Review Article

Current and evolving biomarkers for precision oncology in the management of metastatic colorectal cancer

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Abstract: Colorectal cancer remains one of the most common cancers worldwide and is almost uniformly fatal for those with metastatic disease. Despite this, there is an increasing number of treatments and overall survival has been increasing. Utilizing increasing knowledge of tumor biology, there have been 13 new FDA drug approvals and 5 additional drugs that appear on the National Comprehensive Cancer Network (NCCN) guidelines that await FDA approval since 2002. Still, there is great need for many patients for additional treatment options. In the following text, we review our current clinical and molecular knowledge as it pertains to treatment of patients with metastatic colorectal cancer and future directions regarding therapeutic vulnerabilities.

Keywords: Metastatic colorectal cancer (mCRC); molecular heterogeneity; biomarkers

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Introduction

As the 3rd most common cancer worldwide, colorectal cancer (CRC) is the 2nd leading cause of death, accounting for an estimated 1.8 million new diagnoses and 880,000 deaths globally in 2018. Worldwide, the incidence of CRC diagnoses varies significantly with rates almost 4-fold higher in regions with high human development index (HDI). Three distinct global trends have been observed and linked to levels of regional development with increases in incidence and mortality in Russia, China and Brazil, with increasing incidence but decreasing mortality in the UK and Singapore, and with decreasing incidence and mortality in the United States and Japan (1,2). Rises in incidence have been attributed to worldwide dietary changes, obesity and lifestyle factors. Conversely, declining mortality has been linked to robust screening patterns and adoption of best practices (3-5). While in the United States CRC rates have been decreasing by about 2 percent per year population wide, the incidence of patients with CRC younger than age 50 is increasing. Colon cancer and rectal cancer are estimated to increase by 90 percent and 124 percent, respectively in patients 20–34 years old by 2030 (6,7).

Approximately 50–60% of patients who are diagnosed with CRC will eventually develop metastatic disease (8). Most often, metastases develop after treatment for locoregional disease, however 20–34% of those with CRC will present with synchronous metastatic disease. Unfortunately, the majority of patients who present with metastatic CRC (mCRC) have inoperable disease (9-11). The standard of care for these patients is antineoplastic agents with an ultimate goal of improving quality of life and prolonging survival.

5-fluorouracil represented the mainstay of treatment in patients with mCRC from 1962 until 1996. In the span of the following 6 years, the FDA approved three new cytotoxic

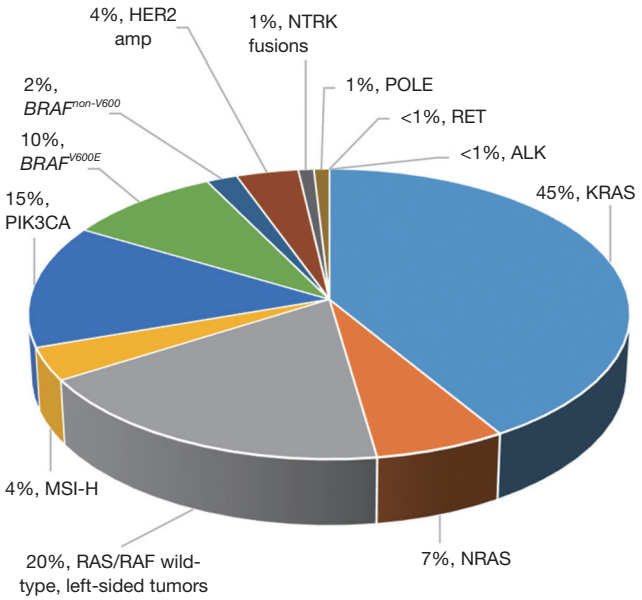

Figure 1 The landscape of molecular heterogeneity in metastatic colorectal cancer. HER2, human epidermal growth factor receptor 2; NTRK, neurotrophic receptor tyrosine kinase; MSI-H, microsatellite-instability high; PIK3CA, phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha.

agents starting with irinotecan, followed by capecitabine in 2001 and oxaliplatin in 2002 (12). Since 2002, there have been 13 new FDA drug approvals and 5 additional drugs that appear on the National Comprehensive Cancer Network (NCCN) guidelines but still await FDA approval. Therefore, the current arsenal of drugs include monoclonal antibodies (mAbs) that target anti-epidermal growth factor receptor (EGFR) (cetuximab and panitumumab), vascular endothelial growth factor/vascular endothelial growth factor receptor (VEGF/VEGFR) (bevacizumab, ramucirumab, and ziv-aflibercept), small molecule tyrosine kinase inhibitors (TKIs) (regorafenib, binimetinib/encorafenib, trametinib/dabrafenib, vemurafenib, larotrectinib), and immune checkpoint inhibitors, including mAbs targeting anti-programmed death-1 (PD-1, nivolumab and pembrolizumab) as well as anti-cytotoxic T-lymphocyte antigen-4 (CTLA-4, ipilimumab). Trifluridine/tipiracil is the only cytotoxic agent that has been approved since 2002 (10).

