Anti-angiogenic Targeting in Metastatic Colorectal Cancer Therapy

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Abstract
Angiogenesis has been identified as a hallmark of cancer. Thus, anti-angiogenic targeting has been evaluated in cancer. Colorectal cancer is one of the entities where this therapeutic principal has been most successfully introduced into the daily care of patients. Today, several anti-angiogenic drugs are approved in all lines of metastasized colorectal cancer (mCRC). In adjuvant settings, anti-angiogenic treatment did not show any benefit. In summary, still overall the benefit from anti-angiogenic treatment is modest and the identification of specific patient subgroups benefiting from this treatment is missing. Several new drugs are in development to further improve the treatment of patients with mCRC. In addition, large efforts were made to identify predictive biomarker, but so far, none of these has entered the clinical routine. Here we present the current status of anti-angiogenic drugs in mCRC and the new drugs in development for this clinical entity.

Keywords
Colorectal cancer • Metastatic • Chemotherapy • Clinical Trial • Bevacizumab • Aflibercept • Ramucirumab • Regorafenib • VEGF • VEGFR

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Introduction
Colorectal Cancer

Colorectal cancer is one of the leading cancer health-care problems worldwide. It is estimated that 1.3 million people were newly diagnosed with colorectal cancer in 2012 and 690,000 died from this disease. Over the last years, the incidence of colorectal cancer has been rising particularly in those countries where this disease traditionally had a low incidence. In several
developed countries with traditional high incidence, mortality of colorectal cancer has decreased during the last decade. This is most likely due to the introduction of effective screening and the development of new treatments for metastatic colorectal cancer (Torre et al. 2015). At initial diagnosis, about 25% of patients present with hematogenous metastasis and additional 25% of patients develop metastases subsequently (Ferlay et al. 2013; Siegel et al. 2014). In the last 20 years, the median overall survival has significantly improved for patients with metastasized colorectal cancer from 12 months when 5-fluorouracil (5-FU) single agent was given (Van Cutsem et al. 2001) to about 30 months as of today with combined chemotherapy and biological agents (Cremolini et al. 2015b). In detail, the first step of treatment improvement was the introduction of the two cytostatic agents oxaliplatin and irinotecan, which allowed for the doublet chemotherapy regimens FOLFOX (5-FU, folinic acid, oxaliplatin) (de Gramont et al. 2000) and FOLFIRI (5-FU, folinic acid, irinotecan) (Douillard et al. 2000), respectively. Both regimens were safe and showed superior activity compared to 5-FU monotherapy. Even the triplet chemotherapy FOLFOXIRI exploiting the synergistic activity of the three chemotherapeutic agents could be applied safely (Falcone et al. 2007). In addition to these chemotherapeutic agents, subsequently biological agents were introduced into the therapy of mCRC, namely, bevacizumab, aflibercept, ramucirumab, regorafenib, cetuximab, and panitumumab, further widening the therapeutic armamentarium for the treatment of mCRC. Along with constantly improving methods of local treatment, multiple options are now available to treat patients with mCRC.

In this chapter, we will focus on the role of anti-angiogenic agents in the treatment of mCRC. In this context, we will discuss approved drugs as well as further anti-angiogenic drugs in development.

The Role of Tumor Angiogenesis in Colorectal Cancer

Tumor angiogenesis is a prerequisite of neoplastic growth and represents a hallmark of cancer (Hanahan and Weinberg 2000). Vascular endothelial growth factor (VEGF) is one of the key regulators of tumor angiogenesis. The VEGF family consists of different member (VEGF A, VEGF B, VEGF C, VEGF D, and placental-growth factor, PlGF). VEGF-A is a survival factor for endothelial cells (EC) and induces proliferation, thus playing a central role in the process of sprouting angiogenesis (Tung et al. 2012). In solid tumors, VEGF is expressed by different cells of the tumor stroma as well as by tumor cells. The role of VEGF expression levels as a prognostic marker in colorectal cancer patients, however, is controversial. Morphologically, in contrast to “normal,” physiological angiogenesis, tumor angiogenesis results in an abnormal blood vessel network. Structurally, vessels within a tumor are often dilated, tortuous, and in an immature state (i.e., EC are not covered by pericytes). In addition, there is marked heterogeneity of distribution of vessels with both hypovascular and hypervascular areas, resulting in an increase in the interstitial fluid pressure (IFP) within tumors. It was demonstrated in several models that an increased IFP decreases the accessibility of chemotherapeutic compounds to the tumor (Carmeliet and Jain 2011). Inhibition of the VEGF pathway was shown to impair sprouting angiogenesis and to normalize the chaotic vascular structure by pruning immature vessels in a number of preclinical models and in patient with rectal cancer treated with the anti-VEGF antibody bevacizumab (Willett et al. 2004). Vascular normalization results in an improvement of the efficacy of chemotherapy (Goel et al. 2012) or irradiation therapy in preclinical models (Winkler et al. 2004). These data in part may serve as an explanation for the fact that in colorectal cancer combinations of anti-angiogenic compounds plus chemotherapy are most effective, while anti-angiogenic monotherapy alone exerts only limited efficacy.

The platelet-derived growth factor (PDGF)-receptor (PDGFR) pathway and the
Angiopoietin-Tie-2-system represent other key signaling pathways involved in tumor angiogenesis. Specifically, pericytes (PC) are dependent on PDGF produced by endothelial cells to support their interaction with EC, thus playing an important role during angiogenesis and vascular maturation (Heldin 2013). Interestingly, in colorectal cancer patients, high tumor expression of the ligand PDGF-BB was associated with significantly poorer survival compared to low PDGF-BB expression (Nakamura et al. 2008).

