Targeted therapy in advanced metastatic colorectal cancer: Current concepts and perspectives

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Targeted therapy in advanced metastatic colorectal cancer: Current concepts and perspectives

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Abstract

The introduction of new cytotoxic substances as well as agents that target vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) signaling has improved clinical outcome of patients with metastatic colorectal cancer (mCRC). In this review we summarize the most relevant clinical data on VEGF and EGFR targeting regimens in mCRC. The effects of available treatment strategies for mCRC are often temporary, with resistance and disease progression developing in most patients. Thus, new treatment strategies are urgently needed. Some GI peptides including gastrin and gastrin releasing peptide, certain growth factors such as insulin-like growth factor-I and II and neuropeptides such as growth hormone releasing hormone (GHRH) are implicated in the growth of CRC. Experimental investigations in CRC with antagonistic analogs of bombesin/gastrin-releasing peptide, GHRH, and with cytotoxic peptides that can be targeted to peptide receptors on tumors, are summarized in the second part of the review.

Key words: Colorectal cancer; Targeted treatment; Vascular endothelial growth factor; Epidermal growth factor receptor; Peptide receptors; Gastrin-releasing peptide; Growth hormone releasing hormone; Lueteinizing hormone-releasing hormone; Cytotoxic analogs

Core tip: Our review evaluates the most recent clinical data on therapeutic reagents designed to target the vascular endothelial growth factor and epidermal growth factor receptor signaling pathways in colorectal cancer. As colorectal cancers express receptors for bombesin/gastrin-releasing peptide, growth hormone-releasing hormone, somatostatin as well as luteinizing hormone-releasing hormone, we review the implications of these pathways in the growth of colorectal cancers and summarize experimental data and clinical studies performed to date with regard to the antiproliferative action of antagonistic peptide analogs of these receptors as well as their cytotoxic analogs and their status as drug candidates for the treatment of metastatic colorectal cancer.
INTRODUCTION

Worldwide estimates of new cases of colorectal cancer (CRC) exceed 1.2 million, with more than 600000 deaths per year[1]. It is estimated that 20% of patients with CRC have metastatic disease at the time of diagnosis; 20%-25% of patients will experience metastases during the course of the disease thus resulting in a relatively high overall mortality rate of 40%-45%[2]. Beside standard chemotherapy (CTX) with 5-fluorouracil (5-FU) based regimens, the incorporation of monoclonal antibodies (mAbs) targeting vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) signaling pathways have further broadened the treatment options for metastatic colorectal cancer (mCRC) patients. Although the survival for all patients with mCRC has improved significantly, the 5-year survival rates still remain low at about 10%, with a median overall survival (OS) of 24 mo. Thus, new approaches to the treatment of mCRC are required. Antagonistic analogs of bombesin/gastrin releasing-hormone (BN/GRP) and growth hormone-releasing hormone (GHRH) as well as targeted cytotoxic analogs of luteinizing hormone-releasing hormone (LHRH) and somatostatin (SST), linked to chemotherapeutic substances, which have been developed in our laboratories over the last two decades, have been shown to be highly effective in suppressing the proliferation of experimental human CRC in vitro and in vitro and represent an entirely new class of antineoplastic agents for the treatment of mCRC. In the first part of the present review the most recent data on currently available biological agents that target the VEGF (bevacizumab, aflibercept and regorafenib) and EGFR pathways ( cetuximab and panitumumab) are highlighted. In the second part, we summarize experimental studies performed so far regarding the antiproliferative action of antagonists of BN/GRP and GHRH as well as targeted cytotoxic analogs of Somatostatin and LHRH against CRC in vitro and in vivo.

