New approaches and targets in advanced colorectal cancer

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Introduction

The prognosis of cancer remains poor in spite of the advances obtained in recent years with new therapeutic agents, new approaches in surgical procedures and new diagnostic methods. An emerging understanding of the molecular pathways that characterise cell growth, cell cycle, apoptosis, angiogenesis and invasion has led to the identification of novel targets for cancer therapy. Numerous proteins have been implicated as having a crucial role in colorectal cancer (CRC). The targets can be grouped according to their cellular localisation such as membrane receptor targets (epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), insulin-like growth factor receptor (IGFR), platelet-derived growth factor receptor (PDGFR), tumour necrosis factor-related apoptosis-inducing ligand receptor (TRAIL-R), and c-Met), intracellular signalling targets (Ras/Raf/MAPK pathway, phosphatidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR), src kinase and p53/Hdm2) and other protein kinases that regulate cell division including aurora kinases and polo-like kinases (Fig. 1). Emerging data from the clinical development of new drugs directed to these targets is providing novel opportunities in the treatment of patients with CRC that will probably translate into efficacy benefits in the coming years.

Agents targeting membrane receptors

Epithelial growth factor receptor inhibitors

The epidermal growth factor receptor (EGFR) is a member of the family of transmembrane protein kinase receptors known as the erbB or HER receptor family: EGFR (HER1 or erbB1), erbB2 (HER2), erbB3 (HER3) and erb4 (HER4). When activated, EGFR phosphorylates and activates other intracellular proteins that affect cell signalling pathways, cellular proliferation, control of apoptosis and angiogenesis. EGFR is overexpressed in 75–95% of CRC and it confers a poor prognosis [1]. While multiples strategies of targeting the EGFR are under development, two modalities are best developed: small molecule inhibitors of the intracellular kinase domain of the EGFR and monoclonal antibodies (MoAbs) designed to block the extracellular ligand-binding domain of EGFR.

Cetuximab, the most advanced anti-EGFR agent in clinical development, and panitumumab are two monoclonal antibodies approved in Europe and in the US for the treatment of metastatic CRC (mCRC) patients [2,3]. Several articles have reported that *K-Ras* mutations are associated with lack of response to anti-EGFR therapy [4–6]. *K-Ras* mutations are found in about 40% of CRC and a high concordance between primary tumour and related metastases has been reported [7,8]. A retrospective analysis recently published suggested that *B-Raf* wild-type is also required for response to anti-EGFR therapy [9]. Recently, the results of randomised studies have confirmed the predictive value of *K-Ras* status for the addition of cetuximab to standard first-line chemotherapy schedules in mCRC [10,11]. In the second-line setting, cetuximab has demonstrated an improvement in median progression free-survival (mPFS) when added to irinotecan, although the predictive value of *K-Ras* status has not been fully reported [12].

Amado and colleagues tested whether the effect of panitumumab on PFS differed by *K-Ras* status. The treatment effect on PFS in the wild-type *K-Ras* group was significantly greater than in the mutant group ($P < 0.001$) [4]. Multiple studies are currently evaluating the efficacy and safety of panitumumab in mCRC. Some of these trials are summarised in Table 1.

There is a large number of tyrosine kinase inhibitors (TKIs) directed to EGFR in clinical development (Table 2). Thus far, three TKIs have been specifically evaluated in mCRC: gefitinib and erlotinib, reversible EGFR-specific TKIs, and EKB-569, an irreversible EGFR-specific TKI. Response and disease control rates observed in some phase I/II combination studies,