With respect to the angiopoietin-Tie-2-system, the ligands angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) bind to the Tie-2 receptor. Binding of Ang-1 to the Tie-2 receptor induces signal transduction leading to a stabilization of the EC/PC interaction. In contrast, Ang-2 binds to the Tie-2 receptor without inducing a signal, thereby inhibiting the activity of Ang-1. Consequently, overexpression of Ang-2, as can be found in different solid tumor types including colorectal cancer, results in a more immature vascular state (Maisonpierre et al. 1997). Based on these key angiogenic pathways, a number of compounds have been developed to target tumor angiogenesis: (i) Monoclonal antibodies targeting the VEGF-pathway (i.e., bevacizumab, ramucirumab); (ii) a fusion construct targeting VEGF A, VEGF B, and placental growth factor (PIGF) (i.e., aflibercept); and (iii) a number of different tyrosine kinase inhibitors (TKI) targeting the VEGF pathway and other pathways involved in tumor angiogenesis.

### Anti-angiogenic Treatment

#### Approved Monoclonal Antibodies and Derived Constructs

##### Bevacizumab

Bevacizumab is a recombinant humanized monoclonal antibody targeting VEGF-A.

##### First-Line Treatment

Chemotherapy Doublets ± Bevacizumab

In the pivotal phase III randomized AVF2107 trial, initially three therapeutic regimens, 5-FU/folinic acid plus bevacizumab, 5-FU, folinic acid and irinotecan (IFL), and IFL plus bevacizumab, were compared in 923 mCRC patients in the first-line setting (Hurwitz et al. 2004). Since the toxicity profile of IFL plus bevacizumab was acceptable at an interim analysis after 300 patients, overall 813 patients were recruited to be treated in the two primary comparison arms IFL +/− bevacizumab. The primary endpoint was overall survival (OS). Progression-free survival (PFS) and overall response rate (RR) were secondary endpoints. Addition of bevacizumab to IFL leads to a significant increase in median OS of almost 5 months from 15.6 to 20.3 months, Hazard Ratio (HR) 0.66; \( p < 0.001 \). Similarly, progression-free survival time was significantly increased from 6.2 to 10.6 months, HR 0.54; \( p < 0.001 \) as well as overall response rate (34.8–44.8%; \( p < 0.001 \)).

The main grade 3/4 toxicity conferred by bevacizumab was hypertension. However, this side effect was easily manageable. The importance of this trial should be underlined as it represents the first randomized phase III trial formally proving the hypothesis that blockade of VEGF by a monoclonal antibody is active in cancer patients improving overall survival when combined with chemotherapy.

In the phase III NO16966 trial following a 2x2 factorial design FOLFOX or XELOX ± bevacizumab was compared in 1401 mCRC patients. The primary endpoint of this study was PFS. The addition of bevacizumab- to oxaliplatin-based first-line chemotherapy significantly improved median PFS from 8.0 months to 9.4 months, HR 0.83; \( p = 0.0023 \). Interestingly, the improvement in median PFS “on-treatment” was even more pronounced (10.4 months in the bevacizumab arm vs. 7.9 months in the placebo arm, HR 0.63; \( p = 0.0001 \)). In contrast, no statistically significant improvement in median OS was found (i.e., 21.3 months in the bevacizumab arms vs. 19.9 months in the placebo arm; HR 0.89; \( p = 0.08 \)). Response rates, were identical in both arms (38%, HR 1.00; \( p = 0.99 \)). The study confirmed the toxicity profile of bevacizumab as no new or unexpected adverse events occurred (Saltz et al. 2008).
discussed. Specifically, median duration of treatment in both arms of the NO16966 study was approximately 6 months. This implicates that treatment was discontinued prior to progression in a relevant number of patients. Censoring of these patients as in the PFS “on-treatment” provided a significantly greater PFS benefit. This finding pointed to the hypothesis that bevacizumab should be continued until final progression of the therapy line, and this hypothesis indeed was later validated and extended in clinical trials investigating the role of bevacizumab continuation beyond progression (see 1.3.3).

As in the NO16966 study, a number of subsequent randomized clinical trials evaluating the addition of bevacizumab to first-line treatment in mCRC failed to show an overall survival benefit, while uniformly demonstrating improved progression-free survival. Interestingly, this effect was most pronounced in fluoropyrimidine monotherapy combinations (Tebbutt et al. 2010; Cunningham et al. 2013a), and this points to the role of bevacizumab in the context of less effective combination therapies (IFL) or fluoropyrimidine monotherapy.

5-FU-Based Chemotherapy ± Bevacizumab

Apart from studies examining the addition of bevacizumab to chemotherapy-doublets, randomized phase II studies have been conducted that combined 5-FU or capecitabine monotherapy +/− bevacizumab. In a small randomized phase II study, published by the group of Kabbinavar in 2003, 104 patients with previously untreated mCRC received first-line treatment with either bolus 5-FU/folinic acid \((n = 36)\) or 5-FU/folinic acid with two doses of bevacizumab: 5 mg/kg, \((n = 35)\) or 10 mg/kg, \((n = 33)\) (Kabbinavar et al. 2003). The primary endpoints of time to disease progression and response rate were reached: Addition of bevacizumab increased the response rate in both arms: Control arm 17%, 5 mg/kg arm 40%, and 10 mg/kg arm 24%. The time to tumor progression was longer in the bevacizumab arms: Control arm, 2.0 months, 5 mg/kg arm 9.0 months and 10 mg/kg arm 7.2 months. OS was also improved: Control arm 13.8 months, 5 mg/kg arm 21.5 months and 10 mg/kg arm 17.3 months. Based on these data the authors recommended a dose of 5 mg/kg every 2 weeks.

