Advances in targeted therapies for metastatic colorectal cancer

Colorectal cancer is one of the most frequently diagnosed malignancies in men and women and despite recent advances the prognosis of metastatic colorectal cancer remains poor. A better understanding of the molecular pathways that characterize tumor growth has provided novel targets in cancer therapy. Several proteins have been implicated as having a crucial role in metastatic colorectal cancer. Targets are defined according to their cellular localization, such as membrane receptor targets, intracellular signaling targets and protein kinases that regulate cell division. In the last 5 years, cetuximab, panitumumab and bevacizumab have been approved for the treatment of metastatic colorectal cancer and emerging data on the clinical development of new drugs, other than EGF-receptor and VEGF inhibitors, are likely to provide novel opportunities in the treatment of this malignancy.

KEYWORDS: metastatic colorectal cancer ■ molecular cancer biology ■ targeted therapies

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Colorectal cancer (CRC) is one of the most frequent diagnosed cancers in men and women. In recent years, CRC mortality has progressively decreased, probably owing to the availability of earlier diagnosis through screening, improvements in surgical therapies and both chemotherapy and radiotherapy approaches. Better knowledge of the molecular cancer biology has also contributed to the better outcomes of the metastatic CRC (mCRC) population, but there is a need to optimize and define the best use of these new approaches.

Targets are defined according to their cellular localization, such as membrane receptor targets (e.g., EGF-receptor [EGFR], VEGF receptor [VEGFR], IGF-receptor [IGFR], PDGF-receptor [PDGFR], TNF-related apoptosis-inducing ligand receptor [TRAIL-R] and hepatocyte growth factor receptor or C-Met), intracellular signaling targets (e.g., Ras/Raf/MAPK pathway, phosphatidylinositol-3-kinase [PI3K]/AKT/mammalian target of rapamycin [mTOR] pathway, src kinase and p53/Hdm2) and protein kinases that regulate cell division, such as aurora kinases (AKs) and polo-like kinases (Plks) (Figure 1).

In this review, we focus on the advances and development status of targeted therapies in the treatment of mCRC.

Agents targeting membrane receptors

■ VEGFR inhibitors

The regulation of angiogenesis is a complex, multistep process resulting from a dynamic balance between proangiogenic and antiangiogenic factors. One of the most important regulators of this process is VEGF and VEGFR. VEGF is a 45-kDa homodimer that belongs to a family of growth factors comprising six different glycoproteins: VEGF-A (commonly referred to as VEGF), -B, -C, -D, -E, and PlGF. Owing to its central role in tumor-associated angiogenesis, VEGF has emerged as an attractive and central therapeutic target in CRC.

Bevacizumab is a humanized anti-VEGF monoclonal antibody (mAb) that binds and neutralizes human VEGF [1]. In 2004, bevacizumab was approved for first-line treatment of mCRC patients. Bevacizumab improved response rate (RR), median progression-free survival (mPFS) and overall survival (OS) when given in combination with irinotecan-based therapy [2]. The results of the NO16966 trial that evaluated the addition of bevacizumab to leucovorin/5-fluorouracil/oxaliplatin (FOLFOX) or capecitabine/oxaliplatin (XELOX) have recently been published. The aim of the study was to demonstrate the noninferiority of XELOX versus FOLFOX and the superiority of bevacizumab when added to oxaliplatin-based treatment. Bevacizumab added statistically significant improvement in mPFS (8 vs 9.4 months, respectively; p = 0.0023; hazard ratio [HR]) = 0.83) [3]. In second-line therapy, the Phase III E3200 study compared FOLFOX plus high-dose bevacizumab (10 mg/kg), FOLFOX alone or bevacizumab alone and showed a statistically significant improvement in OS, mPFS and RR for the combination arm [4]. After these results, bevacizumab was approved

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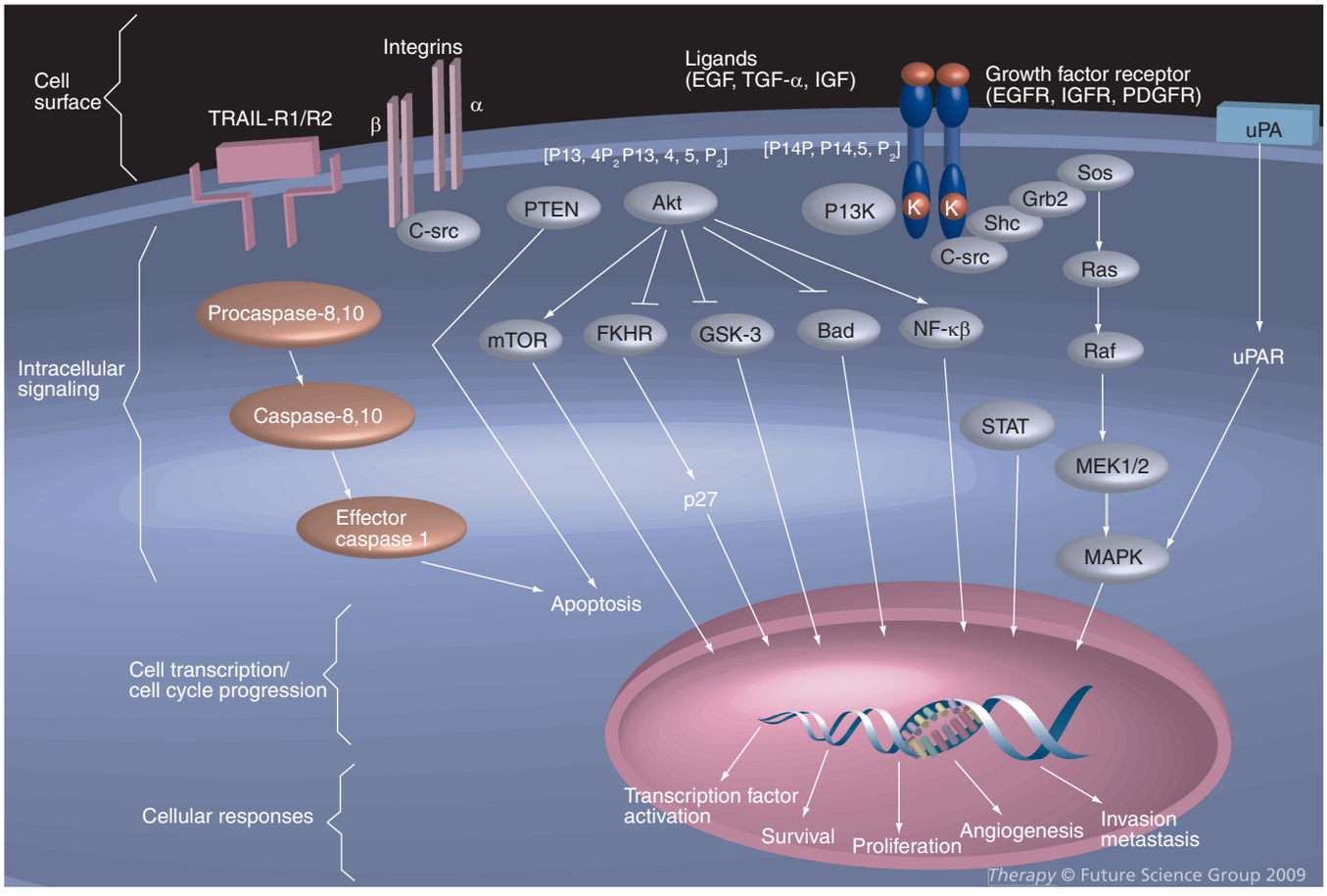

