Review

Right Versus Left Colon Cancer Biology: Integrating the Consensus Molecular Subtypes

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Abstract

Although clinical management of colon cancer generally has not accounted for the primary tumor site, left-sided and right-sided colon cancers harbor different clinical and biologic characteristics. Right-sided colon cancers are more likely to have genome-wide hypermethylation via the CpG island methylator phenotype (CIMP), hypermutated state via microsatellite instability, and *BRAF* mutation. There are also differential exposures to potential carcinogenic toxins and microbiota in the right and left colon. Gene expression analyses further shed light on distinct biologic subtypes of colorectal cancers (CRCs), with 4 consensus molecular subtypes (CMSs) identified. Importantly, these subtypes are differentially distributed between right- and left-sided CRCs, with greater proportions of the "microsatellite unstable/immune" CMS1 and the "metabolic" CMS3 subtypes found in right-sided colon cancers. This review summarizes important biologic distinctions between right- and left-sided CRCs that likely impact prognosis and may predict for differential responses to biologic therapy. Given the inferior prognosis of stage III–IV right-sided CRCs and emerging data suggesting that anti–epidermal growth factor receptor antibody therapy is associated with worse survival in right-sided stage IV CRCs compared with left-sided cancers, these biologic differences between right- and left-sided CRCs provide critical context and may provide opportunities to personalize therapy.

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Colorectal cancer (CRC) is the fourth most commonly diagnosed cancer and the second leading cause of cancer death in the United States,[1] with heterogeneous outcomes and diverse underlying pathobiologic and molecular characteristics. Accordingly, epidemiologic and histologic differences in proximal and distal CRCs have been observed: patients with right-sided CRCs are more likely to be women, be older, and have mucinous, undifferentiated, or signet-ring cell histology compared with those with left-sided CRCs.[2–5] There are also different frequencies of metastasis to different organs, with right-sided CRCs more likely to metastasize to the peritoneum and left-sided CRCs more likely to metastasize to the thorax or, less commonly, bone.[6] The primary tumor site likely serves as a surrogate for underlying biology, including differential pathways of carcinogenesis and varying molecular features.[7] With the availability of genomic platforms capable of broadly surveying gene expression and methylation, as evidenced by The Cancer Genome Atlas,[8] we can now identify genomic subtypes of CRCs that are also differentially distributed between proximal and distal CRCs. This review describes the key molecular and genomic differences between proximal (right-sided) and distal (left-sided) CRCs, shedding light on the heterogeneity of clinical outcomes observed between right- and left-sided CRCs.

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Definition of Right- and Left-Sided CRC

Notably, a consistent definition of the dividing point between right- and left-sided CRCs is not uniformly used. The most common distinction defines cancers proximal to the splenic flexure as right-sided and cancers at or distal to the splenic flexure as left-sided.[7,9,10] This cutpoint is often used because most (proximal two-thirds) of the transverse colon arises embryologically from the midgut, and only the distal one-third arises from the hindgut. Although the midgut also gives rise to other organs, including most of the small bowel and the vermiform appendix,[11] the rare carcinomas arising from these organs have unique biologies with different rates of key driver mutations and are clinically treated distinctly,[12,13] and thus have not been included in most prior studies of CRC. Vascular supply has also been proposed as a defining characteristic of embryologic origin—the superior and inferior mesenteric arteries supply the midgut and hindgut, respectively. However, this information is not routinely recorded during colonic resection. For practical purposes, given the mixed embryologic origin of the transverse colon, some studies exclude the transverse colon from analyses dichotomizing right and left CRCs.[4,14] Rectal cancer is also often excluded,[2,10,15] particularly because localized stage II–III rectal cancers have a different treatment paradigm than colon cancers. However, biologically, based on somatic copy number variation and mRNA and microRNA expression analyses, rectal and colon cancers are indistinguishable.[8] Moreover, systemic therapies for metastatic rectal and colon cancers are identical, and several recent studies have included both in their analyses.[16,17] Indeed, as the frequencies of several important molecular features vary in a gradual continuum spatially from rectum through cecum,[18] dichotomizing the large bowel into right- and left-sided is overly simplistic but does provide an easily captured clinical variable that connotes differing underlying biology, which is described in this review.