Addition of bevacizumab to 5-FU/folinic acid was further evaluated in a randomized phase II trial (Kabbinavar et al. 2005) in elderly \((\geq 65\) years of age) or less fit patients not deemed to be candidates for IFL treatment. Endpoints of this trial were overall survival, progression-free survival, overall response rate, and duration of response along with safety. A total of 209 patients were randomized in this trial. Also in this poor prognostic patient population, addition of bevacizumab improved median overall survival by 3.7 months \((12.9\) vs. 16.6 month, HR 0.79; \(p = 0.16)\), progression-free survival by 3.7 months \((5.5\) vs. 9.2 months, HR 0.5; \(p = 0.0002)\), and response rate from 15.2% to 26%.

The MAX study is the largest trial investigating the addition of bevacizumab to capecitabine (Tebbutt et al. 2010). In this first-line three-arm randomized study, a 1: 1: 1 randomization was applied to compare the following treatment arms: Capecitabine at the standard dose of 2500 mg/m² +/− bevacizumab 7.5 mg/kg every 3 weeks. In a third arm, capecitabine + bevacizumab + mitomycin C was tested. Overall 471 patients were enrolled. Bevacizumab significantly improved PFS compared to capecitabine alone \((HR 0.63; p = 0.001)\) and compared to the mitomycin-containing combination \((HR 0.59; p = 0.001)\). It was demonstrated that the benefit for the addition of bevacizumab was preserved in the subgroup of patients >75 years of age compared to the younger patients and that toxicities were not different (Price et al. 2012). Interestingly, many of the patients enrolled had only received a starting dose of 2.000 mg/m² capecitabine, which did not translate in an inferior clinical outcome, while the bevacizumab effect on PFS was fully preserved.

In the APEX trial, patients aged 70 years and older and not deemed candidates for oxaliplatin or irinotecan combination chemotherapy were randomized to receive capecitabine ± bevacizumab and a total of 280 patients were included (Cunningham et al. 2013a). PFS was significantly improved with bevacizumab/capecitabine compared to capecitabine (median 9.1 months vs. 5.1 months; HR 0.53; \(p < 0.0001)\). Treatment-related
adverse events of $\geq$ grade 3 occurred in 40% of patients in the combination group and 22% in the capecitabine group. The most common $\geq$ grade 3 adverse events related to bevacizumab or chemotherapy were hand-foot syndrome (16% vs. 7%), diarrhea (7% in both arms), and venous thromboembolic events (8% vs. 4%).

Chemotherapy Triplet $\pm$ Bevacizumab
The finding that the use of all available chemotherapeutic drugs (i.e., 5-FU, oxaliplatin, and irinotecan) during the entire treatment course is correlated with improved survival compared to doublets or a monotherapy in mCRC patients led to the development of the triplet chemotherapy regime (FOLFOXIRI) consisting of all three drugs. Initially, a single-group phase 2 study of FOLFOXIRI plus bevacizumab was conducted by the Italian GONO group and safety and activity data of the combination were promising (Masi et al. 2015). This concept was further investigated by the same group in a phase III trial. Specifically, in the TRIBE study a combination of FOLFOXIRI/bevacizumab was compared with FOLFIRI/bevacizumab in 508 patients. The median follow-up was 48.1 months and the median overall survival was 29.8 months in the FOLFOXIRI plus bevacizumab group vs. 25.8 months in the FOLFIRI plus bevacizumab group (HR 0.80; $p = 0.03$). As expected, a better OS and PFS was found in patients with wild-type Ras tumors as compared to patients with mutated Ras tumors. Patients with mutated B-Raf tumors carried the most adverse prognosis. The efficiency of FOLFOXIRI/Bevacizumab, however, was preserved in all molecular groups. Interestingly, in a small subgroup of patients with mutated B-Raf tumors, FOLFOXIRI/bevacizumab compared to FOLFIRI/bevacizumab even resulted in a more pronounced improvement in OS (HR 0.54; 95% CI 0.24–1.20) pointing to a role for the combination of FOLFOXIRI/bevacizumab in patients with mutated B-Raf tumors, see Table 1 (Loupakis et al. 2014; Cremolini et al. 2015a).

