Molecular profiling in the treatment of colorectal cancer: focus on regorafenib

Yiyi Yan
Axel Grothey
Department of Medical Oncology, Mayo Clinic, Rochester, MN, USA

Abstract: Metastatic colorectal cancer (mCRC) is a highly heterogeneous disease. Its treatment outcome has been significantly improved over the last decade with the incorporation of biological targeted therapies, including anti-EGFR antibodies, cetuximab and panitumumab, and VEGF inhibitors, bevacizumab, ramucirumab, and aflibercept. The identification of predictive biomarkers has further improved the survival by accurately selecting patients who are most likely to benefit from these treatments, such as RAS mutation profiling for EGFR antibodies. Regorafenib is a multikinase inhibitor currently used as late line therapy for mCRC. The molecular and genetic markers associated with regorafenib treatment response are yet to be characterized. Here, we review currently available clinical evidence of mCRC molecular profiling, such as RAS, BRAF, and MMR testing, and its role in targeted therapies with special focus on regorafenib treatment.

Keywords: metastatic colon cancer, targeted therapy, molecular profiling, regorafenib

Introduction

Metastatic colorectal cancer (mCRC) is a significant cause of mortality and morbidity in the US. In 2015 alone, an estimated 132,700 newly diagnosed cases of CRC and an estimated 49,700 deaths from CRC were expected. Approximately 25%–30% of patients with newly diagnosed CRC have evidence of metastases upon diagnosis.\(^1\) While some patients with mCRC with limited metastatic spread can be rendered free of disease long-term by a multidisciplinary approach, for the majority of the patients, treatment generally consists of medical therapy with palliative intent. Since the 1980s, 5-fluorouracil based chemotherapy has been the backbone of therapy, with the incorporation of oxaliplatin and irinotecan in the 1990s. Over the past decade, the addition of targeted biologic agents, such as antibodies against the EGFR monoclonal antibodies (mAbs), cetuximab, panitumumab, and inhibitors of the VEGF system like bevacizumab and aflibercept, to combination chemotherapy has changed the landscape of mCRC treatment, with a doubling of the median overall survival (OS) and improved long-term survival. Regorafenib, an oral multikinase inhibitor, was later approved in 2012, providing further treatment options for mCRC patients who failed multiple lines of therapy.

Despite the promising outcome of targeted therapies in mCRC, primary and secondary resistance to therapy remains a clinical challenge in mCRC. As we advance our knowledge in mCRC tumor biology and genetics, it is of special importance to understand the underlying molecular profiles in relationship to targeted therapy. This allows the development of reliable biomarkers to more accurately select patients who will have greatest benefit from such therapy. Although multiple molecular makers have been well established to guide some targeted therapy for mCRC, exemplified by...
RAS testing for EGFR mAbs, there is no validated molecular testing to improve the outcome with regorafenib treatment.

In this review, we summarize existing evidence of the value of molecular profiling in mCRC for targeted therapies with special focus on the currently available clinical evidence on molecular biomarkers associated with regorafenib treatment.

Overview of current biomarkers in CRC treatment

RAS analysis and anti-EGFR treatment in mCRC

*RAS* encodes a family of small GTP-binding proteins that act as self-inactivating signal transducers in response to stimulation of a cell surface receptor, including EGFR. Oncogenic mutations of *KRAS* are found in approximately 40% of mCRC tumors. It results in constitutive activation of the RAS/RAF/ERK pathway, rendering EGFR inhibitor ineffective. *KRAS* and *NRAS* are closely related *RAS* oncogene family members, and CRCs can harbor mutations in either gene, which tend to be mutually exclusive, suggesting functional redundancy. Resistance to anti-EGFR therapies can also be mediated by any activating mutation in exons 2, 3, and 4 of *KRAS* and *NRAS*.

Multiple recent studies have established the role of RAS analysis in identifying patients who are likely to respond to anti-EGFR agents.