With significant advances in systemic chemotherapeutic and biologic options for mCRC, outcomes have improved with median overall survival (OS) now exceeding 30 months (10,11). However, long term outcomes still remain poor with 5-year OS for patients with metastatic disease estimated at only 14.2%, declining to 7.4% in patients older than 65 (13,14). While continual clinical investigation for novel treatment modalities remains imperative, honing in on distinct molecular subsets of CRC has allowed us to leverage targeted therapy in a meaningful way. Over the last several years, more robust research has demonstrated that mCRC is not one entity, but rather consistent with significant molecular heterogeneity (*Figure 1*). These advances in understanding the molecular and cellular mechanisms of mCRC increasingly affect prognosis and treatment decisions. Furthermore, this underlying biologic heterogeneity explains the tremendous variability noted in regards to treatment outcomes, especially in the advanced setting. Today, molecular biomarkers are a critical component of the management of mCRC, serving as both prognostic and predictive tools. In light of this, we are becoming progressively aware of the proper utilization of pharmacologic agents at our disposal. In this review, we will summarize the current literature on molecular biomarkers in CRC and their impact on treatment decision making, as well as highlight evolving biomarkers of increasing clinical significance.

Established molecular biomarkers in CRC

RAS

The mitogen-activated protein kinase (MAPK) pathway, altered in many cancers, is essential in a number of cellular pathways. Dysregulation leads to uncontrolled cellular proliferation, survival and dedifferentiation. The first step in initiation of the MAPK pathway occurs when ligands such as growth factors, cytokines or hormones bind to extracellular membrane receptor tyrosine kinases such as EGFR. Binding activates RAS, which activates BRAF, followed by MEK kinase (MEK1 and MEK2), and finally ERK (15).

The rat sarcoma viral oncogene (RAS) was discovered in 1982 and marked the first discovered mutated gene in disease (16). Since that time, there have been over 500 validated genes in cancer (17), but the three RAS genes (*HRAS*, *NRAS*, and *KRAS*) still represent the most mutated oncogene family in cancer (30%) and an estimated 52% of all CRCs. *KRAS* is the most frequently isolated form, representing 86% of RAS mutations in CRC, followed by 14% *NRAS* mutations (18). The majority of *KRAS* mutations in colon cancer affect codons 12 (30%) and 13 of exon 2 (8%). An additional 6% of mutations are found in

KRAS exons 3 and 4, while 5% of mutations are in *NRAS* exons 2, 3 or 4 (19).

RAS genes encode a 21-kDa monomeric GTPase downstream of EGFR that transduces extracellular signal to intracellular signals by binding growth factors to cell membrane receptors and helps mediate signals related to cell survival, senescence and other cell survival signals.

They are essential components of the EGFR signaling cascade, and mutations can isolate the pathway from EGFR signaling. RAS proteins are GTP-binding proteins, with GTPase functionality that switches between the active and inactive state. Missense mutations in RAS proteins alter binding toward the active state (20). While complex, the two major signaling pathways that RAS proteins affect are the MAPK and the phosphoinositide-3 kinase (PI3K) pathways.

Increasing understanding of CRC carcinogenesis has led to the production of mAbs that target EGFR. EGFR is a commonly expressed transmembrane glycoprotein that is a member of the human epidermal growth factor receptor (HER) tyrosine kinase growth factor receptor family and is encoded by proto-oncogene *c-erb B*. Activation of *c-erb B* proto-oncogene results in expression of EGFR, which prompted interest as a potential target for anticancer therapy (21). Upon extracellular ligand binding to growth factors, receptor dimerization occurs, and the intracellular tyrosine kinase is activated, prompting downstream signaling, including RAS (22,23).

EGFR is often upregulated in CRC and presents an opportunity for therapeutic intervention by preventing signal transduction of dependent pathways involving RAS, PI3K and MAPK pathway. With this strategy in mind, two mAB's, cetuximab and panitumumab, were tested and found to improve OS in patients with metastatic colon cancer. Cetuximab (a chimeric IgG1 mAb) and panitumumab (a fully human mAb) both bind to the extracellular domain of EGFR, and block ligand induced receptor signaling and therefore tumor growth (24,25). Shortly after drug approval, there were a number of early studies that suggested that mutations in *KRAS* conferred resistance to cetuximab (26,27). Post hoc analysis of both CRYSTAL (FOLIRI +/– cetuximab) and PRIME (FOLFOX +/– panitumumab) demonstrated irrevocable resistance when RAS-mutant mCRC patients receive anti-EGFR mAb therapy (27,28). As we have learned, and discuss further below, only 10–20% of patients can expect benefit from EGFR inhibition (EGFRi) (10).