VEGF TARGETING MABS

Bevacizumab

Bevacizumab (Bev), developed in the early 1990s, is a recombinant, humanized IgG1 mAb effective against all isoforms of VEGF-A that disrupts their interactions with VEGFRs[3]. Preclinical studies have demonstrated that Bev exhibits a broad range of antitumor activity[4]. The most relevant clinical studies with Bev in combination with CTX are summarized in Table 1. In the pivotal clinical trial of Hurwitz et al[5], designated AVF2107, patients with untreated mCRC were given a combination of irinotecan, bolus 5-FU, leucovorin (LV) (IFL), and placebo or a combination of IFL and Bev (5 mg/kg biweekly). The study showed a significant benefit in overall response (45% vs 35%, P = 0.004), progression free survival (PFS) (10.6 mo vs 6.2 mo, P < 0.001), and overall survival (OS) (20.3 mo vs 15.5 mo, P = 0.001) for mCRC patients treated with Bev. In another phase 3 clinical trial, designated BICC-C, performed by Fuchs et al[6], patients with mCRC were randomly assigned to receive one of three different irinotecan-containing regimens (irinotecan plus infusional 5-FU and LV (FOLFIRI), irinotecan plus bolus 5-FU/LV (mIFL) and irinotecan plus oral capecitabine (CapeIRI) (designated as period 1). After a protocol amendment, an additional 117 patients were randomly assigned to FOLFIRI plus Bev or mIFL + Bev, whereas, due to toxicity concerns, further enrollment of CapeIRI was discontinued (designated as period 2). The results for both periods 1 and 2 demonstrated that FOLFIRI and FOLFIRI+Bev offered superior activity to their therapeutic alternatives. Furthermore, patients who received FOLFIRI+Bev showed a higher overall response rate (47% vs 54.4%), a longer PFS (11.2 mo vs 7.6 mo) and median OS (28 mo vs 23.1 mo) compared to FOLFIRI alone. The fact that infusional 5-FU showed a significant longer PFS compared to the oral 5-FU prodrug, capecitabine (7.6 vs 5.8, P = 0.0015), led to the recommendation to preferentially use infused 5-FU, instead of its oral prodrug, in combination with irinotecan. However, a subsequently performed phase II trial which assessed the efficacy and safety of Bev plus oral capecitabine and irinotecan or FOLFIRI as first line therapy for patients with mCRC found no difference between the oral and the infused 5-FU regimen regarding the PFS and OS (9 mo and 23 mo)[7]. The convincing results obtained by phase 3 combination studies with irinotecan and Bev led study designers to consider whether Bev could enhance the effect of any CTX regimen. However, subsequent trials with oxaliplatin-based regimens produced less robust differences[8,9]. In the Phase-III trial, NO16966, by Saltz et al[10], the effect of capecitabine and oxaliplatin (XELOX) compared with those of infused 5-FU, LV and oxaliplatin (FOLFOX), with or without Bev, was evaluated in previously untreated patients with mCRC. Although the difference in PFS and OS (both 1.4 mo) was statistically significant for treatment with Bev and Oxalplatin based combinations compared to CTX alone, the additional benefit in PFS and OS was smaller for the oxaliplatin based regimen than that achieved in the study of Hurwitz et al[5] (4.4 mo and 4.8 mo, respectively). Another Phase-III trial performed by Hochster et al[11], the TREE study, investigated the tolerability of oxaliplatin in combination with 3 different 5-FU regimens (continuous infusion, bolus and oral) with (TREE-2 cohort) or without (TREE-1 cohort) Bev as a first-line therapy for mCRC. The study showed a benefit in overall response (52% vs 41%), PFS (9.9 mo vs 8.7 mo) and OS (24.6 mo vs 19.2 mo) in patients treated with FOLFOX6 + Bev compared to CTX.
alone. The addition of Bev to second-line CTX with FOLFOX4, after progression on a CTX regimen without Bev, was evaluated in the ECOG E3200 Phase III trial[11]. The addition of Bev to FOLFOX4 improved response rates, PFS and OS in patients whose tumors had already progressed on irinotecan-containing CTX. These findings led to the approval of Bev in combination with CTX as second-line therapy for mCRC. The first randomized Phase III trial which investigated the efficacy of Bev therapy continuation beyond progression was the ML18147 (TML) study performed by Bennouna et al[12]. In this trial, patients with mCRC who progressed after a Bev containing first-line CTX were randomly assigned to Bev + CTX and CTX alone. Continued use of Bev in combination with a standard 2nd line CTX showed a modest but significant benefit in PFS (5.7 mo vs 4.1 mo, P = 0.0001) and OS (11.2 mo vs 9.8 mo) compared to CTX alone.

**Aflibercept**

Aflibercept is a recently developed, multiple angiogenic factor trap that prevents not only VEGF-A, but also two additional members of the VEGF family, VEGF-B and placental growth factor (PIGF), from activating their native receptors (VEGFR-1)[13,14]. These findings suggest that upregulation of PIGF and VEGF-B with concurrent activation of VEGFR-1 could be a potential mechanism of tumor resistance to therapies such as Bev, which targets VEGF-A only[15,16]. The VELOUR trial evaluated aflibercept plus FOLFIRI vs FOLFIRI alone in patients with mCRC after progression on an oxaliplatin based CTX trial[19]. Addition of Bev significantly improved PFS (6.9 mo vs 4.7 mo, P = 0.0007) and OS (13.5 mo vs 12.06 mo) compared to CTX alone.