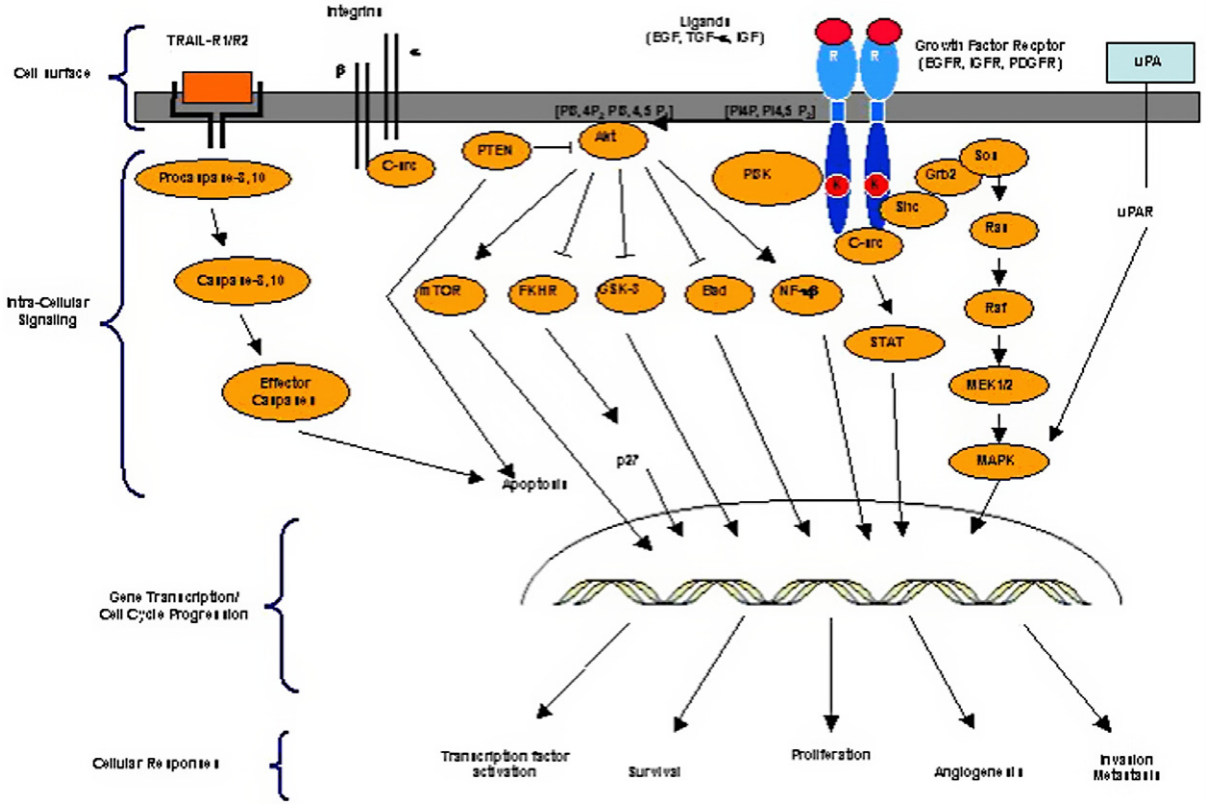

Fig. 1. Intracellular signal transduction pathways. PTEN = phosphatase and tensin homologue; PI3K = phosphatidylinositol 3-kinase; mTOR = mammalian target of rapamycin; $PI_{34}P_2$ = phosphatidylinositol (3,4) biphosphate; $PI_{345}P_3$ = phosphatidylinositol (3,4,5) triphosphate; TRAIL = tumour necrosis factor-related apoptosis-inducing ligand; TRAIL-R = tumour necrosis factor-related apoptosis-inducing ligand receptor; EGFR = epidermal growth factor receptor; EGF = epidermal growth factor; TGF-α = transforming growth factor alpha; IGF = insulin-like growth factor; IGFR = insulin-like growth factor receptor; PDGFR = platelet-derived growth factor receptor; MAPK = mitogen-activated protein kinase; MEK = MAP kinase kinase; FKHR = forkhead transcription factor; GSK-3 = glycogen synthase kinase-3; STAT = signal transducers and activators of transcription protein; NF-kβ = nuclear factor kappa-light-chain-enhancer of activated B cells; SHC = Src homology 2 domain-containing transforming protein; GRB2 = growth factor receptor-bound protein 2; SOS = son of sevenless protein; uPA = urokinase-type plasminogen activator; uPAR = urokinase-type plasminogen activator.