RAS wild type (WT) and tumor sidedness

Primary tumor location in mCRC has emerged as a prognostic and potentially predictive tool. The NCCN defines right sided tumors as those that arise in the cecum to the hepatic flexure, while left sided tumors are those from the splenic flexure to rectum. Initial signals reflecting the impact of sidedness were noted in a first-line chemotherapy study revealing right sided tumors exposed to 5-FU based chemotherapy had worse outcomes than left sided tumors, with a difference of at least 5 months in survival (29). These findings have since been reproduced in a more recent analysis of two randomized phase III trials, AVF2107g and NO16966. Both trials utilized frontline chemotherapy in combination with bevacizumab for treatment-naïve mCRC. Tumor location analysis revealed right sided tumors as a negative prognostic variable irrespective of mucinous histology or mutational status, with agnostic efficacy of bevacizumab (30). A post hoc analysis of RAS WT populations in the CRYSTAL, PRIME, PEAK and FIRE-3 trials found that the benefit of anti-EGFR therapy in terms of OS and progression-free survival (PFS) remained only for patients with left sided tumors (31-34). The most convincing evidence for the predictive lack of response of EGFR inhibitors in regards to tumor sidedness stems from the CALGB/SWOG 80405 study. The authors found that among RAS WT patients treated with chemotherapy + cetuximab, there was a statistically significant reduction in OS of 13.6 months for right sided tumors in contrast to 39.3 months for left sided tumors [P=0.001, hazard ratio (HR) 0.55] (35). Bevacizumab exposure, however, remained agnostic in terms of survival outcomes for RAS WT left *vs.* right sided tumors (P=0.50).

Current guidelines, both from the European Society of Medical Oncology (ESMO) and NCCN, recommend against treating patients with RAS-mutant mCRC with anti-EGFR therapy, either alone or in combination with chemotherapy. In regards to RAS-WT tumors, due to the retrospective findings of CALGB 80405 highlighting sidedness as a reliable biomarker of response and selection for anti-EGFR therapy, a paradigm shift in the management of mCRC has emerged. In light of this practice changing data, the NCCN definitively supports excluding patients with RAS-WT right sided tumors from exposure to EGFR inhibitors in the first-line setting (ESMO and NCCN). However, whether or not right sided RAS-WT patients should be treated with EGFRi in subsequent lines of therapy, remains an area with limited data and therefore a

lack of consensus guidelines. Nonetheless, in the landmark CO.17 trial that confirmed lack of benefit of cetuximab compared to best supportive care for refractory *KRAS* mutant tumors, there was no statistically significant benefit with cetuximab among RAS WT right sided colon cancers compared to left sided colon cancers (36). Additionally, two smaller studies showed a similar trend for lack of benefit with anti-EGFR therapy in second line and beyond for right sided tumors; however, these cohorts are too small for definitive conclusions (37). Further studies are required to elucidate whether anti-EGFR therapy is appropriate in subsequent lines of therapy for patients with RAS WT right sided CRC.

BRAF^{V600E}

BRAF is a serine threonine kinase downstream of RAS in the MAPK pathway and almost always exclusive of RAS mutations (10). BRAF mutations in CRC consists of a valine to glutamic acid change at codon 600 (c.1799T>A or p.V600E), and mutations result in a constitutively active protein, representing 96% of all BRAF V600 mutations (17,38). While *BRAF^{V600E}* mutations are relatively uncommon in CRC with an estimated incidence of 7–14%, their extremely poor prognosis has warranted intense clinical and research focus (10,11,39,40). Of note, *BRAF^{V600E}* mutations have distinct clinicopathologic factors including right sided tumors, high grade, older age, female sex, T4 tumors, mucinous histology, poorly differentiated tumors, and microsatellite instability (41-43). They tend to have higher rates of peritoneal disease, distant nodal involvement and brain metastases, but a lower incidence of lung metastases (44). Tumorigenesis in CRC occurs via specific molecular pathways, most commonly the classical (*APC*, *KRAS*, *p53*) pathway or the germline mutation (Lynch syndrome) pathway. However, the BRAF mutation is associated with a serrated adenoma precursor via the serrated/methylated pathway manifesting in a hypermethylated phenotype, which can result in inactivation of MLH1 causing a mismatch repair (MMR) deficiency and microsatellite instability (40,45). BRAF mutational status remains a strong prognostic factor for OS in both the metastatic setting and early stage disease (46). In the adjuvant setting, patients with *BRAF^{V600E}* CRC tend to have shorter disease-free survival and worse OS after recurrence (47). Unsurprisingly, these patients tend to have diminished response to therapy with a median OS of around 12 months (48-50).

