the combination of cetuximab with single agent irinotecan as second-line treatment also showed an improvement in ORR and median PFS compared with irinotecan alone in patients with mCRC refractory to 5-fluorouracil (5-FU)/oxaliplatin: ORR was 16.4% vs 4.2% (p < 0.001) and median PFS was 4 months vs 2.6 months (HR 0.69, p < 0.001), respectively. Again, due to the crossover design, there was no benefit in OS which was remarkably long, exceeding >10 months in both arms. Therefore, from the clinical perspective, it remains unclear whether this combination should be offered to most of the patients in second-line treatment or whether the sequential strategy with the use of cetuximab/irinotecan after failure of irinotecan should be employed.

Cetuximab in first-line combinations with chemotherapy

In chemotherapy-naive patients, cetuximab has also been shown to improve efficacy parameters in combination with standard chemotherapy regimens such as 5-FU/folinic acid (FA) or capecitabine plus oxaliplatin and irinotecan in numerous phase II trials. ORR according to RECIST ranged from 54% to almost 80%, with OS exceeding 30 months, probably indicating a selection of favourable patients. However, in 2007, results from two randomised trials confirmed the increase of ORR compared with chemotherapy alone, in ‘K-ras-unselected patients’, from today’s perspective. In an international randomised phase II trial, the ORR of the combination of FOLFOX4 with cetuximab (the so-called OPUS trial) was 46% (vs 36% for chemotherapy alone; p = 0.064). However, PFS was not improved. In contrast, the combination of cetuximab with the FOLFIRI protocol (the CRYSTAL trial) resulted in a significant improvement of both the PFS (median PFS from 8 to 8.9 months; HR 0.851, 95% CI 0.726 to 0.998) and ORR (39% vs 47%; p = 0.004) (24; see table 2).

Specific side effects of cetuximab

The most common side effect of EGFR-targeting treatments is skin rash. Papulopustulous rash and

| Table 2 | Randomised trials with cetuximab in treatment of mCRC |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| n | RR (%) | P Value | PFS Median | HR | p Value | OS Median | HR | p Value |
| CRYSTAL First-line, ITT population | | | | | | | | |
| FOLFI | 599 | 39 | 0.004 | 8.0 | 0.85 | 0.048 | 18.6 | 0.93 | 0.31 |
| FOLFI+ cetuximab | 599 | 47 | 8.9 | 19.9 | | | | |
| OPUS | | | | | | | | |
| FOLFOX4 | 168 | 36 | 0.064 | 7.2 | 0.93 | 0.617 | NA | | |
| FOLFOX4-cetuximab | 169 | 46 | 7.2 | 19.3 | | | | |
| CRYSTAL First-line, K-ras population | | | | | | | | |
| FOLFI | 176 | 43 | 0.003 | 8.7 | 0.68 | 0.017 | 21.0 | 0.84 | 0.22 |
| FOLFI + cetuximab | 172 | 59 | 9.9 | 24.9 | | | | |
| FOLFI | 87 | 40 | 0.46 | 8.1 | 1.07 | 0.75 | 17.7 | 1.03 | 0.85 |
| FOLFI + cetuximab | 105 | 36 | 7.6 | 17.5 | | | | |
| OPUS | | | | | | | | |
| FOLFOX4 | 73 | 37 | 0.011 | 7.2 | 0.57 | 0.016 | | | |
| FOLFOX4 + cetuximab | 61 | 61 | 7.7 | 5.5 | | | | |
| FOLFOX4 | 47 | 49 | 0.10 | 8.6 | 1.83 | 0.019 | | | |
| FOLFOX4 + cetuximab | 52 | 33 | 5.5 | 5.5 | | | | |

ITT, intention to treat; mCRC, metastatic colorectal cancer; mut, mutated; NA, not available; OS, overall survival; PFS, progression-free survival; WT, wild type.
exposure. This particular side effect is not detectable in bodies against cetuximab were detectable in a hypersensitivity reaction to cetuximab, IgE antibodies against cetuximab, IgE antibodies against cetuximab were detectable in pretreatment samples. This indicates that patients are likely to be already sensitised to galactose-α,1,3-galactose and develop an allergic reaction upon re-exposure. This particular side effect is not observed with panitumumab, a fully human antibody produced by human hybridoma cells.

**PANITUMUMAB**
Panitumumab is a human monoclonal antibody directed against the EGFR. In a phase III trial a significant improvement in median PFS was demonstrated with panitumumab compared with BSC in an unselected group of patients with chemotherapy-refractory mCRC. (5 weeks in the panitumumab group vs 7.3 weeks in the BSC group; HR, 0.54; 95% CI 0.44 to 0.66, p <0.0001). Tumour response was similar to the findings with cetuximab (10% in the panitumumab group vs 0% in the BSC group). Probably related to the crossover design of the trial, OS did not differ.

In the panitumumab group there were remarkable differences in PFS and tumour response between individual patients, which raised the question of why certain groups of patients do benefit whereas others do not benefit from this concept and whether there might be biomarkers allowing prediction of the response to this treatment. Therefore, this trial opened the box for the further intensive search for biomarkers, with the change of landscape after identification of K-ras.

**PREDICTION OF RESPONSE TO EGFR TARGETING**
**The role of EGFR expression and function in CRC**
Similarly to Her2/neu staining in breast cancer, the expression status of the EGFR as determined by immunohistochemistry (IHC) was initially regarded as a useful prerequisite to predict a response to anti-EGFR treatment in patients with CRC. Consequently, the first clinical trials using anti-EGFR antibodies focused largely on EGFR-expressing CRCs, and positive EGFR staining of the tumour was mandatory for a patient to be eligible for anti-EGFR treatment. However, various clinical trials revealed that the response to cetuximab can be low in patients with EGFR-expressing tumours, whereas significant tumour responses to cetuximab were observed in patients whose tumours did not express EGFRs as determined by IHC. Neither the total number of EGFR-expressing tumour cells nor the number of receptors per cell as indicated by the staining intensity of individual cells correlated with tumour response in both the monotherapy and combinations with chemotherapy, and in pretreated as well as chemotherapy-naive patients. Thus, the IHC analysis of the EGFR status in a tumour specimen does not allow prediction of whether a patient will benefit from anti-EGFR treatment.

The activity of a given intracellular signalling pathway can be determined even in tumour samples given that there are activation-specific antibodies that allow determination of the phosphorylation of critical sites in a kinase that indicate its catalytic activity. The ERK and Akt signalling cascades are important downstream targets of the EGFR, and their activity status does reflect to some degree the activity of the EGFR. The activity of ERKs and Akt in CRCs as determined by phosphospecific antibodies correlates poorly with expression of the EGFR. This reflects to a certain degree the redundancy in the upstream signal transducers that converge on these kinase networks. In addition, the activity of these kinases as well as expression of the EGFR may be relevant only in certain parts of a tumour, for example at the leading edge. This potential heterogeneity would require the examination of a larger part of the tumour, making this type of analysis far more complex.

**EGFR gene copy number**
It has been suggested that an increase in EGFR copy number in the tumour correlates with response to an anti-EGFR treatment. Indeed, examination of EGFR gene copy numbers (GCNs) yields positive predictive values between 40 and 48.3% and negative predictive values between 81 and 96.3% in CRC. A mean GCN >2.83 provides an area under the curve (AUC) of 0.71. However, there is a certain degree of heterogeneity when the mean EGFR-GCN is repeatedly determined within tumours that can result in misclassifications of the FISH (fluorescence in situ hybridisation) status in patients when a certain cut-off is used. Thus, the clinical usefulness of EGFR gene copy number assessment is so far limited and not routinely performed.

**Response prediction of EGFR targeting in CRC: prediction by side effects?**
The major side effect of anti-EGFR antibody treatment is skin toxicity. This is not surprising since the EGF sustains the integrity of the epidermis. Skin rash, fissures and paronychia are frequently observed. Higher grades of skin toxicity involve pain and secondary infections. The degree of skin toxicity seems to mirror the tumour response to anti-EGFR treatment. A subgroup analysis of clinical trials in patients receiving cetuximab monotherapy revealed that there was virtually no tumour response or improvement in median survival in patients that do not develop a skin rash. The precise underlying mechanism for this correlation is as yet unclear. An EGFR intron 1 polymorphism is likely to be associated with response to EGFR inhibitors and may provide...
an explanation as to why the development of skin toxicity is associated with a favourable outcome in patients treated with these agents.\(^{33}\) Modern approaches using ‘pre-emptive’ skin toxicity management can lower the rate of severe skin toxicity in response to anti-EGFR targeting, without compromising the therapeutic efficacy.