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### Table 1  Effect of bevacizumab in phase III Studies in patients with metastatic colorectal cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Phase</th>
<th>Regimen</th>
<th>Patients (n)</th>
<th>Overall response</th>
<th>Median PFS (mo)</th>
<th>Median OS (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hurwit et al[20], AVF2107 trial, 2004</td>
<td>First-line</td>
<td>3</td>
<td>IFL + Bev vs Placebo</td>
<td>402</td>
<td>45%</td>
<td>10.6</td>
<td>20.3</td>
</tr>
<tr>
<td>Fuchs et al[21], BICC-C trial, 2007</td>
<td>First-line</td>
<td>3</td>
<td>FOLFIRI (period 1) vs FOLFIRI + Bev (period 2)</td>
<td>144 vs 57</td>
<td>47% vs 54.4%</td>
<td>7.6 vs 11.2</td>
<td>23.1 vs 28.0</td>
</tr>
<tr>
<td>Saltz et al[22], N016966 trial, 2008</td>
<td>First-line</td>
<td>3</td>
<td>FOLFOX-4 or XELOX + Placebo vs FOLFOX-4 or XELOX + Bev</td>
<td>701 vs 699</td>
<td>38% vs 38%</td>
<td>8.0 vs 9.4</td>
<td>19.9 vs 21.3</td>
</tr>
<tr>
<td>Hochster et al[23], TRE152 study, 2008</td>
<td>First-line</td>
<td>3</td>
<td>mFOLFOX-6 vs XELOX vs mFOLFOX-6 + Bev vs XELOX + Bev</td>
<td>69 vs 71 vs 72 vs 72</td>
<td>41% vs 52% vs 36% vs 36%</td>
<td>8.7 vs 9.9 vs 10.3 vs 10.3</td>
<td>19.2 vs 26.1 vs 24.6 vs 24.6</td>
</tr>
<tr>
<td>Giannontio et al[24], ECOG E3200, 2007</td>
<td>Second-line</td>
<td>3</td>
<td>FOLFIRI + Bev vs Placebo</td>
<td>290 vs 289</td>
<td>22.7% vs 8.6%</td>
<td>7.3 vs 4.7</td>
<td>12.9 vs 10.8</td>
</tr>
<tr>
<td>Bev beyond progression</td>
<td>Second-line</td>
<td>3</td>
<td>Continued use of Bev + standard 2nd-line CTX vs 2nd-line CTX alone</td>
<td>409 vs 411</td>
<td>5.4% vs 3.9%</td>
<td>5.7 vs 4.1</td>
<td>11.2 vs 9.8</td>
</tr>
</tbody>
</table>

| regeneration (mo) | compared to CTX alone. The VELOUR trial evaluated aflibercept plus FOLFIRI vs FOLFIRI alone in patients with mCRC after progression on an oxaliplatin based CTX trial[19]. Addition of Bev significantly improved PFS (6.9 mo vs 4.7 mo, P = 0.0007) and OS (13.5 mo vs 12.06 mo) compared to CTX alone.

**Regorafenib**

Regorafenib is an inhibitor of PDGF receptors, c-KIT, FGFR receptor and VEGF1-3[20]. In the pivotal Phase III study, CORRECT, patients with mCRC who had progressed after all approved drugs were randomly assigned to Regorafenib or placebo[21]. Treatment with Regorafenib significantly prolonged OS (6.4 mo vs 5.0 mo, P = 0.0052) and PFS (1.9 mo vs 1.7 mo, HR = 0.49) compared to placebo.

