Targeted approach to metastatic colorectal cancer: what comes beyond epidermal growth factor receptor antibodies and bevacizumab?

Teresa Troiani, Erika Martinelli, Floriana Morgillo, Anna Capasso, Anna Nappi, Vincenzo Sforza and Fortunato Ciardiello

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Abstract: The prognosis of patients with 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. The discovery of a plethora of cellular targets and the rational generation of selective targeting agents has opened an era of new opportunities and extraordinary challenges. The specificity of these agents renders them capable of specifically targeting the inherent abnormalities of cancer cells, potentially resulting in less toxicity than traditional nonselective cytotoxics. Among the many new types of rationally designed agents are therapeutics targeting various strategic facets of growth signal transduction, malignant angiogenesis, survival, metastasis and cell-cycle regulation. The evaluation of these agents is likely to require some changes from the traditional drug development paradigms to realize their full potential. Inhibition of the epidermal growth factor receptor and the vascular endothelial growth factor have provided proof of principle that disruption of signal cascades in patients with colorectal cancer has therapeutic potential. This experience has also taught us that resistance to such rationally developed targeted therapeutic strategies is common. In this article, we review the role of signal transduction in colorectal cancer, introduce promising molecular targets, and outline therapeutic approaches under development.

Keywords: aurora kinase inhibitors, colorectal cancer, insulin-like growth factor receptor inhibitors, MET inhibitors, new therapies, Src kinase inhibitors

Introduction

Despite continued advances in early diagnosis and improvements of new treatments, colorectal cancer (CRC) remains one of the most common types of human cancer in terms of incidence and mortality. In 2008, CRC was responsible for 212,000 (12.2%) deaths in Europe, representing the second most common cause of cancer death after lung cancer (19.9%) [Ferlay *et al.* 2010]. During the last decade, median duration of survival among patients with metastatic CRC (mCRC) has increased from 12 to 21 months, mainly because of the introduction of new cytotoxic agents such as oxaliplatin and irinotecan [De Gramont *et al.* 2000; Douillard *et al.* 2000]. Improvements in

the knowledge of cancer biology have led to the development of agents that specifically inhibit tumour growth. Several novel targeted agents are being investigated both as single agents and in combination with chemotherapy to assess the potential for increased efficacy. Three of these, cetuximab and panitumumab, monoclonal antibodies (MoAbs) against the epidermal growth factor receptor (EGFR), and bevacizumab, a MoAb against the vascular endothelial growth factor (VEGF) protein, have already shown clinical benefit and are commercially approved for use in advanced CRC [Cunningham *et al.* 2004; Hurwitz *et al.* 2004; Van Cutsem *et al.* 2007]. Thus, agents that target the EGFR and VEGF pathways have been

Correspondence to:
Fortunato Ciardiello, MD, PhD
Dipartimento Medico-
Chirurgico di Internistica
Clinica e Sperimentale 'F.
Magrassi e A. Lanzara',
Seconda Università degli
Studi di Napoli, Via S. Pansini
5, 80131 Naples, Italy
fortunato.ciardiello@unina2.it
Teresa Troiani, MD, PhD
Erika Martinelli, MD, PhD
Floriana Morgillo, MD, PhD
Anna Capasso, MD
Anna Nappi, MD
Vincenzo Sforza, MD
Oncologia Medica and
Immunologia Clinica,
Dipartimento Medico-
Chirurgico di Internistica
Clinica e Sperimentale F.
Magrassi e A. Lanzara,
Seconda Università degli
Studi di Napoli, Napoli, Italy

Figure 1. Intracellular signal transduction pathway.

EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; GRB2, growth factor receptor-bound protein 2; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IGFR, insulin-like growth factor receptor; MAPK, mitogen-activated protein kinase; MET, mesenchymal epithelial transition factor; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3 kinase; PIP2, phosphatidylinositol-biphosphate; PIP3, phosphatidylinositol triphosphate; PTEN, phosphatase and tensin homologue; RAS, rat sarcoma; SOS, son of sevenless protein; STAT, signal transducers and activators of transcription protein; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

clinically benchmarked in this disease. Despite the encouraging results obtained, resistance to such rationally developed targeted therapeutic strategies is common. An emerging understanding of the molecular pathways that characterize 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 mCRC. The targets can be grouped according to their cellular localization, such as membrane receptor targets, intracellular signaling targets and other protein kinases that regulate cell division, including aurora kinases (AKs) (Figure 1). Emerging data from the clinical development of new drugs directed to these targets are providing novel opportunities in the treatment of patients with CRC that will probably translate into efficacy benefits in the coming years.

Membrane receptor targets

Insulin-like growth factor receptor inhibitors

The insulin-like growth factor (IGF) family consists of two ligands (IGF-I and IGF-II), two cell membrane receptors (IGF-IR and IGF-IIR), six IGF binding proteins (IGFBP1–6) and at least five adaptor proteins (insulin receptor substrate IRS1–4 and Shc), and other associated proteins including the insulin-receptor-related-receptor IRR, IGFBP-related proteins and IGFBP proteases [Guo *et al.* 1992]. IGF-I and -II are potent growth factors with a close structural similarity with insulin. IGF-I mediates growth in the adult, whereas IGF-II is expressed in the developing foetus. Both peptides are produced in the liver and in the gastrointestinal tract and act via the IGF-IR, by a number of mechanisms [Rouyer-Fessard *et al.* 1990]. IGF-IR is widely expressed

throughout the gastrointestinal tract with the highest expression detected in the proliferating cells at the bases of the colonic crypts, whereas the type II IGF receptor has an unknown physiological role. IGF-I is a potent mitotic, antiapoptotic peptide and, in addition, has been demonstrated to promote angiogenesis by increasing the production of VEGF [Akagi *et al.* 1998; Bustin *et al.* 2002; Fukuda *et al.* 2002; Warren *et al.* 1996; Wu *et al.* 2002]. IGF-I can also promote cell invasion and motility by a variety of mechanisms and has a significant influence on the interleukin system, inhibiting production of the growth-inhibitory tumour necrosis factor α (TNF α) and increasing production of the antiapoptotic interleukin 8 (IL-8) [Andre *et al.* 2004; Remacle-Bonnet *et al.* 2000; Vallee *et al.* 2003].

The role of the IGF pathway in the pathogenesis of CRC has been demonstrated in different preclinical studies. Wu and colleagues conducted a study involving liver-specific IGF-I-deficient (LID) mice in whose serum the IGF-I level was 25% of that in control mice. In LID mice, the growth of orthotopically transplanted colon adenocarcinoma was significantly less than in controls and the appearance of a palpable caecal tumour was not only slower but also smaller than that in control mice. The frequency of hepatic metastasis was also significantly lower in LID mice. These results support the hypothesis that circulating IGF-I levels play an important role in colon cancer development and metastasis. CRCs often express 10–50 times higher levels of IGF-I and IGF-II than adjacent uninvolved colonic mucosa [Reinmuth *et al.* 2002]. Even more, CRC cells frequently overexpress IGF-IR and when activated by IGF-I they inhibit apoptosis and allow progression through the cell cycle [Ouban *et al.* 2003].