Figure 1. Scheme of intracellular signal transduction pathways. Ligands bind to the extracellular domain of membrane receptors, which are phosphorylated, leading to activation of several cytoplasmatic messengers, which activate transcription factors in the nucleus. The activation of transcription factors in the nucleus involves some target genes which are implicated in the proliferation, angiogenesis, apoptosis and tumor invasion processes.
EGFR: EGF-receptor; FKHR: forkhead transcription factor; GRB2: growth factor receptor-bound protein 2; IGFR: IGF-receptor; mTOR: Mammalian target of rapamycin; PDGFR: PDGF-receptor; $PI_{34}P_2$: Phosphatidylinositol (3,4) biphosphate; $PI_{345}P_3$: Phosphatidylinositol (3,4,5) triphosphate; PI3K: Phosphatidylinositol 3-kinase; PTEN: Phosphatase and tensin homolog; SHC: Src homology 2 domain-containing transforming protein; SOS: Son of sevenless protein; STAT: Signal transducers and activators of transcription protein; TRAIL: TNF-related apoptosis-inducing ligand; TRAIL-R: TRAIL-receptor; uPA: Urokinase-type plasminogen activator; uPAR: Urokinase-type plasminogen activator receptor.

in second-line therapy for mCRC patients. The observational cohort study Bevacizumab Regimens Investigation of Treatment Effects and Safety (BRITE) has recently been published and suggested that continuation of VEGF inhibition with bevacizumab beyond initial progression could prolong OS [5]. Two ongoing prospective randomized trials (ML18147 and Southwest Oncology Group [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.

Box 1. Anti-VEGF adverse effects.

- Hypertension
- Proteinuria
- Bleeding
- Thrombotic effects
- Wound healing
- Gastrointestinal perforation

Hypertension is the most common bevacizumab-related toxicity. Other toxicities of antiangiogenic therapy are summarized in **Box 1**.

A variety of small-molecule tyrosine kinases (TKIs) targeting the VEGFRs are being developed, such as PTK-787 (vatalanib), SU-5416 (semaxanib), SU-11248 (sunitinib), AZD-2171 (cediranib), BAY-43–9006 (sorafenib) and ZD-6474 (vandetanib). Of these, PTK-787 is the most advanced VEGFR TKI in clinical development. The CONFIRM-1 study failed to demonstrate an advantage in PFS when PTK-787 was added to FOLFOX in first-line treatment [6]. The CONFIRM-2 study evaluated the efficacy of PTK-787 in combination with FOLFOX

versus FOLFOX alone in 855 patients with irinotecan-refractory mCRC. PFS was significantly longer in the PTK-787 arm but no improvement in OS was demonstrated [7]. A metanalysis of the CONFIRM-1 and CONFIRM-2 studies showed that PTK-787 significantly improved PFS in patients with high lactate dehydrogenase levels [8]. Whether lactate dehydrogenase could serve as a surrogate marker for hypoxia and a predictive factor of response to antiangiogenic therapy remains nuclear [9]. SU-11248 alone has not demonstrated objective responses in refractory mCRC patients [10]. A randomized Phase IIb study of FOLFOX plus SU-11248 versus FOLFOX plus bevacizumab and a Phase III study of leucovorin/5-fluorouracil/irinotecan (FOLFIRI) with or without SU-11248 in first-line mCRC patients are currently ongoing. At the 2008 American Society of Oncology (ASCO) meeting, a Phase II randomized study (HORIZON-1) of cediranib with FOLFOX versus bevacizumab with FOLFOX in patients with previously treated mCRC was presented [11]. In this study no statistically significant difference in PFS was observed, although the bevacizumab arm showed the numerically longest PFS and patients receiving cediranib had more adverse effects. A Phase II/III study (HORIZON-3) 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-2) in first-line therapy for mCRC patients are currently recruiting.

There is preclinical evidence for combining EGFR inhibitors and VEGF inhibitors. Activation of the EGFR upregulates the production of VEGF in cancer cells. The randomized Phase II Bowel Oncology with Cetuximab Antibody (BOND)-2 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 favorable RR and time to tumor progression compared with previous controls of the BOND-1 study [12]. Two other studies failed to demonstrate synergistic activity for the use of combined anti-VEGF and anti-EGFR agents. The Panitumumab Advanced Colorectal Cancer Evaluation (PACCE) study was designed to evaluate bevacizumab plus chemotherapy (oxaliplatin- or irinotecan-based chemotherapy) with or without panitumumab. The addition of panitumumab to bevacizumab and oxaliplatin- or irinotecan-based chemotherapy resulted in increased toxicity and decreased mPFS [13]. The CAIRO-2 study was designed to evaluate bevacizumab plus chemotherapy (XELOX) with or without cetuximab. Similarly, the combination of cetuximab and bevacizumab resulted in a shorter mPFS [14]. The results of these two studies suggested that there is a lack of biological synergistic effect between mAbs against EGFR (panitumumab or cetuximab) and bevacizumab in combination with chemotherapy in first-line therapy of mCRC patients. A National Cancer Institute-sponsored study (Cancer and Leukemia Group B [CALGB]/SWOG 80404) comparing the addition of cetuximab, bevacizumab or both to standard chemotherapy – either FOLFOX or FOLFIRI, at the physician's choice – in chemonaive 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 (ZD-6474) or AEE-788, are under clinical development. A Phase II randomized 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 whether the combination of vandetanib with FOLFOX is more effective than FOLFOX alone in irinotecan- and fluoropyrimidine-refractory mCRC patients

EGFR inhibitors

The 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). EGFR is a 180-kDa transmembrane glycoprotein. Several EGFR-ligands have been identified including EGF, HB-EGF-like growth factor, TGF, epiregulin, betacellulin and amphiregulin. These ligands induce dimerization and autophosphorylation of the receptor and initiate intracellular signaling pathways linked to cellular proliferation, control of apoptosis and angiogenesis. EGFR is overexpressed in 75–90% of

Box 2. Anti-EGFR adverse effects.