Table 1
Clinical trials with panitumumab

Treatment		Population	Status
FOLFOX + panitumumab or bevacizumab	Phase II	First-line (K-RAS wild-type)	Recruiting
FOLFOX +/– panitumumab (PRIME trial)	Phase III	First-line	Active, not recruiting
Panitumumab +/– AMG-102 or AMG-479	Phase Ib/II	Refractory (K-RAS wild-type)	Recruiting
Irinotecan +/– panitumumab	Phase II	Refractory (K-RAS wild-type)	Recruiting
FOLFIRI + panitumumab or bevacizumab (SPIRITT trial)	Phase II	Refractory (K-RAS wild-type)	Recruiting

FOLFOX = leucovorin/5-fluorouracil/oxaliplatin; FOLFIRI = leucovorin/5-fluorouracil/irinotecan.

in the first-line setting and in the refractory population, are encouraging when compared with the results obtained with standard chemotherapy in the same population, although randomised phase III studies are needed in order to reach definitive conclusions [13–19].

Vascular endothelial growth factor receptor inhibitors

The regulation of angiogenesis is a complex, multistep process resulting from a dynamic balance between pro-angiogenic and antiangiogenic factors. One of

Table 2
Tyrosine kinase inhibitors targeting epidermal growth factor receptors developed in metastatic colorectal cancer

Tyrosine kinase inhibitor	Population	Treatment	No. of patients	Response rate
Gefitinib	Refractory	Gefitinib [13]	28	0%
	First-line	Gefitinib+FOLFOX [14]	12	75%
	First-line	Gefitinib+FOLFOX [15]	39	74%
Erlotinib	Refractory	Erlotinib [16]	25	0%
	Refractory	Erlotinib [17]	41	8%
	Refractory	Erlotinib+FOLFOX4 [18]	15	23%
EKB-569	First-line	EKB-569+FOLFIRI [19]	41	38%

FOLFOX = leucovorin/5-FU/oxaliplatin; FOLFIRI = leucovorin/5-FU/irinotecan.

the most important regulators of this process is the vascular endothelial growth factor (VEGF) and its receptors (VEGFR). VEGF is a 45-kDa homodimer that belongs to a family of growth factors comprising six different glycoproteins: VEGF-A (commonly referred as VEGF), VEGF-B, VEGFC, VEGF-D, VEGF-E, and placenta growth factor (PlGF). Because of its central role in tumour-associated angiogenesis, VEGF has emerged as an attractive and central therapeutic target in CRC.

Bevacizumab is a humanised anti-VEGF monoclonal antibody that binds and neutralises human VEGF [20]. Bevacizumab has been approved for the treatment of patients with mCRC in combination with standard fluoropyrimidine-based chemotherapy after several randomised studies demonstrated its benefit [21–23]. The observational cohort study BRITE has recently been published suggesting that the continuation of VEGF inhibition with bevacizumab beyond initial progression could prolong overall survival (OS) [24]. Two ongoing prospective randomised trials (ML18147 and SWOG S0600) are comparing the value of adding bevacizumab to second-line chemotherapy after failing first-line chemotherapy in combination with bevacizumab in patients with mCRC.

A variety of TKIs targeting the VEGFR are being developed such as vatalanib (PTK787), semaxanib (SU5416), sunitinib (SU11248), cediranib (AZD2171), sorafenib (BAY43-9006) and vandetanib (ZD6474). Of these, vatalanib has reached the most advanced stage of development but it has now been abandoned in the mCRC setting. The CONFIRM-1 study failed to demonstrate an advantage in PFS when vatalanib was added to FOLFOX (5-fluorouracil (5-FU)-leucovorin-oxaliplatin) in first-line treatment [25]. The CONFIRM-2 study evaluated the efficacy of vatalanib in combination with FOLFOX *versus* FOLFOX alone in 855 patients with irinotecan-refractory mCRC. PFS was significantly longer in the vatalanib-containing arm but no improvement in OS was demonstrated [26]. Sunitinib alone has not demonstrated objective responses in refractory mCRC patients although there are hints of clinical benefit with patients having prolonged stable disease [27]. A randomised phase IIb study of FOLFOX plus sunitinib *versus* FOLFOX plus bevacizumab and a phase III study of FOLFIRI (leucovorin/5-FU/irinotecan) with or without sunitinib in first-line mCRC patients have recently been completed; results are awaited. A randomised phase II study (HORIZON I) of cediranib with FOLFOX *versus* bevacizumab with FOLFOX in patients with previously treated mCRC was presented at the 2008 ASCO meeting [28]. 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* FOLFOX or XELOX alone (HORIZON II) in first-line therapy mCRC patients are currently recruiting patients.