**K-RAS MUTATIONS AS A PREDICTIVE MARKER FOR ANTI-EGFR THERAPEUTIC STRATEGIES IN CRC**

K-ras belongs to the group of Ras-GTP-binding proteins that also comprises N-ras and H-ras. Ras-GTPases cycle between an active, GTP-bound and an inactive, GDP-bound state. They play an important role as mediators in the signal transduction pathways induced by receptor tyrosine kinases including the EGFR. K-ras is mutated in numerous tumours. Mutations are found particularly in codons 12 and 13 of the K-ras oncogene and lead to constitutive activation of the K-ras protein that now induces intracellular signalling pathways independently of upstream regulators. In CRC, activating K-ras mutations are detectable in \(-40\%\) of all tumours and occur early during the adenoma–carcinoma sequence.\(^{34} 35\) K-ras mutations can be analysed in formalin-fixed paraffin-embedded tissue. Interestingly, there is a 95\% match in the K-ras status between the primary tumour and metastases, and therefore material from both sources can be used for analysis.\(^{36}\) Even punch biopsies or single tissue slides can be used for the analysis after microdissection. Analysis can be performed by conventional sequencing or quantitative PCR.

Preclinical data suggested that the anti-proliferative effect of an anti-EGFR treatment is compromised upon constitutive activation of the K-ras oncogene. Similar data demonstrating that K-ras mutations in CRC are predictors of resistance to anti-EGFR treatment were also obtained in retrospective analyses of small clinical trials\(^{37}\) and finally confirmed in larger cohorts of patients receiving cetuximab\(^{38}\) or panitumumab.\(^{39} 41\)

In a trial comparing panitumumab with BSC in chemotherapy-refractory patients, the ORR was 17\% in patients with K-ras wild-type tumours and 0\% in patients with K-ras-mutated tumours. Median PFS was 12.3 and 7.4 weeks, for patients with K-ras wild-type or mutated tumours upon treatment with panitumumab. Consequently, in the group with K-ras-mutated tumours, there was no benefit from treatment with panitumumab compared with BSC\(^{42}\) (table 1). Thus, panitumumab was approved by the European Medicines Agency exclusively for the treatment of patients with K-ras wild-type, chemorefractory mCRC.

Similarly, cetuximab failed to induce chemosensitisation upon combination with irinotecan in patients with chemorefractory CRC with K-ras mutations in a small cohort of patients.\(^{38}\) These findings were confirmed in a randomised phase III trial versus BSC in chemorefractory patients\(^{42}\): only the group with K-ras wild-type tumours and cetuximab treatment exhibited an improved prognosis, with a median OS of 9.5 months vs 4.5 months for the patients with K-ras-mutated tumours, which again did not differ from the BSC group.

The studies using cetuximab in combination with a first-line standard chemotherapy regimen, FOLFOX4 (OPUS trial) or FOLFIRI (CRYSTAL trial) addressed in a retrospective analysis the issue of whether K-ras mutations also confer anti-EGFR resistance to chemotherapy-naïve patients with mCRC. The data in the whole study population showed that the addition of cetuximab to both regimens improved the ORR and, in the larger trial, the median PFS. However, a marked improvement in ORR and median PFS was only observed in the K-ras wild-type population in both trials (ORR: cetuximab: OPUS, 61\% vs 57\%, \(p=0.011\); CRYSTAL, 59\% vs 43\%, \(p=0.003\); median PFS: cetuximab: OPUS, 7.7 vs 7.2 months, \(HR=0.57\), \(p=0.016\); CRYSTAL, 9.9 vs 8.7 months, \(HR=0.68\), \(p=0.017\); table 2). In contrast, addition of cetuximab to either FOLFOX or FOLFIRI did not result in any benefit for patients with K-ras-mutated tumours (ORR: cetuximab: OPUS, 33\% vs 49\%, \(p=0.106\); CRYSTAL, 36\% vs 40\%, \(p=0.46\); median PFS: cetuximab: OPUS, 5.5 vs 8.6 months, \(HR=1.83\), \(p=0.0192\); CRYSTAL, 7.6 vs 8.1 months, \(HR=1.07\), \(p=0.47\). Indeed, in the group of patients with K-ras-mutated tumours the median PFS was significantly shorter when cetuximab was combined with the FOLFOX4 regimen.\(^{35} 43\) In both trials the K-ras status did not affect the prognosis of patients receiving chemotherapy alone. Thus, these data suggest that the K-ras status is in this setting predominantly a predictive marker for therapeutic strategies directed against the EGFR.

**How do K-ras mutations affect EGFR signalling?**

At present it is largely unknown why tumours with K-ras mutations are refractory to anti-EGFR therapeutic strategies in CRC. This is likely to be a multifactorial issue. Constitutively active K-ras provides a continuous signalling input to the tumour cells. However, most of the assays currently used to determine activity of a certain pathway are not sensitive enough to provide a clear signalling signature of endogenous K-ras. Many preclinical data on the signalling network of mutated Ras-GTPases have been obtained in models overexpressing mutated ras isoforms. These results have to be viewed critically since an overexpressed oncogene is likely to recruit downstream signalling pathways that are not addressed under physiological levels of expression. Thus, the precise signalling mechanisms induced by endogenously expressed, constitutively active K-ras are poorly understood.

Work in transgenic animals demonstrates substantial phenotypic differences between mutant K-ras and N-ras and suggests that the oncogenic phenotype of mutant K-ras might be mediated by non-canonical effector pathways of the c-Raf serine/threonine specific kinase.\(^{33}\) This opens up the possibility to break anti-EGFR resistance in K-ras-mutated tumours using combinations of targeted treatments. Finally, recent evidence demonstrates that the dynamic range of a signal has a major...
impact on a signalling network and may be even more important that signal strength.\textsuperscript{44} The tonic level of signalling provided by constitutively active K-ras could sensitize cells to certain other signalling inputs and/or desensitize distinct pathways, resulting in a survival or migration advantage for the tumour cells.

FURTHER BIOMARKERS FOR ANTI-EGFR TARGETING

Mutations in the B-raf gene

Mutations in the B-raf gene, in particular the V600E mutation, are found in 8–10\% of CRCs. They appear to be more frequent in tumours with infiltrating lymphocytes, location in the proximal colon, poor histological grade and mucinous appearance. Interestingly, mutations in B-raf and K-ras are mutually exclusive.\textsuperscript{45} Recently, it has been shown that mutated B-raf (V600E) provides proliferation and survival signals in MSI (microsatellite instability) colorectal carcinoma cells.\textsuperscript{46} So far, there is conflicting evidence on whether B-raf mutations in CRCs are predictive for an anti-EGFR treatment. In a retrospective analysis, a B-raf V600E mutation was detected in 11 of 79 K-ras wild-type metastatic CRCs. None of the B-raf-mutated tumours responded to treatment with cetuximab or panitumumab and none of the responders carried B-raf mutations in their tumours. Patients with B-raf-mutated tumours had a significantly shorter PFS (p=0.011) and OS (p<0.0001) than wild-type patients.\textsuperscript{47} However, in a retrospective analysis of the CRYSTAL trial, B-raf mutations were shown to be only prognostic, but not predictive for a response to the treatment with cetuximab.\textsuperscript{48}

PTEN EXPRESSION

Another potentially predictive biomarker for anti-EGFR therapeutic strategies is the PTEN (phosphatase and tensin homologue deleted on chromosome 10) expression status. PTEN is a phosphatase that dephosphorylates phosphoinositide (PI3) and thereby controls activity of the PI3 kinase (PI3K) pathway. Loss of PTEN by monallelic or biallelic inactivation or epigenetic silencing results in constitutive activation of the PI3K signalling network and in apoptosis resistance. In vitro, colon cancer cell lines with activating mutations of PI3K or loss of PTEN are more resistant to cetuximab or panitumumab and none of the responders carried B-raf mutations in their tumours. Patients with B-raf-mutated tumours had a significantly shorter PFS (p=0.011) and OS (p<0.0001) than wild-type patients.\textsuperscript{47} However, in a retrospective analysis of the CRYSTAL trial, B-raf mutations were shown to be only prognostic, but not predictive for a response to the treatment with cetuximab.\textsuperscript{48}

WHAT CONSEQUENCES RESULT FROM THESE DATA?

The K-ras status in CRC is one of the best predictive biomarkers we have so far for any targeted treatment. Before treatment of CRC with EGFR-blocking antibodies the K-ras status of the tumour must be analysed. Only patients with K-ras wild-type CRC should be treated with antibodies directed against the EGFR. This applies to monotherapy as well as any combination of anti-EGFR antibodies with chemotherapy, and for all lines of treatment. Due to the high concordance of the K-ras status between the primary tumour and its metastases, both tissues can be used for K-ras analysis. K-ras mutational status is a negative predictive marker—that is, the K-ras status helps to determine who is not going to benefit from an anti-EGFR antibody treatment. This does not mean that every patient with a tumour lacking a K-ras mutation will benefit from this treatment. A B-raf mutation is likely to constitute a negative prognostic marker for mCRCs, but does not seem to predict the response to an anti-EGFR treatment.