While the negative prognostic role of *BRAF^{V600E}* is clear, historically, its predictive role in the first-line setting is less established. Initial retrospective studies were conflicting in terms of benefit to anti-EGFR therapy (51,52). For example, an unplanned retrospective subset analysis demonstrated that patients whose tumors were treated with FOLFIRI + cetuximab *vs.* FOLFIRI alone who were *KRAS* WT/BRAF mutant, had an improved OS of 14.1 *vs.* 10 months (53). Additionally, a planned subset analysis of the PRIME study demonstrated that *BRAF^{V600E}* mutations were not predictive of benefit to panitumumab added to FOLFOX (54). Conversely results from the COIN trial suggested no benefit with addition of cetuximab to FOLFOX with a trend towards harm (55). In subsequent lines of therapy, the lack of benefit from mABs is clearer. One retrospective study demonstrated that patients with *BRAF^{V600E}* had only an 8.3% response rate to cetuximab (56). Additionally, data from the PICCOLO study suggested harm with the addition of panitumumab to irinotecan in subsequent line settings (57). Of note, a large meta-analysis of 9 phase III and 1 phase II trial (six 1st line trial, two 2nd line trials, two chemotherapy refractory trials) evaluated a total of 463 pooled *BRAF^{V600E}* patients. This analysis revealed that the addition of anti-EGFR therapy to standard chemotherapy or best supportive care did not significantly improve PFS, OS, or overall response rate in patients with *BRAF^{V600E}* mCRC compared to control regimens. Although this analysis remains limited by size, being underpowered, non-randomized and retrospective in nature, there remains a notable signal that exposure to anti-EGFR therapy has limited clinical efficacy both as monotherapy or in combination with chemotherapy in *BRAF^{V600E}* mCRC (58). In line with this data, both the NCCN and ESMO suggest against the utilization of EGFRi in isolation or in combination with chemotherapy for patients with *BRAF^{V600E}* mCRC (10,11).

Unlike *RAS/RAF* WT mCRC, patients who harbor *BRAF^{V600E}* mutation do not benefit from exposure to multiple lines of chemotherapy, with historical median PFS of 2.5 months among those who received second- and third-line treatment (59). Therefore, initial therapeutic decisions for this unique patient population remain critical. There is a growing body of evidence demonstrating viable alternate treatment approaches for these patients. The TRIBE study compared bevacizumab + FOLFIRI to bevacizumab + 5-FU + oxaliplatin + irinotecan (FOLFOXIRI) for treatment naïve unresectable mCRC. The study met its primary endpoint of improved PFS with an increase by 2.4 months for patients

randomized to the intensified triplet arm compared to standard doublet chemotherapy. Of note, there were 28 patients in this study with *BRAF*^{V600E} mutant disease of whom 16 received FOLFOXIRI + bevacizumab (B). While numbers are small in this subgroup analysis, patients in the FOLFOXIRI + B arm had an improved OS of 19 *vs.* 10.7 months in the FOLFIRI + B arm, consistent with historical norms (60). Based on these results, FOLFOXIRI + B remains the best chemotherapeutic option for patients with *BRAF*^{V600E} mutation and adequate performance status in the first-line setting. It is sobering to note that comparatively *RAS/RAF* WT patients exposed to triplet chemotherapy had a median OS of 41.7 months in this study, highlighting the need for novel therapeutic strategies for this chemotherapy refractory subtype.

With encouraging results in melanoma, interest increased in BRAF inhibition in mCRC. Unfortunately, early studies demonstrated very little activity of vemurafenib and encorafenib with response rates of 5% and 0% respectively (61,62). This finding was somewhat unsurprising as there was little benefit in pre-clinical studies. However, it was discovered that while BRAF inhibition transiently induces impairment in the MAPK pathway, rapid reactivation of ERK occurs through activation of RAS and RAF (40). With this mechanism in mind, early phase trials demonstrated modest but improved clinical efficacy with combined BRAF and MEK inhibition with dabrafenib and trametinib and a response rate of 12%, including one complete response (63). Expanding on the concept of dual inhibition, the Southwest Oncology Group (SWOG) 1406 randomized 106 previously treated mCRC patients to irinotecan, and cetuximab, with or without vemurafenib, allowing crossover at progression. The authors found an improved PFS of 4.4 *vs.* 2.0 months and response rates of 16% *vs.* 4% in the vemurafenib arm (64).

More recently the phase III BEACON study, a 3-arm trial for treatment refractory *BRAF*^{V600E} mCRC comparing a BRAF inhibitor (encorafenib) + MEK inhibitor (binimetinib) + cetuximab *vs.* encorafenib + cetuximab *vs.* standard of care (irinotecan/FOLFIRI +cetuximab), demonstrated impressive improvement on previous strategies by meeting primary endpoints of ORR and OS. Of note, based on a press release on 5/21/19, the OS HR was reported at 0.52 with improved response rate of 26% (*vs.* 2% in control) and an even higher response rate noted in 2nd line (34%) (65). Based on these exciting results, the NCCN has recommended the BEACON regimen of encorafenib in combination with binimetinib and cetuximab and triplet regimens of dabrafenib/trametinib/EGFR mAb as available treatment options for *BRAF*^{V600E} mCRC, representing for the first time a completely targeted therapeutic approach for these high-risk patients (10).

Microsatellite-instability high (MSI-H)

MSI-H or deficient MMR (dMMR) tumors represent approximately 4–5% of all mCRC patients with a vastly different prognosis and exciting additional treatment options in the form of immunotherapy (10,11,66,67). Microsatellites are repetitive DNA sequences which represent a large portion of our genome and are susceptible to errors in insertion and deletions during DNA replication. During DNA synthesis, the primer and template strands in a microsatellite may become mismatched and the number of repeating strands may differ between the two strands. In normal functioning cells, these errors are reconciled by the MMR system. When MMR is defective, mistakes are replicated resulting in MSI and hypermutations (68).