### Use of Bevacizumab for Conversion Therapy
Based on the fact that patients with initially unresectable liver metastasis, who show a

<table>
<thead>
<tr>
<th>Trial</th>
<th>Line</th>
<th>N</th>
<th>Treatment</th>
<th>OS</th>
<th>PFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVF2017</td>
<td>1</td>
<td>813</td>
<td>IFL+ (A)/−(B) Bevacizumab</td>
<td>20.3 (A) vs. 15.6</td>
<td>10.6 (A) vs. 6.2</td>
</tr>
<tr>
<td>NO16996</td>
<td>1</td>
<td>1401</td>
<td>XELOX/FOLFOX + (A)/−(B) Bevacizumab</td>
<td>21.3 (A) vs. 19.9</td>
<td>9.4 (a) vs. 8 (B)</td>
</tr>
<tr>
<td>ITACA</td>
<td>1</td>
<td>376</td>
<td>FOLFOX/FOLFIRI + (A)/−(B) Bevacizumab</td>
<td>20.6 (A) vs. 20.6</td>
<td>9.2 (A) vs. 8.4</td>
</tr>
<tr>
<td>AVEX</td>
<td>1</td>
<td>280</td>
<td>Capecitabine +(A)/−(B) Bevacizumab</td>
<td>20.7 (A) vs. 16.8</td>
<td>9.1 (A) vs. 5.1</td>
</tr>
<tr>
<td>CAIRO3</td>
<td>Maintenance</td>
<td>558</td>
<td>CAPOX + Cap/Beva maintenance (A) vs. CAPOX</td>
<td>21.6 (A) vs. 18.1</td>
<td>8.5 (A) vs. 4.1</td>
</tr>
<tr>
<td>E3200</td>
<td>2</td>
<td>829</td>
<td>FOLFOX4 + (A) vs. −(B) Bevacizumab</td>
<td>12.9 (A) vs. 10.8</td>
<td>7.3 (A) vs. 4.7</td>
</tr>
<tr>
<td>ML18147</td>
<td>2</td>
<td>820</td>
<td>FOLFOX or FOLFIRI + (A) vs. −(B) Bevacizumab</td>
<td>11.2 (A) vs. 9.8</td>
<td>5.7 (A) vs. 4.1</td>
</tr>
<tr>
<td>VELOUR</td>
<td>2</td>
<td>1226</td>
<td>FOLFIRI + (A) vs. −(B) Afiblercept</td>
<td>13.5 (A) vs. 12.06</td>
<td>6.9 (A) vs. 4.7</td>
</tr>
<tr>
<td>RAISE</td>
<td>2</td>
<td>1072</td>
<td>FOLFIRI + (A) vs. −(B) Ramucirumab</td>
<td>13.3 (A) vs. 11.7</td>
<td>5.7 (A) vs. 4.5</td>
</tr>
<tr>
<td>CORRECT</td>
<td>3</td>
<td>760</td>
<td>BSC + (A) vs. −(B) Regorafenib</td>
<td>6.4 (A) vs. 5 (B)</td>
<td>1.9 (A) vs. 1.7</td>
</tr>
</tbody>
</table>

AVF2017 (Hurwitz et al. 2004); NO16996 (Saltz et al. 2000); ITACA (Passardi et al. 2015); AVEX (Cunningham et al. 2013a); CAIRO3 (Simkens et al. 2015); E3200 (Giantonio et al. 2007); ML18417 (Bennouna et al. 2013); VELOUR (Van Cutsem et al. 2016b); RAISE (Tabernero et al. 2015); CORRECT (Grothey et al. 2013)
response to systemic chemotherapy, allowing complete resection of metastases, have a far better long-term outcome compared with patients treated with chemotherapy alone, conversion therapy is an approach that aims at rendering technically irresectable metastasis resectable. (Van Cutsem et al. 2016a). There is a large body of evidence for the use of EGFR-antibodies in wild-type Ras mCRC in combination with chemotherapy in this setting and triple chemotherapy regimen have also been shown to increase response as well as resection rates compared with chemotherapy doublets (Van Cutsem et al. 2016a). Table 2 gives an overview on clinical trials having tested bevacizumab combinations with respect to response and resection rates in Ras-unselected patients.

Second-Line Therapy
Bevacizumab was also tested in the second-line treatment of mCRC. Patients pretreated with 5-FU and irinotecan were randomized in a phase III trial to receive FOLFOX4 or FOLFOX4 + bevacizumab (10 mg/kg) or bevacizumab (10 mg/kg) alone as a second-line treatment. A total of 291 patients were randomized to FOLFOX4, 286 to FOLFOX4 + bevacizumab, and 243 to bevacizumab alone. Addition of bevacizumab to chemotherapy significantly increased median overall survival from 10.8 to 12.9 months (p = 0.0011), median progression-free survival from 4.7 to 7.3 month (p < 0.0001), and overall response rate from 8.6% to 22.7%. The bevacizumab mono arm was inferior to the chemotherapy alone arm (OS 10.2 months, PFS 2.7 months, and overall response rate 3.3%) (Giantonio 2007).

In another more recent second-line trial, the dose of bevacizumab (5 or 10 mg/kg) does not seem to have a major effect. In a randomized phase III trial (Iwamoto et al. 2015), patients were treated with FOLFIRI +5 or 10 mg/kg bevacizumab after progression during an oxaliplatin-based chemotherapy as differences in progression-free survival or overall survival were observed (Table 1).

Maintenance Therapy
A potential role for maintenance therapy became evident for the first time, when the results of the NO16966 study demonstrated an improved PFS in the subgroup of patients receiving chemotherapy only arm until disease progression. The Concept Trial in part fits into the concept of maintenance therapy as it compared intermittent versus continuous treatment. Specifically, patients either received FOLFOX7 and bevacizumab 5 mg/kg every 2 weeks (CO-arm) continuously or intermittent oxaliplatin (IO). This IO-arm consisted of 8 cycles of FOLFOX4/
bevacizumab followed by 8 cycles without oxaliplatin (i.e., 5-FU and bevacizumab) and so on. The study had to be terminated prematurely. The time to failure of treatment strategy (TTF) was longer in the IO arm: TTF reached a median of 25 weeks in the IO-arm compared to 18 weeks in CO-arm, HR 0.58; \( p = 0.0025 \) (Hochster et al. 2014).