The best positive predictive marker for anti-EGFR strategies so far is skin toxicity. Skin toxicity most probably corresponds to a specific property of the patient, not of the tumour. This could explain why patients with K-ras-mutated tumours can develop substantial skin toxicity in response to anti-EGFR treatment, but still do not benefit from this treatment.\textsuperscript{36} A number of somatic mutations in the EGFR gene have been identified that are associated with increased activity of EGFR tyrosine kinase inhibitors. However, no EGFR mutations could be detected in CRC.\textsuperscript{54} In patients with CRC, the correlation of skin toxicity with tumour response could be due to polymorphisms of the EGFR receptor. Indeed, polymorphic variations in intron 1 of the EGFR gene, namely the number of CA single sequence repeat in intron 1, have been associated with a high frequency of skin toxicity in patients with CRC receiving an EGFR inhibitor.\textsuperscript{53}

FURTHER RECEPTOR TYROSINE KINASES: THE INSULIN-LIKE GROWTH FACTOR I RECEPTOR (IGF-IR)

The IGF pathway consists of three ligands (insulin, IGF-I and IGF-II), six receptors (with IGF-I and IGF-IIR as the most important) and up to seven ligand–receptor regulating binding proteins (IGFBP 1–7).\textsuperscript{55} IGF-I and IGF-II are major ligands of the IGF-IR and are involved in carcinogenesis, tumour progression and metastasis.\textsuperscript{56} The IGF-IR induces major signalling cascades including the MEK–ERK,
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the Akt/PISK/mTOR (mammalian target of rapamycin) and the Src pathway that stimulate cell cycle progression, proliferation, angiogenesis, cell migration and invasion and prevent apoptosis. IGFs and the IGF-IR are overexpressed in a wide variety of tumours including CRC.\textsuperscript{57} High-level IGF-IR expression in CRC could be initiated by an abnormality of stem cell programmed differentiation in the aberrant crypt focus.\textsuperscript{58} IGF-IR promoter polymorphisms—that is, shorter IGF-1 CA-repeat lengths—have been related to an increased risk for CRC in patients with hereditary non-polyposis colorectal cancer (HNPCC).\textsuperscript{59}

There are various strategies to block the IGF-IR: monoclonal antibodies that block ligand binding, such as CP-751,871, IMC-A12 or AMG479, or tyrosine kinase inhibitors such as NVP-ADW742, BMS-536924, and a non-ATP antagonist INSM18. AMG479\textsuperscript{60} \textsuperscript{61} is currently being examined in a phase II trial in patients with K-ras mutant, chemorefractory mCRC in combination with FOLFIRI (NCT00813605). A further trial is examining another monoclonal antibody against the IGF-IR, CP-751,871 in patients with refractory mCRC versus the combination of FOLFIRI with AMG-655, a fully human agonist monoclonal antibody that binds human TRAIL-R2 (NCT008560560).

Significant cross-talk has been observed between the IGF and EGF pathways. This cross-talk provides a rationale for combined treatments against these pathways, to improve antitumour activity.\textsuperscript{62} A randomised phase II/III study of MK-0646 in combination with cetuximab and irinotecan is currently recruiting (NCT00925015).

**TARGETING TUMOUR ANGIogenesis**

Angiogenesis, the development of novel vessels from pre-existing vessels, plays a role not only in developmental biology but also in cancer. According to Folkman’s hypothesis, tumours establish a vascular network of their own once they reach a critical size (usually ~2 mm\textsuperscript{3}) to provide enough nutrients and oxygen. Already in the 1970s the concept of inhibiting tumour growth by preventing angiogenesis had emerged.\textsuperscript{63} \textsuperscript{64} With the discovery of VEGF, which stimulates growth of endothelial cells via activation of receptor tyrosine kinases,\textsuperscript{65} \textsuperscript{66} an interesting target was available to block tumour angiogenesis.

The major players in tumour angiogenesis are the secreted glycoproteins VEGFA, VEGFB, VEGFC, VEGFD and VEGFE, the placental growth factors (PIGF)-1 and -2 and their respective receptors VEGFR1 (also called Flt-1), VEGFR2 (also called Flk/KDR) and VEGFR3 (or Flt-4). VEGFR2 is mainly expressed in the vasculature and is the key mediator of VEGF-induced angiogenesis. In contrast, VEGFR1 has a higher binding affinity for VEGF but induces less potent intracellular signalling cascades and can thereby act as a negative regulator of angiogenesis. VEGFR3 plays a role in the development of vascular networks during embryogenesis and postnatal lymphangiogenesis.\textsuperscript{66} In addition, there are co-receptors for VEGFRs termed neuropilins that increase the binding affinity of VEGF for VEGFR.

VEGFA has several isoforms due to alternative splicing, and the 164 amino acid VEGFA165 is the predominant isoform.\textsuperscript{66} VEGFA binds to VEGFR1 and VEGFR2 and is secreted by many tumours as well as stroma cells. VEGF promotes proliferation, survival and migration of endothelial cells and thereby angiogenesis. Furthermore, VEGF mediates numerous prosurvival pathways in endothelial cells including induction or activation of Akt, Bcl2, survivin and IAPs (inhibitor of apoptosis proteins). In addition, VEGF increases the permeability of existing vessels (thereby increasing the interstitial pressure in tumour tissues), chemotaxis and homing of bone marrow-derived endothelial precursors as a prerequisite for metastasis. Furthermore, VEGF induces immune suppression and has autocrine effects on tumour cells due to the fact that VEGFRs are also expressed in tumour cells, for example in CRC and pancreatic cancer. Thus, VEGF can act as a direct growth factor on tumours, and VEGF targeting can also have a direct impact on tumours.\textsuperscript{67} \textsuperscript{68}

In the 1990s it could be demonstrated that blocking VEGF using a monoclonal antibody results in a marked inhibition of both tumour angiogenesis and tumour growth in preclinical models.\textsuperscript{69} \textsuperscript{70} In 1998 Folkman and O’Reilly reported the successful treatment of metastatic disease in mice after resection of the primary tumour using angiostatin and endostatin. The humanised version of a VEGF-blocking antibody, bevacizumab, is now widely used in clinical practice for the treatment of CRC.\textsuperscript{65} \textsuperscript{71} However, how inhibitors of angiogenesis work in established tumours and their respective metastases is up to now not entirely understood. In preclinical models, angiogenesis inhibitors have a potent effect on tumour growth and induce apoptosis of endothelial cells. This mechanism could be relevant in those human tumours in which angiogenesis inhibitors induce significant response rates as single agents (eg, renal cancer). However, at least as far as inhibition of angiogenesis is concerned, preclinical models do not reliably reflect the situation of the whole spectrum of human tumours. In mice, there are usually rapidly growing tumours with a high proportion of immature vessels and a high index of proliferating endothelia. In these models, objective response rates can be readily achieved with a single
antiangiogenic agent. In humans there are also comparatively slowly growing tumours with a high proportion of mature vessels and a low index of proliferating endothelia. Clinical trials in these tumours (including CRCs) show an improvement in PFS, but no major tumour shrinkage and/or improvement in response rates. These results provide circumstantial evidence that targeting VEGF can be primarily cytostatic for vessel growth and the ORR may not be the best parameter to judge efficacy of an antiangiogenic agent. In addition, the use of the RECIST criteria might be misleading as there are examples of tumour cavitation and loss of viable tumour in response to antiangiogenic agents that did not result in different tumour measurements. The most recent version of the RECIST criteria takes morphological changes into account.72

A further potential mechanism by which angiogenesis inhibitors act on large tumours is the normalisation of the dysregulated tumour vasculature, which in turn could lead—at least for a certain amount of time—to increased delivery of chemotherapy and better tumour oxygenation. Recent data demonstrate that VEGFA is a negative regulator of pericyte function and vessel maturation by inducing the formation of a VEGFR2–PDGFRb (platelet-derived growth factor receptor b) complex on vascular smooth muscle cells.73 In MTV-PyMT mice that develop mammary tumours, decreased vascular density, shorter, less tortuous vessels and increased pericyte coverage were observed when the mice lacked myeloid VEGFA.74 Tumours in mice lacking myeloid VEGFA were more susceptible to chemotherapy with cyclophosphamide or cisplatin. Other preclinical data also suggest that normalisation of tumour vasculature could be an important mechanism of action of angiogenesis inhibitors.75 76

Data in rectal cancer patients show that treatment with bevacizumab results in increased pericyte coverage of tumour vessels, a significant decrease of the interstitial fluid pressure within the tumour and correspondingly an increased tumour perfusion.77 Results from the use of angiogenesis inhibitors also demonstrated such a ‘window of normalisation’. However, this window was rather variable from patient to patient. Judged from the literature available it is also likely that the extent and the potential benefit of such normalisation—that is, better tumour oxygenation and accessibility for chemotherapeutic agents—varies between individual tumours and even between primary tumours and their respective metastases.