- Skin toxicity (acne-like rash, dry skin, paronychia, photosensitivity and trichomegaly)
- Diarrhea
- Asthenia
- Nausea/vomiting
- Abdominal pain
- Ocular toxicity (conjunctivitis, ocular hyperemia, blepharitis and increased lacrimation)
- Estomatitis-mucosits
- Hypomagnesemia
- Infusion reactions

CRC and confers a poor prognosis [15]. While multiple strategies targeting the EGFR are under development, two modalities have been the most developed: small molecule inhibitors of the intracellular kinase domain of the EGFR and mAbs designed to block the extracellular ligand-binding domain of EGFR.

Cetuximab, the most advanced anti-EGFR agent in clinical development, and panitumumab, a fully human mAb against EGFR, have been approved in the USA and in Europe for the treatment of mCRC patients [16,17]. The most common toxicity reported in clinical trials of cetuximab is a self-limiting acne-like rash. Other toxicities of anti-EGFR therapy are summarized in Box 2. In chemorefractory mCRC (disease progression during or within 6 months following the last administration of fluoropyrimidine, oxaliplatin and irinotecan), panitumumab significantly improved mPFS (8 vs 7.3 weeks, respectively; $p < 0.001$; HR: 0.54) and cetuximab signficantly improved OS (6.1 vs 4.6 months; $p = 0.005$; HR: 0.77) and PFS ($p < 0.001$; HR: 0.68) in comparison with best supportive care alone [16,18]. Currently, several articles have reported that K-RAS mutations are associated with lack of response to anti-EGFR therapy [19–21]. K-RAS mutations are found in approximately 40% of CRC and a high concordance between primary tumor and related metastases has been reported [22,23]. Two retrospective analyses recently published have suggested that B-Raf wild-type and absence of PI3KCA mutations are also required for response to anti-EGFR therapy [24,25]. Recently, Van Cutsem *et al.* reported the results of a Phase III trial (the Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer [CRYSTAL] study) in mCRC patients in first-line therapy. The combination of cetuximab plus FOLFIRI demonstrated a statistically significant increase in mPFS and RR in the K-RAS wild-type population [26]. Similar results have also been observed with the combination of cetuximab plus FOLFOX (the Oxaliplatin and Cetuximab in First-Line Treatment of Metastatic Colorectal Cancer [OPUS] study) [27]. In the second-line setting, the Phase III Erbitus plus Irinotecan in Colorectal Cancer (EPIC) study compared cetuximab plus irinotecan with irinotecan alone and the combination arm showed a statistically significant benefit in mPFR and RR, but no significant difference was observed in terms of OS, probably owing to the crossover therapy [28].

Multiple studies are currently evaluating the efficacy and safety of panitumumab in mCRC. Some of these trials are summarized in Table 1.

There are a large number of TKIs directed to EGFR in clinical development (Table 2). So far, three TKIs have been specifically evaluated in mCRC: gefitinib and erlotinib, reversible EGFR-specific TKIs, and EKB-569, an EGFR-specific and irreversible TKI. Response and disease control rates observed in some Phase I/II combination studies, 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 randomized Phase III studies are needed in order to reach definitive conclusions [29–34].

IGFR inhibitors

The IGF pathway plays a critical role in regulating cell proliferation, differentiation, apoptosis and transformation. IGF-I and IGF-II inhibit apoptosis, promote tumor growth and induce transformation and metastasis in many tumors. The IGF pathway consists of three ligands (insulin, IGF-I and IGF-II), six receptors (IGF-1R and IGF-2R are the most important) and up to seven ligand–receptor regulating binding proteins (IGFBP 1–7) [35].

Interest in the role of IGF-I in CRC is due to the increased risk of this malignancy in patients with acromegaly, a disease characterized by

Table 1. Clinical trials with panitumumab.

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

FOLFOX: Leucovorin/5-fluorouracil/oxaliplatin; FOLFIRI: Leucovorin/5-fluorouracil/irinotecan; PRIME: Panitumumab Randomized Trial in Combination with Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy; SPIRITT: Second-Line Panitumumab Irinotecan Treatment Trial.

Table 2. Clinical studies with EGF-receptor tyrosine kinase inhibitors.

Tyrosine kinase inhibitor	Population	Treatment	Number of patients	Response rate (%)	Ref.
Gefitinib	Refractory	Gefitinib + FOLFOX	27	33	[32]
	First-line	Gefitinib + FOLFOX	43	72	[30]
Erlotinib	Refractory	Erlotinib	31	0	[29]
	Refractory	FOLFOX + erlotinib	24	33	[34]
	Refractory	XELOX + erlotinib	32	25	[33]
EKB-569	First-line	EKB569 + FOLFIRI	47	48	[31]

FOLFOX: Leucovorin/5-fluorouracil/oxaliplatin; FOLFIRI: Leucovorin/5-fluorouracil/irinotecan; XELOX: Capecitabine/oxaliplatin.

elevated levels of IGF-I and pituitary growth hormone. Many alterations in IGF pathways are involved in the neoplasic transformation and progression of CRC and strong overexpression of IGF-1R has been found in most CRC [36]. In human colon cancer cells, IGF-1R blockade with a mAb inhibited cell proliferation [37]. Several antibodies and small molecule inhibitors of the IGFR have recently entered clinical development (**Box 3**). AMG-479 is a fully human mAb against the IGF-1R and a Phase II study of FOLFIRI in combination with AMG-479 or AMG-655 (a fully human agonist mAb that binds human TRAIL-R2) versus FOLFIRI in K-RAS mutant mCRC is currently under development.

Significant crosstalk has been observed between the IGF and EGF pathways. This issue provides a rationale for combined therapies against these different pathways, to improve antitumor activity [38]. A randomized Phase II/III study of MK-0646, another anti-IGF-1R mAb, in combination with cetuximab and irinotecan, is currently recruiting patients.

In preclinical models, treatment of human tumor 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.

PDGFR inhibitors

PDGFR is a transmembrane protein system involved in multiple tumor-associated processes. It has a role in autocrine growth stimulation of tumor cells, regulating tumor stroma fibroblast function and tumor angiogenesis. There are five dimeric PDGF isoforms. PDGF binds to the tyrosine kinase PDGFR, producing PDGFR dimerization and autophosphorylation. This leads to activation of the intracellular signaling pathways [39]. PDGF expression is increased in several solid tumors, including CRC, and some studies have tried to elucidate the role of PDGF in colon cancer angiogenesis [40,41].

