Enhancing new possibilities in metastatic colorectal cancer

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Improving outcomes in metastatic colorectal cancer: is there still room for improvement?

by A. Sobrero, Italy

Advances

Advanced colorectal cancer treatment has improved substantially in the last 2 decades. Median survival is around 30 months in most recent clinical trials. In addition, a growing percentage of patients with one or few metastases in a single organ can be cured by the combined approach of systemic therapy and surgery, meaning that more than 50% of patients live longer than 30 months; it is not uncommon to see patients who live 5 years and beyond. These results are due to a combination of factors, including the use of a growing number of chemotherapeutic drugs and biologic agents, better surgical procedures for the eradication of metastases and the use of other locoregional procedures, and improved diagnostic imaging helping to earlier diagnose stage 4 disease. However, the crucial advancement lies in the contribution of the new agents. With extension of survival from 10 months to the current 30 months and over, the new drugs have encouraged a wider use of local approaches, which have a synergistic effect.

Continuum-of-care concept

The rational use of as many of these families of agents as possible without forgetting the possibility of surgery and/or locoregional treatment constitutes what is called the “continuum of care.” Continuum of care does not mean continued chemotherapy nor does it imply that a patient must receive the same drug or drug combinations until the disease progresses. Continuum of care means that from start to finish, the treating physician must reconcile the two key medical principles—first, do no harm (primum non nocere) and second, help (bonum facere). Thus, the physician should start treatment with a regimen that is more or less aggressive depending on what the clinical condition demands and then adjust the intensity of the treatment, including chemotherapy-free periods, according to what is needed as the disease progresses, stabilizes, or shrinks.

Address for correspondence:
Professor Alberto Sobrero, Medical Oncology, IRCCS Ospedale San Martino IST
Genova, ITALY

(alberto.sobrero@hsanmartino.it)

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Initialization of first-line treatment: four approaches

Currently, there are four classical ways to initialize the systemic treatment of stage 4 unresectable colorectal cancer: (i) a chemotherapeutic doublet plus bevacizumab; (ii) a chemotherapeutic doublet plus an anti-EGFR; (iii) a chemotherapeutic triplet plus or minus bevacizumab; and (iv) single-agent fluoropyrimidine plus bevacizumab. The last treatment option is reserved for very elderly patients or patients who cannot tolerate aggressive regimens because of comorbidities. FOLFOXIRI—a triple chemotherapeutic regimen consisting of fluorouracil-leucovorin, oxaliplatin, and irinotecan—is reserved for exceptional cases where patients have never received adjuvant oxaliplatin-based adjuvant therapy, have a very good performance status, and need the tumor to shrink because of severe tumor-related symptoms or because there is potential for conversion of unresectable liver metastases into resectable conditions. However, the two most used first-line therapies are the chemotherapeutic doublets plus one of the two classes of biologic agents. The debate is about how to choose between a chemotherapeutic doublet plus bevacizumab or a doublet plus one of the two anti-EGFR agents.

Contribution of molecular factors and sidedness to the choice of biologics

The debate around choice of biologics is simplified by the fact that anti-EGFR agents are beneficial only if the tumor is RAS wild-type (RAS being a family of small GTPases involved in cellular signal transduction); otherwise, these antibodies are detrimental. Thus, RAS mutational status must be determined before deciding on a first-line treatment. In general, there is a preference for the anti-EGFR agents if the primary tumor is located on the left side of the colon, whereas bevacizumab is preferred for tumors located on the right or transverse colon. Tumor location as a major driver of treatment choice has only recently been established. The remarkable consistency of the data, at least among the most important recent randomized trials, makes sidedness very important in the decision-making process (sidedness probably represents a surrogate of different molecular assets on the two sides of the colon). The four most recent trials on this topic show that the anti-EGFR agents, when given as first-line treatment in combination with chemotherapy for left-sided tumors, produced an approximate 5- to 10-month gain in overall survival compared with bevacizumab. In contrast, the opposite is true for right-sided tumors, where bevacizumab is more beneficial than anti-EGFR by approximately the same extent.

Third- and fourth-line treatments

If conditions call for third-line treatment, there are two main challenges: (i) how tired patients are of side effects; and (ii) if the extent to which their bone marrow reserves are compromised. These two challenges are recognized as the most relevant decisional factors for the treating physician because the two possibilities that we have are based on two completely different agents: regorafenib and trifluridine tipiracil. Regorafenib poses a particular challenge in terms of toxicity (asthenia and painful callous-like lesions on the hands and feet), whereas trifluridine tipiracil is very light in terms of symptomatic toxicity, but strongly affects leukopenia and thrombocytopenia. Therefore, patients who have received extensive amounts of chemotherapy resulting in a reduced bone marrow reserve should preferably receive regorafenib first and then trifluridine tipiracil. However, patients that had debilitating side effects from previous treatments should receive the drugs in reverse sequence, ie, trifluridine tipiracil first and then regorafenib. It is curious that if the toxicities described develop after treatment with one or the other of these two agents, the efficacy is more pronounced. Trifluridine tipiracil was developed after regorafenib; therefore, whereas we have data showing that trifluridine tipiracil may still work on regorafenib-resistant patients, we have no data regarding regorafenib efficacy in trifluridine-tripiracil-resistant patients.

Future developments

Finally, three avenues seem particularly promising as future developments: (i) checkpoint inhibitors; (ii) the anti–stem-cell compound napabucasin; and (iii) the anti-carcinoembryonic-antigen (CEA) bispecific antibodies. Of these, the checkpoint inhibitors pembrolizumab and nivolumab are the most advanced. Results of initial trials are impressive in that through treatment with these immunological agents, tumor regression
occurred even with extremely advanced tumors. However, there was a complete absence of effect in patients with microsatellite-stable (MSS) colon tumors. The clinical data are strengthened by the plausibility of these effects. In fact, checkpoint inhibitors would probably be more efficacious against tumors with a high mutational load, eg, tumors with microsatellite instability (MSI) have a very high mutational load compared with MSS tumors. Unfortunately, only a low percentage of stage 4 tumors are MSI (3%); therefore, the challenge facing the new immunotherapeutic agents is that a way must be found to change the average colon tumor, which is “cold,” ie, poorly responsive to immunotherapy because of a poor lymphocytic infiltrate, to “hot.” Several approaches are under study, including the use of combinations of checkpoint inhibitors with other immunologic compounds, targeted agents, chemotherapy, and radiotherapy.

Considering the growing success that immunotherapy is achieving in almost all types of cancers, with few exceptions, it is likely that this avenue of research will substantially improve treatment outcomes for colorectal cancer, with longer and longer plateaus in survival and potential cures. ■

Keywords: colorectal cancer; continuum of care; immunotherapy; tumor sidedness

References
A new relevant tool in precision medicine is liquid biopsy, which provides, in a simple and noninvasive way, relevant information about the presence of tumor in the organism even when it is macroscopically undetectable by conventional imaging techniques. Moreover, it could give important information on clonal evolution and molecular mechanisms of resistance to therapy. Liquid biopsies would at least provide us with the possibility to detect minimal residual disease...”

New molecular challenges in metastatic colorectal cancer

Colorectal cancer (CRC) is a leading cause of death worldwide. Despite clear improvements in clinical outcome for metastatic CRC observed over the last decade, treatment remains a challenge and new approaches are urgently needed. Here, we discuss “sidedness” of colorectal tumors as an important factor to consider, as left- and right-sided tumors (though the definition of such is still controversial) have differing molecular profiles, sensitivities to treatment, and survival rates; they also have different patterns of metastasis. These differences could be exploited to help guide choice of therapy. For example, the presence or absence of particular gene mutations (such as RAS and BRAF) along with tumor localization (right vs left) should be considered together when deciding on treatment. Furthermore, a novel subtyping classification system, based on gene expression, may prove useful for predicting potential therapeutic effect from new therapies. The use of molecular classification of CRC will help in personalizing treatment. From molecular analysis, it is suggested that tumors that have a high mutational burden respond better to immunotherapy. Also discussed here are potential novel biomarkers—eg, BRAF mutational status, PIK3CA mutational status, and HER2 amplification status, which may be useful for predicting survival or response to therapy—and new technologies, such as liquid biopsy and patient-derived organoids, which may be useful for guiding the decision-making process. A number of new drugs that are under investigation, eg, Sym004, labetuzumab govitecan, fruquintinib, ensituximab, and temozolomide are also discussed briefly.