Panitumumab is a fully human mAb for EGFR extracellular domain. Its benefit as monotherapy in chemotherapy-refractory mCRC was demonstrated in the 408 trial, comparing panitumumab with best supportive care (BSC) to BSC alone in patients unselected for *KRAS* status. The panitumumab treated population had improved median progression-free survival (PFS) (8 weeks vs 7.3 weeks, hazard ratio [HR], 0.54; 95% confidence interval [CI], 0.44 to 0.66, *P*<0.0001). Post hoc analysis based on the *KRAS* status (exon 2 with codon 12 and codon 13) was later carried out based on the previous observations that mutant *KRAS* might correlate with poor prognosis in mCRC and other types of tumors. This reanalysis showed that the benefit of panitumumab was limited to patients with *KRAS* wild-type (wt) CRC.

Extended *RAS* analysis was also performed on 408 trial data. In *KRAS* wt patients, effect of panitumumab treatment on PFS was studied on multiple genotypes including *NRAS*, *BRAF*, *PIK3CA*, *AKT*, *TP53*, and *CTNNB1*. A favorable PFS benefit with panitumumab treatment was observed among those with wt *NRAS* (HR, 0.39; 95% CI, 0.27–0.56) and wt *BRAF* (HR, 0.37; 95% CI, 0.24–0.55), but not mutant *NRAS* (HR, 1.94; 95% CI, 0.44–8.44, *P*=0.379).

The detrimental impact of panitumumab treatment in patients with *RAS* mutation beyond *KRAS* exon 2 was observed in multiple studies.

For example, in the PRIME trial, the association of *RAS* mutations beyond *KRAS* exon 2 and anti-EGFR treatment efficacy was assessed in patients treated with panitumumab plus FOLFOX4 vs FOLFOX4 alone. Tumors were analyzed for full spectrum of *RAS* mutations (*KRAS* and *NRAS* exon 2, 3, 4) as well as *BRAF* V600E mutation. In patients without any *RAS* mutations, panitumumab plus FOLFOX4 was associated with a significant improvement in PFS and OS as compared to FOLFOX4 alone (median PFS 10.1 vs 7.9 months, *P*=0.004; median OS 26.0 vs 20.2 months, *P*=0.04). In the subgroup of patients carrying *RAS* mutations other than *KRAS* exon 2, shorter PFS and OS was associated with panitumumab combination treatment than with FOLFOX4 alone was shown, consistent with the outcome observed in patients with *KRAS* exon 2 mutated tumors. These results confirmed the role of *RAS* mutations beyond *KRAS* exon 2 as predictive markers for an adverse outcome for panitumumab treatment, suggesting the importance of extended *RAS* testing to provide the greatest treatment benefit with panitumumab.

Another anti-EGFR agent, cetuximab, an IgG1 chimeric monoclonal EGFR antibody was also extensively studied in mCRC treatment. It binds to the EGFR, competitively inhibiting ligand binding and inducing receptor dimerization and internalization. The efficacy of cetuximab vs panitumumab was compared in *KRAS* wt chemotherapy-refractory patients in the ASPECT trial, a non-inferiority Phase 3 study. Panitumumab was demonstrated to be non-inferior to cetuximab, with a median OS of 10.0 months vs 10.4 months, respectively (HR, 0.97; 95% CI, 0.84–1.11).

The efficacy of cetuximab compared to BSC in patients with metastatic CRC was assessed in the NCIC CO.17 trial. Cetuximab improved OS and PFS in patients with detectable EGFR regardless of *KRAS* status. Benefit in OS and PFS with cetuximab treatment was significantly greater in patients with wt *KRAS* (exon 2, codons 12/13) (median OS 9.5 vs 4.8 months; HR, 0.55; 95% CI, 0.41–0.74; median PFS 3.7 months vs 1.9 months; HR, 0.40; 95% CI, 0.30–0.54, *P*<0.001). However, the OS in patients receiving BSC was not affected by *KRAS* mutation status.