Three different PDGFR TKIs, STI-571 (imatinib), BAY-43–9006 (sorafenib) and SU-11248 (sunitinib) have entered into clinical development to treat gastrointestinal malignancies. Imatinib has demonstrated activity in human CRC cells in preclinical studies and Phase II studies [42]. A Phase I/II study of XELOX in combination with bevacizumab and imatinib in first-line mCRC is ongoing. Sorafenib demonstrated clinical activity in the initial Phase I study, but failed to demonstrate any objective response in patients with heavily pretreated mCRC [43]. Currently, 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

HGFR, also known as C-Met, has been shown to be deregulated in several human cancers. HGF is the only known ligand of this receptor. C-Met

Box 3. Anti-IGF receptor-targeted agents in preclinical and clinical development.

Human monoclonal antibody

- MK-0646
- IMC-A12
- AVE-1642
- CP-751871
- AMG-479
- IMC-A14
- EM-164
- R-1507

Tyrosine kinase inhibitor

- BMS-554417
- BMS-536924
- PPP
- NVP-AEW541
- NVP-ADW742

activation by HGF plays an important role in metastatic growth of colon tumor cells in the liver and cooperates with K-RAS mutation to enhance tumorigenicity of colon cancer cells [44,45].

So far two targeted approaches directed to HGF/C-Met have entered clinical development: mAb targeting the ectodomain, such as AMG-102 and AV-299 (SCH-900105), and small molecules TKIs, such as ARQ-197 or XL-880 (a dual C-Met/VEGFR2 inhibitor). A Phase Ib/II study of panitumumab in combination with AMG-102 or AMG-479 in K-RAS wild-type mCRC patients is ongoing.

TRAIL-R

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 proapoptotic receptors. The TRAIL, also known as Apo-2L, induces apoptosis and is a member of the TNF ligand superfamily [46]. Of four receptors identified to date, TRAIL-R1 and TRAIL-R2 mediate downstream signaling upon binding with TRAIL, leading to apoptosis. TRAIL-R3 and TRAIL-R4 have nonfunctional or absent death domains, do not transmit apoptotic signals, and may function as decoy receptors [47]. Upon binding of TRAIL to functional receptors, TRAIL-R1 and -R2 recruit apoptosis-inducing caspases that activate the proapoptotic proteins Bid and Bax, leading to cytochrome c release from mitochondria.

The TRAIL-related pathway has been targeted, either by agonist mAb directed to TRAIL-R1 or R2 or by recombinant variants of the ligand TRAIL itself. AMG-655 is a fully human monoclonal agonist antibody that binds human TRAIL-R2. A Phase Ib study of AMG-655 in combination with FOLFOX and bevacizumab for the first-line treatment of mCRC patients has been recently presented [48] and a randomized Phase II trial of FOLFOX plus bevacizumab with or without AMG-655 is currently in progress.

Agents targeting downstream signaling pathways

PI3K–Akt–mTOR inhibitors

The PI3K/Akt signaling pathway is an integral part of diverse functions, including cellular proliferation, differentiation and survival. The PI3Ks are a family of intracellular lipid kinases whose primary biochemical function is to phosphorylate the 3-hydroxyl group of phosphoinositides. PI3Ks are divided into three classes (I–III) and the functions of class I PI3Ks pertain to growth, proliferation and survival. The class I PI3K is further grouped into two classes. Class IA molecules are heterodimers comprised of a regulatory p85 subunit and a catalytic p110 subunit and class IB are heterodimers comprised of a catalytic p110γ subunit and a regulatory p101 subunit [49]. PI3Ks are 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 phosphoinositol triphosphate (PIP_3). Phosphatase and tensin homolog (PTEN) dephosphorylates PIP_3, therefore acting as a negative regulator of PI3K signaling. 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 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 approximately half of human tumors, although mutated versions of mTOR have not yet been described in human tumors.

Aberrant PI3K/Akt/mTOR-dependent signaling has been observed in many human malignancies [50]. This results in overexpression and mutations of growth factor receptors, amplification and/or overexpression of PI3K and Akt, and loss of the tumor suppressor phosphatase PTEN or loss of the tuberous sclerosis complex. The PI3K signaling pathway is upregulated in many CRCs [51], and this upregulation positively correlates with increased tumorigenic potential of colon adenocarcinoma cell lines. Mutations in PI3KCA (which encodes the p110 catalytic subunit) have been identified in up to a third of CRC specimens.

Rapamycin and its analogs CCI-779 (temsirolimus), RAD-001 (everolimus) and AP-23576 (deferolimus) are macrolids that inhibit mTOR function. These drugs inhibit the growth of several human cancer cells in preclinical models, including gastrointestinal tumor models. On the basis of this preclinical activity, rapamycin and its analogs are being clinically developed as anticancer drugs. Everolimus alone has not demonstrated objective tumor responses in heavily pretreated mCRC patients [52] but the combination of bevacizumab and everolimus has activity in refractory mCRC patients who have progressed on a bevacizumab-based regimen [53].

Another potential target in this pathway is the PI3K inhibition. Several PI3K inhibitors are in clinical development such as BEZ-235 [54], BGT-226, XL-147, BKM-120, GDC-0941 and XL-765 (**Box 4**). Other PI3K inhibitors are still in preclinical development, such as SF-1126 [55] and ZSTK-474. PI3K inhibition could serve to overcome drug resistance to other targeted therapies [56].

Box 4. PI3K inhibitors in clinical development.

PI3K inhibitor

- BKM-120
- XL-147
- GDC-0941

PI3K and mTOR dual inhibitor

- BEZ-235
- BGT-226
- XL-765

PI3K: Phosphatidylinositol 3-kinase; mTOR: Mammalian target of rapamycin.

Src kinase inhibitors

C-src is a nonreceptor 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 [57].

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 [58]. C-src activity increases with the advanced stage of tumor development, for example, the high C-scr activity in dysplasic polyps with high malignant potential compared with more benign adenomas [59]. 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 tumors that also overexpress several receptor tyrosine kinases, indicating the potential role for cross-talk interactions in promoting tumorigenesis. Overexpression of Erb family members in gastrointestinal tumor 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 [60]. C-src can also phosphorylate EGFR. In addition, src proteins regulate molecules associated with angiogenesis.

Numerous C-src inhibitors are entering Phase I–II trials, including SKI-606 (bosutinib), SU-6656, AP-23464, BMS-354825 (dasatinib) and AZD-0530.

Hdm2 inhibitors

p53 tumor 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 transcriptional activity inhibition of p53 [61]. Hdm2 has been shown to be overexpressed in a wide variety of tumor 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 [62].

The first type of drugs developed were small molecules that target the Hdm2–p53 interaction, Nutlins being the most prominent group, which specifically bind and dissociate Hdm2 from p53, saving p53 from degradation and inducing cell-cycle arrest and apoptosis. Nutlin-3 has been the most preclinically developed drug, showing a strong antiproliferative and proapoptotic effect in different tumor types, predominantly in tumors that preserve the wild-type p53 status.

A second small molecule (JNJ-26854165) is currently in Phase I trials and has showed significant activity not only in wild-type p53 tumors but also in mutant cell lines. This molecule is able to stabilize Hdm2-p53 after its ubiquitation and save the complex from proteosomal degradation.