**EGFR TARGETING MABS**

**Cetuximab**

Cetuximab is a recombinant, chimeric, human/murine immunoglobulin (Ig)G1 mAb that binds specifically to the extracellular domain of EGFR in normal and tumor cells, promoting receptor internalization and degradation without receptor phosphorylation and activation[21]. The most relevant clinical studies with cetuximab in combination with CTX are summarized in Table 2. In the pivotal Phase II study, BOND, Cunningham et al[22] randomly assigned patients with mCRC, who where refractory to irinotecan based CTX, to either irinotecan and cetuximab or cetuximab alone. The combination of irinotecan with cetuximab significantly improved overall response (22.9% vs 10.8%), median PFS (4.1 mo vs 1.5 mo) and OS (8.6 mo vs 1.5 mo) compared to cetuximab alone. These findings led to the approval of cetuximab for patients with irinotecan refractory CRC, in the United States and Europe, as well as patients who were refractory to other previous therapies. Several small, retrospective studies have shown an association between KRAS mutation status and responsiveness of a colorectal tumor to cetuximab[23-26].
Table 2 Effect of cetuximab in phase II/III studies in patients with metastatic colorectal cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Phase</th>
<th>Regimen</th>
<th>Patients (n)</th>
<th>Overall response</th>
<th>Median PFS (mo)</th>
<th>Median OS (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cunningham et al[22],</td>
<td>Refractory to</td>
<td>2</td>
<td>Irinotecan + cetuximab</td>
<td>218</td>
<td>22.90%</td>
<td>4.1</td>
<td>8.6</td>
</tr>
<tr>
<td>BOND study, 2004</td>
<td>irinotecan</td>
<td></td>
<td>vs cetuximab alone</td>
<td>211</td>
<td>10.80%</td>
<td>P = 0.0074</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 0.0001</td>
<td>7.6</td>
<td>17.5</td>
</tr>
<tr>
<td>Van Cutsem et al[24],</td>
<td>First-line</td>
<td>3</td>
<td>FOLFIRI + cetuximab</td>
<td>105</td>
<td>36.20%</td>
<td>7.6</td>
<td>15.7</td>
</tr>
<tr>
<td>CRYSTAL trial, 2009</td>
<td>placebo</td>
<td></td>
<td>(K-Ras mutant)</td>
<td>87</td>
<td>40.20%</td>
<td>HR = 0.80</td>
<td>8.1</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>HR = 1.17</td>
<td>17.1</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>P = 0.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FOLFIRI + cetuximab</td>
<td>172</td>
<td>59.30%</td>
<td>9.9</td>
<td>24.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>placebo</td>
<td></td>
<td>(K-Ras wild-type)</td>
<td>176</td>
<td>46.20%</td>
<td>8.7</td>
<td>21.0</td>
</tr>
<tr>
<td>Bokemeyer et al[28,30],</td>
<td>First-line</td>
<td>2</td>
<td>FOLFOX + cetuximab</td>
<td>52</td>
<td>33%</td>
<td>8.6</td>
<td>NR</td>
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<tr>
<td>OPUS trial, 2008</td>
<td>placebo</td>
<td></td>
<td>(K-Ras mutant)</td>
<td>47</td>
<td>49%</td>
<td>HR = 0.80</td>
<td>5.5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.106</td>
<td>HR = 1.17</td>
<td>P = 0.0192</td>
</tr>
<tr>
<td></td>
<td>FOLFOX + cetuximab</td>
<td>61</td>
<td>61%</td>
<td>7.7</td>
<td>NR</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>placebo</td>
<td></td>
<td>(K-Ras wild-type)</td>
<td>73</td>
<td>37%</td>
<td>7.2</td>
<td>NR</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>OR = 2.54</td>
<td>HR = 0.57</td>
<td>P = 0.016</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.011</td>
<td>HR = 0.84</td>
<td>P = 0.016</td>
</tr>
<tr>
<td>Heinemann et al[39],</td>
<td>First line</td>
<td>3</td>
<td>FOLFIRI + cetuximab</td>
<td>297</td>
<td>62%</td>
<td>10.3</td>
<td>28.7</td>
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<tr>
<td>FIRE-3, 2013</td>
<td>FOLFIRI + bevacizumab</td>
<td></td>
<td></td>
<td>295</td>
<td>57%</td>
<td>10.4</td>
<td>25.0</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR = 1.249</td>
<td>HR = 1.04</td>
<td>HR = 0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.18</td>
<td>P = 0.69</td>
<td>P = 0.017</td>
</tr>
</tbody>
</table>

PFS: Progression free survival; OS: Overall survival; NR: Not reported.

In the study of Karapetis et al[37] patients with mCRC refractory to standard treatment were randomly assigned to receive Cetuximab plus best supportive care (BSC) or BSC alone, to detect activating mutations in exon 2 of the KRAS gene. Patients with tumors expressing mutant KRAS did not respond to cetuximab (overall response rate 1.2%), whereas patients with tumors harboring a wild-type KRAS did benefit from cetuximab compared to BSC alone in terms of overall response rate (12.8% vs 0%), PFS (3.7 mo vs 1.9 mo, HR = 0.4, P < 0.001) and OS (9.5 mo vs 4.8 mo, HR = 0.55, P < 0.001). In the patient cohort receiving BSC alone, the mutation status of the KRAS gene was not significantly associated with OS (HR = 1.01)[27]. Retrospective analysis of the KRAS status in the CRYSTAL trial has recently shown statistically significant differences between patients with wild-type KRAS and those with mutant KRAS in response to FOLFIRI plus cetuximab in terms of PFS (9.9 mo vs 7.6 mo) and overall response (59% vs 36%)[28]. In KRAS wild-type patients, treatment of mCRC patients with FOLFIRI plus cetuximab vs FOLFIRI alone significantly prolonged OS (24.9 mo vs 21.0 mo, HR = 0.84)[28]. Data from the OPUS trial showed that the combination of cetuximab and FOLFOX4 has an overall response rate of 61% in patients with wild-type KRAS compared with 33% in those with mutant KRAS[29,30]. In the Phase III study FIRE-3 by Heinemann et al[31], patients with mCRC were randomly assigned to FOLFIRI plus either Cetuximab or Bev. Patients in the cetuximab and Bev arms had similar times to disease progression (10 mo vs 10.3 mo), but those treated with cetuximab had a significant improved OS (28.7 mo vs 25 mo, HR = 0.77, P = 0.01).