VEGF can recruit bone marrow-derived haematopoietic progenitor cells (HPCs) and endothelial progenitor cells (EPCs) to tumours and controls chemotaxis of EPCs. In mice 5–50% and sometimes up to 90% of the tumour endothelial cells are derived from EPCs. In humans <12% of tumour vessels contain bone marrow-derived cells.78 Here, these EPCs are required for the conversion of vascular micrometastases to progressive tumours. However, anti-VEGF treatment can be effective even when vessel count and/or density are unchanged.79

**CLINICAL DATA ON ANGIOGENESIS INHIBITION IN CRC**

The most promising results using angiogenesis inhibitors in CRC have so far been obtained with bevacizumab, a humanised murine monoclonal antibody that binds to and functionally neutralises VEGF. Bevacizumab is barely effective as a single agent in patients with CRC from a clinical perspective. However, in combination with chemotherapeutic agents this compound has demonstrated efficacy in various clinical trials in mCRC.

**Bevacizumab in first-line treatment of mCRC**

In a first randomised phase II trial in patients with metastatic CRC the combination of 5 mg/kg bevacizumab every 2 weeks with 5-FU/FA significantly improved tumour response and median time to tumour progression (TTP) compared with 5-FU/FA alone (without/with bevacizumab: ORR 17% vs 40%; median TTP, 5.2 months vs 9.0 months; HR 0.46).80 Interestingly, the same combination using 10 mg/kg bevacizumab every 2 weeks did not further increase efficacy but was inferior to 5 mg/kg (ORR, 24%; median TTP, 7.2 months). Subsequently, 5 mg/kg bevacizumab was selected as ‘standard’ in first-line mCRC treatment. In a further randomised trial with 209 patients being ‘not optimal candidates for irinotecan’, a 5-FU/FA/bevacizumab combination led to an improvement of PFS and ORR compared with chemotherapy alone. Remarkably, the obtained PFS of 9.2 months is within the range of other PFS data from combination chemotherapy.81 Importantly, the combination of bevacizumab with the former ’standard of care’ chemotherapy regimen of 5-FU/FA plus irinotecan (IFL protocol) was also tested in a randomised phase III trial in patients with mCRC.82 Here again, the addition of bevacizumab prolonged median OS of the patients by an impressive 4.7 months (15.6 months vs 20.3 months; HR 0.66; p<0.001). Also, PFS (median 6.2 months vs 10.6 months, HR 0.54; p<0.01) and ORR (34.8% vs 44.8%; p<0.01) as well as duration of response (IFL, 7.1 months; IFL+bevacizumab, 10.4 months) were significantly longer when bevacizumab was administered.

Another phase III trial including >1400 patients evaluated the addition of bevacizumab to the FOLFOX4 (5-FU/FA/oxaliplatin) or XELOX (capecitabine/oxaliplatin) regimen. The aim of the study was to demonstrate the non-inferiority of both chemotherapy ‘backbone’ regimens and the superiority of bevacizumab when added to either oxaliplatin-based treatment in a 2×2 factorial design. Compared with the chemotherapy—placebo arms, there was a statistically significant improvement in median PFS (8 months vs 9.4 months, HR=0.83; p=0.0025) and a trend to improvement in OS (median 19.8 months vs 21.3 months, HR=0.89; p=0.77) in both bevacizumab arms. However, the ORR was not improved upon addition of the VEGF inhibitor to this highly active chemotherapy regimen (83; table 3).

Gut 2010;59:838–858. doi:10.1136/gut.2009.196006
Recent advances in clinical practice

**Table 3** Randomised phase III trials with bevacizumab

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<th>p Value</th>
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</tbody>
</table>

*Independent review.

FA, folic acid; 5-FU, 5-fluorouracil; IFL, 5-FU/FA plus irinotecan; OS, overall survival; PFS, progression-free survival; WT, wild type.

Numerous phase II trials and two large phase IV observational studies with in total >4500 patients have confirmed that the median PFS regularly exceeds 10 months when bevacizumab is added, independently of the combination chemotherapy chosen (S-FU or capcitabine plus either oxaliplatin or irinotecan; table 2).

**Second-line treatment with bevacizumab**

In a large three-arm phase III trial including 828 patients, the combination of bevacizumab and FOLFOX4 was compared with the FOLFOX4 regimen alone, or bevacizumab alone, for the treatment of patients with irinotecan-refractory CRC. In this trial, bevacizumab was used at 10 mg/kg. FOLFOX4 plus bevacizumab was superior in all efficacy parameters when compared with FOLFOX alone, including median OS (12.9 months vs 10.8 months, HR 0.75; p=0.0011). Interestingly, and in contrast to the first-line FOLFOX/XELOX trial, the ORR was also significantly improved in this setting (21.3% vs 9.2%; p<0.001). The ‘bevacizumab alone’ arm resulted in an ORR of only 2.7%, indicating the lack of cytotoxic capacity when used as single agent. However, the 1-year survival rate of single agent bevacizumab did not differ from the FOLFOX arm (43% and 44%, respectively), indicating that single agent bevacizumab may play a role in disease stabilisation. Therefore, this approach is being currently examined in clinical trials where the antibody is used as a ‘maintenance’ treatment, after induction with cytotoxic combinations.

**Side effects of bevacizumab**

Bevacizumab is generally well tolerated with regard to symptomatic toxicities. Early phase I/II trials reported an increased risk of adverse events in patients receiving bevacizumab, such as bleeding, thrombosis, bowel perforations, high blood pressure, and proteinuria, which might be related to the mechanism of action of a VEGF-blocking agent. However, both phase III trials did not report a significantly higher rate of venous thromboembolic complications upon bevacizumab exposure. However, an increased risk of bleeding, bowel perforations and hypertension, and a doubling of arterial thromboembolic events was noted in the bevacizumab arm. In particular, the incidence of transient ischaemic attacks and strokes was higher upon treatment with bevacizumab. Patients at high risk for these complications were >65 years of age, and/or had previous arterial thromboembolic events (for further information, see: http://www.fda.gov/cder/foi/label/2004/125085lbl.pdf).

Bevacizumab has a half-life in patients of between 17 and 21 days, which may cause problems when surgery is required. Bevacizumab caused no increased risk of wound healing complications when administered 28–60 days after primary cancer surgery. Similarly, preoperative bevacizumab does not significantly increase postoperative complication rates in patients undergoing hepatic surgery for CRC liver metastases when there is an appropriate interval between discontinuation of bevacizumab and surgery.

**FURTHER INHIBITORS OF ANGIOGENESIS IN CRC**

**Anti-PIGF**

PlGF is an interesting target, because it appears to regulate the angiogenic switch in disease, but not in health. Anti-PIGF treatment was also able to inhibit growth of tumours refractory to anti-VEGF treatment and did not lead to induction of the angiogenic rescue programme in the tumours. Anti-PIGF strategies such as neutralising monoclonal antibodies that block its binding to VEGFR1 could hence be an interesting therapeutic approach for anti-VEGF-refractory tumours.

**ANTIANGIOGENIC SMALL MOLECULES**

Another approach to block angiogenesis is small molecules that block the ATP-binding site in the tyrosine kinase domain of the VEGFR. This approach could be even more efficient since some tumour cells have a survival system by which VEGF acts as an internal autocrine or intracrine survival factor through binding to intracellular VEGFR1. Inhibition of extracellular VEGF by antibodies does not affect this intracrine system, whereas cell-permeable small molecules that block all VEGFR tyrosine kinases will hit the intracrine loop.
SELECTIVE VEGFR TYROSINE KINASE INHIBITORS

Vatalanib or PTK787/ZK 222584 is an oral angiogenesis inhibitor targeting VEGFR1/Fit-1, VEGFR2/KDR, VEGFR3/Fit-4 and, to some degree, PDGFR and c-Kit. In preclinical tumour models, vatalanib exhibited potent antiangiogenic effects, but also direct effects on tumour proliferation and apoptosis.59–61 In a randomised, placebo-controlled phase III trial (the CONFIRM-1 trial), vatalanib was examined in combination with the FOLFOX4 protocol for the first-line treatment of CRC. Upon inclusion of ~1200 patients there was a marginally significant improvement in PFS according to the independent reviewing board.62 The CONFIRM-2 study evaluated the efficacy of PTK787 in combination with FOLFOX versus FOLFOX alone in 855 patients with irinotecan-refractory mCRC. PFS was significantly longer in the PTK787 arm, but no improvement in OS was demonstrated.63

It is currently unclear why the clinical data using vatalanib were only marginal compared with the effect of bevacizumab in combination with chemotherapy. Possible explanations could be the schedule of application used in this trial and the half-life of the compound or the duration of treatment related to PFS. Interestingly, only patients with a relatively poor PFS and OS and elevated lactate dehydrogenase (LDH) levels benefited significantly from PTK787 treatment. In this patient cohort, the treatment duration was almost as long as the PFS duration. Alternatively, since various cell types including haematopoetic stem cells are dependent on an intracrine VEGF loop,64 one could speculate that the effect of VEGFR inhibitors on physiological, non-tumour signalling networks might compromise their antitumour effects.