Side matters: the evolution of personalized medicine within the sidedness revolution

Colorectal cancer (CRC) is the second most commonly diagnosed cancer in Europe and a leading cause of death worldwide. Over the last decade, the clinical outcome for patients with metastatic CRC (mCRC) has clearly improved, and median overall survival (OS) is about 30 months.¹

Several molecular classifications have been proposed, aiming to improve treatment and facilitating a personalized approach. Through different analyses, it is evident that left and right colorectal tumors have different characteristics in terms of molecular profile, treatment sensitivity, and survival. The cut-point for defining left and right colon is still under debate. Vascular supply and embryological origins could help clarify the issue; cancers placed proximal to the splenic flexure are considered right-sided,
**Enhancing new possibilities in metastatic colorectal cancer**

**Table I. Differential features according to sidedness in colorectal cancer**

<table>
<thead>
<tr>
<th>Features</th>
<th>Right-sided tumor</th>
<th>Left-sided tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryology</td>
<td>Mid gut</td>
<td>Hind gut</td>
</tr>
<tr>
<td>Vascularization</td>
<td>Superior mesenteric artery</td>
<td>Inferior mesenteric artery</td>
</tr>
<tr>
<td>Differential pattern of dissemination</td>
<td>Peritoneum</td>
<td>Lung</td>
</tr>
<tr>
<td>Precursor lesions</td>
<td>Sessile serrated adenoma</td>
<td>Bone</td>
</tr>
<tr>
<td>Molecular characteristics</td>
<td>Microsatellite instability</td>
<td>Adenomatous polyps</td>
</tr>
<tr>
<td></td>
<td>BRAF-mutated MAPK signaling</td>
<td>HER1, HER2 amplification</td>
</tr>
<tr>
<td>HER-family ligands</td>
<td>Invasive bacterial biofilm: 89%</td>
<td>Familial adenomatous polyposis</td>
</tr>
<tr>
<td></td>
<td>Low EREG and AREG expression</td>
<td>Invasive bacterial biofilm: 12%</td>
</tr>
</tbody>
</table>

whereas cancers at or distal to this point are considered left-sided. Rectal cancer is excluded from this classification; however, it could be considered left-sided. Right-sided tumors are less prevalent and represent one-third of all colon cancers; they are more likely to occur in women and the elderly and to have a mucinous, undifferentiated, or signet-ring cell histology than left-sided lesions. These tumors are often diploid and are more commonly associated with poor prognostic indicators, eg, mutations in RAS and BRAF (B-Raf proto-oncogene, serine/threonine kinase), microsatellite instability (MSI), high-grade CpG island methylator phenotype (CIMP-high), mutagenic metabolites of cytochrome p450, mitogen-activated protein kinase (MAPK) mutations, HER1 and HER2 gene amplification, aneuploidy, and a hypermutant and very heterogeneous gene-expression profile. All these characteristics can be related to differences in sensitivity to epidermal growth factor receptor (EGFR)-targeted antibody therapy for patients diagnosed with left- or right-sided CRC. A different pattern of metastasization is also evident, with right-sided CRC more likely to metastasize to the peritoneum and left-sided cancers more likely to metastasize to the thorax or, less commonly, bone. Moreover, hereditary nonpolyposis CRC is more likely to develop on the right side of the colon, whereas familial adenomatous polyposis is more frequently diagnosed in left-sided tumors. Table I summarizes the main differences between right- and left-sided colon cancers.

One of the most studied aspects that might help to clarify differences in right- and left-sided carcinomas is variation in the microbiome. The entire colon houses a rich microbiome of intestinal bacteria, some of which—eg, Fusobacterium nucleatum, enterotoxigenic Bacteroides fragilis, and Enterococcus faecalis—are believed to accelerate CRC development. No significant differences between colon segments have been observed, suggesting that an individual's microbiome is quite uniform. Interestingly, differences have been observed in the mucosal microbiota, the bacterial biofilm, between patients who developed right- vs left-sided cancer. Invasive bacterial biofilms were found in 89% of right-sided CRCs, but in only 12% of left-sided CRCs. These biofilms were associated with significantly decreased epithelial E-cadherin, increased interleukin-6, and activated signal transducer and activator of transcription 3 (Stat3), along with increased proliferation. The differences in bile acid levels are also a relevant factor potentially related to the development of left- vs right-sided CRC and could contribute to differential mechanisms of carcinogenesis.

A recent retrospective analysis of the impact of tumor location on clinical outcome in patients with chemotherapy-refractory KRAS (KRAS proto-oncogene, GTPase)–wild-type mCRC...
One of the reasons behind such evidence could lie in the differing levels of the EGFR ligands epiregulin (EREG) and amphiregulin (AREG). High tumor expression of EREG and AREG is associated with greater response rates and improved outcomes with anti-EGFR antibody therapy in patients with KRAS exon 2 wild-type, RAS wild-type, and RAS wild-type/BRAF wild-type disease who received EGFR-antibody therapy as part of a systemic treatment approach.17-20 Moreover, the results of a recent trial suggested that the efficacy outcomes for bevacizumab were greatest in patients with right-sided tumors.21 One of the reasons behind such evidence could lie in the differing levels of the EGFR ligands epiregulin (EREG) and amphiregulin (AREG). High tumor expression of EREG and AREG is associated with greater response rates and improved outcomes with anti-EGFR antibody therapy in patients with KRAS– and NRAS (NRAS proto-oncogene, GTPase)-wild-type mCRCs.22-24 EREG and AREG expression is significantly higher in left-sided CRCs, and is inversely correlated with promoter methylation and CIMP-high status.25 This is clinically very relevant because therapeutic regimens and treatment approaches may not be similarly effective across these two tumor types. This is a relatively new concept, and to date, primary tumor localization has not been a factor in guiding the selection of the most appropriate therapy for mCRC patients.

Figure 1. Prevalence of different subgroups in colorectal cancer. Abbreviation: CMS1-4, consensus molecular subtypes 1-4. © 2015, Springer Nature.

Advanced right-sided tumors have a worse prognosis in all reviewed studies. Taking this fact into consideration may help in designing new prospective randomized trials. Tumor sidedness should at least be taken into account as a stratification factor to avoid potential unbalance among allocation to treatment arms. Another important observation is that right-sided location is a negative predictive factor, indicating lack of efficacy of anti-EGFR antibodies even in the absence of RAS or BRAF mutations. In our current practice, RAS or BRAF mutational status should be considered for decision making in parallel with primary tumor location, favoring the use of anti-EGFR antibodies plus chemotherapy in first-line treatment of left-sided tumors. On the other hand, wild-type tumors from the right side should be preferentially treated with chemotherapy and anti-EGFR antibodies plus targeted therapy.

The hope offered by immuno-oncology in metastatic colorectal cancer

A gene expression–based subtyping classification system, based on results of an analysis of 4141 localized CRCs, has been proposed, classifying CRC into four major subtypes: microsatellite instable (MSI)-immune (CMS1), canonical (CMS2), metabolic (CMS3), and mesenchymal (CMS4). Hypermutated tumors belong to the MSI-immune subtype, and they have strong immune activation (Table II).26 Figure 1 depicts the prevalence of these subgroups.26

Within the framework of this new taxonomy, the immune subtype could be of interest because of a potential therapeutic effect of immunotherapy. Patients with MSI-immune tumors,
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Mutational tumor load has been associated with resistance to chemotherapy and with sensitivity to immunotherapy. Tumors bearing a high mutational burden may respond better to immunotherapy. Among patients diagnosed with mCRC, only those who were MSI had a clinical benefit from treatment with a PD-1 inhibitor. MSI CRCs are characterized by the presence of 10 to 100 more somatic mutations that could activate the immune system through the production of neoantigens. Moreover, mismatch repair-deficient (dMMR) CRCs contain prominent lymphocyte infiltrates, a finding consistent with an increase in immune response. To validate the use of an anti-PD1 inhibitor in dMMR CRC tumors, a phase 2 clinical trial assessed immune checkpoint blockade in a phase 2 clinical trial assessed immune checkpoint blockade in a phase 2 clinical trial assessed immune checkpoint blockade in a phase 2 clinical trial assessed immune checkpoint blockade in a phase 2 clinical trial assessed immune checkpoint blockade in a phase 2 clinical trial assessed immune checkpoint blockade in a phase 2 clinical trial assessed immune checkpoint blockade in a phase 2 clinical trial assessed immune checkpoint blockade in a phase 2 clinical trial assessed immune checkpoint blockade in a phase 2 clinical trial assessed immune checkpoint blockade in patients whose tumors were or were not dMMR. Patients with refractory mCRC with both hereditary and sporadic dMMR tumors, patients with mismatch repair-proficient (pMMR) colorectal adenocarcinomas, and patients with dMMR cancers of types other than colorectal were enrolled in this trial to receive pembrolizumab. Among patients with dMMR CRC, median PFS and median OS were not reached. In contrast, in patients with pMMR CRC, median PFS was only 2.2 months, and the median OS was 5.0 months. For patients with dMMR non-CRC, the median PFS was 5.4 months, and the median OS was not reached. The hazard ratio analyses for disease progression or death and for death showed much better results for patients with dMMR CRC. The data extrapolated from this small phase 2 trial with pembrolizumab suggest that in MSI colon cancers, blocking PD-1 is an appropriate therapeutic strategy. In a phase 2 trial, nivolumab showed encouraging activity in patients with tumors that were dMMR with a high degree of MSI (MSI-H). Responses were recorded across all patient subgroups, including those with (>1%) and without (<1%) tumor PD-L1 expression, suggesting that PD-L1 is not a predictive biomarker of response. Additionally, responses were reported in patients with and without a clinical history of Lynch syndrome or KRAS or BRAF mutations. No responses were observed in patients with pMMR mCRC, suggesting dMMR/MSI-H is a marker for response to PD-1 checkpoint inhibition in mCRC.