In the CRYSIAT trial, the efficacy of cetuximab treatment in combination with FOLFIRI vs FOLFIRI alone as first-line therapy in mCRC was investigated. This trial...
demonstrated the benefit of cetuximab in PFS, OS, and tumor response, and these benefits were limited to KRAS wt patients.\textsuperscript{14,15}

Taken together, these clinical trials demonstrated the importance of extended RAS mutation analysis, rather than just in KRAS exon 2, in optimal patient selection to benefit from anti-EGFR therapy. According to current guidelines,\textsuperscript{16} comprehensive mutation testing in KRAS and NRAS exon 2, 3, and 4 is mandated for consideration of anti-EGFR therapy; cetuximab and panitumumab should be avoided for patients with any RAS mutations.

**BRAF mutations and RAF/MEK inhibitor treatment in CRC**

Although extended RAS testing allows identification of appropriate patients to benefit from anti-EGFR treatment, a significant subset of patients with wt RAS fail to show improved outcome from such a therapy. Therefore, recognition of other biomarkers beyond RAS would optimize the outcome of personalized treatment.

In addition to RAS, activating mutations in BRAF, predominantly V600E, are among one of the first events in colorectal carcinogenesis, and are identified in 8\%-10\% of CRC patients. They are considered as mutually exclusive to RAS mutations.\textsuperscript{17} BRAF mutant tumors are associated with typical clinical characteristics, including right-sided, high-grade mucinous histology, high frequency of lymph node and peritoneal metastasis and microsatellite instability (MSI), with distinctive gene expression patterns.\textsuperscript{18,19} A correlation between BRAF V600E mutation and poor prognosis and aggressive phenotype has been well demonstrated.\textsuperscript{15,20}\textsuperscript{21}

Given the aggressiveness of BRAF mutated CRC, intensification of first-line chemotherapy has shown outcome benefit. FOLFOXIRI plus bevacizumab have been shown to improve PFS and OS in BRAF mutated mCRC as compared to FOLFIRI plus bevacizumab, justifying the intensive upfront approach in this subgroup of patients with an unfavorable prognosis.\textsuperscript{22,23}

The predictive value of BRAF mutation status in outcomes of anti-EGFR treatment was also assessed in multiple clinical studies and meta-analyses. In KRAS wt patients, favorable effects of anti-EGFR mAbs on OS, PFS, and overall response rate (ORR) were observed in patients with wt BRAF CRC compared to BRAF mutants.\textsuperscript{5,24}\textsuperscript{25} Additionally, according to a recent meta-analysis examining the impact of cetuximab and panitumumab in BRAF-mutated mCRC patients, there is no overall benefit (OS, PFS, and ORR) with anti-EGFR treatment in patients with BRAF mutated tumors.\textsuperscript{25}

Unlike in melanoma, treatment with BRAF inhibitor failed to provide benefit in BRAF mutated mCRC.\textsuperscript{26} Treatment resistance of CRC to BRAF inhibitor, vemurafenib, was found to be caused by feedback activation of EGFR, mediated through the MAPK/ERK pathway. In vivo and in vitro studies demonstrated synergistic activity of vemurafenib and EGFR inhibitor in BRAF mutated CRC.\textsuperscript{27,28} In addition, hyperactivation of PI3K/Akt/PTEN pathway can also result in BRAF inhibition resistance.\textsuperscript{29,30} These preclinical studies provided rationale supporting combination target therapies in BRAF mutated CRC (Table 1).

Combination regimens including a BRAF inhibitor have been investigated for the treatment of BRAF mutant CRC in multiple recent clinical trials. Dabrafenib, a BRAF inhibitor, combined with the MEK inhibitor, trametinib, showed low activity in ORR and PFS.\textsuperscript{31} Combination of BRAF inhibitor and anti-EGFR mAbs, panitumumab or cetuximab, was also examined by different groups. In a recent pilot clinical trial,\textsuperscript{32} vemurafenib plus panitumumab treatment was able to provide modest clinical benefit. Similar clinical outcome was also demonstrated when cetuximab was combined with vemurafenib.\textsuperscript{33}