Cediranib, or AZD2171, is a potent ATP-competitive inhibitor of the VEGFR tyrosine kinases. Recently, a phase II randomised study (HORIZON I) of cediranib plus FOLFOX versus bevacizumab plus FOLFOX in patients with previously treated mCRC has been presented. Here, the activity of the tyrosine kinase inhibitor was comparable with that of the anti-VEGF antibody bevacizumab.65 As a consequence, a phase II/III study (HORIZON III) of cediranib plus FOLFOX versus bevacizumab plus FOLFOX and a phase III study of cediranib plus FOLFOX or XELOX versus chemotherapy alone (HORIZON II) are currently recruiting patients with mCRC in first-line treatment.

‘MULTITARGET’ TYROSINE KINASE INHIBITORS WITH ANTI-VEGFR ACTIVITY

Sorafenib is an orally available multikinase inhibitor of the VEGFR, the PDGFR and Raf. Sorafenib is the only agent so far that significantly prolongs survival of patients with advanced hepatocellular carcinoma66 and has been approved for the treatment of this tumour. Sorafenib seems to act synergistically with cetuximab, in particular in tumours with a B-raf mutation.47 There is currently one trial examining the combination of sorafenib, cetuximab and irinotecan in patients with advanced or metastatic CRC (NCT00134069).

Sunitinib is an orally available inhibitor of the VEGFR, the PDGFRb, c-Kit and Fli-3. The compound is currently approved for the treatment of renal cell cancer78 and for imatinib-refractory GISTs.90 SU11248 alone has not demonstrated any objective responses in patients with refractory mCRC.91 A randomised phase Ib study of FOLFOX plus SU1128 versus FOLFOX plus bevacizumab and a phase III study of FOLFIRI (leucovorin/5-FU/irinotecan) with or without SU11248 in patients with mCRC in first-line treatment are currently ongoing.

Pazopanib and motesanib are other oral multitargeted tyrosine kinase inhibitors that target VEGFR, the PDGFR and c-Kit, and are currently being examined in phase I/II clinical trials for various tumours.100,101 Motesanib is currently being examined in CRC in combination with FOLFOX4 or FOLFIRI and panitumumab (NCT00101894). AV-951 is another inhibitor of VEGFR1, VEGFR2 and VEGFR3, and induces tumour regression in patients with advanced renal cancer. The compound showed clinical activity and tolerability in other solid tumours including CRC and lung cancer. AV-951 is currently being evaluated in combination with FOLFOX 6 for the treatment of CRC (NCT00660153).

Axitinib, or AG013736, is a small molecule tyrosine kinase inhibitor under development that inhibits VEGFR1, VEGFR2, VEGFR3, PDGFR and c-Kit (CD117). Currently, axitinib is being examined in combination with chemotherapy±bevacizumab in patients with mCRC in two trials (NCT00460603; NCT00615056).

IS THERE RESISTANCE TO ANGIOGENESIS INHIBITORS?

Since antiangiogenic treatment mainly aims at genetically stable endothelial cells, resistance to this concept seems unlikely at first sight. However, there are different modes of resistance to antiangiogenic treatments. In tumour models, intrinsic resistance against anti-VEGF strategies can be conferred by infiltration of the tumour by bone marrow-derived CD11bGr1+ cells. Here, refractoriness to anti-VEGF treatment is determined by the ability of tumours to prime and recruit CD11bGr1+ cells.102 Some tumours may exhibit a primary refractoriness to anti-VEGF treatment due to the use of existing vessels for proliferation that makes these tumours independent of neoangiogenesis. Anti-VEGF resistance can also be acquired. Clinical evidence shows that treatment with antiangiogenic agents prolongs PFS of patients with CRC, but does not prevent tumour progression altogether.103 There is a debate as to whether it is advisable to change the whole therapeutic concept under these circumstances or whether it is sensible to continue the treatment with the angiogenesis inhibitor beyond progress and to change only the chemotherapy. Discontinuation of the antiangiogenic treatment upon tumour progression...
could be detrimental because it could lead to rapid vascular regrowth, boosting tumour growth. There is experimental evidence supporting this argument by showing that remnants after anti-VEGF treatment, empty sleeves of basement membrane and accompanying pericytes, provide a scaffold for rapid revascularisation of tumours after discontinuation of anti-VEGF treatment. However, these experiments were not performed when tumours progressed after a VEGF-blocking treatment for several months, but in tumour-bearing mice that were treated with the angiogenesis inhibitor for only 7 days and still responded to this treatment. There is definitely drug resistance to angiogenesis inhibitors. In a mouse model of cancer, phenotypic resistance to anti-VEGFR2 treatment was demonstrated with vascular regrowth in a VEGF-independent form of angiogenesis involving other proangiogenic ligands, for example from the FGF family. Furthermore, upon bevacizumab treatment, PICF is upregulated in the plasma of patients with CRC which can enhance pathological angiogenesis by initiating cross-talk between VEGFR1 and VEGFR2.

Kopetz and colleagues recently reported a cohort of patients undergoing treatment with FOLFIRI and bevacizumab. In this cohort, serum cytokine levels were repeatedly analysed every 2 weeks until progression. There was a clear increase in the levels of PICF, bFGF and hepatocyte growth factor (HGF)—which may stimulate other angiogenic pathways—prior to clinical progression. Other antiangiogenic agents also induce proangiogenic factors, for example sunitinib induces PICF and VEGF as well as G-CSF (granulocyte colony-stimulating factor) and SDF-1 (stromal cell-derived factor-1) which mobilise circulating endothelial progenitor cells. Rapid vascular remodelling occurs in tumours as a consequence of antiangiogenic treatment. As a consequence, the remodelled vessels are more resistant to antiangiogenic drugs that usually target immature vessels. Furthermore, one has to bear in mind that tumour vascularisation is not only due to angiogenesis, but also to other mechanisms including vasculogenic mimicry and mosaic vessels where tumour cells form a part of the surface of the vessel while the remaining part is covered by endothelium.

Therefore, it is likely that resistance to anti-VEGF treatment occurs. However, as most antiangiogenic treatments are administered together with a cytotoxic chemotherapy, it is unlikely that resistance will regularly occur simultaneously. Therefore, further clinical investigations of continuation of antiangiogenic drugs beyond progression (with an altered chemotherapy regimen) and of individual cytokine profiles predicting resistance by upregulation of concurrent pathways is warranted. A large phase III trial conducted by the German AIO group is currently recruiting patients to examine the treatment with bevacizumab beyond progression in mCRC.

It has also to be considered that prolonged tumour treatment with antiangiogenic agents may change the biological behaviour of the tumour. Recent data obtained in mouse models of tumour angiogenesis suggest that prolonged antiangiogenic treatment by targeting VEGF or multitarget inhibitors can lead to increased local invasion and accelerated distant metastases, suggesting a ‘metastasis conditioning’ of the tumour cells by antiangiogenic treatment despite antitumour effects on the primary tumour. At present, it is unclear whether this mechanism is also be relevant for the treatment of patients with mCRC.

**Biomarkers for Angiogenesis and Its Inhibitors?**

Early reports suggested that circulating VEGF could be a reliable surrogate marker of angiogenic activity and tumour progression in cancer patients. However, recent evidence suggests that VEGF production is already comparatively high even in non-tumour-bearing humans. The contribution of tumour-secreted VEGF becomes significant only when the tumour load is rather large. It is therefore plausible that systemic VEGF levels do not serve as a sensitive surrogate marker when it would be most relevant; that is, when tumour load is low.