Mutations in MMR may be hereditary or acquired. Germline mutations in MMR genes including *MLH-1*, *MSH2*, *MSH6* and/or *PMS2* or *EpCAM* are mutations found in patients with hereditary nonpolyposis CRC, also known as Lynch syndrome, and represent 1–6% of all CRCs (69). Somatic MMR defects are estimated to occur in 19% of all CRC patients and are associated with higher rates of tumor infiltrating lymphocytes (70). As mentioned above, the rates of patients with MSI-H and metastatic disease are much lower with studies ranging from 3.5–5.5% of patients (71,72). Intriguingly, multiple studies suggest improved prognosis for patients with stage II CRC. Specifically, the QUASAR study demonstrated a reduced risk of recurrence of 11% for those with dMMR in contrast to 26% for those with proficient mismatch repair (pMMR) (73).

Tumors with dMMR contain thousands of mutations which allow for easier recognition and targeting by the immune system. With the advent of checkpoint inhibitors, this has made for an intriguing new therapeutic strategy for treating this subset of patients. Specifically, strategies targeting programmed death ligand 1 (PD-L1) on tumor cells or its counterpart PD-1 on T cells have been evolving. Pembrolizumab and nivolumab, two IgG4 mAbs targeting PD-1, have shown promising, practice changing durable activity. Nivolumab was investigated in the Checkmate-142 in two separate cohorts, as single agent or in combination with ipilimumab, a fully human IgG1 mAb that targets the CTLA-4. In the single agent arm, including heavily pre-treated patients, the objective

response rate (ORR) was 31.1% with 69% of patients demonstrating disease control at 12 weeks, with a median OS of 73% at 1 year (74). In the combination cohort, the ORR was 55% with a disease-control rate of 80% and an OS of 85% at 1 year. The combined efficacy came at a cost of 32% grade 3–4 treatment related events (75). Pembrolizumab also has demonstrated activity as a single agent in a recent phase II study. The study investigated pembrolizumab in tumors with MMR deficiency and included 11 patients with dMMR mCRC, 21 patients with pMMR CRC and an additional 9 patients with dMMR in the CRC. In the patients with mCRC dMMR group, a response rate of 40% and a PFS of 78% at 20 weeks were observed (67). This data, in conjunction with the nivolumab data, is indicative that MSI is a predictive marker for response to checkpoint blockade.

With this data in mind, both the NCCN and ESMO guidelines suggest universal testing for MMR or MSI with a dual purpose of identifying patients with Lynch syndrome to provide prognostic information for patients with stage II disease and to advise on use of immunotherapy in the advanced setting. NCCN recommends pembrolizumab, nivolumab or nivolumab plus ipilimumab as recommended treatment options for patients with advanced or metastatic dMMR CRC in second or third-line settings or for those patients "not appropriate for intensive therapy" (10,11).

HER2 amplified

HER2 is a member of the EGFR receptor family, and altered signaling may be caused by genomic amplification of *ERBB2* or mutations. Activation leads to upregulation of the MAPK and PI3K pathways (76). *HER2* amplifications are seen in 3–5% of all CRC, are mutually exclusive from *RAS/RAF* mutations, and have been associated with resistance to EGFR inhibitors (77). *ERBB2* is far more commonly amplified in breast cancer and has been exploited as a target with therapeutic efficacy with mAbs such as trastuzumab and pertuzumab. Similar approaches are emerging in CRC and potentially represent a new therapeutic approach for these patients. The HERACLES study enrolled patients with *KRAS* WT, and *HER2* positivity defined as tumors with 3+ HER2 score in more than 50% of cells by immunohistochemistry or with 2+ HER2 score and a HER2:CEP17 ratio higher than two in more than 50% of cells by fluorescence in situ hybridization (FISH). Patients were treated with trastuzumab (HER2 mAb) and lapatinib (an oral dual HER2/EGFR kinase inhibitor) after failure of standard therapies. Twenty-seven patients were enrolled and 8 patients (30%) had an objective response with one patient obtaining a complete response (4%) (78). More recently, the phase IIa MyPathway, a multiple basket study, reported their data on treatment-refractory, histologically confirmed *HER2*-amplified mCRC treated with dual HER2-targeted therapy with pertuzumab plus trastuzumab. Fifty-seven eligible patients were enrolled with 18 patients having an objective response (32%), one of which obtained a complete response (79). Based on these results the NCCN has now recommended the use of trastuzumab and lapatinib or trastuzumab and pertuzumab for HER2-amplified refractory mCRC patients (10). Considering the data revealing HER2 amplification as a negative predictive biomarker for anti-EGFR therapy, the SWOG1613 (NCT03365882) is an ongoing phase II clinical trial for anti-EGFR naïve *RAS/RAF* WT mCRC patients who have received at least 2 prior lines of therapy, randomizing patients to trastuzumab and pertuzumab *vs.* cetuximab and irinotecan.