Based on the in vitro evidence that combined EGFR and MEK inhibitors synergize to inhibit BRAF mutated CRC,\textsuperscript{27} dabrafenib plus panitumumab was evaluated with or without trametinib.\textsuperscript{34} Triplet treatment demonstrated a response rate (RR) of 26\% compared to 10\% in the dabrafenib plus panitumumab group with tolerable toxicity profile.\textsuperscript{34} Given the important role of PI3K/Akt/PTEN pathway in the BRAF inhibitor treatment resistance, a specific PI3K inhibitor, alpelisib, was studied in combination with encorafenib, 

### Table 1: Studies of BRAF-targeted therapies in mCRC

<table>
<thead>
<tr>
<th>Studies (Author)</th>
<th>Treatment</th>
<th>ORR</th>
<th>PFS (months)</th>
</tr>
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<tbody>
<tr>
<td>Kopez et al\textsuperscript{26}</td>
<td>Vemurafenib</td>
<td>5%</td>
<td>2.1</td>
</tr>
<tr>
<td>Falchook et al\textsuperscript{34}</td>
<td>Dabrafenib</td>
<td>10%</td>
<td>NR</td>
</tr>
<tr>
<td>Corcoran et al\textsuperscript{25}</td>
<td>Dabrafenib + trametinib</td>
<td>12%</td>
<td>3.5</td>
</tr>
<tr>
<td>Yaeger et al\textsuperscript{32}</td>
<td>Vemurafenib + panitumumab</td>
<td>13%</td>
<td>3.2</td>
</tr>
<tr>
<td>Tabernero et al\textsuperscript{33}</td>
<td>Vemurafenib + cetuximab</td>
<td>20%</td>
<td>3.2</td>
</tr>
<tr>
<td>Atreya et al\textsuperscript{34}</td>
<td>Dabrafenib + panitumumab</td>
<td>10%</td>
<td>3.4</td>
</tr>
<tr>
<td>Atreya et al\textsuperscript{34}</td>
<td>Dabrafenib + panitumumab + trametinib</td>
<td>26%</td>
<td>4.1</td>
</tr>
<tr>
<td>Tabernero et al\textsuperscript{35}</td>
<td>Encorafenib + cetuximab</td>
<td>23%</td>
<td>4.0</td>
</tr>
<tr>
<td>Tabernero et al\textsuperscript{35}</td>
<td>Encorafenib + cetuximab + alpelisib</td>
<td>32%</td>
<td>4.4</td>
</tr>
<tr>
<td>Hong et al\textsuperscript{36}</td>
<td>Vemurafenib + cetuximab + irinotecan</td>
<td>35%</td>
<td>7.7</td>
</tr>
</tbody>
</table>

**Abbreviations:** mCRC, metastatic colorectal cancer; ORR, overall response rate; PFS, progression-free survival; NR, not reported.
a BRAF inhibitor, and cetuximab. Addition of alpelisib improved RR to 32% compared to 23% in encorafenib plus cetuximab only group.\textsuperscript{35} A triplet regimen including standard chemotherapy resulted in remarkably long PFS (median PFS of 7.7 months) and a high RR of 35% when irinotecan was added to vemurafenib plus cetuximab.\textsuperscript{36} These significant improvements in treatment of this aggressive subtype of mCRC substantiate the importance of further understanding and identification of biomarkers in CRC.

**MSI and PD-1 blockade in mCRC**

Over the last decade, the understanding of the immune checkpoint pathways, such as the PD-1 pathway, has paved the way for immunotherapy in solid tumors.