Significant cross talk has been observed between the IGF pathway and several other receptors. There is a crucial 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 IGFBP3 and increase the ability of free IGFs. This interaction provides a rationale for combined therapy against these different pathways with the goal to improve antitumour activity [Burtrum *et al.* 2003]. Several MoAbs and small inhibitors of IGF-R have recently entered into clinical development (Table 1). IMC-A12, a fully human MoAb

currently in clinical development, binds to IGF-IR with high affinity and inhibits ligand binding with a half maximal inhibitory concentration (IC₅₀) of 0.6–1 nM [Burtrum *et al.* 2003]. In xenograft tumour models, IMC-A12 results in significant growth inhibitions of breast, pancreatic and colon tumours [Burtrum *et al.* 2003]. The safety and efficacy of IMC-A12 has been evaluated in a phase II randomized open-label study with or without cetuximab in patients with metastatic refractory anti-EGFR MoAb CRC [Reidy *et al.* 2010]. In this trial, IMC-A12 alone or in combination with cetuximab did not demonstrate meaningful antitumour activity and of 64 patients treated, only one patient achieved a durable partial response to the combination treatment. The limited clinical efficacy of IMC-A12 in this study suggests that additional preclinical work will be required to identify predictors of IGF-IR dependence in CRCs. Another fully humanized MoAb directed against IGF-IR, CP-751,871, is in advanced clinical development. In experimental models, it inhibits IGF-I binding to cells, inhibits IGF-I-induced receptor phosphorylation, and results in downregulation of IGF-IR expression at the plasma membrane through internalization of the receptor.

Inhibition of tumour growth has been documented in multiple xenograft models, and its combination with standard chemotherapeutic agents enhances its antitumour efficacy. The combination of CP-751,871 with 5-fluorouracil (5-FU) in a Colo-205 xenograft model resulted in improved antitumour activity compared with either agent given alone [Cohen *et al.* 2005]. Ganitumab (AMG 479) is a fully human MoAb against IGF-IR and a phase II trial has been presented by Eng and colleagues comparing panitumumab with panitumumab plus ganitumab (AMG 479) and panitumumab plus rilotumumab (AMG 102), an antibody against hepatocyte growth factor (HGF). In preliminary data, ganitumab as a single agent and in pretreated patients failed to generate any signal in this randomized trial [Eng *et al.* 2011]. In addition, a phase II randomized study with leucovorin, 5-FU and irinotecan (FOLFIRI) plus ganitumab or AMG655 *versus* FOLFIRI in Kirsten rat sarcoma (KRAS)-mutant mCRC is currently in progress.

Dalotuzumab (MK-0646), another humanized immunoglobulin G1 antibody, has shown

Table 1. Membrane receptor targets and drugs.

Target	Category	Drug	Development stage in CRC	Population	Treatment
IGFR	MoAb	IMCA12	Phase II	Refractory	IMCA12 ± cetuximab
		Ganitumab (AMG479)	Phase II	Refractory KRAS wild type	Panitumumab ± AMG479
		Dalotuzumab (MK-0646)	Phase II	Second-line KRAS mutant	FOLFIRI ± ganitumab
			Phase II/III	Refractory	Irinotecano + cetuximab ± dalotuzumab
c-MET	TKI	NVP-AEW541	Preclinical	–	–
	MoAb	Rilotumumab (AMG102)	Phase Ib/II	Refractory KRAS wild type	Panitumumab ± rilotumumab
	TKI	Tivantinib	Phase I/II	Refractory KRAS wild type	Irinotecan + cetuximab ± tivantinib
TRAIL	MoAb	LBY135	Preclinical	–	–
		CS-1008	Phase II	Second line	Irinotecan ± CS-1008
		Mapatumumab	Phase II	Refractory	Single agent
		Lexatumumab	Phase I	Refractory	FOLFIRI + lexatumumab
		AMG 655	Phase I/II	First line	FOLFOX + bevacizumab ± AMG655
			Phase II	Refractory KRAS wild type	Panitumumab ± AMG655
		HGS-TR2J	Preclinical	–	–
		Apomab	Preclinical	–	–

c-MET, mesenchymal epithelial transition factor; CRC, colorectal cancer; IGFR, insulin-like growth factor receptor; KRAS, Kirsten rat sarcoma; MoAb, monoclonal antibody; TKI, tyrosine kinase inhibitor; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand.

efficacy in a xenograft colon cancer model and a favourable toxicity profile in a phase I trial [Scartozzi *et al.* 2010]. A multicentre, double-blind, randomized phase II/III study of dalotuzumab in mCRC has been conducted [Watkins *et al.* 2011]. Eligible patients were those whose condition had previously failed to respond to both irinotecan and oxaliplatin and had progressed on or within 3 months of their last therapy. Patients were randomized to receive either dalotuzumab 10 mg/kg weekly or 15 mg/kg loading followed by 7.5 mg/kg every 2 weeks or placebo combined with cetuximab and irinotecan at standard dose/schedule. The trial was stopped at the first interim analysis with 345 wild-type (wt) KRAS patients enrolled as the two dalotuzumab-dosing regimens did not achieve proof of concept. Median progression-free survival (PFS) for all randomized wt KRAS status patients [per intention to treat (ITT)] was 3.3 months in the weekly dalotuzumab group and 5.4 in the 2-weekly group *versus* 5.6 in the placebo group. Median overall survival (OS) for wt KRAS patients (per ITT) was 10.8 and 11.6 months

in the two dalotuzumab groups respectively *versus* 14.0 in the placebo arm. The addition of dalotuzumab to cetuximab and irinotecan worsened PFS and OS in patients with chemorefractory wt KRAS mCRC. Based on these results a comprehensive, retrospective analysis has been performed to identify possible biomarkers predictive to cetuximab resistance and eventually dalotuzumab responsiveness [Watkins *et al.* 2012]. In particular, high IGF-I expression was found to be associated with resistance to cetuximab and sensitivity to dalotuzumab. Moreover, dalotuzumab was not effective in tumours with high IGF-II expression. Based on these data dalotuzumab will be further evaluated in a molecularly selected population of mCRC. This phase II/III study with dalotuzumab was performed without selection of patients with mCRC on KRAS status. KRAS-mutated mCRC is inherently resistant to EGFR-targeted therapy and carries an inferior prognosis to wt disease. For this reason the activity of dalotuzumab has been investigated in KRAS-mutant preclinical colon cancer models [Sathyanarayanan *et al.* 2012]. Moreover the

efficacy data relating to a subset of patients with KRAS-mutant mCRC enrolled in a randomized phase II/III study were reevaluated. In preclinical KRAS-mutant models, dalotuzumab was found to be effective in inhibiting IGF-I-mediated cellular growth. Furthermore, in CRC xenograft models high IGF-I was found to identify a subset of dalotuzumab-responsive tumours. Combination studies demonstrated that irinotecan exposure resulted in the activation of IGF-IR and phosphatidylinositol 3 kinase (PI3K) signalling pathways, representing a possible cellular survival mechanism. In xenograft models the combination of dalotuzumab with irinotecan produced lasting tumour growth inhibition that persisted even after treatment withdrawal. Interestingly, efficacy data of 69 patients with KRAS-mutant mCRC enrolled in the randomized study were evaluated based on this preclinical information. Efficacy data demonstrated differential activity between colon and rectal tumours with evidence of activity in the rectal subgroup. Molecular analysis suggests that this observation may be mediated by differential IGF-I expression. These data suggest that dalotuzumab in combination with irinotecan may have utility in the treatment of patients with KRAS-mutated CRC. Small molecules that selectively inhibit the tyrosine kinase domain of IGF-IR without significant effect on the insulin receptor are under development (Table 1). NVP-AEW541, a kinase inhibitor, has shown induction of apoptosis and cell cycle arrest in two CRC cell lines, HT29 and HCT116, resulting in dose-dependent inhibition of proliferation. Combining this agent with either 5-FU or cetuximab resulted in additive growth inhibition. NVP-AEW541 alone inhibited proliferation in primary cancer cell cultures of tumours of eight patients with primary CRC [Hopfner *et al.* 2006].