Later, the MACRO trial (Díaz-Rubio et al. 2012) examined a bevacizumab-based maintenance strategy applying a noninferiority design. Following induction chemotherapy of six cycles of XELOX/bevacizumab in the first-line treatment of mCRC, XELOX/bevacizumab was compared with bevacizumab monotherapy given until disease progression. The primary endpoint was PFS, and secondary endpoints were OS, objective RR, time to response, duration of response, and safety. A total of \( n = 480 \) patients were included. After a median follow-up of 29 months, no significant differences in PFS and OS were found between the arms. The median PFS was 10.4 months in the continuous XELOX/bevacizumab arm and 9.7 months in the bevacizumab mono arm (HR 1.10; 95% CI 0.89–1.35, ns). Median survival was 23.2 months in XELOX/bevacizumab arm and 20.0 months in the bevacizumab-mono arm (HR 1.05; \( p = 0.65 \)). Importantly, second-line therapies were well balanced between the two arms (72% and 74% of patients received at least one second-line treatment). Thus, although the noninferiority of bevacizumab versus XELOX plus bevacizumab could not be formally confirmed statistically, a median PFS detriment >3 weeks could be excluded, pointing to a possible role of bevacizumab monotherapy as a maintenance concept. In this respect two further trials have recently broadened the data base.

In the AIO 0207 study and the CAIRO-3 study, the concept of maintenance therapy was further evaluated including treatment arms of bevacizumab monotherapy during maintenance (Simkens et al. 2015). The AIO 0207 trial was an open-label, noninferiority, randomized phase III trial. Following 24 weeks of induction therapy (fluorouracil/leucovorin, oxaliplatin plus bevacizumab or capecitabine, oxaliplatin plus bevacizumab), patients without disease progression during induction therapy were randomly assigned to the following treatment arms: fluoropyrimidine plus bevacizumab, bevacizumab alone, or no treatment at all. At first progression, re-induction with all drugs of the induction treatment was a planned part of the protocol. The primary endpoint was “time to failure of strategy,” defined as time from randomization to second progression after maintenance (and if applicable re-induction), death, or initiation of further treatment including a new drug. Consequentially, for patients who did not receive re-induction, time to failure of strategy was equivalent to time to first progression. A total of 472 patients were randomized. Median time to failure of strategy was 6.9 months for the fluoropyrimidine/bevacizumab arm, 6.1 months for the bevacizumab-mono arm, and 6.4 months for the no treatment arm. Bevacizumab monotherapy was noninferior to standard fluoropyrimidine/bevacizumab (HR 1.08; 95% CI [0.85–1.37]; \( p = 0.53 \)), whereas no treatment was not (HR 1.26 [0.99–1.60]; \( p = 0.056 \)). OS was similar in both arms in this trial (Hegewisch-Becker et al. 2015). The results of this study support the concept of maintenance therapy. Accordingly, discontinuation of oxaliplatin following induction phase and maintenance with a fluoropyrimidine and bevacizumab has evolved as a practical approach in the clinic to prevent oxaliplatin related neurotoxicity.

In the phase III CAIRO-3 study, induction treatment consisted of six 3-weekly cycles of capecitabine, oxaliplatin, and bevacizumab. Patients without disease progression were then randomized to either maintenance treatment with capecitabine/bevacizumab or observation. On first progression (PFS1), patients in both groups were to receive the induction regimen again until second progression (PFS2), which was the study’s primary endpoint. Median PFS2 was significantly improved in the maintenance group (11.7 months vs. 8.5 months in the observation group, HR 0.67; \( p < 0.0001 \)). Maintenance therapy was well tolerated; however, the rate of hand foot skin reactions was increased (23% all grades). Maintenance treatment resulted in a nonsignificant absolute increase in median overall survival.
of 3.5 months (from 18.1 to 21.6 months). This study again underlined the role of maintenance therapy with a fluoropyrimidine and bevacizumab in the first-line treatment of mCRC patients.

Finally, the DREAM trial (Tournigand et al. 2015) conducted by the French GERCOR working group study evaluated the combination of erlotinib with bevacizumab as a maintenance therapy. In this randomized phase III study, patients received induction chemotherapy consisting of a chemotherapy doublet plus bevacizumab. If at least stable disease was reached, they were randomized between bevacizumab monotherapy (7.5 mg/kg every 3 weeks) or a combination of bevacizumab and erlotinib (150 mg/day). The primary endpoint was PFS during maintenance therapy. Of 694 patients who started induction therapy, 446 patients were randomized. Maintenance PFS was improved from 4.57 to 5.75 months (HR 0.72; \(p = 0.005\)). Even the PFS from registration was significantly improved (10.2 vs. 9.23 months, HR 0.73; \(p = 0.0045\)). As expected, diarrhea (all grades 58% versus 12% and skin rash 85% versus 8%) was the main side effect attributable to the addition of erlotinib. This concept, however, has not gained a role in clinical practice due to increased toxicities and the fact that erlotinib is not approved for the treatment of mCRC (Table 1).