So far, there are no sensitive predictive biomarkers for angiogenesis inhibitors such as bevacizumab. The efficacy of an antiangiogenic treatment in CRC appears to be independent of the K-ras status of the tumour. This is plausible since this treatment is primarily not directed against the tumour bearing the mutation, but against the endothelium which does not exhibit tumour-specific mutations. However, it has been argued that K-ras mutations act angiogenically and thereby antagonise anti-EGFR targeting by neutralising its antiangiogenic properties.

Plasma VEGF levels, VEGF levels in the primary tumour, downstream targets of VEGFRs such as c-Raf and p53, or angiogenic mediators such as TSP-2 are also not predictive for anti-VEGF treatment strategies. However, the increase of specific cytokines during the treatment course may be a indicator for upregulation of concurrent antiangiogenic pathways and occurrence of resistance.

There is preliminary evidence that gene expression profiling might yield some gene ontologies (such as mitosis-, cell motility- and VEGFR activity-associated genes) that could be helpful in predicting tumour response to bevacizumab plus chemotherapy. Given the multiple biological functions of VEGF and consequently the multiple and diverse actions of angiogenesis inhibitors, useful predictive markers are likely to be specific for a particular function and potentially even for particular tumours.

**Response Evaluation of Antiangiogenic Treatment by Dynamic Contrast-Enhanced MRI (DCE-MRI)**

In the absence of predictive biomarkers for antiangiogenic strategies, various imaging techniques are being examined as potential pharmacodynamic markers, including DCE-MRI. Several clinical trials describe a decrease in tumour perfusion in response
to antiangiogenic treatment. However, some of the effects detected with DCE-MRI may simply be due to vasoconstriction as a result of VEGF inhibition. VEGF induces nitrous oxide and prostacyclin, both of which are potent vasodilators that are induced in tumour vessels and are lacking upon inhibition of VEGF. The kinetics of the decrease in tumour blood flow is rapid, making it unlikely to be due to destruction of vessels. Finally, the variability of the DCE-MRI measurements appears to be high, results are still conflicting and there is so far no standardised protocol. Thus, DCE-MRI should be further evaluated in clinical trials, but is not ready to be used in clinical practice.

Vasoconstriction in response to angiogenesis inhibition is also seen in non-tumour vessels, resulting in one frequent side effect of this treatment, hypertension. There is a debate as to whether hypertension might be a surrogate marker for efficacy of an antiangiogenic treatment. Preliminary data suggest that this might be the case.

**MULTIPLE TARGETING: OPTIMISING EFFICACY?**

Most solid tumours are characterised by multiple genetic changes. Therefore, targeting only one signal transduction pathway may not be sufficient to hit the tumour substantially. Multiple signalling networks in the tumour exhibit a certain degree of redundancy and are additionally modulated by signalling strength and kinetics. The challenge is to determine whether there are critical ‘hubs’ in the tumour signalling networks that constitute optimum targets. EGFR signalling and angiogenesis constitute such ‘hubs’. Alternatively, one has to establish combinations of several targeted treatment that specifically counteract the signalling redundancy in the tumours, but ideally have no or little negative impact on physiological signalling processes. Such strategies could also be used to overcome drug resistance in cases of genetic alterations such as anti-EGFR resistance in K-ras-mutated CRC.

The first trial examining a double targeting concept in CRC was a small phase II trial in 43 patients with irinotecan-refractory CRC. In this trial, patients received irinotecan plus cetuximab and bevazcizumab (CBI) or a combination of the molecular agents cetuximab and bevacizumab alone (CB). Median TTP in the CBI arm was 7.3 months and the ORR was 37%. In the CB arm, median TTP was 4.9 months, with an ORR of 20%. OS in this trial was reported as 14.5 months and 11.4 months for CBI and CB, respectively. Compared with historical controls of cetuximab or cetuximab plus irinotecan, addition of bevacizumab seemed to increase activity substantially. The toxicity was similar to what could be expected from the two agents alone. There are no data on the K-ras status of the tumours in this trial.

Consequently, double targeting strategies combining bevacizumab and anti-EGFR antibodies were examined in previously untreated patients with CRC: the CAIRO2 trial examined the combination of capecitabine, oxaliplatin and bevacizumab without (COB) or with cetuximab (COBCet). The combination of all four agents did not improve median PFS and OS compared with capecitabine plus oxaliplatin plus bevacizumab in the study population (median PFS/OS: COB, 10.7/20.4 months; COBCet, 9.6/20.3 months; table 4).

The analysis of the K-ras status in the tumours revealed that median PFS was inferior in patients with K-ras-mutated tumours receiving COBCet compared with patients receiving only COB. There was no substantial increase in toxicity in the group receiving the quadruple therapy, which might have been an explanation if lowered dose intensities could have resulted. A similar study, the PACCE trial, evaluated the addition of panitumumab to standard chemotherapy doublets (modified FOLFOX or FOLFIRI) and bevacizumab. An interim analysis revealed a statistically significant difference in PFS favouring the control arm. In this

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**Table 4 Triple combination with/without EGFR inhibitors**

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<tr>
<td>XELOX–Bev–Cet</td>
<td>368</td>
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</tr>
<tr>
<td><strong>PACCE 82% ITT of population</strong></td>
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<tr>
<td>5-FU/FA/Ox–Bev</td>
<td>WT</td>
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<td>56 NA</td>
<td>11.5</td>
<td>1.36</td>
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<tr>
<td>5-FU/FA/Ox–Bev–Pan</td>
<td>WT</td>
<td>201</td>
<td>50</td>
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<td>12.5</td>
<td>1.50</td>
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<tr>
<td>5-FU/FA/Ki–Bev–Pan</td>
<td>WT</td>
<td>57</td>
<td>54</td>
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<td>11.9</td>
<td>1.9</td>
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</tr>
<tr>
<td>5-FU/FA/Ox–Bev–Pan</td>
<td>mut</td>
<td>125</td>
<td>44</td>
<td>11.0</td>
<td>1.25</td>
<td>NS</td>
<td>19.3</td>
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<tr>
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<td>136</td>
<td>47</td>
<td>10.4</td>
<td>11.9</td>
<td>1.9</td>
<td>20.5</td>
</tr>
<tr>
<td>5-FU/FA/Ki–Bev–Pan</td>
<td>mut</td>
<td>47</td>
<td>30</td>
<td>9.3</td>
<td>11.9</td>
<td>1.9</td>
<td>20.5</td>
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Bev, bevacizumab; Cet, cetuximab; EGFR, epidermal growth factor receptor; FA, folic acid; 5-FU, 5-fluorouracil; KI, irinotecan; ITT, intention to treat; mut, mutated; NA, not available; NS, non-significant; OS, overall survival; Ox, oxaliplatin; Pan, panitumumab; PFS, progression-free survival; WT, wild type.
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There was an increase in the incidence of grade 3 diarrhoea, dehydration and infections, and an increased incidence of pulmonary embolism in the panitumumab arm, including one death. Thus, in this setting, addition of panitumumab to bevacizumab and oxaliplatin- or irinotecan-based first-line chemotherapy did not improve efficacy and even increased toxicity.

The reason for the obvious difference in efficacy of double targeting concepts in previously untreated patients versus patients with chemorefractory mCRC is unclear at present. The first-line data have been obtained in large, randomised trials, whereas the data in chemorefractory patients were obtained in rather small trials with a probably higher selection of patients. In addition, the targeting agents used could also be more effective in chemorefractory tumours by exerting a chemosensitising effect that translates into a higher efficacy that is obviously not detectable when the tumour is still chemosensitive. Further trials and insight into the mechanism of action of these agents in chemosensitive as compared with chemorefractory tumours will be required to clarify this issue. These data also stress the importance of well-designed clinical trials to demonstrate an effect of targeting agents in CRC before they enter clinical practice despite the fact that the concept per se seems to be plausible.

INTEGRATING EGFR AND VEGF TARGETING INTO CLINICAL PRACTICE: CONTINUATION OF CARE AND CONVERSION TREATMENT

The integration of bevacizumab and both EGFR inhibitors, panitumumab and cetuximab, into clinical practice has extended the armamentarium against mCRC: now that three (to four) chemotherapy drugs and two molecular mechanisms are available, there is an upcoming debate how to integrate this optimally into the treatment strategy. There are two major strategies: conversion treatment to enable secondary resection of liver or lung metastases for a small, but relevant subset of patients; and the ‘continuum of care’ for the rest of the patients with metastatic disease.

Conversion to resectability

Beyond the traditional end points of OS and PFS, it is also useful to consider the additional advantages these highly active combination treatments can have on broader treatment goals, such as rates of resection in patients for whom surgery was not previously an option.