Panitumumab
Panitumumab (Vectibix®) is a fully human, recombinant IgG2 mAb that binds specifically and with high affinity to the extracellular domain of EGFR in normal and tumor cells. Through competitive binding to EGFR ligands, panitumumab prevents EGFR dimerization, autophosphorylation and signaling, thereby inhibiting proliferation and promoting apoptosis[32]. The most relevant clinical studies with panitumumab in combination with CTX are summarized in Table 3. In a phase-3 trial Van Cutsem et al[33] randomly assigned patients refractory to standard treatment, to treatment with panitumumab and BSC vs BSC alone. Objective response rates favored panitumumab over BSC (10% vs 0%). Panitumumab significantly prolonged PFS (8 wk vs 7.3 wk, HR = 0.54) but did not influence OS (HR = 1.00). A Phase III study, PRIME, evaluated the combination of panitumumab with FOLFOX4 vs FOLFOX4 alone as first-line treatment of metastatic CRC[34]. The combination therapy significantly improved PFS compared to CTX alone in patients with KRAS wild type (9.6 mo vs 8.0 mo, P =
and increased overall response rates (55% vs 48%). A non-significant increase in OS was also observed for panitumumab-FOLFOX4 vs CTX alone (23.9 mo vs 19.7 mo, respectively, \( P = 0.072 \)). Peeters et al[35] randomly assigned patients with mCRC pretreated with one CTX, to panitumumab plus FOLFIRI vs FOLFIRI alone. In wild-type KRAS exon 2 mCRC patients a significant improvement in PFS (5.9 mo vs 3.9 mo, \( P = 0.004 \)) and response rates (35% vs 10%) was observed with the addition of panitumumab compared to CTX alone. In patients with mutant KRAS exon 2, there was no difference in efficacy. In order to assess the efficacy and safety of panitumumab plus FOLFOX4 as compared with FOLFOX4 alone according to the KRAS (exon 2-4) and NRAS (exon 2-4) mutation status data of the PRIME study were updated[36]. In patients without any RAS mutation (KRAS2-4/NRAS exon 2-4 wild-type) treatment with panitumumab significantly prolonged PFS (10.1 mo vs 7.9 mo, \( P = 0.004 \)) and OS (26.0 mo vs 20.2 mo, \( P = 0.043 \)) compared to CTX alone. In the trial designated PEAK, Schwartzberg et al[37] randomly assigned untreated patients with mCRC to FOLFOX4 plus either panitumumab or Bev. Again, RAS status was assessed. In RAS wild-type stratum combination of panitumumab with FOLFOX4 improved PFS (13.5 mo vs 9.5 mo, HR = 0.65, \( P = 0.03 \)) and OS (HR = 0.61, \( P = 0.09 \)) compared to Bev with the

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Phase</th>
<th>Regimen</th>
<th>Patients</th>
<th>Overall response</th>
<th>Median PFS (mo)</th>
<th>Median OS (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Cutsem et al[33], 2007</td>
<td>Panitumumab + BSC vs BSC</td>
<td>Refractory to standard CTX</td>
<td>3</td>
<td>231</td>
<td>10%</td>
<td>2007</td>
<td>232</td>
</tr>
<tr>
<td>Douillard et al[34], PRIME-trial, 2010</td>
<td>K-Ras WT FOLFOX4 + panitumumab FOLFOX</td>
<td>First-line</td>
<td>3</td>
<td>325</td>
<td>55%</td>
<td>2010</td>
<td>331</td>
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<tr>
<td></td>
<td>K-Ras MT FOLFOX4 + panitumumab FOLFOX</td>
<td></td>
<td></td>
<td>221</td>
<td>40%</td>
<td></td>
<td>219</td>
</tr>
<tr>
<td>Peeters et al[35], 2010</td>
<td>K-Ras WT FOLFIRI + panitumumab FOLFIRI</td>
<td>Second-line</td>
<td>3</td>
<td>303</td>
<td>35%</td>
<td>2010</td>
<td>294</td>
</tr>
<tr>
<td></td>
<td>K-Ras MT FOLFIRI + panitumumab FOLFIRI</td>
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<td>238</td>
<td>13%</td>
<td></td>
<td>248</td>
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<td>K-Ras WT/MT other Ras FOLFOX4 + panitumumab</td>
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<td>Prime-trial, 2013</td>
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<td>2013</td>
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PFS: Progression free survival; OS: Overall survival; BSC: Best supportive care; CTX: Chemotherapy; WT: Wild-type; MT: Mutant; NR: Not reported.
same combination.