There is strong preclinical evidence of the additive or synergistic effect of combining EGFR inhibitors and VEGF inhibitors. Activation of the EGFR upregulates the production of VEGF in cancer cells. The randomised phase II BOND2 study evaluated the concurrent administration of bevacizumab and cetuximab alone or in combination with irinotecan in the refractory setting. The addition of bevacizumab appeared to be synergistic, with favourable response rates (RR) and time to tumour progression compared to previous controls of the BOND-1 study [29]. However, two large phase III studies have failed to demonstrate synergistic activity for the use of combined anti-VEGF and anti-EGFR agents in the first-line setting. The PACCE study was designed to evaluate the effect of the addition of panitumumab to bevacizumab plus chemotherapy (oxaliplatin- or irinotecan-based chemotherapy). The addition of panitumumab resulted in increased toxicity and decreased PFS and OS [30]. The CAIRO 2 study was designed to evaluate the effect of the addition of cetuximab to bevacizumab plus chemotherapy (XELOX

(capecitabine-oxaliplatin)). Similarly, the addition of cetuximab resulted in a shorter mPFS [31]. The results of these two studies suggested that there is a lack of biological synergistic effect between antibodies against the EGFR (panitumumab or cetuximab) and bevacizumab in combination with chemotherapy in first-line therapy of mCRC patients. A National Cancer Institute-sponsored study (CALGB/SWOG 80404) comparing the addition of cetuximab, bevacizumab or both to standard chemotherapy – either FOLFOX or FOLFIRI at the physician's discretion – in chemotherapy-naïve mCRC patients is ongoing and should help to define the role of these two agents used in combination in the therapeutic armamentarium.

Small molecule dual inhibitors of VEGFR and EGFR such as vandetanib (ZD6474) or AEE788 are under clinical development. A phase II randomised study of two doses of vandetanib in combination with FOLFIRI *versus* FOLFIRI alone in oxaliplatin- and fluoropyrimidine-refractory mCRC patients has recently completed its recruitment. A similar phase II trial is evaluating if the combination of vandetanib with FOLFOX is more effective than FOLFOX alone in irinotecan- and fluoropyrimidine-refractory mCRC patients.

Insulin-like growth factor receptor inhibitors

The insulin-like growth factor (IGF) system plays a critical role in regulating cell proliferation, differentiation, apoptosis, and transformation. IGF-I and IGF-II inhibit apoptosis, promote tumour growth, and induce transformation and metastasis in many tumours. There are two types of cell membrane receptors (IGF-1R and IGF-2R). IGFs exert their actions by interacting with IGF-1R and this interaction is regulated by a group of specific binding proteins (IGFBP-1 through IGFBP-6) [32]. Evidence of IGF-1R signalling in cancer has emerged from various studies demonstrating high expression levels of multiple components of the IGF signalling system in diverse tumour types. The gastrointestinal system may be one of the major targets of IGF action. For example, there is increasing evidence that many alterations in IGF signalling are involved in the neoplasic transformation and progression of CRC. Even more, strong overexpression of IGF-1R has been found in most CRCs [33]. In human colon cancer cells, IGF-1R blockade with a MoAb inhibited cell proliferation [34]. Several MoAbs and small molecule inhibitors of the IGF-R have recently entered into clinical development (Table 3). AMG 479 is a fully human MoAb against the IGF-1R and a phase II study with FOLFIRI in combination with AMG-479 or AMG-655 (a fully human agonist MoAb that binds human TRAIL-R2) *versus* FOLFIRI in *K-Ras* mutant mCRC is currently under development.