The IGF-IR signalling system is now recognized as an important component of cancer development, progression and response to treatment across a wide spectrum of tumour types. Early stage clinical trials suggest that the main anticipated toxicities can be managed effectively. Further work is required to ascertain the efficacy of IGF-IR-targeted therapy in the clinical setting and to elucidate biomarkers of response to treatment. The role of IGF-IR-targeted treatment in CRC, which appears to respond due to autocrine production of IGF-I, likewise needs to be elucidated. The optimal treatment combinations with chemotherapy and other targeted agents

and sequence of treatment also need to be established.

c-MET

The mesenchymal epithelial transition factor (MET) gene encodes a high-affinity receptor for the HGF, also known as scatter factor, and consists of an extracellular α chain disulfide bonded to a membrane-spanning β chain [Giordano *et al.* 1989]. HGF binds to MET, induces receptor homodimerization, and leads to phosphorylation of the cytoplasmic tyrosine kinase domain at two specific sites (Y1234 and Y1235) and activation of MET-mediated signalling [Longati *et al.* 1994]. These events are essential during embryogenesis and also play an important role for development, homeostasis and tissue regeneration, such as mitogenesis and morphogenesis in a wide range of cell types, including epithelial, endothelial and haematopoietic cells, neurons, melanocytes and hepatocytes [Furge *et al.* 2000]. Deregulation of the MET pathway has been observed in many human malignancies, and the effects of sustained MET activation have been extensively characterized in preclinical models [Iyer *et al.* 1990]. *In vivo* studies have shown that activation of HGF/MET signalling promotes cell invasiveness and triggers metastases through direct involvement of angiogenic pathways [Zhang *et al.* 2003]. A variety of mechanisms that lead to aberrant MET signalling have been characterized and these include overexpression of HGF or MET, MET gene amplification, mutations or structural rearrangements. Importantly, MET expression has been found in 55–78% of primary colon tumours. Moreover, MET amplification has been detected in patients with CRC, whereas no mutations involving the tyrosine kinase domain have been described in those patients [Umeki *et al.* 1999]. The prognostic role of HGF and MET has also been examined in several studies. Whereas the majority of these studies show a poor outcome for individuals in whom tumour HGF or MET overexpression was detected [Sawada *et al.* 2007; Shattuck *et al.* 2008; Siegfried *et al.* 1997], a few studies failed to show a prognostic role for MET overexpression [Nakamura *et al.* 2007; Resnich *et al.* 2004].

The prevalence of HGF/MET pathway activation in human malignancies has driven rapid growth in drug development programmes. Agents currently under development as HGF/MET pathway inhibitors can be broadly divided

into biologicals and low molecular weight synthetic compounds. Biologicals, or protein-based agents, act through a variety of mechanisms and possess target selectivity and pharmacokinetic properties that are predictable and often desirable. Nonetheless, their size typically restricts their action to extracellular events and their complexity impacts drug manufacture, routes of administration and shelf life. Thus, it is not surprising that synthetic, low molecular weight tyrosine kinase inhibitors (TKIs) presently outnumber every other class of HGF/MET therapeutic. Among HGF/MET-targeted biologicals, the most advanced drug candidates are MoAbs directed against either HGF or MET (Table 1). Neutralizing MoAbs against human HGF, such as rilotumumab (AMG102), are currently being developed in patients with mCRC. Rilotumumab *in vitro* has been shown to bind the HGF light chain with a K_d of 0.22 nM and to block HGF/MET binding with an IC_{50} of 2.1 nM [Burgess *et al.* 2006; Giordano, 2009]. In a phase I clinical trial, it was well tolerated up to the planned maximum dose of 20 mg/kg, and the maximum tolerated dose (MTD) was not reached. Treatment-related adverse events (AEs) were moderate in severity, with the most frequently occurring being fatigue, constipation, anorexia, nausea and vomiting [Gordon *et al.* 2010]. Results of a phase Ib/II study of rilotumumab in combination with panitumumab in patients with wt KRAS mCRC have recently been presented [Van Cutsem *et al.* 2010]. In the dose-finding portion of the study, the standard dose of panitumumab and 10 mg/kg of rilotumumab every 2 weeks was tested. There were no dose-limiting toxicities reported in the first six patients. That dose was therefore set for the randomized phase II portion of the study. A total of 142 patients with wt KRAS metastatic colorectal tumours previously pretreated with irinotecan or oxaliplatin chemotherapy were enrolled. With a median follow up of nearly 7 months, 31% of patients in the panitumumab–rilotumumab arm showed a partial response, compared with 21% in the panitumumab–placebo arm. The median duration of response with the experimental arm was 5.1 months compared with 3.7 months for panitumumab alone. Median PFS showed a trend for improvement in the combination treatment at 5.2 months compared with 3.7 months in the panitumumab–placebo arm. Adverse events increased in the combination arms, and were within an acceptable range [Van Cutsem *et al.* 2011].

Several selective and nonselective c-MET kinase inhibitors have been developed and tested in different tumour types (Table 1). Among these, the one that is in clinical development for patients with mCRC is tivantinib, an oral, highly selective, nonadenosine triphosphate (ATP)-competitive c-MET inhibitor. Results from a phase I/II study of tivantinib in combination with irinotecan and cetuximab in patients with wt KRAS mCRC have been presented [Bessudo *et al.* 2011]. This study is based on the hypothesis that adding tivantinib to irinotecan plus cetuximab may decrease resistance to cetuximab treatment and improve patient outcomes. Patients with locally advanced CRC or mCRC who received more than one prior line of chemotherapy, were wt KRAS and had Eastern Cooperative Oncology Group performance status less than 2 were included in the study. Patients were treated with irinotecan (180 mg/m²) and cetuximab (500 mg/m²) every 2 weeks along with escalating doses of tivantinib (120, 240, 360mg) twice daily. Preliminary toxicity and efficacy data for nine patients showed no dose-limiting toxicities (DLTs) and grade 3/4 AEs included neutropenia (grade 4 in one patient), fatigue (grade 3 in two patients) and one case each of grade 3 leukopenia, acneiform rash, vomiting, diarrhoea, anaemia and syncope. In nine patients with evaluable responses, best responses included one complete response (after four cycles), two partial responses (after two cycles), five stable disease and one progressive disease. The randomized phase II portion of the study continues to accrue data for the recommended phase II dose of 360 mg tivantinib twice daily.

In closing, the wealth of basic knowledge about HGF/MET biology has enabled an accurate assessment of the pathway's oncogenic potential and provided the insight needed to develop potent and selective inhibitors and use them with relative safety in humans. Patient selection, which is of primary importance, will advance as more robust methods are developed to analyse the many known potential diagnostic biomarkers of pathway activity. Methods that rely on DNA or RNA (e.g. detecting MET gene amplification or mutation) are now faster and more sensitive than those available for quantitating MET protein content and phosphorylation state, but efforts to improve both are under way. Similarly, the need for pharmacodynamic markers that track drug effect and patient response is recognized and clinical pharmacodynamic marker studies currently under way reveal solid candidates. Finally, although the

complexity of cancer and the risk of acquired resistance may limit the use of HGF/MET molecular therapeutics as single agents for subgroups of patients, much evidence suggests that pathway involvement is widespread and critical for metastasis. Thus for HGF/MET pathway inhibitors in particular, combinatorial phase II trials with small, carefully selected patient groups may be the most expedient path to more effective cancer treatment.