Differences in Normal Right and Left Colon

Distinct Embryologic Etiologies

As described earlier, distinct embryologic origins exist for the right and left sides of the colon. These distinct origins and the processes of cell migration and differentiation required during normal embryologic development require distinct gene expression patterns within the midgut and hindgut. Notably, there are differential gradients of various homeobox (HOX) and other genes establishing craniocaudal polarization.[4,11] Furthermore, the rostrocaudal colonization of the intestine by enteric neuron precursors is influenced by specific protein expression, along with the associated topography and biological responses.[19] Therefore, the distinct embryologic underpinnings of right and left colon likely contribute to differences in the underlying biology.

Distinct Environmental Milieus

Distinct Microbiota: The colon houses a rich microbiome of intestinal bacteria, and the presence of several bacterial strains, including *Fusobacterium nucleatum*,[20,21] enterotoxigenic *Bacteroides fragilis*,[22] and *Enterococcus faecalis*,[23] is associated with CRC development. Only slight gradations of differences in the mucosa-associated microbial population distribution in different parts of the colon have been observed,[24,25] indicating that within one individual the colonic microbiome is relatively uniform at different sites along the normal colon. However, there is a marked difference in the mucosal microbiota between patients who develop right- versus left-sided CRCs,[26] including in the presence of bacterial biofilms, defined as mucin layers with admixed bacteria on the luminal surface of the colonic epithelium. Invasive bacterial biofilms were found in 89% of right-sided CRCs (proximal to the hepatic flexure) but in only 12% of left-sided CRCs (distal to the hepatic flexure).[27] Patients who had biofilms on their tumors nearly all had similar invasive biofilms overlying normal colon tissue, even distant from the tumor. These biofilms were associated with significant decreases in epithelial E-cadherin and increased interleukin-6 and activated Stat3, along with increased proliferation.[27] Presence of these biofilms was also associated specifically with increased levels of the pro-proliferative polyamine metabolite N^1, N^{12}-diacetylspermine, indicating a unique symbiosis between the bacterial biofilm and the host cancer.[28] Thus, the organization of colonic microbes into invasive biofilms, with concomitant procarcinogenic epithelial responses, has been hypothesized to contribute to

the development of right-sided CRCs. Although additional work is required, this unique microbiome-associated pathobiology may provide a novel biomarker or a rationale for therapies to modulate the microbiome or target the metabolic or inflammatory pathways that appear to be significant features of these biofilm-associated CRCs.

Differential Bile Acid Levels: The luminal level of bile acids and their metabolites, which are putatively carcinogenic, varies with colonic location and is modulated by microbial enzymatic reactions. Bile acids are normally synthesized and conjugated with glycine and taurine residues by hepatocytes and secreted in bile. These conjugated primary bile acids undergo enzymatic deconjugation, followed by conversion to secondary bile acids such as deoxycholic acid (DCA) by anaerobic colonic microbes. Secondary bile acids are passively reabsorbed through the colonic epithelium, where they cause DNA damage through reactive oxygen and nitrogen species.[29] Epidemiologic studies correlated elevated fecal bile acid concentration with increased CRC incidence,[30,31] and increased DCA consumption in mice induces an increased number of colon cancers.[32] Notably, there is a >10-fold greater concentration of the primary bile acid conjugated cholic acid in the right colon compared with the left colon, and there is greater DCA-forming enzymatic activity in cecal aspirates than in rectal fecal samples.[33] The differential concentrations of bile acids in the right and left colon may therefore contribute to differential mechanisms of carcinogenesis.

Differential Gene Expression and Methylation in Normal Right and Left Colon

There are also differential gene expression profiles between normal right and left colon epithelium. Normal right colon has higher expression of cytochrome P450 family genes than the left colon, suggesting differential exposure to metabolites of ingested substances.[4,34] Similarly, there are significant differences in patterns of gene methylation between the right and left colon.[35] Notably, the prevalence of promoter methylation of the mismatch repair gene *hMLH1* and the O-6-methylguanine-DNA methyltransferase *MGMT* is significantly greater in normal right colon mucosa, especially in older women,[36] suggesting epigenetic aberrations in preneoplastic right colon mucosa that may be reflected in subsequent right-sided adenocarcinoma biology.