Microsatellite stable CRC, in general, should not be considered as an immunogenic tumor, and many strategies have been assessed to overcome this problem. For RAS–wild-type tumors, the combination of chemotheraphy plus cetuximab should be considered a standard of care, achieving response rates of about 60%. Cetuximab, apart from blocking EGFR, can induce immunogenic cell death. For this reason, the evaluation of FOLFOX (folinic acid [leucovorin], 5-fluorouracil [5-FU], oxaliplatin) and cetuximab in combination with avelumab in first-line treatment of mCRC is of particular interest. In a phase 1 trial, patients who had progressed to three lines of previous treatment, including trifluridine/tipiracil, were enrolled to receive pegilodecakin (AM0010), a drug that stimulates TILs and activates CD8+ T-cells. The median OS of 11.7 months is encouraging in this advanced CRC population.

Another recently presented trial evaluated the role of CEA CD3 TCB (RG7802, RO6958688), a novel T-cell bispecific antibody targeting CEA on tumor cells and CD3 on T-cells. In preclinical models, CEA CD3 TCB displays potent antitumor activity, leads to increased intratumoral T-cell infiltration and activation, and upregulates PD-1/PD-L1. Evidence of antitumor activity was observed with CEA CD3 TCB monotherapy in ongoing dose escalation. Activity appeared to be enhanced with doses in combination with atezolizumab, with a manageable safety profile. The results obtained with durvalumab, a human monoclonal antibody that inhibits binding of PD-L1 to its receptor (PD-1), in combination with tremelimumab, an inhibitor of cytotoxic T-lymphocyte-associated protein 4 (CTLA4), have also been recently presented and may need further exploration.

The future of personalized medicine: new biomarkers, new technologies, and new drugs

There are many molecular alterations detected in CRC. Figure 2 shows a summary of the prevalence of these diverse molecular alterations.

- **BRAF mutation**

  BRAF mutations are found in approximately 7% of all human cancers, and CRCs harbor such mutations in about 10% of cases, with the V600E mutation (valine substituted for glutamic acid at residue 600) being the predominant recognized alteration. Patients with these mutations are generally older and female. MSI (higher grade) and lymph node involvement are more frequently observed. BRAF-mutated tumors also have a striking predilection for proximal tumor locations, as well as early peritoneal and distant lymph node metastasis. BRAF mutational status is considered a strong predictor for OS in both localized and metastatic settings. In advanced unresectable disease, the difference between patients with wild-type and mutated BRAF is even more relevant; with standard cytotoxic chemotherapy, the median OS for patients with BRAF mutations is approximately one-third that of patients with BRAF wild-type tumors. A benefit in PFS was observed in patients who were treated with an intense regimen of FOLFOXIRI (folinic acid [leucovorin], 5-FU, oxaliplatin, and irinotecan) + bevacizumab, achieving an improvement in PFS that was approximately 2.5 months longer than in the control group. Unfortunately, even with this regimen, survival for patients with BRAF-mutated CRC was still less than half that of patients with wild-type tumors (median OS, 19.0 months vs 41.7 months). B-Raf inhibitors have been tested in this population with poor outcomes, suggesting that mutational status alone was
The combination of B-Raf and EGFR inhibition with CRC. Approximately mutation status for predicting outcome. Prevalence of 105 New technologies PI3K and colorectal cancer NHANCING NEW POSSIBILITIES IN METASTATIC COLORECTAL CANCER MEDICOGRAPHIA, Vol 40, No. 3, 2018 HER2 and CRC concluding that stage IV patients with exon 9 and/or exon 20, making one of the wild-type mCRC patients. The inhibition of this pathway to overcome both primary and acquired resistance should be considered a critical target. The inhibition of this way in preclinical models, a new approach with a B-Raf inhibitor in combination with a mitogen-activated protein kinase kinase (MEK) inhibitor, trametinib, was tested; however, results were not positive. One of the possible explanations for the lack of response is a dramatic increase in EGFR activation that occurred when CRC cells were exposed to B-Raf inhibition; this encouraged testing of the association of B-Raf and EGFR inhibitors. The combination of B-Raf and EGFR inhibition with a third agent, such as a MEK or an inhibitor of phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA), are currently under investigation. One interesting approach that has already shown intriguing results is the combination of B-Raf and EGFR inhibition with cytotoxic chemotherapies. For BRAF--mutated patients, the MAPK pathway should be considered a critical target. The inhibition of this pathway to overcome both primary and acquired resistance by combining B-Raf, EGFR, and MEK inhibition is tolerable, with promising activity in patients with BRAFmut CRC. 

**PI3K and colorectal cancer**

Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) is one of the crucial kinases in the PI3K/AKT1/mTOR pathway (AKT1, RAC-alpha serine/threonine-protein kinase; mTOR, mammalian target of rapamycin), playing a role in the cellular growth, proliferation, and survival of multiple solid tumors. Approximately 15% to 20% of CRCs harbor activating mutations in PIK3CA exon 9 and/or exon 20, making PIK3CA one of the most frequently mutated genes in CRC. These mutations are related to various clinical and tumor molecular features, including associations with KRAS mutations and proximal tumor location, which may be due to a varying biogeographical influence of the host-microbiota-tumor interaction along the colorectal axis. Three previous systematic reviews have outlined the PIK3CA mutation status for predicting outcome in mCRC, concluding that stage IV patients with PIK3CA not predictive of response to B-Raf inhibitors. Because of the lack of an observed inhibition downstream in the MAPK pathway in preclinical models, a new approach with a B-Raf inhibitor in combination with a mitogen-activated protein kinase kinase (MEK) inhibitor, trametinib, was tested; however, results were not positive. One of the possible explanations for the lack of response is a dramatic increase in EGFR activation that occurred when CRC cells were exposed to B-Raf inhibition; this encouraged testing of the association between B-Raf and EGFR inhibitors. The combination of B-Raf and EGFR inhibition with a third agent, such as a MEK or an inhibitor of phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA), are currently under investigation. One interesting approach that has already shown intriguing results is the combination of B-Raf and EGFR inhibition with cytotoxic chemotherapies. For BRAF--mutated patients, the MAPK pathway should be considered a critical target. The inhibition of this pathway to overcome both primary and acquired resistance by combining B-Raf, EGFR, and MEK inhibition is tolerable, with promising activity in patients with BRAFmut CRC.

**HER2 and CRC**

HER2 amplification has recently been identified as a potential mechanism of resistance to anti-EGFR treatment in RAS--wild-type CRC patients. Immunohistochemical analysis positive for HER2 amplification is detectable in about 5% of cases. In a phase 2 clinical trial, patients were shown to benefit from a combined treatment with anti-HER2 drugs: lapatinib and trastuzumab. The overall response rate (ORR) was 30.3%. Similar results were recently observed in the My Pathway trial (NCT02091141), which reported a 38.2% ORR with trastuzumab and pertuzumab in 34 HER2-positive patients. These emerging data support the consideration of HER2 as a biomarker in mCRC. With an incidence of 3%, HER2-positive mCRC represents an uncommon but clinically relevant fraction of patients.

**New technologies**

The most important end point in personalized oncology is the possibility to select the best treatment for each patient. So, the analysis of the molecular changes that can occur as a result of tumor heterogeneity or anticancer treatment, is fundamental. Although tumors remain the cornerstone for diagnosis and characterization of the somatic genomic features of each tumor, it may not accurately reflect the actual molecular profiling of the tumor owing to intratumor and intertumoral heterogeneity. A relevant tool in precision medicine is liquid biopsy, which could improve our understanding of the biological characteristics of cancer and to help optimize treatment. Liquid biopsies provide, in a simple and noninvasive way, relevant information about the presence of tumor in the organism even when it is macroscopically undetectable by conventional imaging techniques. Moreover, it could give important information on clonal evolution and molecular mech-
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To permit a dynamic study of the disease, three-dimensional patient-derived cellular models, organoids (PDOs), have been used to evaluate tumoral biological characteristics and to predict drug response. PDOs obtained from patients with metastatic gastrointestinal tumors have been studied for their ability to predict response to treatment. There was an observable overlap in mutational spectrum between these models and the original tumors. PDOs have been adopted as drug-screening tools. It has been shown that response to chemotherapy, cetuximab, regorafenib, and trifluridine/tipiracil observed in the PDO and in PDO orthotopic xenografts is comparable to that in treated patients. These data suggest that PDOs can be exploited for functional genomics to simulate cancer behavior and treatment response ex vivo, permitting transposition into the decision-making process of early-phase clinical trials (Figure 2).44

New drug development

In recent years, new drugs have been developed and approved. Regorafenib and trifluridine/tipiracil have been demonstrated to prolong OS in pretreated mCRC patients, leading to registration as third-line treatments.45-47 In MSI patients, pembroliuzumab and nivolumab improved both PFS and OS. Despite this evidence, treatment for metastatic patients remains a challenge, and the identification of new drugs is urgently needed.