NTRK fusions

Neurotrophic receptor tyrosine kinase fusion genes (NTRK) encode for TrkA, TrkB, and TrkC receptor tyrosine kinases that are important in the function of the nervous system in human neuronal tissue. Activation of these proteins leads to activation of the MAPK and PI3K pathways (80). Biologic models found that fusions lead to oncogenic addiction irrespective of tissue of origin. These gene fusions are rare, found in an estimated 0.2% to 2.4% of all CRCs, but may be enriched in subpopulations, specifically the MSI-H cohort (3%) (81). The landmark 2018 *NEJM* paper evaluated the efficacy of larotrectinib, a highly selective small molecule inhibitor of three TRK proteins, in 55 adult and pediatric patients with NTRK fusions. Among all patients, the overall response rate was 75% with 55% remaining progression free at 1 year. Three patients had colon cancer, 2 of whom had an objective response. Following receipt of this data, the FDA granted accelerated approval of larotrectinib for all solid tumors. This is only the second time the FDA has approved a drug, independent of cancer type. The NCCN has since followed suit and now recommends testing for NTRK gene fusions and has incorporated larotrectinib as a possible treatment option in guidelines due to its emergence as a biomarker of predictive response in mCRC (82). While we agree with the recommendations to test, more outcomes data is needed for patients with CRC. Furthermore, considering the

extremely low prevalence in CRC, one way to increase the yield of identifying this biomarker is to strategically screen in MSI-H patients.

Emerging molecular biomarkers in CRC

Consensus molecular subtypes (CMS)

As the diverse biology in CRC is increasingly appreciated, so are our efforts to further classify molecular subtypes to inform individualized treatment efforts. With this in mind, The Cancer Genome Atlas (TCGA) evaluated >276 colorectal carcinoma samples using exome sequencing, DNA copy number, promoter methylation, messenger RNA and microRNA expression. Their findings demonstrated that 16% of CRC are hyper mutated, with most of these representing MSI-H primarily with hyper methylation and *MLH1* silencing. However, 25% of these patients had somatic MMR gene and *POLE* mutations. In all other patients, the TCGA found recurrent alterations in the WNT, MAPK, PI3K, TGF-β and p53 pathways. Molecular alterations in the WNT pathway were found in 94% of patients, most prominently APC. Regardless of mutations, nearly 100% of tumors had alterations in MYC transcription and confirmed an important role of MYC in CRC (83).

Building on these efforts, the International Colorectal Cancer Consortium classified patients into 4 defined CMS, CMS-1, CMS-2, CMS-3, and CMS-4 (84). Data was assembled from six CRC subtyping algorithms which were each developed independently using gene expression sets. CMS-1, the MSI immune, represents 14% of all patients and have higher rates of MSI, CpG island methylator phenotype (CIMP) high, hypermutation, and *BRAF* mutations. While the landscape is changing, these patients have historically had worse survival rates after relapse. CMS-2, or canonical subtype, represents 37% of patients, and they typically have higher rates of somatic copy number alterations and high rates of WNT and MYC activations. CMS-3, the metabolic subtype, represents 13% of all patients. This group represents those with lower rates of copy number alterations, high rates of *KRAS* mutations and metabolic dysregulation. CMS-4, the mesenchymal subtype, represents 23% of all patients and are those with high rates of TGF-β activation and angiogenesis. CMS-4 class tends to have worse OS (84). An additional 13% of patients did not fit into a specific subtype.

The development of CMS subtyping reflects an international collaboration to characterize mCRC in a more robust format, one that moves beyond isolated mutational status to a transcriptomic based model that accounts for the interplay between various critical molecular pathways (72). Previous work has revealed the emerging prognostic and potential predictive implications of CMS classification. One study reports CMS-4 as a poor prognostic group irrespective of receipt of adjuvant chemotherapy (85). Additional retrospective analyses also highlight CMS-4 as a potential predictive biomarker due to limited efficacy noted with oxaliplatin and EGFRi, irrespective of *RAS* status (86). Furthermore, a single hospital series applying CMS subtyping to 409 CRCs, stages I–IV, revealed CMS-4 has a 5-year relapse free survival rate of 47% compared with 67% for CMS-1–3 (87). These prognostic findings highlight the need for innovative biomarker driven trial design for the CMS4 subtype.

Poor outcomes in CMS-4 may be a reflection of the high expression of genes associated with T regulatory cells, myeloid-derived suppressor cells, monocyte-derived cells, and TH17 cells in addition to TGF-β activation, all promoting an immune-excluded tumor microenvironment (88). Therefore, understanding this biology will be critical in designing appropriate immunotherapy-based trials for refractory CMS-4, microsatellite stable (MSS) mCRC patients moving forward. To that end, an increasing number of clinical trials that categorize patients and treatments on the basis of their molecular subtype are planned, with one such effort currently underway (NCT03436563).