The PD-1 receptor is expressed on the cell surface of activated T-cells under normal conditions. By binding to its ligand (PD-L1 and PD-L2), PD-1 downregulates T-cell activation and therefore dampens and excessive immune responses, including autoimmunity.\textsuperscript{37,38} The interaction between PD-1 expressed on tumor and stromal cells and PD-1 on T-cells can trigger inhibitory signaling pathways that reduce effector cell functions and T-cell-killing capacity,\textsuperscript{39} enabling tumor cells to evade immune surveillance. Blocking the PD-1/PD-L1 interaction by antibodies has led to remarkable antitumor response in different types of solid tumors, including non-small-cell lung cancer, renal cell carcinoma, and bladder cancer.\textsuperscript{40–45} PD-1 blockade also demonstrated activity towards CRC, although with a low response rate compared to in other types of tumors.\textsuperscript{43,46} Success in PD-1 blockade has led to a search for molecular and genetic biomarkers that correlate to treatment response. Although an association was observed between high baseline PD-L1 expression and clinical response, patients with low/negative PD-L1 expression also benefit from PD-1 blockade.\textsuperscript{40} The presence of pretreatment tumor-infiltrating CD8 T-cells has later been shown to tightly correlate with anti-PD-1 response.\textsuperscript{47,48} However, this correlation lacks tumor specificity.

Deficiency in the expression of MMR enzymes can be seen in a small fraction of mCRC patients, which results in MSI and a higher somatic mutation burden than in MMR proficient mCRC.\textsuperscript{49,50} These mutations expressed by tumors can not only be exploited as a basis for targeted therapies, but can also stimulate anti-tumor immunogenicity through the expression of tumor specific antigens (neoantigens). Previous studies have shown that MSI is associated with more prominent lymphocyte infiltration and that tumors with higher somatic mutations are more responsive to immunotherapy.\textsuperscript{43,50}

MMR status was recently demonstrated to be associated with a response to anti-PD-1 treatment in CRC.\textsuperscript{51,52} In a recent Phase 2 trial, clinically relevant activity was observed with pembrolizumab, a humanized antibody against PD-1 in MMR-deficient CRC, with immune-related ORR of 40% and immune-related PFS at 20 weeks of 78%, respectively, compared to 0% and 11% for MMR-proficient CRCs. Response rate and disease control rates by “response evaluation criteria in solid tumors (RECIST)” criteria were 40% and 90% in MMR-deficient CRC, and 0% and 11% in MMR-proficient CRC.\textsuperscript{52,53} This study demonstrated a role of MSI testing as a potential molecular biomarker to select CRC patients for individualized immune treatment.

**Molecular profiling and regorafenib**

**Regorafenib in treatment of mCRC**

Regorafenib is an oral multikinase inhibitor approved for the treatment of patients with mCRC who have previously been treated with fluoropyrimidine-, oxaliplatin-, and irinotecan-based chemotherapy; anti-VEGF therapy, and anti-EGFR agent if KRAS wt.\textsuperscript{54} It potently inhibits both intracellular and membrane-bound angiogenic and stromal RTKs that promote tumor neovascularization and vessel stabilization in the microenvironment, including VEGF receptor 1–3, PDGFR-β, TIE2, and FGF receptor 1. It also inhibits oncogenic RTKs, such as RET, KIT, and wt and V600E mutated BRAF (Table 2).\textsuperscript{55} Anti-proliferative effects through RAF/MEK/ERK signaling and anti-tumor activity were observed in vitro assays in CRC, breast cancer, and renal cell carcinoma mouse xenograft models.\textsuperscript{55} In the murine metastatic CRC model, regorafenib showed strong anti-angiogenic, anti-tumorigenic, and anti-metastatic effects, suggestive of its potential in the treatment of advanced CRCs.\textsuperscript{56}

A Phase 1/1b study to assess the pharmacokinetics, toxicity, and response of regorafenib was conducted in 38 patients