Tumour necrosis factor-related apoptosis-inducing ligand receptor

The evolution of a cancer cell is dependent on six essential alterations, including self sufficiency in growth signals, insensitivity to growth-inhibitory signals, limitless replicative potential, sustained angiogenesis, tissue invasion and evasion of apoptosis [Hanahan and Weinberg, 2000]. Similar to the cell division cycle, the pathways that lead to apoptosis are complex and consist of a fine homeostatic balance between cell death blockers and inducers [Reed, 1999]. Because apoptosis is a physiological death culminating in fragmentation of cells cleared by phagocytosis, inflammatory reaction or tissue scarring usually does not occur. Defects in apoptosis can prolong cellular lifespan and contribute to neoplastic cell expansion and can create a permissive environment for genetic instability that can contribute significantly to carcinogenesis. The ability to directly induce apoptosis in cancer cells is a novel approach to cancer treatment that has recently begun to be evaluated. One emerging area of research is the evaluation of agents which activate the TNF death receptors [TNF-related apoptosis-inducing ligand (TRAIL)-R1 or DR4 and TRAIL-R2 or DR5], members of the TNF receptor superfamily that, when activated, directly induce programmed cell death in cancer cells. TRAIL induces apoptosis and is a member of the TNF ligand superfamily [Wiley *et al.* 1995]. Of four receptors identified to date, TRAIL-R1 and TRAIL-R2, upon binding with TRAIL, mediate downstream signalling, leading to apoptosis [MacFarlane *et al.* 1997]. TRAIL-R3 and TRAIL-R4 have nonfunctional or absent death domains, do not transmit apoptotic signals and may function as decoy receptors [Pan *et al.* 1997]. Upon binding of TRAIL to functional receptors, TRAIL-R1 and TRAIL-R2 recruit apoptosis-inducing caspases that activate the proapoptotic proteins Bid and Bax, leading to cytochrome C release from mitochondria [Li

et al. 1998]. Strater and colleagues evaluated the expression of TRAIL and its cognate receptors on normal colon mucosa, colon adenomas and resected stage II/III colon carcinomas. They found TRAIL-R1 expression to be an independent predictor of disease-free survival, with increased expression associated with improved clinical outcome. In a severe combined immunodeficient mouse xenograft model prepared from fresh surgical colon cancer specimens, administration of TRAIL resulted in growth inhibition [Naka *et al.* 2002]. Notable synergy was evident with concurrent administration of either 5-FU or irinotecan and TRAIL. Similar results have been noted in xenograft models with established cell lines [Gliniak and Le 1999].

The TRAIL-related pathway has been targeted by agonist MoAbs directed to TRAIL-R1 or TRAIL-R2 or by recombinant variants of the ligand TRAIL itself. Currently one chimeric (LBY135) and six human (CS-1008, mapatumumab, lexatumumab, AMG 655, HGS-TR2J and apomab) monoclonal agonistic antibodies against TRAIL-R1 and TRAIL-R2 are in phase I/II clinical trials. LBY135, lexatumumab, AMG 665, HGS-TR2J and apomab are TRAIL-R2 agonists (Table 1). Generally, results to date indicate some antitumour activity, good safety profile with no hepatotoxicity, linear pharmacokinetics and relatively long half-life times (10–20 days) with no generation of antihuman antibodies [Oldenhuis *et al.* 2008]. In preclinical studies, lexatumumab has been demonstrated to induce apoptosis in a variety of tumour models, including the xenograft Colo205 model [de Bono *et al.* 2004]. In phase I studies, lexatumumab was safely administered up to a 10 mg/kg dose in a range of solid malignancies with stable disease observed in 12 patients (32%) that lasted a median of 4.5 months [Plummer *et al.* 2007]. The safety of lexatumumab in combination with gemcitabine, pemetrexed, doxorubicin or FOLFIRI is also being examined in a phase Ib trial [Wakelee *et al.* 2010]. Tumour shrinkage has been observed, including confirmed partial responses in the FOLFIRI and doxorubicin arms. More detailed results on patient responses are awaited. AMG655 is a fully human monoclonal agonist antibody that binds human TRAIL-R2. A phase Ib study of AMG655 in combination with FOLFOX6 (Oxaliplatin 85 mg/m² (IV), Leucovorin 200 mg/m² (IV), fluorouracil 400 mg² (IV push), and fluorouracil 2400 mg² (cont. infusion over 46 hours)) and bevacizumab for the

first-line treatment of patients with mCRC has recently been presented [Saltz *et al.* 2009] and a randomized phase II trial of FOLFOX6 plus bevacizumab with or without AMG655 is currently in progress. Mapatumumab (HGS-ETR1, TRM-1 MoAb) is an agonistic fully human MoAb against TRAIL-R1. Preclinical data demonstrated growth inhibition of human colon cancer xenograft models in nude mice [Tolcher *et al.* 2007]. Phase I trials in patients with solid malignancies refractory to standard therapy concluded that it can be safely administered up to 10 mg/kg every 14 days. No adverse reactions were observed and out of the 49 patients enrolled, 19 had stable disease with two lasting 9 months. A phase II study of mapatumumab in advanced CRC has recently been published [Trarbach *et al.* 2010]. This trial was designed to evaluate the efficacy and safety of mapatumumab in patients with mCRC whose condition had failed to respond, who were intolerant, or not candidates for fluoropyrimidine, oxaliplatin and irinotecan-based regimens. No clinical activity of single-agent mapatumumab was observed in patients with advanced refractory CRC; in fact 32% of patients achieved stable disease for a median of 2.6 months with a median PFS of 1.2 months. However, on the basis of its favourable safety profile and preclinical evidence of potential synergy in combination with agents commonly used in the treatment of CRC, further evaluation of mapatumumab in combination with chemotherapy is warranted.

TRAIL is now in phase II clinical trials. Whether used as a single agent or in combination therapy, it is hoped that sooner rather than later, it will contribute to improving patient survival. Many questions remain unanswered. Top of the list is why some cells remain resistant to TRAIL. Could the combination of TRAIL receptor agonists with other therapeutics restore cancer cell sensitivity or inadvertently result in the death of normal cells? Why is it that certain tumour cells preferentially transduce apoptosis through one death-inducing TRAIL receptor but not the other; or preferentially induce the expression of only one TRAIL receptor in response to a given anticancer agent? Unfortunately, we are still unable to predict which TRAIL receptors are functional in which tumours and so, as yet, cannot foresee which TRAIL receptor-targeting treatment would be the most appropriate. Another essential question to ask is whether the differences in the pharmacokinetics and pharmacodynamics of the recombinant

humanized TRAIL variants are problematic *in vivo*. Will a 'safe TRAIL variant be found with potent antitumour effects? Given time, through translational research and more extensive clinical trials, we envisage that many of these questions will be answered. In the meantime, however, it appears that we are certainly on the right TRAIL.

Resistance to epidermal growth factor receptor target therapy: human epidermal growth factor receptor 2 activation

The development of drugs directed against EGFR in colon carcinoma began on the basis of findings suggesting that EGFR and its ligands are involved in the pathogenesis of human CRC. Although EGFR is expressed in the majority of CRC, responses to treatment with anti-EGFR MoAbs used as single agents has been observed in approximately only 10% of patients with mCRC. Well established causes of *de novo* cetuximab resistance include activating mutations in KRAS, BRAF, PI3KCA and NRAS. Recently a study conducted by Yonesaka and colleagues identified a new mechanism of *de novo* resistance to cetuximab therapy *via* increased signalling through human epidermal growth factor receptor 2 (HER2), a member of the EGFR family. Two mechanisms were found to be involved: HER2 gene amplification or increased secretion of the HER2 ligand heregulin. Both of these mechanisms caused acquired resistance to cetuximab treatment of human cancer cell lines by leading to persistent activation of extracellular signal-related kinase (ERK) signalling. More importantly, HER2 gene amplification or increased heregulin secretion were found to be associated with acquired resistance to cetuximab therapy in a retrospective analysis of chemotherapy-refractory patients with mCRC. In fact, expression of HER2 protein has been reported in up to 20% of patients with mCRC whereas HER2 gene amplification is a rare event in this disease, occurring in approximately 2% of cases [Yonesaka *et al.* 2011].