Despite this prognostic signal, CMS classification is not yet currently included in staging or consensus guidelines with any recommendations based on specific subtypes. However, we expect that CMS subtyping will continue to refine trial design, fostering biologically defined rational drug combinations aimed at patients who are most likely to have a favorable response.

PIK3CA

Phosphatidylinositol-4,5-bisphosphonate 3-kinases (PI3K) are lipid kinases that regulate signaling pathways downstream of EGFR. The PI3K signaling pathway is important in carcinogenesis of multiple cancers. Particularly, *PIK3CA* (the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene) increases downstream AKT-mTOR signaling pathways which promotes cell proliferation, motility and survival (89). Additionally, upregulation of PI3K increasing prostaglandin E2 synthesis,

results in inhibition of apoptosis in colon cancer cells (90). In CRC, *PIK3CA* mutations are found in 10–20% of tumors and are associated with MSI-H, *KRAS* mutations and poorer prognosis (91,92). Currently genetic profiling is not routinely recommended; however, it is included in broad genomic sequencing and has gained interest as a potential therapeutic target.

Intriguingly, aspirin use has been associated with improved survival in patients with *PIK3CA* mutated tumors but not in patients who are *PIK3CA* WT. A retrospective review of two prospective cohort studies found that aspirin was associated with a significantly longer cancer-specific survival (multivariate HR for cancer-related death, 0.18) (93). However, the data is somewhat mixed, and the NCCN believes that colon cancer survivors may consider taking a 325 mg aspirin, although the reduction in recurrences must be weighed against the increase in gastrointestinal (GI) bleeding and hemorrhagic stroke (10).

There are an emerging number of strategies investigating inhibitors targeting PI3K/mTOR pathway. Our institution has demonstrated that the PI3K pathway is associated with increasing immune infiltration and upregulation of immune checkpoints. Current strategies to combine PI3K inhibition with immunotherapy are underway (94). There are a number of additional studies ongoing; however, targeted therapies directed at this pathway are at this point purely investigational.

Non-TRK fusions (ALK, RET)

RET fusions have been described in various solid tumor including thyroid, non-small cell lung cancers and in <1% of CRCs (95). With impressive responses in the other tumor types, there is enthusiasm for use of multi-targeted TKIs with drugs such as regorafenib and cabozantinib or more targeted agents such as RXDX-105. Early data suggests that patients with RET rearrangements have a worse prognosis compared to RET negative patients with a median OS of 14 *vs.* 28 months. In that same study, a single patient with MSI-H, and a RET fusion received the novel selective inhibitor RXDX-105 and has had a CR and is progression free at 19 months (96). Clearly more data is required, but preliminary data has been encouraging.

ALK rearrangements were first discovered in anaplastic lymphoma but were later discovered in numerous other cancers—most prominently non-small cell lung cancer. ALK inhibitors including crizotinib and ceritinib have become standard of care for patients with lung cancer harboring ALK fusions with superior efficacy compared to cytotoxic medications (97). While less frequent, ALK rearrangements have been observed in 0.05–2.5% of mCRC patients. However, there is intrigue, similar to NTRK, as their presence is felt to represent oncogenic drivers exclusive of RAS or BRAF mutations (98). At this juncture, use of ALK inhibitors is limited to case reports, with encouraging early reports (97). However, widespread use is not yet warranted. Clinical trials are ongoing evaluating their use (NCT03792568).

BRAF $^{non\text{-}V600}$

Atypical, non-V600 BRAF (*aBRAF)* mutations are a rare molecular subset of mCRC distinct from *BRAF^{V600E}* (Class I). Pre-clinical data categorized BRAF into those with intermediate-high kinase activity without RAS dependency (class II) and those with low kinase activity that are RAS dependent (class III) (99,100). Alterations in class II or III are less frequent and account for 2.2% of all patients tested or 21.6% of all BRAF mutations in CRC. In contrast to *BRAF^{V600E}*, patients with *aBRAF* are more often MSS, left sided, lower grade, not mutually exclusive from RAS mutations and have decreased rates of peritoneal metastatic disease. In the most robust study to date characterizing *aBRAF*, these patients have a median OS of 60.7 months in contrast to *BRAF^{V600E}* OS of only 11.7 months (101). There is emerging pre-clinical data that suggests that patients with class III mutations may be sensitive to EGFRi (100). However, retrospective data has been less encouraging to date, with one study demonstrating no responses to EGFRi, regardless of class, with class II emerging as a negative predictive biomarker and detection of *aBRAF* in circulating tumor DNA (ctDNA) potentially reflecting a novel mechanism of resistance (102). Currently, there is not enough data to fully support the use of EGFRi for both class II and III *aBRAF* until more prospective data is available. Innovative trial design with novel agents and rational approaches is an area of active investigation and timely for this rare subset of CRC moving forward.