Table 3
Anti-insulin-like growth factor receptor targeted agents in preclinical and clinical development

MK-0646	Human MoAb
IMC-A12	Human MoAb
AVE-1642	Human MoAb
CP-751871	Human MoAb
AMG-479	Human MoAb
IMC-A14	Human MoAb
EM164	Human MoAb
R1507	Human MoAb
BMS-554417, BMS-536924	TKI
PPP	TKI
NVP-AEW541, NVP-ADW742	TKI

TKI: Tyrosine kinase inhibitors, MoAb: Monoclonal antibodies.

Significant cross-talk has been observed between the IGF pathway and many other proteins known to be involved in cancer. There is critical interaction between the IGF and EGF pathways. EGF is able to stimulate IGF-II and vice versa. In addition, EGF can suppress the expression of IGFBP-3 and increase the availability of free IGFs. This issue provides a rationale for combined therapy against these different pathways with the goal to improve anti-tumour activity [35]. MK-0646, another anti-IGF-1R MoAb, is being developed in a phase II/III study in patients with *K-Ras* wild type tumours in combination with cetuximab in the refractory setting.

In preclinical models, treatment of human tumour cells with anti-IGF-1R therapies could enhance or interfere with the cytotoxic effects of some chemotherapeutic agents, depending on the order of the drug exposure. Thus, sequencing of conventional cytotoxic agents with IGF-1R inhibitors might need to be considered when designing combination clinical trials.

Platelet-derived growth factor receptor inhibitors

Platelet-derived growth factor receptor (PDGFR) is a transmembrane protein system involved in multiple tumour-associated processes. It has a role in autocrine growth stimulation of tumour cells, regulating tumour stroma fibroblast function and tumour angiogenesis. There are five dimeric PDGF isoforms. PDGF binds to the tyrosine kinase PDGFR, producing PDGFR dimerisation and autophosphorylation. This leads to activation of the intracellular signalling pathways [36]. PDGF expression is increased in several solid tumours including CRCs. Some studies have

tried to elucidate the role of PDGF in colon cancer angiogenesis [37,38].

Three different PDGFR TKIs have entered into clinical development to treat gastrointestinal malignancies. Imatinib (STI571) has demonstrated activity in human CRC cells in preclinical studies and phase II studies [39]. A phase I/II study of XELOX in combination with bevacizumab and imatinib in first-line mCRC is ongoing. Phase II/III studies are evaluating the activity of sunitinib and sorafenib in patients with mCRC. Sorafenib showed clinical activity in the initial phase I study; however, it failed to demonstrate any objective response in patients with refractory mCRC in a phase II study [40]. Nevertheless, there are several clinical trials that examine the activity of sorafenib in combination with oxaliplatin- and irinotecan-based chemotherapy and with other targeted therapies, such as cetuximab, in mCRC patients.

c-MET

After binding to the cellular membrane receptor tyrosine kinase, known as c-Met, the hepatocyte growth factor (HGF) stimulates several important cellular functions for development, homeostasis, and tissue regeneration, such as mitogenesis, motogenesis and morphogenesis in a wide range of cellular types including epithelial, endothelial and haematopoietic cells, neurons, melanocytes and hepatocytes. HGF signalling also contributes to oncogenesis and tumour progression in several human cancers and promotes cellular invasiveness linked to tumour metastasis.

So far, two targeted approaches directed to HGF/c-Met have entered clinical development: monoclonal antibodies targeting the HGF ligand such as AMG102 and AV-299 (SCH-900105), and TKIs of the c-Met like ARQ197 or XL880 (a dual c-MET/VGFR2 inhibitor). A phase Ib/II study of panitumumab in combination with AMG102 (HIF inhibitor) or AMG-479 in *K-Ras* wild-type mCRC patients is ongoing.

Tumour necrosis factor-related apoptosis-inducing ligand receptor

Beside the proliferative pathways, there are multiple pro- and anti-apoptotic pathways in cancer cells. A novel approach has emerged attempting direct stimulation of apoptosis via engagement of a family of membrane-bound pro-apoptotic receptors. The tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis and is a member of the tumour necrosis factor (TNF) ligand super-family. Of four receptors identified to date, TRAIL-R1 and TRAIL-R2 mediate downstream signalling, leading to apoptosis, upon binding with TRAIL. TRAIL-R3 and TRAIL-R4 have non-functional or absent death domains, do not transmit apoptotic signals, and may function as decoy receptors [41]. Upon binding of TRAIL to functional receptors, TRAIL-R1 and -R2 recruit apoptosis-inducing caspases that activate the pro-apoptotic proteins Bid and Bax, leading to cytochrome C release from mitochondria. The TRAIL-related pathway has been targeted either by agonistic monoclonal antibodies directed to TRAIL-R1 or -R2 or by recombinant variants of the ligand TRAIL itself.