The role of HER2 in *de novo* resistance to anti-EGFR therapy in mCRC has also been demonstrated by Bertotti and colleagues. In this study, the authors identified HER2 gene amplification as a potential mechanism of resistance to cetuximab in mCRC cases that harbour normal, wt KRAS/NRAS/BRAF/PI3KA genes. In fact, HER2 amplification was found to occur in only 2% of unselected mCRC cases and at a significantly greater frequency in patients with KRAS wt tumours that

did not benefit from treatment with anti-EGFR MoAbs. Analysis of HER2-amplified tumour xenografts in 'xenopatiens' confirmed the role of HER2 gene amplification in cetuximab resistance and suggested that the combined inhibition of HER2 and EGFR by treatment with selective inhibitors could induce long-lasting tumour regression [Bertotti *et al.* 2011]. The summarized finding indicates that blockade of HER2 might prevent or revert resistance to anti-EGFR MoAbs in selected patients. Although these data are exciting and open new possibilities for the treatment of patients with resistance to anti-EGFR drugs, prospective clinical trials are required to assess whether these findings can be translated into effective anticancer treatments in patients with mCRC.

Antiangiogenesis agents

The regulation of angiogenesis is a complex, multistep process regulated by a dynamic balance between proangiogenic and antiangiogenic factors. One of the most important regulators of this process is VEGF and its receptors (VEGFR) [Kerbel, 2008]. The VEGF family of growth factors is composed of six members, VEGF-A-E, and placenta growth factor 1 and 2, with VEGF-A (commonly referred to simply as VEGF) being the most prominent mediator of angiogenesis [Hicklin and Ellis, 2005]. Activation of the VEGF pathway results in the initiation of a number of intracellular signalling cascades that result in endothelial cell survival, proliferation, migration, differentiation and increased vascular permeability. Because of its central role in tumour-associated angiogenesis, VEGF has emerged as an attractive and central therapeutic target in CRC. Almost 40 years ago, Folkman hypothesized that the development of an agent that prevents angiogenesis could have important implications for cancer treatment [Folkman, 1971]. While it took several decades to understand the underlying biology, that hypothesis is beginning to bear fruit, to the clinical benefit of patients.

A number of antiangiogenesis agents have been approved or are undergoing clinical testing. Bevacizumab is a humanized anti-VEGF MoAb that binds and neutralizes human VEGF [Ferrara *et al.* 2004]. Bevacizumab has been approved for the treatment of patients with mCRC in combination with standard fluoropyrimidine-based chemotherapy after several randomized studies demonstrated its benefit [Giantonio *et al.* 2007; Saltz *et al.* 2008]. Several other antiangiogenesis

agents are currently in development. One of these, aflibercept, is a fully humanized recombinant fusion protein that is composed of the extracellular domains of VEGFR-1 and VEGFR-2, fused to the constant region of human immunoglobulin G1. It functions as a decoy VEGFR, binds VEGF-A, VEGF-B, and placental growth factors 1 and 2 with high affinity, prevents their binding to native VEGF receptors and therefore inhibits angiogenesis [Chu, 2009]. In cell-free systems, this molecule binds with higher affinity to VEGF-A than bevacizumab [Holash *et al.* 2002]. Aflibercept demonstrated single-agent activity in a phase II trial of 51 patients with refractory mCRC, with a disease control rate at 4 months of approximately 30% [Kesmodel *et al.* 2008]. Several phase I trials have proven this agent to be safe when combined with standard chemotherapy, and trials assessing the efficacy of combination therapy have been conducted. Recently, results have been presented of a large, multinational randomized trial (VELOUR). In this trial patients who have mCRC and advanced CRC progressed despite first-line chemotherapy with 5-FU, leucovorin, and oxaliplatin (FOLFOX) were randomly assigned to receive conventional infusional treatment with the FOLFIRI regimen plus or minus aflibercept every 2 weeks until progression [Van Cutsem *et al.* 2011]. The primary endpoint of OS was reached, with a hazard ratio of 0.82 ($p = 0.0032$). Median OS in the ITT population was 12.1 months with FOLFIRI alone and 12.5 months with FOLFIRI plus aflibercept. The PFS improved by about 2–3 months and the response rate doubled, from 10% with chemotherapy alone to 20% in the combination arm. Moreover, subgroup analyses confirmed the consistency of the benefit of aflibercept on OS and PFS. The analyses considered the impact of stratification factors (performance status, prior bevacizumab treatment) and patient characteristics (age, gender, geographic region, prior hypertension, number of metastatic sites, disease confined to the liver, location of primary tumour) supported the consistency and robustness of the efficacy results across all domains, including prior treatment with bevacizumab. The greatest treatment effect was seen among patients who had metastases confined to the liver compared with metastases elsewhere or no liver metastases. Among patients with liver-only metastases, the risk of death was reduced by 35% compared with an 11% risk reduction among the other groups. The results for PFS were similar. Patients who received bevacizumab previously fared as well on

aflibercept as patients who had not received bevacizumab. Median PFS was 6.7 months for patients with prior bevacizumab and OS was 12.5 months, similar to the overall study results. The benefit of aflibercept was not dependent on number of metastatic sites, location of the primary tumour, performance status, occurrence of hypertension, geographic region or patient age. The drug was well tolerated, with the frequency of grade 3/4 AEs in keeping with the anti-VEGF class of agents. Toxicity was not worse for patients with prior exposure to bevacizumab.

Several oral agents are also in development, though for reasons that are unclear, the oral VEGFR antagonists do not seem to add much benefit to standard chemotherapy. Of these, vatalanib, an inhibitor of VEGFR-1, -2 and -3, 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 in first-line treatment, though response rates were slightly higher in patients treated with vatalanib [Hecht *et al.* 2005]. 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 [Koehne *et al.* 2006]. Sunitinib is an oral multi-TKI targeting the VEGFRs, platelet-derived growth factor receptor (PDGFR), c-KIT, RET and LT3. In a phase II trial in patients with refractory mCRC, only 1 of 84 patients responded (1.1%) while 13 had stable disease for at least 6 months [Saltz *et al.* 2007]. In a randomized phase II study of irinotecan, cetuximab and either bevacizumab or sunitinib, the response rate (8% *versus* 0%), PFS (8.7 *versus* 3.2 months) and OS (12.1 *versus* 8.7 months) were significantly higher in patients who received bevacizumab, while the addition of sunitinib was moderately more toxic. A randomized phase II study (HORIZON I) of cediranib with FOLFOX *versus* bevacizumab with FOLFOX in patients with previously treated mCRC was presented at the 2008 American Society of Clinical Oncology (ASCO) meeting. A phase II/III study (HORIZON III) of cediranib plus FOLFOX6 *versus* bevacizumab plus FOLFOX6 in patients with first-line mCRC has recently been presented [Robertson *et al.* 2009]. Clinical activity was observed in the cediranib arm of the study and there was no statistically significant difference between treatment

arms on the efficacy endpoints examined. However, the efficacy did not meet the prespecified criteria for the primary endpoint of noninferiority in PFS. A second study evaluating the efficacy of cediranib in first-line mCRC has been presented at the European Society for Medical Oncology (ESMO) meeting this year. HORIZON II is a phase III study of cediranib plus FOLFOX or XELOX (Oxaliplatin 130 mg/m² day 1 and capecitabine 1000 mg/m² twice a day) *versus* FOLFOX or XELOX alone. PFS and OS were coprimary endpoints. The addition of cediranib to chemotherapies met the coprimary endpoint of PFS prolongation, but there were no significant differences in OS, overall response rate or liver resection rate. The overall cediranib AE profile was consistent with previous studies, but the higher incidence of AEs in this arm reduced chemotherapy delivery [Hoff *et al.* 2010].