POLE

POLE mutations are an emerging biomarker that may predict response to immunotherapy. The *POLE* gene is located in 12q24.33 and encodes the proofreading exonuclease domain of polymerase epsilon (103). Pathogenic somatic *POLE* mutations occur in an estimated 1.0% of CRCs and are

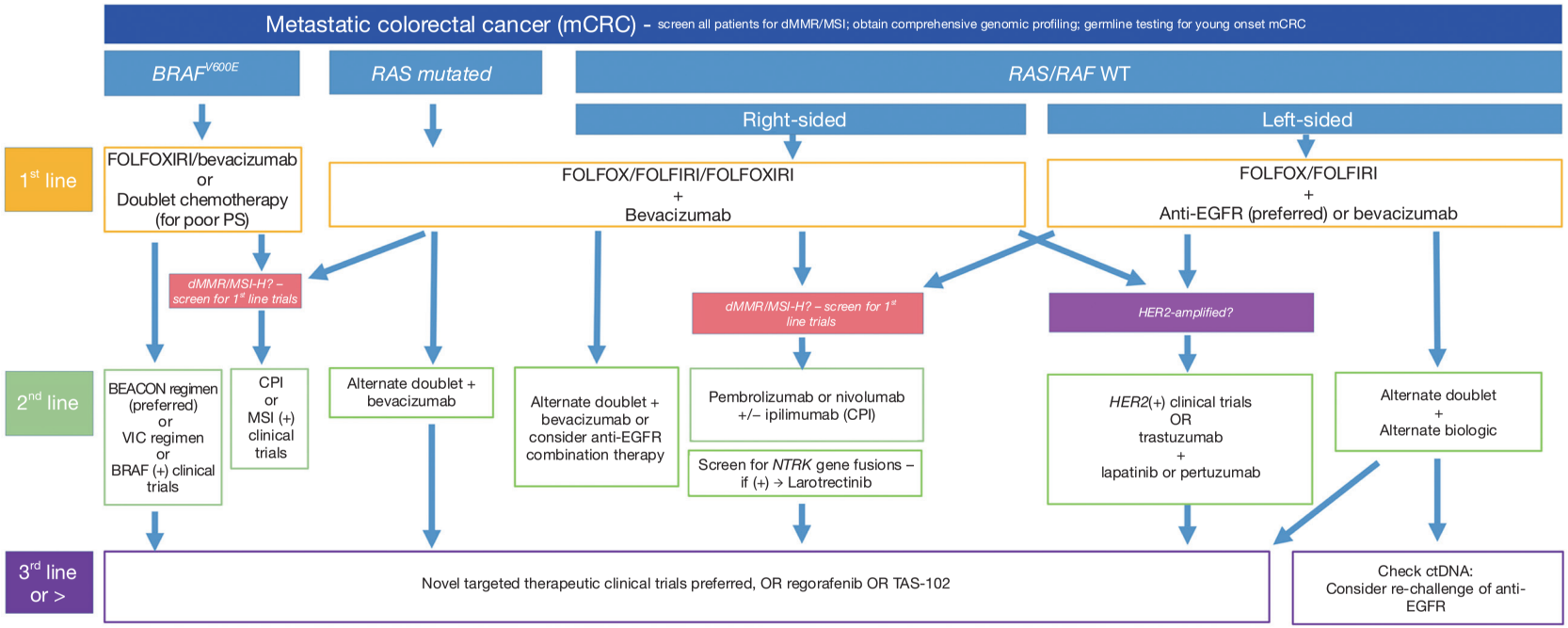

Figure 2 A rationale approach to the management of metastatic colorectal cancer. dMMR, deficient mismatch repair; WT, wild type; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; ctDNA, circulating tumor DNA; NTRK, neurotrophic receptor tyrosine kinase; MSI, microsatellite instability; MSI-H, microsatellite-instability high; CPI, checkpoint inhibitor; PS, performance status; VIC, vemurafenib, irinotecan, cetuximab; FOLFOX, 5-fluorouracil, leucovorin, oxaliplatin; FOLFIRI, 5-fluorouracil, leucovorin, irinotecan; FOLFOXIRI, 5-fluorouracil, leucovorin, oxaliplatin and irinotecan.

mutually exclusive of patients with dMMR (104). Patients who harbor these mutations tend to have hypermutated tumors that harbor increased neoantigen load that may predict a response to immunotherapy (105). Still, there is limited anecdotal data regarding treatment, and as a result, there are no guidelines to date for patients with these mutations.

Conclusions

The presence of distinct molecular biomarkers in mCRC influences clinical presentation, histology, guide treatment decisions and therefore directly impact patient outcomes. Identification of established molecular subtypes such as *RAS*, *BRAF*^{V600E}, *MSI-H*, and *HER2* amplification in CRC is standard of care, highlights the heterogeneity of this disease and supports the use of precision oncology for refined management (*Figure 2*). Ongoing studies to unravel and therapeutically target additional biomarkers of clinical and molecular significance such as CMS subtypes, novel amplifications, non-TRK fusions, *aBRAF* and *POLE* mutations are paramount to moving the needle forward for this malignancy. Future investigation and novel clinical trial design are necessary to allow for thorough exploitation of emerging molecular biomarkers in terms of identifying their predictive, prognostic and therapeutic potential.

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Footnote

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