AMG655 is a fully human monoclonal agonist antibody that binds human TRAIL-R2. A phase Ib study of AMG655 in combination with FOLFOX and bevacizumab for the first-line treatment of mCRC patients has recently been presented [42] and a randomised phase II trial of FOLFOX6 plus bevacizumab with or without AMG655 is currently in progress.

Agents targeting downstream signalling pathways

PI3K-Akt-mTOR inhibitors

The PI3K/AkT/mTOR pathway controls many cellular processes that are important for the formation and progression of cancer, including apoptosis, transcription, translation, metabolism, angiogenesis and cell cycle progression. PI3K, a heterodimeric protein composed of a catalytic subunit (p110α) and a regulatory subunit (p85), is activated by growth factor receptor tyrosine kinases. The survival mechanism initiated by these proteins is executed through downstream effectors of this kinase: Akt, mTOR and p70S6 kinase. Akt is a serine/threonine kinase that is activated by recruitment to the plasma membrane through direct contact with phosphatidylinositol triphosphate (PIP_3) [43]. Phosphatase and tensin homologue (PTEN) dephosphorylates PIP3, thus acting as a negative regulator of PI3K signalling. With the involvement of the PI3K/Akt/TSC/Rheb pathway and in the presence of mitogens and sufficient nutrients, mTOR relays a signal to translational regulators, resulting in the specific enhancing of the translation of messenger RNAs (mRNAs) encoding proteins essential for cell growth and cell cycle progression through G1 to S transition. mTOR has numerous regulatory functions including activation of p70S6 kinase. As a result of its position within this signal transduction pathway, mTOR is an important target for new anticancer drug development. The mTOR complex signals to, among others, two downstream effectors: the ribosomal protein S6 kinase 1 and the translational repressor

protein eukaryotic initiation factor 4E-binding protein 1 (eIF4E-4EBP1). After a final phosphorylation, 4EBP1 dissociates from eiF4E, thereby enabling the reconstitution of a translationally competent initiation factor complex eIF4F and eiF4G.

The mTOR-related pathway is aberrantly activated in around half of human tumours [44]. Although mutated versions of mTOR have not yet been described in human tumours so far, aberrant PI3K/Akt/mTOR dependent signalling has been observed in many human malignancies. This results from overexpression and mutations of growth factor receptors, amplification and/or overexpression of PI3K and Akt, and loss of the tumour suppressor phosphatase PTEN or loss of the tuberous sclerosis complex 2 (TSC2), as well as other effects.

The PI3K signalling pathway is upregulated in many CRCs [45] and this upregulation positively correlates with increased tumourigenic potential of colon adenocarcinoma cell lines. Mutations in PI3KCA (which encodes the p110α catalytic subunit) have been identified in up to one third of CRC specimens.

Rapamycin and its analogues (CCI-779 (tensirolimus), RAD001 (everolimus) and AP23573 (deforolimus)) are macrolids that inhibit mTOR function. These drugs inhibit the growth of several human cancer cells in preclinical models including gastrointestinal tumour models. On the basis of this preclinical activity, rapamycin and its analogues are being developed clinically as anticancer drugs. Everolimus alone has not demonstrated objective tumour responses in heavily pretreated mCRC patients but the combination of bevacizumab and everolimus has activity in refractory mCRC patients who have progressed on a bevacizumab-based regimen [46].

Another potential target in this pathway is the PI3K inhibition. Several PI3K inhibitors are in clinical development such as BEZ235 [47], SF1126 [48], BGT226, XL147, BKM120, GDC-0941 and XL765. Other PI3K inhibitors are still in preclinical development, such as ZSTK474. PI3K inhibition could serve to overcome drug resistance to other targeted agents [49].