**Aflibercept**

Aflibercept is a soluble decoy receptor fusion protein consisting of the second IgG-like domain of VEGFR1, the third IgG-like domain of VEGFR2, and the human IgG1 constant region. Due to this specificity, aflibercept is able to bind VEGF-A, VEGF-B, and PIGF. PIGF has been shown to be increasingly expressed during anti-angiogenic treatment (Cao 2009; Van de Veire et al. 2010; Yao et al. 2011). Antibodies against PIGF are able to block such an angiogenic rescue program (Giampieri et al. 2016). As the decoy receptor aflibercept binds VEGF-A, VEGF-B, and PIGF, second-line treatment following a prior bevacizumab challenge appeared a promising strategy, thus defining the clinical setting in which this new drug could be preferentially evaluated. First early trials exploring aflibercept monotherapy as well as aflibercept, in combination with chemotherapy, confirmed the safety of this drug (Gaya and Tse 2012; Van Cutsem et al. 2013). In a subsequent phase II trial, 75 patients with refractory metastatic colorectal cancer were treated with single agent aflibercept at a dose of 4 mg/kg (Tang et al. 2012). In the bevacizumab-naïve control group, the tumor control rate after 16 weeks was 20%, whereas in the bevacizumab-
experienced group, the tumor control rate was only 11.8%, hence indicating limited activity of single agent aflibercept. At the same time, a randomized phase III trial was launched to investigate aflibercept in combination with irinotecan-based chemotherapy after failure of oxaliplatin-based chemotherapy (Van Cutsem et al. 2012). In this VELOUR trial, 1226 patients were randomized to FOLFIRI +/- aflibercept. Addition of aflibercept lead to an increase in median overall survival from 4.7 to 6.9 months (HR 0.758; p < 0.0001), median overall survival from 12.6 to 13.5 months (HR 0.817; p = 0.0032) and response rate of 11.1–19.8% (p < 0.0001) (Van Cutsem et al. 2012). Interestingly, the improvement in survival was independent of pretreatment with bevacizumab (Tabernero et al. 2014). Aflibercept treatment was not only associated with side effects typically attributed to anti-angiogenic drugs such as hypertension, hemorrhage, or thromboembolism but also side effects typically attributed to chemotherapy such as diarrhea and neutropenia. In contrast, addition of aflibercept to an oxaliplatin-based chemotherapy in first-line colorectal cancer did not improve patient outcome (Folprecht et al. 2016). In summary, aflibercept is a new anti-angiogenic drug available for the treatment of recurrent metastatic colorectal cancer that recently broadened the therapeutic armamentarium of this disease (Table 1).

Ramucirumab
In contrast to bevacizumab and aflibercept, ramucirumab is an antibody that specifically binds to VEGF receptor 2. Ramucirumab prevents binding of VEGF to its natural receptor expressed on target cells, thereby inhibiting the activation of the downstream signaling cascades. In preclinical models, ramucirumab demonstrated antitumor activity (Fontanella et al. 2014). In a phase I trial exploring ramucirumab in patients with solid tumors, a reduction of vascularity and perfusion was observed (Spratlin et al. 2010). To further evaluate the therapeutic potential of ramucirumab in colorectal cancer, a phase III randomized trial was launched in patients with recurrent metastatic disease. In this trial, ramucirumab (8 mg/kg) was added to a FOLFIRI-based chemotherapy. A total of 1072 patients were enrolled. Addition of ramucirumab increased median overall survival from 11.7 months to 13.3 months (HR0.84; p = 0.0219). Progression-free survival increased from 4.5 months to 5.7 months (HR0.79; p = 0.0005). The toxicity profile was typical of anti-angiogenic drugs with hypertension being a leading adverse event. The results of this trial lead to the recent approval of ramucirumab for second line treatment of colorectal cancer (Tabernero et al. 2015).

To date no direct comparison between the different anti-angiogenic drugs exist. In first-line treatment, only bevacizumab has proven beneficial, whereas in second-line treatment three different anti-angiogenic drugs are available. Of these, bevacizumab has also been shown to be beneficial when added to an oxaliplatin-based chemotherapy while for ramucirumab and aflibercept improved outcome has only been demonstrated so far for a combination with irinotecan-based chemotherapy (Table 1).

Approved Tyrosine Kinase Inhibitor Regorafenib
Regorafenib is a multitryosine kinase inhibitor that potently targets several kinases involved in tumor angiogenesis. As many patients with metastatic colorectal cancer remain in relatively good performance status even after two lines of chemotherapy, regorafenib was evaluated in third-line treatment. In the CORRECT trial, 760 patients were randomly assigned to regorafenib or best supportive care. The primary endpoint was overall survival. Regorafenib increased the overall survival from 5 to 6.4 months (HR = 0.77; p = 0.0052). However, a significant number of patients experienced clinically meaningful side effects, mainly mucositis (Grothey et al. 2013). Nevertheless, on the basis of the available data, regorafenib was approved for the treatment of recurrent metastatic colorectal cancer.

The data were later validated in an Asian study cohort in the CONCUR trial with very similar results. Median OS was 8.8 months in the
regorafenib arm compared to 6.3 month in the placebo arm (HR0.55; p = 0.00016). Again, considerable toxicity was seen (Table 1) (Li et al. 2015a).

**Anti-angiogenic Drugs in Development in mCRC**

Several new anti-angiogenic drugs were more recently developed clinically in colorectal cancer where we are still awaiting final clinical results (Tampellini et al. 2016).