To try to overcome anti-EGFR antibody resistance, Sym004—a mixture of two anti-EGFR monoclonal antibodies—was tested in a phase 2 clinical trial. This open-label, multinational, three-arm (1:1:1) study including 254 anti-EGFR–pretreated mCRC patients compared two regimens (12 mg/kg or 9 mg/kg loading dose followed by 6 mg/kg) of weekly Sym004 vs investigator choice of 5-FU, capetabine, or best supportive care. Although the study was negative in the intent-to-treat population, treatment with Sym004 was associated with a remarkable response when compared with any fourth-line treatment of mCRC. The promising results in the molecularly selected population provide rationale for design of a pivotal trial (Figure 2).

Results have been published for an expanded phase 2 trial investigating treatment with labetuzumab govitcanc, an antibody-drug conjugate targeting carcinoembryonic antigen–related cell adhesion molecule 5 (CEACAM5) for tumor delivery of 7-ethyl-10-hydroxycamptothecin (SN-38), in patients with relapsed or refractory mCRC. Monotherapy with labetuzumab govitcanc demonstrated a manageable safety profile and therapeutic activity in heavily pretreated mCRC patients.48 Another drug, which seems to be active in this setting of patients (ie, with relapsed or refractory mCRC) is fruquintinib, an oral kinase inhibitor selectively targeting vascular endothelial growth factor receptors. In an Asian double-blind, placebo-controlled, multicenter trial, fruquintinib significantly improved median OS (9.30 vs 6.57 months). Statistically significant benefits were also seen with fruquintinib in all secondary end points, such as PFS, objective response rate, and disease control rate. The most frequent fruquintinib-related adverse events included hypertension (21.6%), hand-foot skin reaction (10.8%), proteinuria (3.2%), and diarrhea (3.2%).

Recently, the role of mucin 5 (MUC 5) in both CRC and pancreatic cancer has been recognized. A phase 1/2 clinical trial with ensituximab—a novel chimeric monoclonal immunoglobulin G1 (IgG1) antibody derived from an immunogenic neocanterm with sequence homology to mucin 5 subtype AC (MUCSAC)—was conducted in pretreated colorectal and pancreatic patients. To be included, patients had to present with at least 20% expression of the tumor antigen, assessed by immunohistochemistry. In this population, in which all patients were heavily pretreated, ensituximab demonstrated excellent tolerability and encouraging OS.

In CRC, alterations have been observed in the DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT). MGMT promoter methylation is associated with loss of MGMT expression and response to temozolomide in many solid tumors. CRC patients with tumors presenting with methylation of MGMT were treated with temozolomide. MGMT protein was detected in 13 of 24 colorectal tumor samples. Both ORR (29%) and PFS (4.3 vs 1.6 months, HR=0.38) were improved in patients with MGMT protein levels below a cutoff of 200 amol/ug. OS appeared to be improved, though this was not statistically significant (8.9 vs 6.9 months).

Conclusion

Despite the advances made in treatment of mCRC over the past decade, there remains an urgent need for new approaches to improve therapy, for example, through more personalized treatment options. Taking sidedness and tumor locale into consideration along with mutational status, as well as utilizing a new subtyping classification system to help inform treatment choices appears to be useful approaches. Further personalization of therapy through use of potential biomarkers, eg, BRAF mutation, PIK3CA mutation, and HER2 amplification status; new technologies, such as liquid biopsy and use of PDOs, allowing better prediction of treatment response and tracking tumor evolution throughout treatment; and eventually adding new drug options to the therapeutic arsenal are also promising.
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Enhancing new possibilities in metastatic colorectal cancer


Keywords: classification; immunotherapy; metastatic colorectal cancer; mutation; tumor sidedness
Recent stratification studies have led to the start of more efficient treatment designs, where the RAS mutation status, the sidedness of the primary tumor, and the subsequent sequence strategies are now considered to improve the prognosis of patients with metastatic colorectal cancer (mCRC). In this context, more patients are fit for treatment continuation, and thus, there is an increasing need for effective and well-tolerated third-line therapies. Before the recent advances in the development of novel targeted therapies that significantly improved the patients’ prognosis in this setting, such as tyrosine kinase inhibitors, there had been an unmet need for well-tolerated drugs to overcome acquired treatment resistance. The introduction of trifluridine/tipiracil, an oral drug composed of trifluridine plus tipiracil hydrochloride, has improved the prognosis for heavily pretreated mCRC patients, and it has an acceptable toxicity profile to maintain the patients’ performance status. The RECOURSE trial (REfractory COloREctal cancer Study), a prospective, randomized phase 3 trial, showed that this cytotoxic antimetabolite controls the disease efficiently, thereby prolonging progression-free survival and overall survival. Notably, the toxicity is manageable in pretreated mCRC patients. This paper reviews both recent phase 3 trials that are exploring the use of approved agents and early phase trials that are investigating new drugs for chemotherapy-refractory metastatic colorectal cancer.
Contrary to previous lines of treatment, such as polyneuropathy, diarrhea, and skin reactions, including hand-foot syndrome. In the prospective, placebo-controlled, phase 3 trial RECURFENT (REfractory COloRECTal cancer Study), trifluridine/tipiracil, an oral drug consisting of trifluridine plus tipiracil hydrochloride, improved patient survival rates. Therefore, trifluridine/tipiracil is currently approved in 50 countries, including the United States (US), the European Union, and Japan, for the treatment of adult patients with mCRC who have been previously treated with, or are not considered candidates for, available therapies, including fluoropyrimidine-, oxaliplatin-, and irinotecan-based chemotherapies, anti-VEGF agents, and anti-EGFR agents.

There is an unmet need to address the molecular drivers of an individual’s disease to overcome treatment resistance. Insights from genetic studies have led to ongoing studies on novel targeted agents for the treatment of CRC. In addition, individualized treatment is required to address the molecular mechanisms for malignant transformation, tumor growth, angiogenesis, and metastasis formation. Therefore, an extension of treatment protocols focusing on the patient’s molecular profile and a combination of novel chemotherapeutic agents and biological products is needed.

This review focuses on current and future strategies in the continuum of care beyond second-line treatment in mCRC. Recent phase 3 trials are summarized in relation to the continuum of care approach for the patients’ benefit.

Trifluridine/tipiracil

In 2014, a new agent, trifluridine/tipiracil, was introduced in Japan, which was followed by US Food and Drug Administration approval in the United States in 2015 and by the European Medicines Administration in Europe in 2016. Trifluridine/tipiracil has been shown to provide a significant overall survival benefit for patients with refractory mCRC. The tipiracil hydrochloride component of this oral drug improves the bioavailability of trifluridine (a reversible inhibitor that binds to the active site of thymidylate synthase) by inhibiting its catabolism by thymidine phosphorylase. The primary mode of action of trifluridine/tipiracil is to incorporate trifluridine into the DNA, which induces DNA dysfunction, including DNA strand breaks. Fluoropyrimidines, such as fluorouracil (5-FU), may also be incorporated into the DNA, but they are rapidly cleaved by uracil-DNA glycosylases, which reduces the damaging effects to the DNA. Moreover, the tipiracil hydrochloride component may enhance the durability of the response to trifluridine.

In the RECURFENT trial, 800 mCRC patients were randomized 2:1 to either trifluridine/tipiracil or placebo. Patients included in the trial were all resistant or intolerant to standard chemotherapies, including oxaliplatin, irinotecan, fluoropyrimidine, bevacizumab, and, 1) BRAF or KRAS wild-type tumors, anti-EGFR antibodies. The patients had received ≥2 previous standard chemotherapy regimens. The median overall survival was 7.1 months with trifluridine/tipiracil vs 5.3 months with placebo, representing a 32% mortality risk reduction (hazard ratio [HR], 0.68; 95% confidence interval [CI], 0.58-0.81; P<0.001). These robust signs of clinical activity were further supported by the fact that trifluridine/tipiracil significantly reduced the risk of progression by 52% (HR, 0.48; 95% CI, 0.41-0.57; P<0.001). Besides its clinical activity in improving survival of heavily pretreated mCRC patients, the time to deterioration in the performance status from ECOG (Eastern Cooperative Oncology Group [scale]) 0 or 1 to ECOG 2 was significantly longer for patients treated with trifluridine/tipiracil (5.7 months vs 4.0 months; HR, 0.66; 95% CI, 0.56-0.78; P<0.001) (Figure 1).