**Table 2** Biochemical activities of regorafenib in in vitro kinase assays

<table>
<thead>
<tr>
<th>Kinase targets\textsuperscript{55}</th>
<th>Regorafenib IC\textsubscript{50} (nM) ± SD</th>
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<tbody>
<tr>
<td>VEGFR1</td>
<td>13±0.4</td>
</tr>
<tr>
<td>TIE2</td>
<td>311±46</td>
</tr>
<tr>
<td>PDGFR-β</td>
<td>22±3</td>
</tr>
<tr>
<td>FGFR1</td>
<td>202±18</td>
</tr>
<tr>
<td>KIT</td>
<td>7±2</td>
</tr>
<tr>
<td>RET</td>
<td>1.5±0.7</td>
</tr>
<tr>
<td>RAF-1</td>
<td>2.5±0.6</td>
</tr>
<tr>
<td>B-RAF</td>
<td>2.5±0.10</td>
</tr>
<tr>
<td>B-RAF\textsuperscript{v600e}</td>
<td>19±6</td>
</tr>
</tbody>
</table>

**Abbreviations:** IC\textsubscript{50}, inhibitory concentration of 50%; SD, standard deviation.
with heavily pretreated CRC, including anti-EGFR antibody in KRAS wt patients (Table 3). One partial response and 19 stable diseases were seen among 27 evaluable patients. Median PFS was 107 days (95% CI, 66–161). The most commonly seen treatment-related toxicity included hand-foot skin reaction, fatigue, and rash.37,58

The CORRECT trial, a multinational, multicenter, randomized, placebo-controlled, Phase 3 trial was conducted based on the Phase 1/1b safety and efficacy data, with a primary endpoint of OS.59 A total of 760 patients were randomized to either regorafenib or placebo in a 2:1 ratio. Improved median OS was observed in the regorafenib group compared to the placebo group, 6.4 months vs 5.0 months, respectively (HR, 0.77; 95% CI, 0.64–0.94, one-sided P=0.0052).

This benefit of regorafenib in OS was also demonstrated in a second multinational multicenter randomized Phase 3 trial, CONCUR trial, conducted in 204 Asian patients.60 Patients were randomized to either regorafenib or placebo in a 2:1 ratio. After a median follow-up of 7.4 months, a significant prolonged OS was observed associated with regorafenib treatment compared to placebo (HR, 0.55; 95% CI, 0.44–0.77, one-sided P=0.00016); with median OS of 8.8 months in the regorafenib-treated group vs 6.3 months in the placebo group.

Interestingly, in the CORRECT trial, although no difference in median PFS between the regorafenib or placebo group (1.9 vs 1.7 months, respectively) was seen, a PFS HR of 0.49 (95% CI, 0.42–0.581, P=0.0001), adjusted for stratification factors, was highly statistically significant, indicating a 51% reduction in the hazard of disease progression or death for patients receiving regorafenib. The Kaplan–Meier curves diverged after around 50% of the patients had progressed at the first scan obtained at 8 weeks.59 A similar observation was made in the CONCUR trial,60 suggesting a refined subgroup of patients is likely to benefit from such therapy.

Despite the statistically significant benefit of regorafenib observed in Phase 3 clinical trials, a substantial group of patients failed to respond to or continue to progress with the treatment. The toxicity profile can also make treatment with regorafenib challenging, in particular in the palliative salvage therapy setting. Therefore, further identification and validation of potential molecular markers might help to refine the patient population that will most likely benefit from regorafenib and to avoid unnecessary adverse effects in patients who are not likely to respond.

**Molecular markers in oncogenic pathways**

RAS/RAF/PI3K/AKT signaling pathways are essential in EGFR-mediated CRC oncogenesis. Multiple biomarkers identified in these pathways have improved the success of other targeted therapies, and their associations with regorafenib response have been recently investigated.

In the CORRECT trial, 57% of patients harbored a KRAS mutation; however, KRAS status was not predictive of OS or PFS associated with regorafenib.59 Seventy percent of patients were assessed for KRAS status, and 21% were analyzed for BRAF mutation status in the CONCUR trial. Consistent with the findings in the CORRECT trial, neither KRAS nor BRAF was predictive for a response to regorafenib.60

Further biomarker analysis on CORRECT trial data also confirmed that regorafenib provided clinical benefits in various mutational subgroups.61 Correlative analysis based on mutational status by BEAMing62 of circulating plasma DNA demonstrated regorafenib benefit across patient groups defined by KRAS and PIK3CA mutational status, and that no significant difference was revealed between wt and mutant tumors for either KRAS or PIK3CA, consistent with additional anti-angiogenesis activity of regorafenib.63 The correlation between the circulating DNA level and clinical outcome was also assessed in the same study. Although a high circulating DNA level does not predict regorafenib clinical response, it is associated with shorter OS than that in patients with a lower baseline circulating DNA level regardless of treatment.