An oral multikinase inhibitor, regorafenib, with activity against selected tyrosine kinases (VEGF-R2, VEGF-R3, TIE-2 (tyrosine kinase endothelial receptor 2), PDGFR, fibroblast growth factor receptor, RET and c-KIT) as well as a signal transduction inhibitor of the RAF/MEK/ERK pathway has demonstrated preclinical and clinical activity in mCRC [Kies *et al.* 2010]. Based on these findings, regorafenib was investigated in a phase III trial, designated CORRECT (patients with mCRC treated with regorafenib or placebo after failure of standard therapy), for single-agent activity in refractory CRC [Grothey *et al.* 2012]. This placebo-controlled study has already completed patient accrual and the result of a preplanned interim analysis conducted by an independent data monitoring committee of the CORRECT were presented at the 2012 meeting of ASCO on gastrointestinal cancer [Grothey *et al.* 2012]. The CORRECT trial is an international, multicentre, randomized, double-blind, placebo-controlled study that enrolled 760 patients with mCRC whose disease had progressed after approved standard therapies. Patients were randomized to receive regorafenib plus best supportive care (BSC) or placebo plus BSC. Treatment cycles consisted of 160 mg of regorafenib (or matching placebo) once daily for 3 weeks on, 1 week off plus BSC. The primary endpoint of this trial was OS. Interim analysis of data showed that the addition of regorafenib to BSC significantly prolonged median OS, the primary endpoint, from 5.0 to 6.4 months and disease progression during or within 3 months after their last standard therapy ($p = 0.0052$, one

sided). This translated into a 23% reduction in the risk of death with regorafenib. Even if the benefit was just 1.4 months compared with placebo and BSC, it is important to note that all participants were running out of options after the failure of standard therapies, including bevacizumab (Avastin, Genentech, CA, USA) and EGFR inhibitors in those who had KRAS wt tumours. Regorafenib also significantly prolonged median PFS from 1.7 months to 1.9 months when added to BSC ($p < 0.000001$, one sided). The median difference in PFS was again small, at just 0.2 months, but this corresponded to a 51% reduction in the risk of progression events (hazard ratio 0.49; $p < 0.000001$). The PFS curve clearly identifies that the median difference in PFS does not fully reflect the efficacy of this drug in this patient population. These curves run together for about 50% of patients but then spread out wide. The response rate was similar between regorafenib and placebo (1.0% *versus* 0.4%), but regorafenib distinguished itself with a much higher disease control rate than placebo (45% *versus* 15%). So the strength of this drug is more in delaying tumour progression than inducing responses. The side-effect profile was similar to that observed in the drug's phase I trial and included grade 3 hand-foot skin reactions, fatigue, anorexia and a class effect of hypertension that was controlled with dose reductions. The proportion of patients experiencing AEs leading to treatment discontinuation was 8.2% with regorafenib and 1.2% with placebo. It is extremely important to underline the type of population included in the trial to really appreciate the significant results of the study. The patients had a median age of 61 years and a total of 60% had received four or more lines of prior therapy. Moreover, 57% had tumours with a KRAS mutation. Interestingly, KRAS status did not seem to be predictive of response to regorafenib in patients with CRC, which suggests that this agent might be effective in the 30–40% of patients with mCRC with mutant KRAS.

Intracellular downstream signalling targets

PI3K/AKT/mammalian target of rapamycin inhibitors

The PI3K/AKT/mammalian target of rapamycin (mTOR) pathway provides an alternative signal transduction cascade with pleiotropic biological effects that are important for the formation and progression of cancer, including apoptosis, transcription, translation, metabolism, angiogenesis

and cell cycle progression [Testa and Bellacosa 2001]. PI3K, a heterodimeric protein composed of a catalytic subunit (p110 α) and a regulatory subunit (p85), is activated by growth factor receptor tyrosine kinases. The survival mechanism initiated by these proteins is executed through the downstream effectors of this kinase: Akt, mTOR and p70S6 kinase. Upon binding of ligands to various membrane growth factor receptors, the corresponding receptor tyrosine kinases activate PI3K, which results in recruitment of PI3K to the cell membrane and production of phosphatidylinositol (3,4,5)-triphosphate (PIP3) [Raffioni and Bradshaw, 1992; Thompson and Thompson, 2004]. PIP3 subsequently recruits AKT to the plasma membrane where it is phosphorylated and activated. Phosphatase and tensin homologue dephosphorylates PIP3, thus acting as a negative regulator of PI3K signalling. Activated AKT exerts antiapoptotic activity by preventing release of cytochrome C from mitochondria, inactivating forkhead transcription factors known to induce expression of proapoptotic factors which results in transcription of antiapoptotic genes [Cross *et al.* 1995]. AKT activation also affects cell cycle progression, through regulation of cyclin D stability, inhibition of p27Kip1 protein levels and regulation of mRNA translation via control of phosphorylation of 4E-BP1 and its dissociation from the mRNA cap binding protein eIF4E [Muise-Helmericks *et al.* 1998; Collado *et al.* 2000; Sonenberg and Gingras, 1998]. Finally, AKT mediates the activation of endothelial nitric oxide synthase (an important modulator of angiogenesis), promotes tumour invasiveness and enhances telomerase activity [Fulton *et al.* 1999; Kim *et al.* 2001; Kang *et al.* 1999; Vivanco and Sawyers, 2002].

In colon cancer, AKT phosphorylation and PI3K activation directly correlate with tumourigenic potential. PI3K activity is elevated in the vast majority of CRCs compared with normal mucosa, and mutations in the p110 α subunit of PI3K are found in approximately one-third of CRCs [Khaleghpour *et al.* 2004; Samuels *et al.* 2004]. Inhibition of PI3K and AKT confers antitumour activity in preclinical models [Meuillet *et al.* 2004; Ihle *et al.* 2004]. Further support for the development of AKT inhibitors in CRC is provided by a clinical study in patients treated with an EGFR inhibitor, in which responses were restricted to those tumours that showed complete abrogation of AKT phosphorylation after treatment. Despite the appeal of AKT as a target, however,

Table 2. Downstream intracellular signalling targets and drugs.

Target	Drug	Development stage in CRC	Population	Treatment
PI3K	BEZ235	Phase I	Refractory	MEK162 + BEZ235
	SF1126	Phase I	Refractory	Single agent
	BGT226	Phase I	Refractory	Single agent
	XL147	Phase I	Refractory	XL147 + MSC1936369B
	BKM120	Phase I	Refractory	BKM120 + irinotecan
	GDC-0941	Phase I	Refractory	Single agent
	XL765	Phase I	Refractory	Single agent
mTOR	Temsirolimus (CCI-779)	Phase I	Cetuximab -	Temsirolimus + cetuximab
	RAD001	Phase I	Refractory	RAD001 + OSI906
		Phase II	Refractory	RAD001 + irinotecan +
		Phase Ib/II	Refractory	cetuximab
		Phase I	Refractory	Irinotecan + cetuximab + RAD001
Src	AP23573	Phase I	Refractory	5-Fluorouracil + RAD001
			Refractory	Bevacizumab + AP23573
	Bosutinib (SKI-606)	Phase I	Refractory	Capecitabine + bosutinib
	SU6656	Preclinical	-	-
	AP23464	Preclinical	-	-
Aurora kinase	Dasatinib (BMS-354825)	Phase I	Refractory wild type	Dasatinib + FOLFOX + cetuximab
	Saracatinib (AZD0530)	Phase II	Refractory	Single agent
	MK0457	Phase I	Refractory	Single agent
	PHA-739358	Phase I	Refractory	Single agent
	MLN8054	Phase I	Refractory	Single agent
	MLN8237	Phase I	Refractory	Single agent
	MK0457	Phase I	Refractory	Single agent

CRC, colorectal cancer; FOLFIRI, leucovorin, 5-fluorouracil and irinotecan; FOLFOX, 5-fluorouracil, leucovorin, and oxaliplatin; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3 kinase.

development of specific AKT inhibitors has been challenging and candidate drugs for clinical trials have only recently become available [van Ummersen *et al.* 2004]. Several PI3K inhibitors are in clinical development, such as BEZ235 [Maira *et al.* 2008], SF1126 [Garlich *et al.* 2008], BGT226, XL147, BKM120, GDC-0941 and XL765 (Table 2). Other PI3K inhibitors are still in preclinical development, such as ZSTK474 [Maira *et al.* 2008; Garlich *et al.* 2008].