**ANTIPROLIFERATIVE EFFECT OF BN/GRP ANTAGONISTS IN CRC**

In addition to polypeptide growth factors, such as EGF family members, much evidence supports the autocrine involvement of specific neuropeptides, such as gastrin-releasing peptide (GRP), in the proliferation, local invasion, metastasis and angiogenesis of many tumors including CRC\(^ {38-42}\). GRP is a member of the bombesin (BN)-like peptide family and normally functions as a gastrointestinal hormone and neurotransmitter\(^ {43}\). From an oncologic point of view, GRP affects the growth and differentiation of a number of human tumors including CRC\(^ {38-44,46-48}\). Four receptor subtypes associated with the BN-like peptide family have been identified and cloned\(^ {38,67}\). Receptor subtype 1, termed GRP-R, binds BN and GRP with high affinity. Subtype 2 prefers neuromedin B and subtype 3 is classified as an orphan receptor because its natural ligand is not yet identified. A fourth subtype has a higher affinity for amphibian BN than for GRP. These receptors are coupled to G-protein via their intracellular domains and, thus, belong to the G-protein receptor superfamily. Studies have shown that receptors for GRP (GRPs) are overexpressed in human CRC and human CRC cell lines when compared with normal colonic epithelial cells\(^ {48-53}\). Approaches to inhibit the autocrine growth effect of GRP-like peptides on tumor growth in human and animal studies include receptor antagonists, monoclonal antibodies, vaccination against GRP, antisense oligonucleotides or bispecific molecules\(^ {60}\). During the past decade, a large number of BN/GRP antagonists were synthesized in our laboratories. Among these compounds were RC-3095 and RC-3940-II, both of which showed strong inhibitory effects on several experimental cancers including CRC in vitro and in mouse xenografts in vivo\(^ {40,41,54,57,60}\). The tumor-inhibitory mechanism of BN/GRP antagonists appears to be more complex than a simple competitive action on the receptor and is incompletely understood\(^ {40,50}\). In xenografts of HT-29 human CRC inhibition of tumor growth by BN/GRP antagonist, RC-3095, was linked with a significant down-regulation of EGF receptor\(^ {60}\). In another experiment we showed that combined treatment with RC-3940-II and a chemotherapeutic agent, such as 5-FU or irinotecan, resulted in a synergistic growth inhibition of experimental human colon cancers xenographed into nude mice\(^ {53}\). Cell cycle analysis of in vitro material revealed that BN/GRP antagonist, RC-3940-II, led to an increase in the number of cells blocked in S and G\(_2\)/M phase and fewer cells with G\(_0\)/G\(_1\) DNA content\(^ {54}\). A Phase I clinical trial with BN/GRP antagonist, RC-3095, in 25 heavily pretreated patients with advanced solid malignancies, including 2 patients with mCRC, showed no objective tumor response at the dosage used\(^ {61}\). In conclusion, BN/GRP antagonists have shown impressive preclinical antitumor activity and should be further investigated in clinical trials.