Currently, several trials are ongoing with these compounds and planned Phase II trials in specific tumor types alone or in combination with cytostatics will be initiated in the near future.

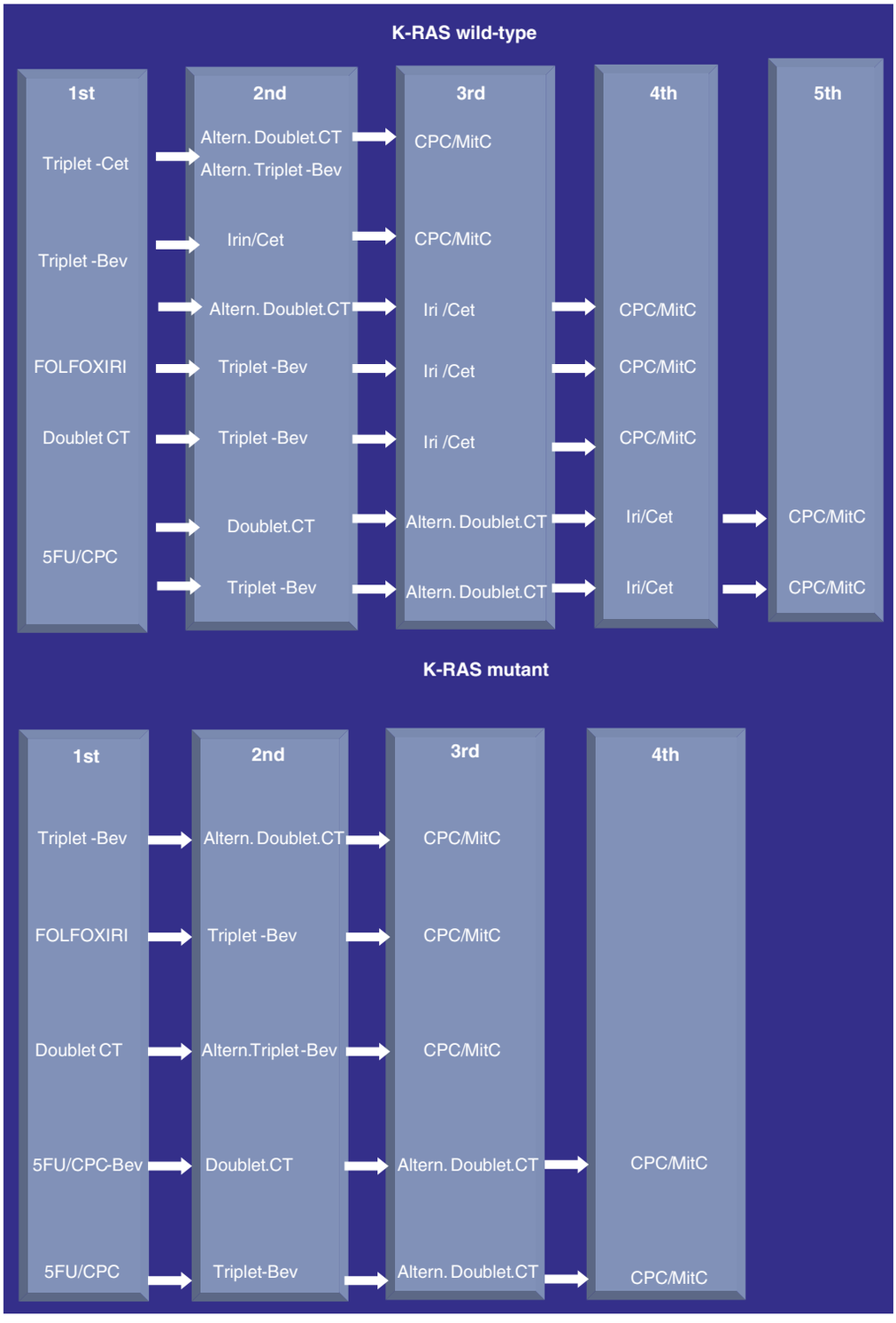

Figure 2. Treatment algorithm for metastatic colorectal cancer.
5-FU: 5-Fluorouracil; Altern.: Alternative; Bev: Bevacizumab; Cet: Cetuximab; CPC: Capecitabine; Doublet.CT: 5-fluorouracil or capecitabine + oxaliplatin or irinotecan; FOLFOXIRI: Oxaliplatin/5-fluorouracil/irinotecan/leucovorin; Iri: Irinotecan; MitC: Mitomycin; Triplet-Bev: 5-fluorouracil or capecitabine + oxaliplatin or irinotecan + bevacizumab ; Triplet-Cet: 5-fluorouracil or capecitabine + oxaliplatin or irinotecan + cetuximab.

Executive summary

Targets according to their cellular localization

- Membrane receptor targets.
- Intracellular signaling targets.
- Protein kinases that regulate cell division.

New anti-EGF-receptor-based regimens & predictive factors of response

- K-RAS, B-RAF and PI3KCA mutations are associated with lack of response to anti-EGF-receptor therapy.
- The CRYSTAL and OPUS studies investigated a combination of FOLFOX/FOLFIRI with or without cetuximab. The combination showed a statistically significant increase in response rate and median progression-free survival (mPFS) in first-line metastatic colorectal cancer (mCRC) (K-RAS wild-type population).
- EPIC study. Irinotecan ± cetuximab. The combination showed a statistically significant benefit in response rate and mPFS in oxaliplatin-refractory mCRC.

New antiangiogenic-based regimens & 'antiangiogenic therapy beyond progression concept'

- NO16966 study. FOLFOX/XELOX ± bevacizumab. The combination showed a statistically significant benefit in mPFS in first-line mCRC and no statistically significant difference between FOLFOX and XELOX was observed.
- HORIZON-1 study. FOLFOX + bevacizumab vs FOLFOX + cediranib in first-line mCRC. No difference in mPFS was observed.
- BRITE observational study. Continuation bevacizumab beyond progression could prolong overall survival.

Dual VEGF–EGF-receptor inhibition-based regimens

- CAIRO study. XELOX + bevacizumab ± cetuximab. The combination of cetuximab and bevacizumab resulted in a shorter mPFS.
- PACCE study. Chemotherapy (oxaliplatin- or irinotecan-based regimens) + bevacizumab ± panitumumab. The combination of panitumumab and bevacizumab resulted in increased toxicity and shorter mPFS.

New targeted therapies in development

- PI3K inhibitors.
- C-Met inhibitors.
- TRAIL agonists.
- Hdm2 inhibitors.
- Aurora and polo-like kinases inhibitors.