Src kinase inhibitors

C-src is a non-receptor tyrosine kinase protein. C-src is composed of a carboxy-terminal tail containing a negative-regulatory tyrosine residue, four src homology domains and an amino terminal domain. The autophosphorylation site is located in the SH1 kinase domain and is required for full src activation. *In vitro* observations have led to the hypothesis that in addition to increasing cellular proliferation, a primary role for c-src in cancer is to regulate cell adhesion, invasion and motility [50].

C-src is overexpressed and activated in many human cancers and is associated with advanced-stage and distant metastases. Increased C-src activity has been demonstrated in CRC [51]. C-src activity increases with the advanced stage of tumour development, for example, the high C-src activity in dysplastic polyps with high malignant potential compared with more benign adenomas [52]. C-src activity is an independent indicator of poor clinical prognosis in CRC. C-src is also of particular interest in CRC because it is overexpressed and/or activated in a wide range of tumours that also overexpress several receptor tyrosine-kinases, indicating the potential role for cross-talk interactions in promoting tumourigenesis. Overexpression of the Erb family members in gastrointestinal tumour cells leads to C-src activation. Interactions with ligand-activated receptor tyrosine-kinases, such EGFR, PDGFR or HER2, can result in augmented C-src activation. C-src can also phosphorylate EGFR [53]. In addition, src proteins regulate molecules associated with angiogenesis. In summary, C-src has an important role in oncogenic processes which provides a rationale for C-src as a therapeutic target. Numerous C-src inhibitors are entering phase I/II trials, including SKI-606, SU 6656, AP 23464, BMS-354825 (dasatinib), AZD0530 and SKI-606 (bosutinib).

Hdm2 inhibitors

The p53 tumour suppressor gene plays a central role in many cancer types mainly as a transcription factor regulating the pathways of cell-cycle arrest, apoptosis and DNA repair. Hdm2 is a key regulator of p53 inducing the proteosomal degradation and the inhibition of transcriptional activity of p53 [54]. Hdm2 has been shown to be overexpressed in a wide variety of tumour types. Many peptides have been developed to inhibit Hdm2 but only two compounds have demonstrated sufficient ability to penetrate the cell membrane and target Hdm2 [55]. The first type of drugs developed were small molecules that target the Hdm2-p53 interaction, Nutlins being the most prominent group, that specifically bind and dissociate Hdm2 from p53, saving p53 from degradation and inducing cell-cycle arrest and apoptosis. Nutlin-3 has been the most developed drug preclinically showing a strong antiproliferative and pro-apoptotic effect in different tumour types, predominantly in tumours that preserve the wild-type p53 status [56].

A second small molecule (JNJ-26854165) is currently in phase I trials and has showed significant activity not only in wild-type p53 tumours but also in mutant cell lines [57]. This molecule is able to stabilise Hdm2-p53 after its ubiquitation and save the complex from the proteosomal degradation. Currently, several trials are ongoing with these compounds and planned phase II trials in specific tumour types alone or in combination with cytostatics will be initiated in the near future.

Other protein kinases that regulate cell division

Aurora-kinase inhibitors

Aurora is the name given to a family of seronine/threonine protein kinases that regulate many processes during cell division. Three Aurora kinase (AK) family members have been identified in mammalian cells: A, B, and C. These proteins are implicated in several vital events in mitosis and play a critical role as regulators of genomic stability [58]. AKs are frequently overexpressed in human tumours. Deregulation of cell-cycle machinery can have an important impact on cellular proliferation. This observation has led to an interest in this family of kinases as potential drug targets for new anti-cancer therapies. The first data to implicate this family of kinases in tumourigenesis came with the observation that AK A DNA was amplified and its RNA was overexpressed in more than 50% of primary CRC specimens. This overexpression was correlated with poor prognostic in patients with CRC [59,60].