**ANTIPROLIFERATIVE EFFECT OF GHRH ANTAGONISTS IN CRC**

Growth hormone-releasing hormone (GHRH) belongs to the family of related peptides that includes: vasoactive intestinal polypeptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP), secretin and glucagon\(^ {45}\). GHRH released by the hypothalamus, regulates the secretion of growth hormone (GH) by binding to specific receptors for GHRH (GHRH-R) in the pituitary gland\(^ {61,64}\). In turn, GH induces the production of hepatic insulin-like growth factor (IGF-I), which is a known mitogen and has been linked with malignant transformation, tumor progression, and tumor metastasis\(^ {60}\). In addition to its neuroendocrine action, GHRH functions as an autocrine/paracrine growth factor in various cancers, including CRC\(^ {40,64}\). Antagonistic analogs of GHRH, developed our laboratories, strongly suppress the growth in vitro and in vivo of many experimental cancers including CRC\(^ {40,41,54,56-61}\). The antitumor effects of GHRH antagonists were initially thought to be exerted only indirectly through the inhibition of serum IGF-I levels. However, evidence suggested, that the principal antiproliferative effects of GHRH antagonists are exerted directly through the blocking of the stimulatory loop formed by GHRH and its receptors on tumor cells. Our group demonstrated the presence of the pituitary type GHRH-receptor and four truncated splice variants (SVs) of GHRH-R in human cancer specimens and cancer cell lines including CRC\(^ {40,60,65}\). Of the four isoforms, SV1 has the greatest structural similarity to the GHRH-R and it probably mediates, in concert with GHRH-R, the effect of GHRH and its antagonists on tumors. We also examined the protein and mRNA expression of GHRH-R and SV1 in normal human tissues and human CRC tissue by immunohistochemical staining and RT-PCR\(^ {70}\). The main finding was that the expression of GHRH-R and SV1 was absent in normal colonic mucosa but significantly increased in tubulovillous adenomas and in colorectal cancers. We assume that this aberrant expression of GHRH-R and SV-1 in colorectal cancers may provide a molecular target for a therapeutic approach based on GHRH antagonists\(^ {70}\). We showed that GHRH antagonist, JMR-132, significantly decreased the volume of HT-29, HCT-116, and HCT-15 experimental human colon carcinomas grown as xenografts in athymic nude mice by up to 75% and also extended tumor doubling times compared to controls\(^ {61}\). In other studies, combined treatment in vivo with JMR-132 plus chemotherapeutic agents 5-FU, irinotecan or cisplatin resulted in an additive tumor growth suppression of HT-29, HCT-116 and HCT-15 human colon cancer xenografts\(^ {68}\). Cell cycle analysis revealed that treatment of HCT-116 human colon cancer cells with GHRH antagonist, JMR-132, in vitro was accompanied by a cell cycle arrest in S-phase. Thus, we suggest that JMR-132 enhances antiproliferative effects of S-phase specific cytotoxic drugs by causing accumulation of tumor cells in S-phase\(^ {68}\). The molecular mechanisms...
involved in the antiproliferative effects of GHRH antagonists on tumor cells have not been completely elucidated. We showed in HCT-116 human colon cancer cells in vitro, that treatment with GHRH antagonist, JM-132, causes significant DNA damage as measured by an increase in olive tail moment and loss of inner mitochondrial membrane potential. Western blotting demonstrated a time-dependent increase in protein levels of phosphorylated p53(Ser46), Bax, cleaved caspase-9, -3, cleavage of PARP and a decrease in Bel-2 levels. Also, an augmentation in cell cycle checkpoint protein p21(Waf1/Cip1) was accompanied by a cell cycle arrest in S-phase. DNA fragmentation visualized by the comet assay and by the number of apoptotic cells increased time dependently as determined by flow cytometric annexin-V and PI staining assays. Thus we suggest that GHRH antagonists exert their antiproliferative effects on experimental colon cancer cells through p21(Waf1/Cip1) mediated S-phase arrest along with apoptosis involving the intrinsic pathway. So far GHRH antagonists have not been clinically tested. However, the impressive preclinical activity merits further investigations in clinical trials.

ANTIPROLIFERATIVE EFFECT OF CYTOTOXIC ANALOGS OF SOMATOSTATIN, BN/GRP AND LHRH IN CRC

On the basis of the presence of specific receptors for hypothalamic peptides on various human cancers including CRC, our group developed targeted cytotoxic analogs of somatostatin (SST) and LHRH linked to doxorubicin or 2-pyrrolinodoxorubicin.

Cytotoxic somatostatin analogs, AN-238 and AN-162

The hypothalamic neuropeptide SST exists in two main active forms: a 14-amino acid peptide and an amino terminally extended version consisting of 28 amino acids. Both forms are present in the gastrointestinal tract inhibiting the secretion of many hormones including growth hormone, insulin, glucagon, gastrin, secretin and cholecystokinin. At least five distinct SST receptor subtypes, SSTR1-5, have been characterized. These receptors are distributed in both normal and cancerous tissues, but found in higher density in the latter as well specifically in human colon cancer cell lines. While native SST shows high affinities to SSTRs, synthetic octapeptides such as RC-160 and RC-121, synthesized in our laboratory, bind preferentially to SSTR2 and SSTR3, moderately affinity to SSTR1 and with low affinity to SSTR5 and SST6. In our endeavour to develop chemotherapy targeted to SSTR, we synthesized two cytotoxic hybrids of SST, AN-238, AN-162, containing DOX or the strongly active derivative of DOX, 2-pyrrolino-DOX, the latter conjugated to the octapeptide SST analog, RC-121. Both cytotoxic analogs AN-238 (containing 2-pyrrolino-DOX) and AN-162 (containing DOX), significantly inhibited tumor growth of experimental human colon cancer xenografted into nude mice. Cell cycle analysis showed that treatment of HCT-116 human colon cancer cells with AN-162 caused a significantly greater increase in the number of S-phase cells and apoptotic cells as compared to treatment with doxorubicin alone. We hypothesize that the lesser effect of unconjugated doxorubicin compared to AN-162 could be the reduction of intracellular drug accumulation caused by increased drug efflux when Dox alone is used. Cellular resistance (multi drug resistance, MDR) to doxorubicin is often related to its rapid efflux from the intracellular environment by membrane transporters termed p-glycoproteins (Pgp), products of the multiple drug resistance gene 1 (MDR-1). To proof this concept, we treated the doxorubicin resistant mouse leukemic cell line P388/R84, which overexpresses the membrane transporter Pgp, with AN-162 and compared to unconjugated doxorubicin. Cell cycle analysis revealed that AN-162 compared to doxorubicin caused a progressive accumulation of P388/R84 cells in S and G2 phase with an increase in the number of apoptotic cells with < G0/G1 content. Thus, treatment efficacy with targeted cytotoxic peptides may be related to overcoming chemoresistance.