These results are consistent with the manageable safety profile of the drug. Although grade 3+ adverse events were recorded in 69% of patients, most side effects were due to hematotoxicity, meaning that 38% of patients had grade 3+ neutropenia, whereas febrile neutropenia was recorded in only 3.7% of all patients treated with trifluridine/tipiracil. Routine blood cell count testing, dose delays, and/or dose reductions are likely to prevent severe adverse events. In contrast with many other active agents that are routinely used for the treatment of mCRC, trifluridine/tipiracil is very unlikely to cause polyneuropathy (0%), hand-foot syndrome (0%), skin reactions (0%), stomatitis (0.4%), diarrhea (3%), or pulmonary embolism (1.5%). Due to its activity and tolerability, trifluridine/tipiracil was rapidly incorporated into the treatment recommendations from the European Society of Medical Oncology (ESMO), the National Comprehensive Cancer Network (NCCN), and the National Institute for Health and Care Excellence (NICE). Regorafenib

On the basis of data from the CORRECT trial (Regorafenib Monotherapy for Previously Treated Metastatic Colorectal Cancer), regorafenib was introduced in 2013 at a time when there was a clear unmet need for new treatment options. The CORRECT trial was a prospective randomized phase 3 trial, which randomized 760 CRC patients who were resistant to standard...
Intravenous therapy options 2:1 to receive either the oral multi-kinase inhibitor regorafenib or best supportive care only. Regorafenib targets both angiogenic and stromal tyrosine kinases, including tyrosine kinase with immunoglobulin-like and EGF-like domains 2, human VEGF receptor 2, fibroblast growth factor receptor 1, platelet-derived growth factor receptor, and oncogenic kinases, such as KIT, RET, and BRAF. The activity of and results with regorafenib led to its recommendation in major oncology guidelines, including those by ESMO13 and the NCCN.14

In the CORRECT trial, prolongation of the median overall survival was observed in regorafenib recipients with a 23% reduction in the risk of death vs best supportive care alone (median 6.4 months for regorafenib vs 5.0 for placebo; HR, 0.77; 95% CI, 0.64-0.94; one-sided P=0.0052). Furthermore, the risk of progression was reduced by half with regorafenib with a median progression-free survival of 1.9 months for regorafenib and 1.7 months for placebo (HR, 0.49; 95% CI, 0.42-0.58; P<0.0001). Notably, patients were heavily pretreated, with half of the patients having received ≥4 treatments before regorafenib for mCRC.6

The CONCUR trial (Asian Subjects With Metastatic Colorectal Cancer Treated With Regorafenib or Placebo After Failure of Standard Therapy), another double-blind, placebo-controlled, phase 3 trial, showed that regorafenib is also beneficial in Asian patients.15 This trial was exclusively performed in Asia, confirming a survival benefit in pretreated CRC patients with a median overall survival of 8.8 months for regorafenib vs 6.3 months for placebo (HR, 0.55; 95% CI, 0.40-0.77; P=0.00016). However, the phase 3b CONSIGN trial (Regorafenib in Subjects With Metastatic Colorectal Cancer Who Have Progressed After Standard Therapy), an open-label, expanded-access study conducted in 2872 patients, recorded the safety profile of this agent,16 showing that 57% of patients reported treatment-related grade 3+ adverse events. The most common (>5%) grade 3+ treatment-related adverse events were hypertension (15%), hand-foot skin reaction (14%), and fatigue (13%). The safety profile was consistent with all phase 3 regorafenib trials in mCRC patients.

Rechallenge with anti-EGFR treatment
In RAS wild-type patients, the anti-EGFR antibodies cetuximab and panitumumab have demonstrated clinical activity when combined with chemotherapy in first- and second-line settings. Small, prospective, but not randomized, trials provided the first evidence that rechallenge with anti-EGFR antibodies after failure of second-line treatment might be effective in patients pretreated with anti-EGFR drugs in earlier lines. In a prospective phase 2 trial that analyzed the rechallenge with cetuximab in combination with an irinotecan-based chemotherapy, Santini et al4 reported that 39 patients, who were pretreated with and benefited from an anti-EGFR containing regimen, benefited from a rechallenge with cetuximab in combination with an irinotecan-based chemotherapy. The results demonstrated a disease control rate of almost 90% and a partial or complete response rate in 53.8% of the patients. Furthermore, the activity was sustained, leading to a median progression-free survival of 6.6 months.

Our retrospective analysis of the first-line treatment trials PRIME (Panitumumab Randomized Trial In Combination With Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy) and PEAK (Panitumumab Plus mFOLFOX6 vs. Bevacizumab Plus mFOLFOX6 for First Line Treatment of Metastatic Colorectal Cancer Patients With Wild-Type KRAS Tumors) revealed that rechallenge with anti-EGFR antibodies in RAS wild-type mCRC patients led to a median overall survival from the start of treatment to >45 months. The patients who underwent rechallenge were healthier at baseline and had a better response to first-line treatment than those who did not undergo rechallenge.17

However, recent evidence suggests that anti-EGFR treatment frequently leads to acquired resistance toward anti-EGFR treat-
ment by clonal selection of preexisting clones containing a molecular mutation in the EGFR-/RAS-/RAF-/MAPK-signaling pathways. In their analysis of cell-free circulating tumor DNA from the plasma of patients, Bettegowda et al found a selection of novel clones that were resistant to anti-EGFR treatment. The authors described a large number of mutations in codon 61 of either the KRAS or NRAS gene. In this particular study, 15 of 24 patients (62.5%) had at least 1 mutation in codon 61, and these mutations comprised 45% of the total mutations (n=69).

In summary, rechallenge with anti-EGFR antibodies for patients who had a benefit in earlier lines of treatment with this strategy might work for a certain subgroup of patients, but real-time molecular profiling might be required due to the unstable cancer genome. A liquid biopsy might allow for a better selection of patients who may benefit from two lines of treatment with anti-EGFR antibodies. So far, the small series of prospective rechallenge trials have provided only a low level of evidence for treatment recommendation and further studies are urged.

**Future perspectives**

**◆ Targeting HER-2 in mCRC**

In a molecular profile analysis of pretreated mCRC patients, a subgroup of tumors revealed amplification of the human epidermal growth factor receptor 2 (HER2/neu), HER2 overexpression is an effective target for trastuzumab in breast and gastric cancers, but leads to resistance to anti-EGFR antibodies. So far, the small series of prospective rechallenge trials have provided only a low level of evidence for treatment recommendation and further studies are urged.

**◆ BRAF mutation in mCRC**

Constitutive B-Raf activation via mutations occurs in approximately 5% to 10% of mCRC patients. These mutations are thought to be driver mutations because they lead to intracellular signaling that can be associated with aggressive tumor behavior. This tumor cell activation is reflected by a worse prognosis, leading to a lower chance of a response and a reduction in overall survival. While a subgroup analysis of larger phase 3 trials suggested that BRAF-mutated CRC patients might have a benefit from aggressive folic acid (leucovorin)/5-fluorouracil (5-FU)/irinotecan (FOLFIRI) plus bevacizumab treatment, a small series of prospectively observed BRAF-mutated mCRC patients could confirm the efficacy of this approach.

The first approaches to target BRAF-mutated tumors with B-Raf inhibitors resulted in low clinical activity. Only 5% of patients had a partial response with this strategy, and the progression-free survival was 2.1 months. In addition, a double blockage of B-Raf and its downstream signaling molecule MEK using dabrafenib plus trametinib showed a marginally higher efficacy (9% response rate) and a median progression-free survival of 3.5 months. Thus, the first evidence suggested that blocking upstream EGFR was an effective strategy to overcome constitutively activated B-Raf. Subsequent phase 1/2 trials have explored the addition of anti-EGFR monoclonal antibodies to B-Raf inhibitors, with varying results; for example, a 10% relative risk and progression-free survival of 3.5 months was observed with dabrafenib-panitumumab. The triple blockage of EGFR, B-Raf, and MEK has also been analyzed, showing an improved efficacy with a 26% relative risk and progression-free survival of 4.1 months. Based on the data from these recent combination trials targeting B-Raf with a specific serine/threonine-protein kinase inhibitor plus upstream as well as downstream signaling inhibition using a MEK inhibitor and an anti-EGFR antibody, this triplet combination seems to be an effective approach. Currently, the large, prospective, phase 3 trial BEACON-CRC is addressing the question of the most effective way to overcome B-Raf activation (A Multicenter, Randomized, Open-label, 3-Arm Phase 3 Study of Encorafenib + Cetuximab Plus or Minus Binimetinib or Iritinotecan/Cetuximab or Infusional 5-Fluorouracil [5-FU]/Folinic Acid [FA]/Iritinotecan [FOLFIRI]/Cetuximab With a Safety Lead-in of Encorafenib + Binimetinib + Cetuximab in Patients With BRAF V600E-mutant Metastatic Colorectal Cancer; NCT02928224).