Several AK inhibition drugs are in clinical development: ZM447439 [61], Hesperadin [62], VX-680 [63], AZD1152, MLN 8054 [64] and MLN8237 [65]. The first three inhibit phosphorylation of histone H3 on serine 10 and also inhibit cell division. However, they do not inhibit cell cycle progression nor do they selectively inhibit one single kinase. Although it is not yet clear which AK is inhibited to mediate the anti-tumour effects of these three drugs, AK B is probably the primary target. VX-680 inhibits the kinase activity of AK A, B, C, and FLT3. Treatment of nude mice and rats carrying tumour xenografts, derived from either human colon tumours or pancreatic tumours, demonstrated dose-dependent tumour-growth inhibition and, in some cases, regression. MLN 8054 is a selective, orally administered small molecule inhibitor of AK A. It competes with adenosine triphosphate (ATP) binding and therefore reversibly inhibits AK A. MLN 8054 displays anti-tumour activity against three different human colon cancer xenografts. Taken together, the results from studies using VX-680 and MLN 8054 provide a strong rationale for further investigations of AK inhibitors in oncology. Currently, AK inhibitors are being tested in early clinical development in patients with advanced malignancies.

Polo-like kinases family

Polo-like kinases (Plk) are serine/threonine protein kinases and members of the mitotic complex. There are four human Plks: Plk1, Plk2 (Snk), Plk3 (Fnk/Prk) and Plk4 (Sak). Plk1 is the most widely studied family member. Plks are associated with several mitotic structures and play an essential role in centrosome separation, chromosome alignment and segregation, and cytokinesis. Inhibition of these kinases results in abnormal mitotic events and eventually leads to apoptosis [66]. Plk1 is overexpressed in a wide variety of tumours [67]. Some data suggests that deregulation of Plk1 may be an early event in oncogenesis. In fact, overexpression of Plk1 alone was sufficient to induce tumour formation in a nude mouse model. Because of the biological consequences of inhibiting Plks, a number of small-molecule Plk1 selective inhibitors have been developed and are under evaluation in clinical trials.

Conclusions

In recent years, the incorporation of new cytotoxic and molecular agents for the treatment of mCRC has improved overall survival in this group of patients. However, the prognosis of advanced mCRC continues to be poor and most of the patients will ultimately die of their disease.

The demonstration that overexpression and activation of kinase proteins is associated with CRC development as well as poor prognosis has suggested that these kinase proteins are promising targets for the development of inhibitors as potential therapeutic agents for CRC, alone or in combination with chemotherapeutic agents or other new targeted molecules.

Clinical and molecular predictive of response markers are under evaluation, by pharmacodynamic, genomic and proteomic studies, to better select patients that can benefit from these treatments. As a result of the data emerging from the studies recently published, *K-Ras* mutational status has been accepted as a predictive marker for resistance to anti-EGFR MoAbs in mCRC. Moreover, the regulatory approval these compounds have in Europe mandates the need to perform *K-Ras* testing to exclude the presence of

mutations when patients with mCRC are considered for treatment with anti-EGFR MoAbs. However, wild type *K-Ras* does not guarantee the benefit from anti-EGFR MoAbs. Emerging data suggest that mutations of *B-Raf* as well as deregulation of the PI3K/PTEN/AKT/mTOR pathway may also predict the lack of effect of these compounds in patients with mCRC. A prospective validation of these and other biomarkers within the context of prospective randomised trials is required by some regulatory agencies, like the FDA, in order to definitively accept the value of these biomarkers in selecting the patients that will more likely benefit from anti-EGFR MoAbs and other targeted therapies. Indeed, the development of predictive biomarkers of activity is an urgent need for all targeted therapies. In this direction, some academic studies are trying to elucidate which biomarkers could more likely predict a more incremental effect of anti-angiogenesis drugs as well to identify the secondary resistance to these compounds.

In the coming years, emerging data on the new targeted agents that are currently in clinical development will probably translate in an expansion of the therapeutic armamentarium against mCRC. Nevertheless, this expected setting will only occur if we are able to identify predictive biomarkers of either activity or primary and/or secondary resistance that may allow us to identify not only the population of patients with mCRC that will benefit from these treatments but also the timing of its best benefit in the time course of the advanced disease.

Conflict of interest statement

Josep Tabernero has received honoraria for consultancy/advisory boards from Merck-Serono, Amgen, Roche, MSD, Onyx, Bayer, Sanofi-Aventis and Novartis.

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