**Fruquintinib**

Fruquintinib is a tyrosine kinase inhibitor that targets VEGFR1-3 and is administered orally. In early clinical trials in metastatic colorectal cancer, a dose with manageable toxicity profile was established. These trials showed also promising efficacy of this drug with a disease control rate of 83% and a progression-free survival at 16 weeks of 65% (Cao et al. 2016). A randomized phase II trial with fruquintinib was performed in metastatic colorectal cancer refractory to previous treatments. This study met its primary endpoint with an increase in progression-free survival from 1 to 4.7 months (HR 0.3; p < 0.001) and an increase in disease control rate from 20.8% to 68.1% (Li et al. 2015b). We are awaiting results from a randomized phase III trial that is still recruiting.

**Nintedanib**

Nintedanib is a multikinase inhibitor targeting VEGFR 1–3, FGFR 1–3, as well as PDGFA and PDGFRB. In a randomized phase I/II trial, nintedanib was compared with bevacizumab when added to FOLFOX in previously untreated metastasized colorectal cancer. In this study, bevacizumab was slightly superior concerning the primary endpoint of PFS rate at 9 months: 62.1% (95% CI 50.2–73.9) in the nintedanib group and 70.2% in the bevacizumab arm), although the response rate was higher in the nintedanib group (63.5% and 56.1%) (Van Cutsem et al. 2015). Currently, nintedanib is evaluated as a single agent in last line treatment of colorectal cancer in a randomized design with comparison to best supportive care (Van Cutsem et al. 2016c).

**Famitinib**

Famitinib is a multikinase receptor inhibitor targeting VEGFRs 2 and 3, KIT, PDGFRa, and RET. In a phase I trial, good tolerability was observed (Zhou et al. 2013). In a 2:1 randomized phase II trial in refractory metastatic colorectal cancer, an increase in median progression-free survival from 1.5 to 2.8 months (HR 0.58 p = 0.0034) and disease control rate from 30.9% to 57.5% (p = 0.0023) was observed. The safety profile was favorable (Xu et al. 2015).

**Brivanib**

Brivanib is an orally available tyrosine kinase inhibitor which blocks VEGFR-2 and FGFR-1/FGFR-2 signaling. After promising results in early clinical trials in colorectal cancer, brivanib was investigated in a randomized phase III trial in KRAS wild-type recurrent mCRC after prior treatment in combination with cetuximab (Siu et al. 2013). This trial did not meet its primary endpoint overall survival although progression-free survival was prolonged by adding brivanib. Importantly, because a higher rate of gastrointestinal and dermal toxicity as well as increased hypertension rates was observed, this concept was no longer pursued.

**Cediranib**

Cediranib is a tyrosine kinase inhibitor with high affinity to all VEGF receptors and additionally some activity to PDGF and KIT. In early trials, cediranib was combined with FOLFOX and demonstrated manageable toxicity profiles (Satoh et al. 2012). Subsequently, several randomized clinical trials were launched evaluating the addition of cediranib to oxaliplatin-based chemotherapy in first-line metastatic colorectal cancer. In the HORIZON I trial, different doses of cediranib added to FOLFOX were tested in a phase II design. No difference in progression-free survival was observed (Cunningham et al. 2013b). Further evaluation in a phase III trial recruiting 502 patients receiving FOLFOX/CAPOX with or without cediranib resulted in a minimal
increase of progression-free survival of 0.3 months but no improvement in overall survival or response rate (Hoff et al. 2012). The HORIZON III trial was designed as a double-blind randomized phase III trial. FOLFOX + bevacizumab was compared with FOLFOX + cediranib in 1422 patients. No major difference was seen in the efficacy of these two treatments, but cediranib lead more often to delays of treatment (Schmoll et al. 2012). As of today, no further trials are planned to develop cediranib in colorectal cancer.

**Lenvatinib**

Lenvatinib is a multityrosine kinase inhibitor targeting VEGFRs 1–3, FGFRs 1–4, PDGFRA, KIT, and RET. The drug has been successfully applied in radio-iodine refractory metastasized thyroid cancer. No trial has specifically evaluated lenvatinib in metastasized colorectal cancer so far.

**Linifanib**

Linifanib is a tyrosine kinase inhibitor targeting VEGFRs 1–3 and PDGFRB. Linifanib showed single agent activity in a variety of solid tumors. Therefore, a randomized trial was performed in colorectal cancer, evaluating two doses of linifanib added to standard FOLFOX and compared to FOLFOX + bevacizumab. This trial did not meet its primary endpoint (PFS) nor did it demonstrate relevant differences in response rate or overall survival between treatment arms. Slightly increased toxicity was observed in the linifanib arm, leading to a higher rate of treatment discontinuation (O’Neil et al. 2014). Another phase II trial explored the response rate tolinifanib given as single agent in recurrent colorectal cancer. In 23 evaluated patients, the response rate was 0% (NCT01365910).

**Motesanib**

Motesanib is an orally available multikinase inhibitor targeting VEGFRs 1–3 and KIT together with several other kinases. When given at the maximum tolerated dose of 125 mg, some activity has been observed in advanced solid tumors. The toxicity profile of motesanib was tested in a larger phase I trial, in which it was combined either with FOLFOX or FOLFIRI plus panitumumab in a total number of 119 patients. Unfortunately, response rates were relatively low (24% in first line, 14% in second line). A substantial benefit of motesanib for this refractory patient population therefore seems unlikely (Tebbutt et al. 2015).