**Immunotherapies**

Recently, hypermutated colorectal tumors have been treated efficiently with anti-programmed-cell-death-protein-1 (anti-PD-1) antibodies. Most of these tumors are characterized by a mismatch-repair deficiency or mutations in DNA polymerase ε (POL-E) or DNA polymerase δ (POL-D). In this context, a recent study by Le et al demonstrated that pembrolizumab was effective in heavily pretreated MSI-high tumors. The im-
mune-related objective response rate was seen in 4 out of 10 mismatch repair–deficient mCRC patients (40%), whereas none of the 18 mismatch repair–proficient mCRC patients (0%; 0/18 patients) had clinical activity with this strategy. The prospective, phase 2 trial Checkmate 142 (An Investigational Immunotherapy Study of Nivolumab, and Nivolumab in Combination With Other Anti-cancer Drugs, in Colon Cancer That Has Come Back or Has Spread) demonstrated that nivolumab has comparable activity in mismatch repair–deficient/MSI-high mCRC patients. Therefore, the addition of the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitor ipilimumab to nivolumab yielded an increased response rate; however, as expected, higher rates of toxicity were observed.

As only 5% to 10% of stage 4 CRC tumors are mismatch-repair deficient, it is important to understand how to enhance the immunotherapy susceptibility in patients with mismatch repair–proficient tumors. In this context, the first evidence suggests that blocking MEK alone can result in intratumoral T-cell accumulation and major histocompatibility complex (MHC-I) upregulation, and synergizes with an anti–PD-L1 agent, to promote durable regression of mismatch repair–proficient tumors. Data from the phase 3 trial IMblaze370 (A Study to Investigate Efficacy and Safety of Cobimetinib Plus Atezolizumab and Atezolizumab Monotherapy Versus Regorafenib in Participants With Metastatic Colorectal Adenocarcinoma; NCT02788279) were first presented at the ESMO World Congress on Gastrointestinal Cancer 2018. The trial failed to show superiority compared with regorafenib.

### Summary
The recent introduction of novel drugs and a better understanding for treatment-option stratification have led to a median overall survival that is greater than 30 months in mCRC patients. In first-line treatment, a deep response on the tumor load is the primary aim to improve patient prognosis; however, in later lines of treatment, quality of life and disease stabilization becomes an increasingly important, but often unmet, need (Table I). In this context, the introduction of the novel drug trifluridine/tipiracil accomplished both by increasing the overall survival and by maintaining performance status. Tabernero et al recently demonstrated, using a quality-adjusted time without toxicity and symptoms (Q-TWIST) global score analysis, that trifluridine/tipiracil improves progression-free survival time above the time spent with treatment-related toxicity. Aside from an increase in hematotoxicity, trifluridine/tipiracil treatment has a low rate of grade 3 or higher nonhematological adverse events, thereby sparing hand-foot-skin reaction. Thus, trifluridine/tipiracil is thought to be a potential combination partner for other active drugs in mCRC treatment; this is currently being tested in clinical phase 1 to 3 trials.

Beside novel antimetabolic drugs, a deeper understanding of the underlying mechanism of the tumor’s innate or acquired resistance toward anticancer drugs is a prerequisite for novel and efficient treatment strategies for CRC. As more patients are fit for a continuum of care approach, there is a need for novel and individualized therapies in the third-line setting and beyond. Modern techniques to characterize somatic mutations from tissue or cell-free tumor DNA are resulting in a reconsideration of known targets, such as MSI, HER2, and B-Raf, for individual treatment concepts.

### References
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### Table I. Lines of treatment for metastatic colorectal cancer.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First-line</th>
<th>Second-line</th>
<th>≥ Third-line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall risk ratio</td>
<td>38%-65%</td>
<td>5%-35%</td>
<td>1%-17%</td>
</tr>
<tr>
<td>Progression-free survival</td>
<td>9-12 months</td>
<td>4-7 months</td>
<td>2-4 months</td>
</tr>
<tr>
<td>Treatment arm</td>
<td>Response, PFS</td>
<td>PFS, OS</td>
<td>OS, QOL</td>
</tr>
<tr>
<td>Receiving treatment</td>
<td>100%</td>
<td>69%</td>
<td>44%</td>
</tr>
</tbody>
</table>

Abbreviations: OS, overall survival; PFS, progression-free survival; QOL, quality of life.
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Keywords: metastatic colorectal cancer; RECURSE; regorafenib; trifluridine/tipiracil
THE QUESTION

For years the role of primary tumor sidedness (i.e., left/right) as a prognostic factor in metastatic colorectal cancer was overlooked, but it has recently regained importance. In this section, Anelisa K. Coutinho, Jakob Eberhard, Juan Manuel O’Connor, and Cornelis J. A. Punt present the current evidence about the impact of tumor sidedness in metastatic disease and discuss what they believe are the implications in clinical practice of taking tumor sidedness into account or not.

Is primary tumor sidedness relevant in deciding how to treat metastatic colorectal cancer?

1. A. K. Coutinho, Brazil
2. J. Eberhard, Sweden
3. J. M. O’Connor, Argentina
4. C. J. A. Punt, The Netherlands
1. **A. K. Coutinho, Brazil**

For many years, the side location of a primary colorectal tumor was almost only ever mentioned as a prognostic curiosity. However, in May 2016, after three consecutive oral presentations at the American Society of Clinical Oncology Meeting, this simple perspective changed. An analysis of the CALGB/SWOG 80405 trial showed that the primary tumor location affected overall survival with a 14-month difference between left-sided and right-sided tumors (33 months and 19 months, respectively; \( P < 0.0001 \)).\(^1\) In addition, the authors made an exploratory analysis of outcomes between sidedness and the monoclonal antibody used, showing that anti-EGFR treatments (cetuximab) had the best overall survival rate in patients with RAS wild-type left-sided tumors (39 months) vs patients with right-sided tumors (13 months). Differences also existed for an anti-vascular endothelial growth factor (VEGF) treatment, the first-line treatment arm (bevacizumab); however, it was smaller (32 months in left-sided tumors vs 29 months in right-sided tumors) than anti-EGFR treatments.

Following this analysis, other retrospective analyses evaluated the sidedness effect with data from important randomized trials, such as FIRE-3,\(^4\) PRIME,\(^5\) PEAK,\(^5\) study 181, and CRYSTAL.\(^4\) All of these trials obtained the same findings, ie, a huge prognostic difference between left-sided and right-sided tumors and an impressive discrepancy in the results, especially when anti-EGFRs were used for left-sided vs right-sided tumors.

FIRE-3 showed a better overall survival with FOLFIRI + cetuximab in RAS wild-type patients with left-sided vs right-sided primary tumors (38 months vs 18 months) and with FOLFIRI + bevacizumab (28 months vs 23 months) (Table I). In the PRIME study, the RAS wild-type patients with left-sided tumors had a better overall survival with FOLFOX + panitumumab vs patients treated with FOLFOX alone (30.3 months vs 23.6 months; HR, 0.73; \( P = 0.0112 \)). However, in patients with right-sided tumors, there was no significant differences in overall survival with the addition of panitumumab (11.1 months vs 15.4 months with and without panitumumab, respectively [HR, 0.87; \( P = 0.5398 \)].

However, what is so different in the anatomy of each side to explain these findings? The embryological origins of the sides of the colon are diverse; the left side is derived from the embryonic hindgut and the right side from the embryonic midgut. Each side also has different blood supplies, innervations, and

<table>
<thead>
<tr>
<th>Trial</th>
<th>Left side (approx. 76%)</th>
<th>Right side (approx. 24%)</th>
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<tbody>
<tr>
<td>FIRE3</td>
<td>FOLFIRI + bevacizumab 28 months</td>
<td>FOLFIRI + cetuximab 38 months</td>
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<tr>
<td></td>
<td>HR: 0.63; ( P = 0.002 )</td>
<td>HR: 1.31; ( P = 0.28 )</td>
</tr>
<tr>
<td>CALGB 80405</td>
<td>FOLFIRI/FOLFOX + bevacizumab 32.6 months</td>
<td>FOLFIRI/FOLFOX + cetuximab 39.3 months</td>
</tr>
<tr>
<td></td>
<td>HR: 0.77; ( P = 0.04 )</td>
<td>HR: 1.36; ( P = 0.10 )</td>
</tr>
<tr>
<td>Crystal</td>
<td>FOLFIRI + cetuximab 28.7 months</td>
<td>FOLFIRI + placebo 21.7 months</td>
</tr>
<tr>
<td></td>
<td>HR: 0.65; ( P = 0.002 )</td>
<td>HR: 1.08; ( P = 0.76 )</td>
</tr>
<tr>
<td>PRIME</td>
<td>FOLFOX + panitumumab 30.3 months</td>
<td>FOLFOX + placebo 23.6 months</td>
</tr>
<tr>
<td></td>
<td>HR: 0.73</td>
<td>HR: 0.87</td>
</tr>
<tr>
<td>PEAK</td>
<td>FOLFOX + panitumumab 43.4 months</td>
<td>FOLFOX + bevacizumab 32.0 months</td>
</tr>
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<td></td>
<td>HR: 0.84</td>
<td>HR: 0.45</td>
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</table>

**Table I.** Retrospective exploratory data on median overall survival and sidedness in the different treatment arms of several main trials.
lymphatic drainage pathways. Looking at the molecular features, some alterations, such as a BRAF mutation, microsatellite instability (MSI), or hypermutation, are found more frequently in the right side of the colon than in the left side. In addition, an active EGFR signaling pathway and a high gene expression of epiregulin are more frequent in patients with left-sided tumors. Thus, the anatomical distinction in sides is probably just a surrogate marker for many biological and molecular alterations that can be predictive of benefit from the choice of monoclonal antibody regarding sidedness.