Protein kinases that regulate cell division

AK inhibitors

Aurora is the name given to a family of seronine/threonine protein kinases that regulate many processes during cell division. Three 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 a regulators of genome stability [63]. AKs are frequently overexpressed in human tumors. Misregulation 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 anticancer therapies.

The first data to implicate this family of kinases in tumorigenesis 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 prognosis in patients with CRC [64,65].

Several AK inhibition drugs are in clinical development: ZM-447439 [66], Hesperadin[67], VX-680 [68], AZD-1152, MLN-8054 and MLN-8237. The first three inhibit phosphorylation of histone H3 on serine 10 and also inhibit cell division. However, they do not inhibit cell cycle progression and neither do they selectively inhibit a single kinase. Although it is not yet clear which AK is inhibited to mediate the anti-tumor 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 tumor xenografts derived from either human colon tumors or pancreatic tumors underwent a dose-dependent tumor-growth inhibition, and in some cases, regression. MLN-8054 is a selective, orally administered small molecule inhibitor of AK A. It competes with ATP binding and, therefore, reversibly inhibits AK A. MLN-8054 displays antitumor activity against three different human CRC 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.

Plk family

Plks are serine/threonine protein kinases 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 member of the family. Plks are associated with several mitotic structures and play an essential role in centrosome separation, chromosome

alignment and segregation, and cytokinesis [69]. Inhibition of these kinases results in abnormal mitotic events and eventually leads to apoptosis.

Plk1 is overexpressed in a wide variety of tumors [70] and some data suggest that dysregulation of Plk1 may be an early event in oncogenesis. In fact, overexpression of Plk1 alone was sufficient to induce tumor formation in a nude mouse model. Owing to the biological consequences of inhibiting Plks, a number of small-molecule Plk1 selective inhibitors have been developed and are under evaluation in clinical trials.

Conclusion

In recent years, better knowledge of human cancer biology and the development of new targeted therapies have improved the outcome of cancer patients.

So far, three targeted therapies have been approved for the treatment of mCRC patients (FIGURE 2). The FDA and the European Medicines Agencies (EMEA) have approved bevacizumab in combination with fluoropyrimidine-based chemotherapy for the first- and second-line treatment of mCRC patients. Cetuximab has been approved in combination with chemotherapy in a first-line setting and alone or in combination with irinotecan in the refractory setting. Whereas the US FDA's approval is for the whole population, the EMEA has restricted the license to K-RAS wild-type mCRC patients. Finally, the FDA has approved panitumumab as a single agent in mCRC patients refractory to other chemotherapy regimens and EMEA exclusively for K-RAS wild-type refractory patients.

With the increasing number of new targeted agents that will appear in the near future there is a need for better patients selection to reduce the economic impact that this accelerated development will produce. Clinical and molecular markers predictive of response are under evaluation by pharmacodynamic, genomic and proteomic studies, to a better selection of the patients that can be benefit from these treatments. Advances in these fields will facilitate the integration of tumor biology, and functional and clinical data in patients' treatment, allowing a better understanding of how the biology of this disease may impact clinical decisions.

Future perspective

The future of medicine lies in personalized medicine, based on using individual genetic profiles to choose the best therapeutic option.

Knowledge of molecular cancer biology will provide the discovery of new targeted therapies and we need to develop and validate clinical and molecular markers predictive of response to allow better selection of patients who will be able to benefit from these treatments.

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Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Ferrara N, Hillan KJ, Gerber HP, Novotny W: Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nat. Rev. Drug Discov.* 3, 391–400 (2004).

2 Hurwitz H, Fehrenbacher L, Novotny W *et al.*: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N. Engl. J. Med.* 350, 2335–2342 (2004).

▪▪ **Based on the results of this study, bevacizumab was approved for first-line treatment of metastatic colorectal cancer (mCRC).**

3 Saltz LB, Clarke S, Diaz-Rubio E *et al.*: Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. *J. Clin. Oncol.* 26, 2013–2019 (2008).

▪ **Bevacizumab improved median progression-free survival (mPFS) but no differences in response rate and overall survival were observed. Many patients were not managed according to protocol in this study.**

4 Giantonio BJ, Catalano PJ, Meropol NJ *et al.*: Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. *J. Clin. Oncol.* 25, 1539–1544 (2007).

▪▪ **Based on the results of this study, bevacizumab was approved for second-line mCRC.**

5 Grothey A, Sugrue MM, Purdie DM *et al.*: Bevacizumab beyond first progression is associated with prolonged overall survival in metastatic colorectal cancer: results from a large observational cohort study (BRiTE). *J. Clin. Oncol.* 26, 5326–5334 (2008).

▪ **Could continuation of bevacizumab beyond progression prolong overall survival?**

6 Hecht JR, Trarbach T, Jaeger E *et al.*: A randomized, double-blind, placebo-controlled, phase III study in patients (Pts) with metastatic adenocarcinoma of the colon or rectum receiving first-line chemotherapy with oxaliplatin/5-fluorouracil/leucovorin and PTK787/ZK 222584 or placebo (CONFIRM-1). *J. Clin. Oncol.* 23, 3 (2005).

7 Koehne C, Bajetta E, Lin E *et al.*: Results of an interim analysis of a multinational randomized, double-blind, phase III study in patients (pts) with previously treated metastatic colorectal cancer (mCRC) receiving FOLFOX4 and PTK787/ZK 222584 (PTK/ZK) or placebo (CONFIRM 2). *Proc. Am. Soc. Clin. Oncol.* 25, 3508 (2006).

8 Major P, Trarbach T, Lenz H *et al.*: A meta-analysis of two randomized, double-blind, placebo-controlled, phase III studies in patients (pts) with metastatic colorectal cancer (mCRC) receiving FOLFOX4 and PTK/ZK to determine clinical benefit on progression-free survival (PFS) in high LDH pts. *J. Clin. Oncol.* 24, 3529 (2006).

9 Harris AL: Hypoxia – a key regulatory factor in tumour growth. *Nat. Rev. Cancer* 2, 38–47 (2002).

10 Saltz LB, Rosen LS, Marshall JL *et al.*: Phase II trial of sunitinib in patients with metastatic colorectal cancer after failure of standard therapy. *J. Clin. Oncol.* 25, 4793–4799 (2007).

11 Cunningham D, Wong R, D'haens G *et al.*: A phase II, double-blind, randomized multicenter study of cediranib with FOLFOX versus bevacizumab with FOLFOX in patients with previously treated metastatic colorectal cancer (mCRC): Final PFS results. *Proc. Am. Soc. Clin. Oncol.* 26, 4028 (2008).

12 Saltz LB, Lenz HJ, Kindler HL *et al.*: Randomized phase II trial of cetuximab, bevacizumab, and irinotecan compared with cetuximab and bevacizumab alone in irinotecan-refractory colorectal cancer: the BOND-2 study. *J. Clin. Oncol.* 25, 4557–4561 (2007).