Another potential therapeutic target in the PI3K/AKT signalling cascade is mTOR, which lies downstream of AKT. mTOR is phosphorylated in response to stimuli that activate the PI3K/AKT pathway and regulates protein translation through phosphorylation of ribosomal S6 kinase, eukaryote initiation factor 4E-binding protein 1 and p70 kinase [Hidalgo and Rowinsky, 2000; Hara *et al.* 1997; Kumar *et al.* 2000]. Inhibition of mTOR by

the naturally produced macrolide antibiotic sirolimus (rapamycin) results in inhibition of the oncogenic effects of AKT and PI3K [Aoki *et al.* 2001; Sehgal *et al.* 1975]. Several mTOR inhibitors with superior pharmaceutical properties to rapamycin are currently undergoing clinical investigation [Clackson *et al.* 2003; O'Reilly *et al.* 2002; O'Donnell *et al.* 2008, Atkins *et al.* 2004; Punt *et al.* 2003]. There have been several early studies of the mTOR inhibitors suggesting some benefit in patients with mCRC (Table 2). In one phase I trial of RAD-001, another mTOR inhibitor, one partial response was seen in a patient with CRC (lasting 5.3 months with a disease control period of 9 months) [Tabernero *et al.* 2008]. In the subsequent phase II study, a disease control rate (defined as response rate plus rate of stable disease) of 25%, with an OS of 5.9 months was achieved. The combination was somewhat toxic, though, with fatigue, cytopenias and nausea/

vomiting/dehydration being the main AEs. Recently a phase Ib/II study of RAD-001 in combination with tivozanib, a highly potent, selective oral inhibitor of VEGFR-1, -2 and -3, in refractory mCRC was presented [Wolpin *et al.* 2012]. The combination of RAD-001 plus tivozanib was well tolerated with MTD of RAD-001 of 10 mg/day and tivozanib 1 mg/day. The phase II trial has completed enrolment using these doses. The mTOR inhibitors will likely be most effective when combined with traditional chemotherapy, and in one phase I clinical trial, the combination of RAD-001 with 5-FU in patients with refractory CRC resulted in one partial response lasting 7.4 months [Punt *et al.* 2003]. Several phase I and II studies of the mTOR inhibitors in combination with chemotherapy are currently under way.

Src kinase inhibitors

The nonreceptor tyrosine kinase Src was the first identified proto-oncogene, composed of a carboxy-terminal tail containing a negative-regulatory tyrosine residue, four Src homology domains and an amino terminal domain. Recent attention has been garnered by Src as an interesting and versatile target, with location at the nexus of a number of critical signalling pathways which acts as an intermediary between growth factor binding to the receptor and downstream signalling important for a number of cellular processes, including proliferation, differentiation and survival [Summy and Gallick 2000]. Src promotes tumour growth and invasion both through activation of downstream signal transducers and activators of transcription protein (STATs) and through modulation of the actin cytoskeleton focal adhesions and integrins [Brunton *et al.* 2005; Cao *et al.* 1996; Courter *et al.* 2005]. Enhanced Src activity is observed in 80% of CRCs [Talamonti *et al.* 1993]. The activity increases with the advanced stage of tumour development, for example, the high Src activity in dysplastic polyps with high malignant potential compared with more benign adenomas [Aligayer *et al.* 2002]. Src is also of particular interest in colon cancer because of its intimate connections with EGFR signalling. Upon binding of ligand to the EGFR, Src associates with EGFR and phosphorylates downstream targets [Muthuswamy and Muller, 1995]. Src can phosphorylate the EGFR itself, following activation by EGFR ligand binding, and through lateral activation via other growth factor receptor pathways [Biscardi *et al.* 2000]. Src activity has been correlated with shortened

survival of patients with colon cancer [Aligayer *et al.* 2002]. Briefly, Src has an important role in oncogenic processes, which provides a rationale for Src as a therapeutic target.

Numerous Src inhibitors are entering phase I/II trials, including SKI-606 (bosutinib), SU 6656, AP 23464, BMS-354825 (dasatinib), saracatinib (table 2). Among these, the two that have been tested in CRC are saracatinib and dasatinib. Saracatinib has demonstrated preclinical activity against colon cancer metastases *in vivo* and is now undergoing phase II testing in patients with mCRC. In the phase I trial, 81 patients were tested, 28 of whom had CRC [Baselga *et al.* 2010]. Eleven patients were on study for over 12 weeks, five of whom had CRC, demonstrating some promise of activity, at least of disease stabilization in this patient population. Furthermore, a phase II study of the approved Src inhibitor dasatinib has been conducted in patients with unresectable, previously treated mCRC. The study was terminated after the first stage due to lack of efficacy. In fact, there were no objective responses and only one patient had stable disease for 7 months. Moreover, the median PFS rate was 1.6 months and median OS was 5.1 months [Sharma *et al.* 2011]. Src inhibitors are logical combination partners for EGFR inhibitors given the extent of cross talk between the two pathways. Building on this hypothesis, a combination study of dasatinib with FOLFOX and cetuximab was developed. A phase I, dose-finding trial in patients with previously treated mCRC has been conducted. The objectives of this study were to determine the MTD, toxicity and pharmacodynamics of the combination. Dasatinib at a dose of 100 mg daily in combination with FOLFOX and cetuximab was safe with promising early signs of clinical activity in previously treated patients [Kopetz *et al.* 2008].

Intranuclear targets

Aurora kinase inhibitors

Within the complete cell-cycle process, mitosis constitutes one of the most critical steps. A copy of the duplicate genome is segregated by the microtubule spindle system into the two resulting cells. Errors in this process can lead to genomic instability, a condition associated with cancer. Contrary to its potential to induce cancer, mitosis has emerged as an important target for anticancer therapies. In fact, inducing aberrant mitosis in

tumour cells leads to mitotic arrest and consequently, although not always, to cell death [Nigg, 2001]. Aurora is a name given to a family of serine/threonine protein kinases that are key regulators of mitosis essential for accurate and equal segregation of genomic material from parent to daughter cells. 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 regulators of genomic stability [Keen and Taylor, 2004]. AKs are frequently overexpressed in human tumours. Deregulation of cell-cycle machinery can have an important impact on cellular proliferation. The first data that linked tumorigenesis with the AK family came with the observation that AK-A DNA was amplified and its RNA was overexpressed in more than 50% of primary CRC specimens. Moreover this overexpression was associated with poor prognosis in patients with CRC [Bischoff *et al.* 1998; Katayama *et al.* 1999]. Recently, a study of the phenomena involved in the progression from colorectal adenoma to carcinoma identified that several oncogenes were potentially responsible for this malignant transformation [Carvalho *et al.* 2009]. These oncogenes were significantly amplified in carcinomas compared with adenomas, suggesting a potential role of the gene of AK-A in the development of colorectal carcinomas. Unlike AK-A, the role of AK-B in tumorigenesis has been less studied. The reasons for this less extensive evaluation are not clear, although the pattern of expression of AK-B is not much different from that of AK-A. Increased levels of phosphorylation of histone H3 were correlated with overexpression of AK-B in colorectal tumour cell lines [Ota *et al.* 2002]. Like the other members of the AK family, AK-C has been shown to be overexpressed in multiple tumour cell lines; however, little is known about the role of its expression in human cancer specimens and prognosis [Fidalgo *et al.* 2009].