So now, with the acquisition of this data, should we take into account the sidedness of the primary tumor in daily practice to choose the ideal first-line treatment for metastatic colorectal cancer? Alternatively, is it reasonable to leave the information aside and continue to choose regardless of the sidedness until prospective randomized trials clarify this point? The main arguments against considering sidedness as an important decision-making tool include the fact that all of the data was based on exploratory retrospective analyses, which need to be confirmed, and the lack of statistical significance in the reviewed trials for most of the right-sided outcome results, which is probably secondary to the small proportion of patients in this group. However, the arguments in favor of considering sidedness for clinical decisions include: (i) the similar results obtained from many retrospective analyses that showed big differences in overall survival and progression-free survival depending on the class of monoclonal antibody used; (ii) the proposed correlation with molecular characteristics for each side, which suggest a biological origin; and (iii) the fact that we already have the option to choose between a double or even triple chemotherapy regimen (oxaliplatin and/or irinotecan) combined with an anti-EGFR or anti-VEGF treatment as first-line options for RAS wild-type patients. The current regimen selection is based on a collection of information, including the intention of the treatment, volume of the disease, performance status, comorbidities, patient’s desire, and other molecular characteristics, such as BRAF mutational status. Therefore, we will not cause “harm” by including laterality as another item in the decision-making process.

Considering the large numeric differences in overall survival when using an anti-EGFR treatment (either cetuximab or panitumumab) as a first-line therapy for RAS wild-type patients with left-sided vs right-sided tumors, it seems like there is no benefit, based on retrospective observations, for the addition of anti-EGFR for right-sided tumors. Both the CRYSTAL trial and the PRIME trial, which compared FOLFIRI + cetuximab with FOLFOX and FOLFOX + panitumumab with FOLFOX, respectively, for first-line therapy in RAS wild-type patients, showed similar or even worse results for overall survival with anti-EGFR in patients with right-sided tumors (18.5 months vs 15.0 months in CRYSTAL [HR, 1.08; P=0.76] and 11.1 months vs 15.4 months in PRIME [HR, 0.87]).

For the anti-VEGF treatment bevacizumab, the imbalance between the two sides of the colon also favors the left side, although with a smaller difference. Even though an anti-EGFR treatment achieved a better numeric overall survival in left-sided tumors, a retrospective comparison with an anti-VEGF treatment showed that bevacizumab reached reasonable overall survival rates (over 23 months for both left-sided and right-sided tumors) and it showed no detrimental effects in either side. However, the effects of both anti-EGFR and anti-VEGF treatments in second-line therapy, as related to the site of primary tumor, cannot be predicted yet.

In conclusion, in light of the current data, I personally would cautiously favor inclusion of laterality as one of the variables to be considered for treatment selection for first-line treatment in RAS wild-type patients, while using common sense, coherence, and observing individual context, until prospective data becomes available.

References
Is primary tumor sidedness relevant in deciding how to treat metastatic colorectal cancer?

The answer to this specific question is yes, primary tumor sidedness matters or should matter in the decision-making process of how to treat metastatic colorectal cancer. However, it is—of course—not the sidedness per se that matters, but more that it is the surrogate marker for a different biology, and left-sided cancers and right-sided cancers should perhaps be considered as different diseases.

There may be a solution, or at least a partial solution, to this difficult riddle. In 2014, Heinemann et al presented data from the FIRE-3 study, which randomized patients with metastatic colorectal cancer to FOLFIRI + cetuximab or FOLFIRI + bevacizumab as a first-line treatment. While no between-group differences were observed in the primary end point, ie, an objective response, there was a significant difference in survival in favor of the FOLFIRI + cetuximab in patients with KRAS wild-type tumors, indicating that the FOLFIRI + cetuximab regimen would be preferable.¹ The results from FIRE-3 could not be confirmed in the phase 3 CALGB/SWOG 80405 study, which compared FOLFIRI or leucovorin/5-FU/oxaliplatin (FOLFOX) with bevacizumab or cetuximab in the same patient population as in FIRE-3. There was no difference in survival between patients receiving chemotherapy + cetuximab and those receiving chemotherapy + bevacizumab; therefore, both regimens were considered adequate first-line treatments for patients with metastatic, palliative colorectal cancer and RAS wild-type tumors.²

To help us understand why these well-designed and well-conducted studies gave divergent results, the goal is now to identify subgroups, both on a molecular and clinical basis, that can, in the long term, identify characteristics that will enable a better selection of patients who will obtain a greater benefit with one regimen vs another. Due to differences in the biology and embryonic origin for each side of the colon, an analysis of the CALGB study was made by distinguishing between the right and left side of the colon; the transverse colon and rectum were excluded. The results, presented at the 2016 American Society of Clinical Oncology meeting, showed that there is a clinical difference in patients with KRAS wild-type tumors and there was a significantly longer total survival and progression-free survival in patients with a left-sided tumor than in patients with a right-sided tumor. In the KRAS wild-type patients with a left-sided tumor who received cetuximab, the overall survival and progression-free survival were extended, which was the same for KRAS wild-type patients with a right-sided tumor who received bevacizumab. It also appeared that KRAS wild-type patients with a right-sided tumor who received cetuximab had poorer outcomes than did patients with a right-sided tumor and a KRAS mutation. Venook et al, who made the compilation, also had access to the data from FIRE-3 and also presented merged results from both studies. The outcome was the same in both trials; therefore, it could at least partially explain the differences in the results between the CALGB and FIRE-3 studies.

A meta-analysis, recently published by Holch et al,³ analyzed all first-line randomized controlled trials and prospective clinical trials that evaluated the importance of tumor location. The analysis shows that we can say clearly and beyond a doubt that sidedness is an obvious prognostic marker with an inferior prognosis for right-sided tumors and vice versa for left-sided tumors. There is also strong evidence that the addition of an anti-EGFR therapy provides a clear survival benefit for patients with left-sided tumors, whereas this is more controversial and doubtful for patients with right-sided tumors. It also seems that sidedness could be a selector and predictor of treatment choice, considering that an anti-EGFR treatment is more beneficial in left-sided tumors in terms of overall survival and progression-free survival than an anti-VEGF treatment. When it comes to objective risk reduction, it could be different when using an anti-EGFR to shrink the tumor in both left-sided and right-sided tumors, which is relevant mainly in patients with a curative intent of the treatment where maximum shrinkage is a requirement for additional surgery.⁴ All of this information has already been taken into consideration in most treatment proposals and algorithms.

All of these results continue to suggest that tumor localization matters. From a biological point of view, it is important to be aware of the different distributions, prognoses, and frequencies of microsatellite instable / microsatellite stable tumors and tumors with BRAF mutations in the right vs the left side of the colon. Additional subgroup analyses are ongoing, indicating that the distinction goes further than deciding whether treatment with anti-EGFR or anti-VEGF is most suitable. In addition to having the worst prognosis, patients with right-sided tumors have more BRAF mutations and a higher extent of microsatellite instability, and they are more likely to develop in patients who have a genetic predisposition. Sidedness and the different biology between the sides of the colon will probably have an impact on the choice of other targeted drugs as well as new immunotherapies, such as PD1/PDL1 inhibitors.
The sidedness difference is a surrogate marker for different tumor biology, which is important. In the future, all studies should take into account the location of the tumor in the colorectum and secure tumor samples for extended biomarker analysis.

References
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3. J. M. O’Connor, Argentina

Juan Manuel O’CONNOR, MD, FRCP
Instituto Médico Especializado Alexander Fleming, Buenos Aires
ARGENTINA
(email: juanmanuel.oconnor@gmail.com)

Since 2016, the role of sidedness has regained importance as a prognostic factor in colorectal cancer. The results of a subanalysis of the CALGB/SWOG 80504 study were presented at the 2016 American Society of Clinical Oncology meeting. This subanalysis compared chemotherapy + bevacizumab with chemotherapy + cetuximab in patients with advanced colorectal cancer and RAS wild-type tumors, and it showed that there was a median survival of 36 months for patients with left-sided tumors vs 16 months for patients with right-sided tumors. A recent systematic review and meta-analysis of 66 clinical trials, which included more than 1 400 000 patients, demonstrated a significant impact on survival, with a 20% reduction in mortality for patients with left-sided tumors (HR, 0.82; 95% CI, 0.79-0.84; P<0.001), regardless of the initial stage, race, year of the study, number of subjects, adjuvant therapy, and the quality of the studies.\(^3\)

However, the question remaining concerned whether sidedness was also a predictive factor for treatment response. In this respect, several analyses were conducted in an attempt to prove the interaction between sidedness and prediction of a response to biological treatment. Although no interaction was initially observed in the efficacy between different angiogenic agents, such as bevacizumab, the location of the primary tumor seems to play a role in the effect of anti-EGFR therapy, such as cetuximab or panitumumab.