**Tivozanib**

Tivozanib also targets VEGFRs 1–3, KIT, and PDGFRB and is orally available. Safety of tivozanib in combination with FOLFOX was evaluated in a phase I clinical trial in metastasized colorectal cancer. In this early trial, some clinical activity was assumed as one of 30 patients experienced a complete remission and then a partial response (Oldenhuis et al. 2015). In a subsequent randomized phase II trial, tivozanib was compared with bevacizumab in patients with metastasized colorectal cancer receiving FOLFOX as the chemotherapy backbone. No significant difference in response rate or progression-free survival was observed (Benson et al. 2016). The safety profile of this agent appeared very similar to the other multikinase inhibitors described above.

**Trebananib**

Trebananib is an angiopoietin-1 and angiopoietin-2 neutralizing peptide. Angiopoietins play an important role in pathological vascular remodeling, thus making trebananib a promising agent for treatment of cancer. After defining a maximum tolerated dose in a phase I trial in refractory solid tumors (Peeters et al. 2013). Trebananib was evaluated in a randomized phase II trial in combination with FOLFIRI in patients with recurrent colorectal cancer after oxaliplatin-based chemotherapy. This trial did not meet its primary endpoint with no significant difference in progression-free survival (3.5 vs. 5.2 months, \( p = 0.33 \)). No greater grade III/IV toxicities were observed (Tampellini et al. 2016).

**Vandetanib**

Vandetanib is a multikinase inhibitor with primary affinity to EGFR and VEGFR2 and additionally to RET, EPH receptor, and SRC family members. After vandetanib had demonstrated preclinical activity in several tumor models including
colorectal cancer, its toxicity profile in combination with chemotherapy was evaluated specifically in metastasized colorectal cancer. Toxicity of vandetanib in combination with FOLFOX and FOLFIRI was manageable, but efficacy of this combination in randomized phase II trials was not promising (Tampellini et al. 2016). The combination of vandetanib with cetuximab and irinotecan was feasible but demonstrated no favorable efficacy (Meyerhardt et al. 2012). In contrast, combination of vandetanib with bevacizumab and capecitabine with oxaliplatin demonstrated an unfavorable toxicity profile (Cabebe et al. 2012).

**Vatalanib**
Vatalanib targets VEGFRs as an orally available tyrosine kinase inhibitor. Combination with FOLFOX demonstrated a favorable toxicity profile, but efficacy was low in randomized phase II trials in refractory colorectal cancer. Therefore, this drug is not further developed in colorectal cancer (Hecht et al. 2011; Sobrero and Bruzzi 2011; Van Cutsem et al. 2011).

**Additional New Anti-angiogenic Agents in Early Clinical Development**
Additional new agents are in early clinical development and still await clinical evaluation in colorectal cancer. For example, cabozantinib as a multikinase inhibitor is approved for medullary thyroid cancer. A phase I trial is performed to evaluate the tolerability of cabozantinib in combination with panitumumab in colorectal cancer (NCT02008383). Sevacizumab is a new antibody against VEGF-A and currently evaluated in a phase I trial. This drug will be further explored specifically in metastatic colorectal cancer (NCT02453464). A new antibody against VEGF-C (VGX-100) is currently evaluated in phase I trials in combination with bevacizumab. Apatinib is another multikinase inhibitor which has shown clinical activity mainly in gastric cancer. It is now evaluated in metastatic colorectal cancer in an open label randomized phase II trial (NCT01531777). Vanucizumab is a bispecific antibody against angiopoietin-2 and VEGF-A. In an early phase I trial, a safe dose was established with mostly hypertension, headache, and asthenia occurring as side effects. Currently a phase II trial is performed in metastatic colorectal cancer comparing bevacizumab with vanucizumab when added to FOLFOX (NCT02141295).

**Summary**
Altogether these trials indicate that there is a benefit for the addition of anti-angiogenic drugs to standard chemotherapy in mCRC in first-line, maintenance, second-line treatment and for a treatment beyond progression. The beneficial effect is modest in an unselected population but the low toxicity profile warrants this anti-angiogenic treatment. Due to the successful introduction of anti-angiogenic drugs in the treatment of colorectal cancer, many companies have tried to establish new agents in this disease. However, the majority of these drugs have failed to show sufficient efficacy while exerting relevant toxicity or are still in clinical development.

Today the differential role of the different anti-angiogenic drugs in mCRC remains to be established. In addition, there is still a major need for the identification of subgroups particularly sensitive to antiangiogenetic targeting.

**Cross-References**
- Angiogenics in Gastroesophageal Cancer Therapy
- Anti-Angiogenesis and Cytotoxics
- Anti-Angiogenesis in Head and Neck Cancer Therapy
- Anti-Angiogenic Targets: VEGF and VEGF-Receptors
- Anti-Angiogenics in Pancreatic Cancer Therapy
- Inhibition of Tumor Angiogenesis in GIST Therapy
References


Price TJ et al (2012) Bevacizumab is equally effective and no more toxic in elderly patients with advanced colorectal cancer: a subgroup analysis from the AGITG MAX trial: an international randomised controlled trial of Cetirizabine, Bevacizumab and Mitomycin C. Ann Oncol 23:1531–1536


Van Cutsem E, Joulain F, Hoff PM et al (2016b) Aflibercept plus FOLFIRI vs. placebo plus FOLFIRI in second-line metastatic colorectal cancer: a post hoc analysis of survival from the phase III VELOUR study subsequent to exclusion of patients who had recurrence during or within 6 months of completing adjuvant Oxaliplatin-based therapy. Target Oncol 11:383–400. doi:10.1007/s11523-015-0402-9