▪ **Study supports the concomitant use of anti-EGF-receptor (EGFR) and anti-VEGF agents.**

13 Hecht JR, Mitchell E, Chidiac T *et al.*: A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. *J. Clin. Oncol.* 27, 672–680 (2009).

▪ **Study does not support the concomitant use of anti-EGFR and anti-VEGF agents.**

14 Tol J, Koopman M, Cats A *et al.*: Chemotherapy, Bevacizumab, and Cetuximab in Metastatic Colorectal Cancer. *N. Engl. J. Med.* 360, 563–572 (2009).

▪ **Study does no support the concomitant use of anti-EGFR and anti-VEGF agents.**

15 Hemming AW, Davis NL, Kluftinger A *et al.*: Prognostic markers of colorectal cancer: an evaluation of DNA content, epidermal growth factor receptor, and Ki-67. *J. Surg. Oncol.* 51, 147–152 (1992).

16 Van Cutsem E, Peeters M, Siena S *et al.*: Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. *J. Clin. Oncol. 25*, 1658–1664 (2007).

17 Cunningham D, Humblet Y, Siena S *et al.*: Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N. Engl. J. Med.* 351, 337–345 (2004).

▪▪ **Based on the results of this study, cetuximab was approved in irinotecan-refractory mCRC.**

18 Jonker DJ, O'Callaghan CJ, Karapetis CS *et al.*: Cetuximab for the Treatment of Colorectal Cancer. *N. Engl. J. Med.* 357, 2040–2048 (2007).

19 Amado RG, Wolf M, Peeters M *et al.*: Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J. Clin. Oncol.* 26, 1626–1634 (2008).

▪▪ **K-RAS mutation status is the most important predictive factor of response to anti-EGFR therapy.**

20 Lievre A, Bachet JB, Boige V *et al.*: KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J. Clin. Oncol.* 26, 374–379 (2008).

21 Lievre A, Bachet JB, Le Corre D *et al.*: KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res.* 66, 3992–3995 (2006).

22 Andreyev HJ, Norman AR, Cunningham D *et al.*: Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. *J. Natl Cancer Inst.* 90, 675–684 (1998).

23 Santini D, Loupakis F, Vincenzi B *et al.*: High concordance of KRAS status between primary colorectal tumors and related metastatic sites: implications for clinical practice. *Oncologist* 13, 1270–1275 (2008).

24 Di Nicolantonio F, Martini M, Molinari F *et al.*: Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J. Clin. Oncol.* 26, 5705–5712 (2008).

▪ **B-RAF mutation status could be a predictive factor of response to anti-EGFR therapy.**

25 Sartore-Bianchi A, Martini M, Molinari F *et al.*: PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. *Cancer Res.* 69, 1851–1857 (2009).

▪ **PI3KCA mutation status could be a predictive factor of response to anti-EGFR therapy.**

26 Van Cutsem E, Lang I, D'haens G *et al.*: KRAS status and efficacy in the first-line treatment of patients with metastatic colorectal cancer (mCRC) treated with FOLFIRI with or without cetuximab: The CRYSTAL experience. *Proc. Am. Soc. Clin. Oncol.* 26, 2 (2008).

▪▪ **Based on the results of this study, cetuximab has been approved for first-line treatment of K-RAS wild-type mCRC.**

27 Bokemeyer C, Bondarenko I, Makhson A *et al.*: Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J. Clin. Oncol.* 27, 663–671 (2009).

28 Sobrero AF, Maurel J, Fehrenbacher L *et al.*: EPIC: Phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. *J. Clin. Oncol.* 26, 2311–2319 (2008).

29 Townsley CA, Major P, Siu LL *et al.*: Phase II study of erlotinib (OSI-774) in patients with metastatic colorectal cancer. *Br. J. Cancer* 94, 1136–1143 (2006).

30 Fisher GA, Kuo T, Ramsey M *et al.*: A phase II study of gefitinib, 5-fluorouracil, leucovorin, and oxaliplatin in previously untreated patients with metastatic colorectal cancer. *Clin. Cancer Res.* 14, 7074–7079 (2008).

31 Folprecht G, Tabernero J, Kohne CH *et al.*: Phase I pharmacokinetic/pharmacodynamic study of EKB-569, an irreversible inhibitor of the epidermal growth factor receptor tyrosine kinase, in combination with irinotecan, 5-fluorouracil, and leucovorin (FOLFIRI) in first-line treatment of patients with metastatic colorectal cancer. *Clin. Cancer Res.* 14, 215–223 (2008).

32 Kuo T, Cho CD, Halsey J *et al.*: Phase II study of gefitinib, fluorouracil, leucovorin, and oxaliplatin therapy in previously treated patients with metastatic colorectal cancer. *J. Clin. Oncol.* 23, 5613–5619 (2005).

33 Meyerhardt JA, Zhu AX, Enzinger PC *et al.*: Phase II study of capecitabine, oxaliplatin, and erlotinib in previously treated patients with metastastic colorectal cancer. *J. Clin. Oncol.* 24, 1892–1897 (2006).

34 Hanauske AR, Cassidy J, Sastre J *et al.*: Phase 1b dose escalation study of erlotinib in combination with infusional 5-fluorouracil, leucovorin, and oxaliplatin in patients with advanced solid tumors. *Clin. Cancer Res.* 13, 523–531 (2007).

35 Yu H, Rohan T: Role of the insulin-like growth factor family in cancer development and progression. *J. Natl Cancer Inst.* 92, 1472–1489 (2000).

▪ **IGF pathway may play an important role in colon cancer.**

36 Bustin SA, Jenkins PJ: The growth hormone-insulin-like growth factor-I axis and colorectal cancer. *Trends Mol. Med.* 7, 447–454 (2001).

37 Lahm H, Amstad P, Wyniger J *et al.*: Blockade of the insulin-like growth-factor-I receptor inhibits growth of human colorectal cancer cells: evidence of a functional IGF-II-mediated autocrine loop. *Int. J. Cancer* 58, 452–459 (1994).

38 Baselga J, Arteaga CL: Critical update and emerging trends in epidermal growth factor receptor targeting in cancer. *J. Clin. Oncol.* 23, 2445–2459 (2005).

39 Pietras K, Sjoblom T, Rubin K *et al.*: PDGF receptors as cancer drug targets. *Cancer Cell* 3, 439–443 (2003).

40 Sundberg C, Ljungstrom M, Lindmark G *et al.*: Microvascular pericytes express platelet-derived growth factor-β receptors in human healing wounds and colorectal adenocarcinoma. *Am. J. Pathol.* 143, 1377–1388 (1993).

41 Takahashi Y, Bucana CD, Liu W *et al.*: Platelet-derived endothelial cell growth factor in human colon cancer angiogenesis: role of infiltrating cells. *J. Natl Cancer Inst.* 88, 1146–1151 (1996).