The high response rates seen with the triple combinations, indicating the higher percentage of patients with relevant tumour shrinkage, may enable secondary resections of metastases in initially not resectable patients. This conversion from the initial purely palliative intention of treatment to a potentially curative approach represents a benefit per se, as surgical resection provides a chance for cure. Surgery is, however, a viable option for only 15% of patients by the time of mCRC diagnosis, and, as recently shown in the CELIM trial where a blinded surgical panel assessed resectability of liver metastases after pretreatment with cetuximab-based combinations, approximately another 25% of patients with metastases confined to the liver may also convert to resectability.20 However, the number of patients examined in randomised trials with resectability as the predefined end point to date is too small to draw any conclusion on a favourable regimen, also bearing in mind the lack of long-term data reflecting recurrence-free survival. However, trials with cetuximab have consistently shown high ORR, which might be correlated with a higher chance of resectability,123 whereas the data for bevacizumab combinations are less clear (table 5).

Interestingly, recent data from patients undergoing resection of liver metastases have indicated significant reductions of viable tumour cells in the resected specimen after treatment with bevacizumab.124 125 Interestingly, a protective effect from

### Table 5 Comparison of ‘conversion treatments’ in mCRC with molecular agents

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>RR (%)</th>
<th>RR (%) liver only patients</th>
<th>R0 rate (%) all patients</th>
<th>R0 (%) liver only mets.</th>
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<td>FOLFOX+cetuximab</td>
<td>56</td>
<td>68</td>
<td>70*</td>
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<td>Folprecht et al, CELIM trial28</td>
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<tr>
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<tr>
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<td>57</td>
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<tr>
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<td>49</td>
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<tr>
<td>IFL+bevacizumab</td>
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<td>45</td>
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<td>NA</td>
<td>NA</td>
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<tr>
<td>FOLFIRI+bevacizumab</td>
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<td>38</td>
<td>NA</td>
<td>8.4†</td>
<td>17.7†</td>
</tr>
<tr>
<td>Saltz et al87</td>
<td></td>
<td></td>
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</tr>
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</table>

*Kras wild-type population.
†All resections.
mets., metastases; mCRC, metastatic colorectal cancer; NA, not available; R0, resection.
chemotherapy-associated steatohepatitis is also discussed for bevacizumab.124

**Continuum of care**

With the integration of the EGFR antibodies, a new treatment line was established—and, to date, CRC is the only disease with three proven treatment lines versus BSC, at least for K-ras wild-type patients. This requires a strategic consideration of which patient should be treated with each of these drugs in the different treatment lines: cetuximab has proven efficacy in first- to third-line treatments and bevacizumab in first- and second-line treatments. Different algorithms in national guidelines have addressed the selection of treatment strategies, sequentially or simultaneously, and today clinical characteristics and treatment aims define the intensity of a chosen treatment in every line. Notably, efficacy in second- and third-line treatment may also be adjusted to clinical need—for example, after failure of a standard 5-FU/oxaliplatin combination with or without bevacizumab, a salvage strategy could consist of FOLFIRI (ORR ~4%) or FOLFIRI+cetuximab, with much higher ORR.10

Bevacizumab has consistently prolonged PFS up to 10–11 months in first-line treatment. While the combination treatment may be an appropriate first-line treatment for certain patients, this does not mean that it should necessarily be given for as long as the patient can tolerate such treatment or the tumour progresses. Rather, aggressive combination treatment can be envisaged as a form of ‘induction’ treatment, given for a limited period with the aim of achieving disease control and/or allowing resection of metastases. According to this paradigm, induction treatment would be followed by less intensive ‘maintenance’ treatment; more aggressive treatment could then be considered again upon disease progression. Induction treatment is likely to involve doublet chemotherapy, possibly combined with a targeted agent. As the optimal duration of induction treatment has not yet been defined and the question of whether further intensification is appropriate when a patient has failed to respond to the initial combination regimen remains unanswered, patients should be evaluated following the initial treatment, and resection or ablation can then be performed if possible. If these treatments are not an option, maintenance treatment or even a complete ‘drug holiday’ can be considered.

Further work is required to define more rigorously the baseline characteristics of patients who are most likely to benefit from a chemotherapy-free interval as well as to define the most effective agents and the best duration of chemotherapy prior to cessation. In the Combined Oxaliplatin Neurotoxicity Prevention Trial (CONCEPT) study, intermittent oxaliplatin administration was associated with increased time on treatment and prolonged time to treatment failure in patients receiving FOLFOX plus bevacizumab, presumably by mitigating cumulative neurotoxicity.125 Results from CONCEPT and other trials suggest that de-escalation of the relatively toxic combination treatment after some months and continuing with 5-FU/FA and bevacizumab may maintain the benefits of these combinations.

**UPCOMING TARGETED TREATMENTS**

**Inhibition of Src tyrosine kinases**

The Src family of non-receptor protein tyrosine kinases plays critical roles in a variety of biological responses. The exact mechanisms by which Src kinases contribute to individual tumours remains to be defined. Src kinases appear to be important for various aspects of tumour progression including proliferation, disruption of cell–cell contacts, migration, invasiveness, apoptosis resistance and angiogenesis. Constitutively, activated members of this kinase family, including the viral oncoproteins v-Src and v-Yes, can induce malignant transformation of various cell types. Src family kinases are frequently overexpressed and/or aberrantly activated in cancers. Src is also a downstream target of many growth factor receptors overexpressed in cancer. Its activation is detectable in ~80% of all CRCs.127 The extent of Src kinase activity often correlates with the malignant potential of a tumour, a metastatic phenotype and patient survival. Thus, Src family kinases are attractive targets for anti-cancer therapeutics.128 There are several clinical trials ongoing in CRC using Src inhibitors. An orally available Src inhibitor, AZD0530, is currently undergoing phase II testing in patients with mCRC having shown promising activity in a phase I trial.130 Numerous other Src inhibitors are entering in phase I/II trials in mCRC, including SKI-606 (bosutinib), SU6656, AP25464 and BMS-584525 (dasatinib). Furthermore, interactions with ligand-activated receptor tyrosine kinases, such as EGFR, PDGFR or HER2, can result in augmented c-Src activation.130 C-Src can also phosphorylate EGFR and also regulates molecules associated with angiogenesis. Therefore, Src inhibitors are an attractive partner for further combinations with the established molecular agents in mCRC.

**mTOR inhibition**

mTOR, a serine-threonine kinase, is a major downstream target of the the PI3K/Akt signalling pathway.131 The kinase is activated in response to growth factor receptor activation or to nutritional stimuli. Activation of mTOR results in the phosphorylation of translational regulators, such as eukaryotic initiation factor 4E-binding protein and p70S6 kinase which are critical for cell cycle progression and protein synthesis. Inhibition of mTOR results in a late G1 arrest (via down-regulation of cyclin D and accumulation of the cell cycle inhibitor p27) and apoptosis. mTOR inhibitors also block proliferation of endothelial and vascular smooth muscle cells and decrease VEGF expression, resulting in reduced angiogenesis. Finally, inhibition of mTOR leads to apoptosis. mTOR inhibitors are analogues of rapamycin and act by binding to the immunophilin FK506/rapamycin-binding protein that binds to mTOR and inhibits its activity. mTOR inhibitors are particularly active in...
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tumours lacking the FTEN tumour suppressor gene. Currently, the mTOR inhibitor temsirolimus is approved by the European Medicines Agency for the treatment of patients with ‘poor risk’ metastatic renal cell cancer, whereas everolimus, another orally available mTOR inhibitor, has demonstrated significant activity in renal cell cancer patients refractory to multitarget tyrosine kinase inhibitors.

In mCRC, everolimus alone has not demonstrated objective tumour responses in heavily pretreated patients with mCRC, but the combination of bevacizumab and everolimus has activity in patients with refractory mCRC who have progressed on a bevacizumab-based regimen.152

A current trial is examining the addition of everolimus to irinotecan and cetuximab for the treatment of patients with mCRC progressing on prior chemotherapy (NCT00822665). Another trial is examining the combination of temsirolimus and cetuximab in cetuximab-refractory mCRC (NCT00593060).

PI3K/Akt signalling pathway

Aberrant PI3K/Akt/mTOR-dependent signalling has been observed in many human malignancies including CRC.153 It is the result of overexpression and mutations of growth factor receptors, amplification and/or overexpression of PI3K and Akt, loss of the tumour suppressor phosphatase FTEN or loss of the tuberous sclerosis complex 2 (TSC2). The PI3K signalling pathway is upregulated in many CRCs, and this upregulation positively correlates with increased tumourigenic potential of colon adenocarcinoma cell lines.154

Mutations in the p110α catalytic subunit of PI3K have been identified in up to one-third of CRC specimens.49 PI3K inhibition could contribute to overcome drug resistance, as shown in tumours refractory to the kinase inhibitor lapatinib.155 156

Several PI3K inhibitors are in clinical development, such as BEZ235, BGT226, XL147, BKM120, GDC-0941 and XL765, and other PI3K inhibitors are in preclinical development (eg, SF1126 and ZSTK474).137 138

INDUCTION OF APOPTOSIS

Smac

Smac acts proapoptotically by binding in the Smac-binding groove of XIAP, thereby blocking its interaction with caspase 9.139 Smac mimetics bind tightly in this groove in many IAPs and sensitize tumour cells to proapoptotic signals. The Smac-mimetic GDC-0152 is already being used in a clinical trial.