Gene expression profile (eg, chromosomal instability, deficient MMR, KRAS mutant) was obtained in tumors from

<table>
<thead>
<tr>
<th>Studies (Author/Year)</th>
<th>Design</th>
<th>Treatment</th>
<th>Median PFS (months)</th>
<th>Median OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strumberg et al57 (2012)</td>
<td>Phase 1, non-randomized, n=38</td>
<td>60-220 mg daily (21 days on/7 days off)</td>
<td>3.6 (95% CI, 2.2–5.6)</td>
<td>NR</td>
</tr>
<tr>
<td>Grothey et al59 (2013)</td>
<td>Phase 3, randomized (regorafenib/placebo =2:1, n=760)</td>
<td>160 mg daily (21 days on/7 days off)</td>
<td>1.9 vs 1.7 (PFS HR, 0.49; 95% CI, 0.42–0.581, P&lt;0.0001)</td>
<td>6.4 vs 5.0 (HR, 0.77; 95% CI, 0.64–0.94, one-sided P=0.0052)</td>
</tr>
<tr>
<td>Li et al58 (2015)</td>
<td>Phase 3, randomized</td>
<td>160 mg daily (21 days on/7 days off)</td>
<td>3.2 vs 1.7 (PFS HR, 0.31; 95% CI, 0.22–0.44, P&lt;0.0001)</td>
<td>8.8 vs 6.3 (HR, 0.55; 95% CI, 0.40–0.77, P=0.00016)</td>
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</table>

**Abbreviations:** mCRC, metastatic colorectal cancer; PFS, progression-free survival; OS, overall survival; CI, confidence interval; HR, hazard ratio; NR, not reported.
patients, suggesting a prognostic value of these plasma clinical outcomes was detected in the placebo-treated mCRC association between high IL-8 and PIGF levels and poor Oncology, 2015). Also consistent with previous findings, observations (Tabernero et al manuscript in press in Lancet higher regorafenib benefit in OS, consistent with previous of soluble protein TIE-1 were found to be associated with increased risk of progression. concentrations of angiopoietin and soluble TIE2, a crucial step of tumor growth and metastasis in CRC.\(^\text{66–69}\) It is a complex network of pathways controlled by naturally-occurring growth factors, including VEGF, FGF, and PDGF system. It is also regulated by HIF-1, a transcription factor that induces VEGF expression. Anti-angiogenic therapies, such as bevacizumab, sorafenib, and sunitinib, have shown benefit in various solid tumors, including mCRC. Regorafenib is among the small molecule kinase inhibitors with anti-angiogenic activity.

The identification and validation of molecular markers for successful angiogenesis inhibition is an ongoing challenge, with most of the evidence based on bevacizumab treatment in the pre-regorafenib era. Despite extensive research, no validated angiogenesis biomarkers have yet been established. For example, studies have failed to show the predictive value of tumor VEGF expression levels or baseline circulating VEGF level in bevacizumab treatment response.\(^\text{66–69}\)

The association between regorafenib response and the level of plasma biomarkers involved in angiogenesis has been previously reported in a small clinical study.\(^\text{70}\) Increased VEGF-family ligands concentration (eg, PIGF), and decreased TIMP2 and soluble VEGF receptor 2 and TIE1 levels, were detected in response to regorafenib. Higher baseline TIMP2 and soluble TIE1 were correlated with tumor shrinkage with regorafenib treatment, while higher baseline concentration of angiopoietin and soluble TIE2, a crucial regulator of angiogenesis, seemed to be associated with an increased risk of progression.\(^\text{70}\)

Angiogenesis biomarkers were also analyzed in correla- tive studies embedded in the CORRECT trial. Higher levels of soluble protein TIE-1 were found to be associated with higher regorafenib benefit in OS, consistent with previous observations (Tabernero et al manuscript in press in Lancet Oncology, 2015). Also consistent with previous findings,\(^\text{71,72}\) an association between high IL-8 and PIGF levels and poor clinical outcomes was detected in the placebo-treated mCRC patients, suggesting a prognostic value of these plasma proteins. These findings will, however, need to be validated in further studies.