42 Attoub S, Rivat C, Rodrigues S *et al.*: The c-kit tyrosine kinase inhibitor STI571 for colorectal cancer therapy. *Cancer Res.* 62, 4879–4883 (2002).

43 Ratain M, Flaherty K, Stadler W: Preliminary antitumor activity of BAY-43–9006 in metastatic renal cell carcinoma and other advanced refractory solid tumors in a Phase II randomized discontinuation trial. *Proc. Am. Soc. Clin. Oncol.* 4501 (2004).

44 Herynk MH, Stoeltzing O, Reinmuth N *et al.*: Down-regulation of c-Met inhibits growth in the liver of human colorectal carcinoma cells. *Cancer Res.* 63, 2990–2996 (2003).

45 Long IS, Han K, Li M *et al.*: Met receptor overexpression and oncogenic Ki-ras mutation cooperate to enhance tumorigenicity of colon cancer cells *in vivo*. *Mol. Cancer Res.* 1, 393–401 (2003).

46 Pan G, O'Rourke K, Chinnaiyan AM *et al.*: The receptor for the cytotoxic ligand TRAIL. *Science* 276, 111–113 (1997).

▪ **TRAIL, also known as Apo-2L, induces apoptosis and is a member of the TNF-ligand superfamily.**

47 Wang S: The promise of cancer therapeutics targeting the TNF-related apoptosis-inducing ligand and TRAIL receptor pathway. *Oncogene* 27, 6207–6215 (2008).

48 Saltz L, Infante J, Schwartzberg L *et al.*: Phase Ib study of AMG 655 in combination with modified FOLFOX6 (mFOLFOX6) and bevacizumab (B) for the first-line treatment of patients with metastatic colorectal cancer (mCRC). Presented at: ASCO Gastrointestinal Cancers Symposium. San Francisco, CA, USA 15–17 January (2009) (Abstract 290).

49 Vivanco I, Sawyers CL: The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat. Rev. Cancer* 2, 489–501 (2002).

▪ **Many of PI3K inhibitors are under preclinical and clinical development. PI3KCA mutation status could be a predictive factor of response to anti-EGFR therapy.**

50 Samuels Y, Wang Z, Bardelli A *et al.*: High frequency of mutations of the PIK3CA gene in human cancers. *Science* 304, 554 (2004).

51 Khaleghpour K, Li Y, Banville D *et al.*: Involvement of the PI 3-kinase signaling pathway in progression of colon adenocarcinoma. *Carcinogenesis* 25, 241–248 (2004).

52 Fuchs C, Tabernero J, Hwang J *et al.*: Multicenter phase II study of RAD001 in patients with chemotherapy-refractory metastatic colorectal cancer (mCRC). Presented at: ASCO Gastrointestinal Cancers Symposium. San Francisco, CA, USA 15–17 January (Abstract 446).

53 Bullock K, Hurwitz H, Uronis H *et al.*: Bevacizumab (B) plus everolimus (E) in refractory metastatic colorectal cancer (mCRC). Presented at: ASCO Gastrointestinal Cancers Symposium. San Francisco, CA, USA 15–17 January (2009) (Abstract 462).

54 Maira SM, Stauffer F, Brueggen J *et al.*: Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent *in vivo* antitumor activity. *Mol. Cancer Ther.* 7, 1851–1863 (2008).

55 Garlich JR, De P, Dey N *et al.*: A vascular targeted pan phosphoinositide 3-kinase inhibitor prodrug, SF1126, with antitumor and antiangiogenic activity. *Cancer Res.* 68, 206–215 (2008).

56 Eichhorn PJ, Gili M, Scaltriti M *et al.*: Phosphatidylinositol 3-kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3-kinase inhibitor NVP-BEZ235. *Cancer Res.* 68: 9221–9230 (2008).

57 Yeatman TJ: A renaissance for SRC. *Nat. Rev. Cancer* 4, 470–480 (2004).

▪ **C-Src is overexpressed and activated in many human cancers and is associated with advanced-stage and distant metastases.**

58 Talamonti MS, Roh MS, Curley SA, Gallick GE: Increase in activity and level of pp60c-src in progressive stages of human colorectal cancer. *J. Clin. Invest.* 91, 53–60 (1993).

59 Aligayer H, Boyd DD, Heiss MM *et al.*: Activation of Src kinase in primary colorectal carcinoma: an indicator of poor clinical prognosis. *Cancer* 94, 344–351 (2002).

60 Tice DA, Biscardi JS, Nickles AL, Parsons SJ: Mechanism of biological synergy between cellular Src and epidermal growth factor receptor. *Proc. Natl Acad. Sci. USA* 96, 1415–1420 (1999).

61 Chen J, Marechal V, Levine AJ: Mapping of the p53 and mdm-2 interaction domains. *Mol. Cell Biol.* 13, 4107–4114 (1993).

62 Klein C, Vassilev LT: Targeting the p53-MDM2 interaction to treat cancer. *Br. J. Cancer* 91, 1415–1419 (2004).

▪ **MDM2 is a key regulator of p53 and is overexpressed in a wide variety of tumor types.**

63 Keen N, Taylor S: Aurora-kinase inhibitors as anticancer agents. *Nat. Rev. Cancer* 4, 927–936 (2004).

▪ **Aurora is the name given to a family of seronine/threonine protein kinases that regulate many processes during cell division and are frequently overexpressed in human tumors.**

64 Bischoff JR, Anderson L, Zhu Y *et al.*: A homologue of Drosophila aurora kinase is oncogenic and amplified in human colorectal cancers. *EMBO J.* 17, 3052–3065 (1998).

65 Katayama H, Ota T, Jisaki F *et al.*: Mitotic kinase expression and colorectal cancer progression. *J. Natl Cancer Inst.* 91, 1160–1162 (1999).

66 Ditchfield C, Johnson VL, Tighe A *et al.*: Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. *J. Cell Biol.* 161, 267–280 (2003).

67 Hauf S, Cole RW, LaTerra S *et al.*: The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. *J. Cell Biol.* 161, 281–294 (2003).

68 Harrington EA, Bebbington D, Moore J *et al.*: VX-680, a potent and selective small-molecule inhibitor of the Aurora kinases, suppresses tumor growth *in vivo*. *Nat. Med.* 10, 262–267 (2004).

69 Barr FA, Sillje HH, Nigg EA: Polo-like kinases and the orchestration of cell division. *Nat. Rev. Mol. Cell Biol.* 5, 429–440 (2004).

▪ **Inhibition of these kinases results in abnormal mitotic events and eventually leads to apoptosis.**

70 Takahashi T, Sano B, Nagata T *et al.*: Polo-like kinase 1 (PLK1) is overexpressed in primary colorectal cancers. *Cancer Sci.* 94, 148–152 (2003).