A subgroup analysis of six randomized controlled trials, which included patients with a diagnosis of advanced colorectal cancer, showed that, for the patients with RAS wild-type tumors, there were differences in the efficacy of anti-EGFR treatments according to the location of the primary tumor. While anti-EGFR-based treatments showed a significant benefit for patients with left-sided tumors (HR, 0.69; 95% CI, 0.58-0.83), no differences were seen for patients with right-sided tumors (HR, 0.96; 95% CI, 0.68-1.35) (P interaction=0.103). These analyses have some limitations, eg, the inclusion of the phase 2 trial PEAK, the retrospective analysis of the data, using definitions of primary tumor location that were not prespecified, including only patients with a RAS wild-type genotype, and not including the triple combination therapy with or without bevacizumab,\(^4\) as clearly shown by the study design.

A retrospective analysis of the CRystal and FItE-3 trials,\(^5\) which assessed the location of the primary colorectal tumor as a predictive factor, showed that, although the treatment arms compared were different, there were differences observed for progression-free survival, overall survival, and objective response rate in favor of the combined therapy with cetuximab in left-sided tumors. For example, FIRE-3 reported that, for patients with left-sided tumors, the median survival was 38 months when they were treated with FOLFIri + cetuximab vs 28.3 months in those treated with FOLFIri + bevacizumab (P=0.002). However, the median survival for patients with right-sided tumors treated with FOLFIri + bevacizumab was 18.3 months vs 23 months for those treated with FOLFIri + cetuximab (P=0.28). The simplest conclusion based on the analysis of these two studies shows that right-sided primary tumor location is a negative predictive factor for a response to anti-EGFR therapy.

**How can these differences be explained?**

Left-sided tumors show more dependency on EGFR, including a higher number of EGFR copies, a larger number of endogenous EGFR ligands, such as amphiregulin (AREG) and epiregulin (EREG), and a canonical phenotype that is based on the new consensus molecular subtype 2 (CMS2) classification.\(^6\) Moreover, there are some molecular alterations that might account for resistance to treatment with anti-EGFR in right-sided tumors, such as the BRAFV600E mutation, a Cpg island, mutations in phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit \(\alpha\) isoform (PIK3CA), and a higher representation of the immune subtype CMS1 with the phenotype of microsatellite instability.\(^7\)

**Is primary tumor sidedness (left – right) relevant in deciding how to treat metastatic disease?**

The short answer is “yes.” We should consider that, although the analysis of the CRystal and FItE-3 trials was retrospective and not prespecified, the findings were consistent with first-line therapies, mainly in patients with advanced colorectal cancer with RAS, BRAF, and wild-type tumors. Some changes have already been made in the 2017 National Comprehensive Cancer Network guidelines, which exclude the possibility of using anti-EGFR therapy as a first-line therapy for RAS wild-type patients with right-sided tumors.\(^8\) The combined analysis of different studies shows that patients with right-
Controversial Question

Is primary tumor sidedness relevant in deciding how to treat metastatic colorectal cancer?

sided tumors have a poor prognosis and that right-sided tumors have a negative predictive value in terms of response to anti-EGFR treatment. Moreover, it is clear that tumor location must be considered as a stratification factor in future clinical trials. The challenge is to identify the molecular pattern accounting for these differences in the sensitivity to anti-EGFR therapy according to the primary tumor location.

References
Is primary tumor sidedness relevant in deciding how to treat metastatic colorectal cancer?

The prognosis of patients with metastatic colorectal cancer (mCRC) has significantly improved over the past decades, which is primarily due to the availability of more effective drugs and a more frequent use of local treatments for metastases. With more data on the biology of mCRC, it is clear that mCRC is a heterogeneous disease consisting of many subgroups, which have different prognoses and may require different treatments. In addition to well-known clinical prognostic factors, such as performance status and number of metastatic sites, the use of molecular markers is gradually being implemented in the standard of care.

Established prognostic markers are the RAS and BRAF mutational status of the tumor in mCRC, and mismatch repair status in the early stages of the disease. The RAS and BRAF mutational status is also predictive for the use of anti–epidermal growth factor receptor (EGFR) treatments (cetuximab, panitumumab). Promising predictive markers include the HER2 and mismatch repair status in mCRC for treatment with anti-HER2 antibodies and immune checkpoint inhibitors, respectively. These markers may not only provide targets for novel drugs, but may also allow for a better selection of patients for treatment with established drugs to prevent unnecessary exposure to their toxicity and to reduce costs. Therefore, with most research being focused on molecular subtypes, it came somewhat as a surprise that a clinical characteristic, such as sidedness of the primary tumor, resurfaced last year as a topic of intense debate in mCRC.

Although the prognostic value of primary tumor sidedness in mCRC was recognized in 2001 and confirmed in 2015, it was rarely, if ever, used in clinical practice, eg, for stratification or subgroup analyses in prospective randomized trials. Early data from 2015 on its predictive value regarding the use of anti-EGFR antibodies as a late-line monotherapy had little clinical impact at the time. However, data from more recent analyses on the predictive value of primary tumor sidedness can no longer be ignored, and the implications of using expensive drugs probably played a role.

While data on the predictive value for anti-EGFR treatment appear quite robust, it is not expected that tumor sidedness will tell the whole story. Embryologically, the right side of the colon is derived from the midgut, whereas the left side of the colon and rectum develop from the hindgut, and there are genomic differences between these tissues that might underlie the observed difference in outcome. Therefore, further studies on molecular-marker profiles should elucidate this issue. Since factors, such as BRAF mutational status, sex, and prior adjuvant therapy, have been included in multivariate analyses, they cannot account for the observed effect of sidedness. However, even BRAF wild-type tumors may contain a BRAF mutant–like gene expression signature, which is most often present in right-sided mCRC.

In conclusion, sidedness of the primary tumor in patients with mCRC has a strong prognostic value, which should be included in the design of future clinical trials. The predictive results on tumor sidedness should be interpreted with caution due to the retrospective nature of the analyses, which were performed in the cecum, ascending colon, hepatic flexure, and transverse colon; whereas, left-sided tumors are commonly defined as tumors originating in the splenic flexure, descending colon, sigmoid, and rectum. As to its predictive value for anti-EGFR treatment in (K)RAS and BRAF wild-type tumors, all retrospective analyses that have been performed to date show that the benefit of anti-EGFR treatment is limited to mCRC patients with left-sided primary tumors, with no benefit being shown for mCRC patients with right-sided tumors. These results apply to anti-EGFR treatment + chemotherapy as a first- and second-line therapy and as a monotherapy in late-line treatment. The situation is more complex regarding the use of chemotherapy + bevacizumab, an antibody against vascular endothelial growth factor (VEGF), vs anti-EGFR treatment. Currently, the results of three randomized trials—PEAK, FIRE-3, and CALGB/SWOG 80405—on chemotherapy + bevacizumab vs chemotherapy + anti-EGFR are available for this analysis, where: (i) PEAK was a randomized phase 2 trial; (ii) FIRE-3 showed an unexpected benefit in median overall survival for the anti-EGFR treatment arm, which may be related to the use of salvage treatments and therefore not to the comparison of primary interest, while the results on response rate and median progression-free survival were highly comparable; and (iii) the results of CALGB 80405 did not confirm the overall survival benefit observed in FIRE-3. A meta-analysis of these trials showed a superior efficacy for chemotherapy + bevacizumab in right-sided tumors and a preference for chemotherapy + anti-EGFR treatment in left-sided tumors. However, this latter conclusion leans heavily on the effect of sidedness on median overall survival, which is strongly influenced by the FIRE-3 data, which, with respect to overall survival, is questionable.

C. J. A. Punt, The Netherlands

C. J. A. Punt, MD, PhD
Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105AZ Amsterdam
THE NETHERLANDS
(email: c.punt@amc.uva.nl)
formed on subpopulations of patients included in these trials, and because none of these studies contemplated a full treatment sequence strategy. However, currently available data strongly suggest that mCRC patients with right-sided primary tumors should not be treated with anti-EGFR antibodies irrespective of RAS/BRAF mutational status. In these patients as well as in mCRC patients with left-sided RAS/BRAF/V600E mutated tumors, chemotherapy + bevacizumab is the treatment of choice. In patients with left-sided RAS/BRAF wild-type tumors, both chemotherapy + bevacizumab and chemotherapy + anti-EGFR are valid treatment options, and any possible preference for the latter option should be confirmed in further trials.

References