Death receptor 5 (DR5)

DR5 is a cell surface receptor of the tumour necrosis factor (TNF) receptor superfamily and is expressed in a broad range of cancers. Antibodies mimicking the natural ligand TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) bind to DR5, which in turn can directly activate the extrinsic apoptosis pathway and indirectly induce the intrinsic apoptosis pathway in tumour cells. There are phase I/II clinical trials examining human monoclonal antibodies that selectively bind to DR5 in chemorefractory CRC in combination with the FOLFIIRI regimen (AMG 655, NCT00815605) or in combination with cetuximab and irinotecan (apomab).140–143

Aurora kinase inhibitors

Aurora kinases (AKs) are members of a family of serine-threonine kinases that regulate many processes during cell division. Until now, three AKs (A–C) have been identified in mammalian cells. These proteins are implicated in several vital events in mitosis and play a critical role as regulators of genome stability. AKs are frequently overexpressed in human tumours. Misregulation of the cell cycle machinery can have an important impact on cellular proliferation.144 This observation has led to an interest in AKs as new anticancer targets. The first data came a decade ago with the observation that AK-A mRNA was overexpressed in >50% of primary CRC specimens. This overexpression was correlated with poor prognosis in patients with CRC.145 Several AK inhibitors are currently in clinical development: ZM447439, VX-680, AZD1152, MLN8054 and MLN8237. Although it is not yet clear which AK is inhibited to mediate the antitumour effects, AK-B is probably the primary target.146 VX-680 inhibits the catalytic activity of AK-A, -B, -C and Fli-1. MLN 8054 is a selective, orally administered small molecule inhibitor of AK-A. It competes with ATP binding and therefore reversibly inhibits AK-A. MLN8054 displays antitumour activity against three different human CRC xenografts.

Proteasome inhibitors

The 26S proteasome is responsible for the degradation of ubiquinated proteins targeted for destruction. The inhibition of the proteasome could lead to apoptosis by inhibiting the breakdown of proapoptotic molecules and/or through prevention of the breakdown of inhibitors of antiapoptotic proteins. In addition, proteasome inhibitors generate a stress response through the altered protein milieu that itself could lead to apoptosis. They also reduce the levels of antiapoptotic nuclear factor (NF)-κB by preventing the degradation of its inhibitor IκB, and increase the levels of proapoptotic proteins such as Bax and p53.147 So far, proteasome inhibitors such as bortezomib do not seem to have a substantial activity in solid tumours including CRC when used as a single agent.148 However, there are current trials examining the combination of chemotherapies such as irinotecan with proteasome inhibitors in CRC.

Targeting epithelial cell adhesion molecule (EpCAM)

EpCAM is a human cell surface glycoprotein expressed on some normal and most neoplastic epithelial cells that mediates cell adhesion. It is frequently upregulated in CRC and plays an important role in CRC biology. EpCAM interferes with E-cadherin expression by disrupting the link between β-catenin and
The upregulation of EpCAM in colon carcinomas may therefore directly downregulate the local immunity and thus support the escape from immune surveillance. In this view, an anti-EpCAM antibody may contribute as a stimulator for a normalisation of immune surveillance and facilitate a clearance of the tumour. In this setting, a novel, completely humanised IgG1 monoclonal antibody, MT201, has entered into clinical trials in different tumour entities—including adjuvant treatment in resected CRC metastases. Furthermore, newer agents with improved affinity, less chimerism, and improved delivery are being used in clinical trials, including EMD 273066 and tucrutumab celmoleukin.

Antisense and small interfering RNA (siRNA)
Apart from antibodies and small molecules, antisense and siRNA technology is increasingly used specifically to target genes that are regarded as critical for carcinogenesis or tumour promotion. Currently there are preclinical trials and already the first clinical trials are examining antisense strategies directed against Bcl2,154 survivin and XIAP155 in various solid tumours.

As far as these strategies are concerned there are limited data available regarding the best way to deliver the oligonucleotides, their tissue distribution and their activity in specific organs.

Radioimmunotherapy using cancer-specific antibodies
The humanised monoclonal antibody A33 (huA33) targets the A33 antigen which is expressed on 95% of CRCs. It has been shown that this antibody has excellent tumour-targeting properties. Consequently, this antibody coupled to151 has been used for radioimmunotherapy.156 A current study is examining the application of [131I]huA33 in combination with capecitabine in patients with mCRC (NCT00291486).

CONCLUSIONS
Complex solid tumours that are defined by multiple genetic alterations such as CRC are a therapeutic challenge. In recent years we have seen substantial improvement in the treatment of mCTR which is due to more effective chemotherapies, but also due to the introduction of targeted treatments into the therapeutic algorithm. In particular, EGFR-blocking antibodies improve ORR in K-ras wild-type CRC, can lead to more curative resections of liver metastases and act as chemosensitisers in chemorefractory CRC. VEGF-blocking antibodies have been demonstrated to improve PFS in patients substantially and to improve efficacy particularly of less intensive chemotherapies.

Figure 1 shows the improvement of mOS in 1st line mCRC treatment by targeted therapies.

**Box 3 Suggested mechanisms of anti-angiogenic treatment in established tumours**

- Starvation of the tumour by a decrease of tumour vasculature
- Normalisation of dysregulated tumour vasculature leading to a decrease of interstitial pressure and increased accessibility for chemotherapy.

To the human leucocyte immunoglobulin-like receptor 1, which provides a link to immune regulation via NK cells, lymphocytes and dendritic cells. The upregulation of EpCAM in colon carcinomas may therefore directly downregulate the local immunity and thus support the escape from immune surveillance. In this view, an anti-EpCAM antibody may contribute as a stimulator for a normalisation of immune surveillance and facilitate a clearance of the tumour. In this setting, a novel, completely humanised IgG1 monoclonal antibody, MT201, has entered into clinical trials in different tumour entities—including adjuvant treatment in resected CRC metastases. Furthermore, newer agents with improved affinity, less chimerism, and improved delivery are being used in clinical trials, including EMD 273066 and tucrutumab celmoleukin.
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Figure 2 Improvement of median progression-free survival compared with increase in costs in first-line first-line treatment of metastatic colorectal cancer (mCRC) using targeted treatments. mos, months; BSA, (PFS) body surface area.

The mechanism of action of these compounds in tumours of patients. This shows that the model systems used so far to test these compounds are of limited value when it comes to clinical application. The only marker so far that allows prediction of the efficacy of a targeted treatment in CRC is the K-ras mutation status of a tumour when an EGFR-targeting treatment is considered. However, K-ras status is a negative predictive marker that can tell us who is not going to benefit, but does not indicate a subgroup in which all patients benefit from this treatment. For other targeted treatments used in CRC, there are not even negative predictive markers. The elucidation of such markers as well as better ways to evaluate target inhibition in patients is now the great challenge for various reasons. First of all, even targeted treatments do have side effects that do not correlate with efficacy of the drug.

Ideally, one would like to avoid exposing patients who do not benefit from the treatment to side effects. The so-called ‘financial toxicity’ of targeted treatments is also an important issue when resources are limited. If we succeed in identifying those patients who really take advantage of a certain treatment and those who do not, public healthcare systems may be able to provide these drugs to all suitable patients now and in the future.

Figures 2 and 3 demonstrate the increase in costs versus improvement of mPFS in first-line mCRC treatment upon introduction of targeted treatments to the German market.

There is an impressive pipeline of novel targeted treatments, some of which already entered clinical trials at various phases. There is a good chance that some of these novel agents will further improve treatment of mCRC. Novel strategies also include the combination of targeted treatments. In this case, the availability of predictive markers will be even more important since the currently available data suggest that some combinations may also lead to antagonistic rather than synergistic effects in patients. Proteomics and metabolomics might help us to identify such biomarkers or biomarker signatures in the near future. Thus, despite multiple targeted treatment and novel concepts such as tumour-initiating stem cells, a lot of work needs to be done to achieve a higher rate of conversion of mCRC to a resectable stage or a true ‘chronification’ of the disease when the tumour cannot be removed by surgery.

Competing interests None.

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REFERENCES

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Targeted treatments in colorectal cancer: state of the art and future perspectives

Dirk Arnold and Thomas Seufferlein

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