Cytotoxic LH/RH analogs

The hypothalamic hormone, LHRH, also known as gonadotropin-releasing hormone is the primary regulator of gonadal function and reproduction in vertebrates. Receptors for LHRH have been demonstrated in healthy sex organs, as well as in breast, ovarian, endometrial and prostate cancers and cell lines of colorectal cancer. On the basis of the presence of receptors for LHRH on these tumors, we have developed a new class of targeted antitumor agents, AN-152 (AEZS-108) and AN-207, by linking cytotoxic radicals to LHRH agonists. Thus Dox was coupled to LHRH to form the targeted cytotoxic analog AN-152 (AEZS-108) and AN-207, by linking cytotoxic radicals to LHRH agonists. AN-152 (AEZS-108) has been successfully tested in one Phase I and two Phase II studies in patients with heavily pretreated LHRH-R positive recurrent ovarian and endometrial cancers. Phase I / II studies with AEZS-108 in castration-resistant prostate cancer and refractory bladder cancer are presently in progress with promising results. In our experimental studies, all 5 human CRC cell lines evaluated expressed LHRH receptors. Currently, there are no clinical data on the expression of LHRH receptors in CRC. However, a common practice in clinical trials with cytotoxic LHRH analog AN-152 on prostate, bladder, ovarian and endometrial cancers is to first evaluate the expression of the LHRH receptor in the tumors of patients by immunohistochemistry. Cytotoxic LHRH analog, AEZS-108, may be a useful agent for the treatment of LHRH receptor positive advanced colorectal cancer.
cancer. On the basis of our results, patients with mCRC could be considered for the inclusion in future clinical trials with cytotoxic LHRH analog AEZS-108, after establishing the presence of LHRH receptors in biopsy samples.

CONCLUSION

The current management of mCRC involves various active drugs, either in combination or as single agents: 5-FU/LV, capcitabine, irinotecan, oxaliplatin, bevacizumab, aflibercept, regorafenib, cetuximab and panitumumab. The choice of therapy is based on consideration of the goals of therapy, the type and timing of prior therapy, the different toxicity profiles of the constituent drugs and the molecular characteristics of the tumor. Treatment regimens with Bev are independent of the RAS mutation status and show greater response rates, up to 10%, and significantly longer PFS and OS in combination with an irinotecan based CTX. Treatment of Bev with oxaliplatin based regimens seems to have a more moderate benefit in PFS and OS. Beyond progression after a Bev containing regimen, continued use of Bev in combination with a standard second-line CTX significantly improves PFS and OS. Bev or aflibercept, when given with second-line CTX, have comparable outcomes, each adding 1.4 mo of survival time. Regorafenib has been approved as a treatment option for patients with good performance status and who have received all available agents leading to a modest OS advantage of 1.4 mo. Recently published data from the FIRE-3, PRIME and the PEAK trial suggest, that cetuximab based regimens may lead to improved OS compared to Bev containing regimens. The observed survival benefit of EGFR targeting agents may be partially a result of excluding patients with mutated RAS metastatic colorectal cancers as performed in the PEAK trial and the updated PRIME study. Therefore, especially for patients with RAS wild-type mCRC a cetuximab-based treatment may be more beneficial and should be offered as first line therapy. However, response to treatment is usually temporary in patients with mCRC and leads to a median survival of 24 mo. Thus receptors for certain peptide hormones, which are highly expressed in CRC, may be investigated as therapeutic targets. Targeted cytotoxic LHRH analog AEZS-108 (AEZS-108), should be examined for treatment of patients with LHRH receptor positive CRC.

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