The evidence linking AK overexpression and cancer has supported the hypothesis that AK inhibition could be a target for cancer therapy. In fact, as AKs are only expressed and active as kinases during mitosis, nonproliferating cells would not be adversely affected by these drugs. In support of this hypothesis, proliferating tumour cells treated with ZM447439, a pan-AK inhibitor, were killed whereas nonproliferating cells were not affected by this agent [Ditchfield *et al.* 2003]. Given that most normal cells in the

body do not proliferate rapidly, AK inhibitors could have a broader therapeutic index than non-specific cytotoxic agents, such as some alkylating agents that act in a noncell-cycle specific manner. The mechanism of inhibition of the AKs could be to interfere with their ability to interact with their coactivating proteins or binding agents, such as by targeting the catalytic binding site of the ATP. This is the mechanism of action of a number of AK inhibitors.

Several AK inhibition drugs are in preclinical and clinical development in the CRC setting: MK0457, PHA-739358, MLN8054, MLN8237. MK0457, initially developed by Harrington and colleagues with the name of VX-680, is a diaminopyrimidine that targets the ATP-binding site common to all AKs [Harrington *et al.* 2004] (Table 2). VX-680 is an inhibitor of the three AKs with a more than 100-fold selectivity for AKs over a panel of 55 other kinases, with the exception of *fms*-like tyrosine kinase receptor 3 [Tyler *et al.* 2007]. In its first phase I clinical trial, MK0457 was given as an intravenous continuous infusion over several days to patients with previously treated solid tumours. The principal DLT was grade 3 neutropenia, accompanied by some nonspecific side effects, including nausea and fatigue [Rubin *et al.* 2006]. Another AK inhibitor is PHA-739358 which is a pan-aurora inhibitor with documented antitumour activity in multiple tumour xenograft models, including colon cancer, which have shown sustained tumour growth inhibition after discontinuation of treatment [Carpinelli *et al.* 2007]. In a preliminary assessment, DLT was reported to be grade 3–4 neutropenia. No tumour responses were observed but 8 of 40 patients had stable disease for at least 4 months. Pretreatment and post-treatment skin biopsies showed downregulation of phosphorylated histone H3Ser10 levels in eight of nine patients tested. Recently, the final results of the pharmacokinetics and pharmacodynamics of phase I with PHA-739358 were communicated [Steeeghs *et al.* 2009]. As in the previous study, the drug was administered intravenously on days 1, 8 and 15, but in 6 h or 3 h infusions. Dose levels from 45 mg/m² in 6 h and from 250 mg/m² in 3 h were studied. Fifty patients with advanced or metastatic solid tumours were treated. For the 6 h infusion the most frequently reported adverse effects were neutropenia (55%), nausea (25%) and anorexia (23%). In the 3 h infusion they were fatigue (70%), neutropenia (60%) and diarrhoea (50%). The MTD was 330 mg/m² for the 6 h infusion and not

identified for the 3 h infusion. This systemic exposure to PHA-739358 increased linearly with dose. Biomarker analyses showed inhibition of histone H3 phosphorylation indicative of AK-B inhibition at doses of 190 mg/m² or greater. Stable disease was observed in 23.7% of patients and disease stabilization occurred in 6 or more months in five patients. A small-molecule inhibitor of AK with relative specificity for AK-A is MLN8054. It is an ATP competitor and reversible inhibitor. This compound induced G2/metaphase accumulation and spindle defects and inhibits proliferation in multiple tumour cell lines. Growth of colon tumour xenografts in nude mice was dramatically inhibited after oral administration of MLN8054 at well tolerated doses. Moreover, the tumour growth inhibition was maintained after discontinuing MLN8054 treatment. This molecule demonstrated activity against a broad spectrum of human xenografts, including colon cancer [Manfredi *et al.* 2007; Huck *et al.* 2006]. Toxicity in animals was reversible and AEs included myelosuppression, cataracts and gastrointestinal mucosa damage. In a recent phase I trial, 43 patients with advanced solid tumours were treated [Macarulla *et al.* 2010]. In the two initial cohorts treated with 10 or 20 mg of MLN8054 once daily on days 1–5 and 8–12 of 28 days, patients experienced benzodiazepine-like AEs, mainly somnolence, so subsequent cohorts in this trial were treated with 25, 35, 45, 55, 60, 70 and 80 mg/day in four divided doses on days 1–14 with the largest dose at night. To manage somnolence, methylphenidate was administered in some patients with daytime doses. Dose escalation was stopped at 80 mg due to DLTs, including G3 somnolence (one patient) and G3 transaminitis (one patient). Pharmacokinetics showed a rapid absorption with a long half life (30–40 h) and pharmacodynamic studies confirmed that higher doses of MLN8054 corresponded with an increased mitotic count in tumour and skin biopsies and reduced chromosome alignment and spindle bipolarity in the mitotic cells in these biopsies. Stable disease was observed in three patients with colorectal and non-small cell lung cancer, and melanoma. A second-generation oral inhibitor, more potent than MLN8054, is MLN8237, which is a competitive and reversible inhibitor of the ATP of AK-A. Like MLN8054, this new inhibitor has activity for the binding site of the receptor of γ -aminobutyric acid α 1; however, this interaction occurs at a level of dose much higher than the DLT. These advantages over MLN8054 have led to stopping the development of MLN8054, focusing efforts on the

MLN8237. In preclinical, *in vivo* studies, MLN8237 has demonstrated anticancer activity in xenografts with colon cancer with a maximal inhibition of the tumour growth greater than 90%. Doses and maximal efficacy schedules in nude mice models varied from 10 to 30 mg/kg. A phase I trial testing this compound included 27 patients with advanced solid tumours [Cervantes-Ruiperez *et al.* 2009]. MLN8237 was administered orally and once or twice daily for 7 days followed by a 14-day recovery period. Patients were treated in three once daily cohorts with 5, 80 or 150 mg, and four twice daily cohorts with 50, 60, 75 and 100 mg. DLTs observed at 60–100 mg twice daily included neutropenia, pancytopenia, stomatitis and somnolence. Somnolence (of any grade) was seen with once daily dosing but was reduced with divided doses each less than 100 mg. MLN8237 was tolerable with twice daily dosing for 7 days and exhibited favourable pharmacokinetics and pharmacodynamics.

Summary

New cytotoxic and molecular agents for the treatment of mCRC have improved OS in this group of patients. As we are entering into this second decade of personalized therapy for mCRC, an increasing number of new targeted agents are being tested in clinical trials. All these agents have demonstrated some degree of preclinical activity. Unfortunately many of them have not shown confirmed antitumour activity in early clinical trials. As a result, dozens of clinical trials of novel targeted therapies are ongoing. Typically these are single-agent studies, often designed to demonstrate some degree of clinical activity in refractory patients to move forward into first- or second-line treatment. In the coming years, emerging data on the new targeted agents that are currently in clinical development will probably translate into an expansion of the therapeutic armamentarium against mCRC. Nevertheless, this expected setting will only occur if we are able to identify predictive biomarkers of activity, or primary or secondary resistance that allow us to define the population of patients with mCRC who will benefit from these treatments and when the most benefit will be achieved in the time course of advanced disease.

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Conflict of interest statement

The authors declare no conflict of interest in preparing this article.

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