Genotyping in VEGF and VEGFR was also used to identify single nucleotide polymorphisms to determine regorafenib treatment outcome in mCRC. In a recent study, 138 samples from mCRC patients were tested for single nucleotide polymorphisms in VEGF-A, VEGF-C, and VEGFR1-3.\(^\text{73}\) and VEGF-A rs2010963 showed a correlation with PFS and OS, with HR of 0.49 (95% CI, 0.33–0.81) and 0.52 (95% CI, 0.34–0.99), respectively, indicating its use as patient selection tool for consideration for regorafenib treatment.

**Future directions**

The understanding of the molecular profile and the identification of biomarkers associated with targeted therapies’ outcomes is crucial for the success of personalized medicine. Despite ongoing investigation, no validated biomarkers are currently available to guide the use of regorafenib; therefore, further research efforts are essential to delineate its role in the treatment of mCRC.

Regorafenib is currently used as a treatment option in mCRC after progression on other standard therapies, a disease with high biologic heterogeneity. Given the multiple kinase inhibition profile of regorafenib, a full understanding of the complex network of pathways, especially the cross-talk and overlapping of angiogenesis and oncogenesis pathways, is critical for the continued search for meaningful molecular markers. It is very likely that a biomarker signature, rather than a single marker, will predict the activity of regorafenib in individual patients. The potential molecular markers might also need to be evaluated in combination with biomarkers involving other biological pathways in order to demonstrate the abilities to predict clinical outcomes.

The lack of available angiogenesis biomarkers also makes molecular profiling of mCRC treated with regorafenib more challenging. Multiple potential molecular markers in angiogenesis were evaluated in previous studies, including circulating soluble KIT levels as potential markers for sunitinib treatment in gastrointestinal stromal tumors (GIST),\(^\text{74}\) CD31 and PDGFR-A in bevacizumab treated breast cancer,\(^\text{75}\) cytokine TNF-α in sunitinib treated renal cell carcinoma,\(^\text{76}\) and polymorphisms in IL-8 allele in bevacizumab treated ovarian cancer,\(^\text{77}\) which will benefit our future research efforts. In the era of next generation sequencing, extended genetic analysis will also facilitate our understanding of the genetic landscape of mCRC.

One of the main challenges of targeted therapy is secondary resistance to treatments. Biomarker-guided patient
selection might help to increase the success rate of such treatments. However, a substantial group of patients eventually develop secondary therapy resistance and progress, which reflects additional molecular and genetic modifications of tumors, suggesting a role of further, longitudinal biomarker monitoring and the identification of secondary biomarkers to guide the next line of treatment. This is of high importance for regorafenib treatment since most patients conceivably have already developed resistance towards other previous targeted therapies and the majority of patients who initially respond to regorafenib later show disease progression. Reevaluation of the molecular profiling upon secondary treatment failure might identify potential therapeutic targets with potential to improve the outcomes on regorafenib.

Understanding the treatment of mCRC at the molecular level is an actively growing field. Treatment outcomes have been significantly improved over the last decade with the availability of biomarkers associated with treatment response. Identification of new robust molecular markers based on our knowledge of mCRC biology will further improve survival outcome through accurate patient selection, not only in regorafenib treatment, but also in other novel therapies for mCRC.

Disclosure
The Mayo Clinic Foundation received grant funding from Bayer Oncology for research led by author AG. The authors report no other conflicts of interest in this work.

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515(7528):568–571.