Premalignant Tumors Vary by Site

Reflecting the different predisposing factors, premalignant lesions leading to CRC vary substantially between the right and left colon. Sessile serrated adenomas are more prevalent in the right colon. Conversely, conventional tubular and tubulovillous adenomas are more uniformly distributed throughout the colon, although they are more likely to have high-grade dysplasia or associated adenocarcinoma at smaller sizes in the right colon.[37] These adenomas have distinct molecular features and likely serve as precursors of biologically distinct types of CRCs (Table 1). Related partly to the differing biology, physical characteristics, and technical features, colonoscopies and polypectomies are associated with a significant decrease in the prevalence of subsequent left-sided CRCs but not right-sided CRCs.[38,39]

Biologic Characteristics of Colon Cancers That Vary by Side

CIMP, Microsatellite Instability, and Chromosomal Instability

Alternative paths of CRC carcinogenesis that are significantly correlated with either right- or left-sided CRCs have been identified. CRCs that have widespread genome-wide hypermethylation causing epigenetic gene silencing, termed *CIMP-high*, tend to be mutually exclusive from chromosomal instability tumors, defined by marked aneuploidy.[40,41] CIMP-high CRCs are particularly enriched in tumors with microsatellite instability (MSI)–high due to epigen-

Table 1. Molecular Features of Preneoplastic Lesions and CRC by Site

	CIMP-High	MSI-High	*MLH1* Methylation	*BRAF* Mutation	CIN
Preneoplastic lesions					
Sessile serrated adenoma (right-sided)	+	+/–	+/–	+	–
Conventional adenoma (right and left-sided)	–	–	–	–	+
Colorectal cancers					
Right-sided CRC	High prevalence	High prevalence	High prevalence	High prevalence	Low prevalence
Left-sided CRC	Low prevalence	Low prevalence	Low prevalence	Low prevalence	High prevalence

Abbreviations: CIMP, CpG island methylator phenotype; CIN, chromosomal instability; CRC, colorectal cancer; MSI, microsatellite instability.

etic silencing of the *MLH1* mismatch repair gene.[41–43] These CIMP-high/MSI-high CRCs are more likely to be right-sided,[8,43,44] and tumors with chromosomal instability are more likely to be left-sided.[4,8]

CIMP and MSI are prognostic and thus contribute to the clinical differences between right- and left-sided CRCs. CIMP-high CRCs are associated with worse survival,[45–47] particularly in the 50% to 60% of CIMP-high cancers that are microsatellite stable (MSS).[48] The prognostic impact of MSI depends on stage. In stage II and III cancers, MSI-high tumors have superior prognosis compared with MSS tumors.[49–51] Conversely, though only 4% of stage IV CRCs are MSI-high,[52] these tumors have historically been associated with inferior survival.[53] Notably, this inferior prognosis compared with MSS CRCs remained significant in the *BRAF* wild-type subgroup, although there was a uniformly poor prognosis in *BRAF*-mutant tumors that was not significantly altered by microsatellite status.[53] However, given emerging evidence for the efficacy of immune checkpoint antibodies, such as anti–programmed death-1 (PD-1), in the treatment of MSI-high CRC,[54] the prognosis associated with MSI in stage IV disease may soon drastically change. Nevertheless, given the enrichment of CIMP-high and MSI-high cancers in right-sided CRCs and their known prognostic impact, these factors likely contribute to historical differential outcomes by side.

Mutation Profiles

There are different rates of mutations in key oncogenes and tumor suppressors between right- and left-sided CRCs (Table 2).[8] Notably, mutations in *BRAF* V600E, which are associated with significantly inferior survival in stage IV CRC,[53,55,56] are significantly more common in right-sided CRCs.[57] Conversely, mutations in *APC* and *TP53* are enriched in left-sided CRCs.[8] Recently, different patterns of mutations in *APC*, *TP53*, and *KRAS* were identified as conferring differential prognoses in CRC,[58] although given the differences in mutation frequency by site, additional analysis via right or left primary site is warranted. Besides point mutations, potentially targetable amplifications of receptor tyrosine kinases, such as *ERBB2* and epidermal growth factor receptor (*EGFR*), are also more common in left-sided CRCs.[4] These underlying variations in mutation and genomic patterns may explain differential outcomes to therapy or offer potential new therapies for which site would be an important consideration.

Table 2. Rates of Mutations in Key Oncogenes and Tumor Suppressors by Primary Site in The Cancer Genome Atlas Dataset

	Total (n=276)	Right[a] (n=92)	Left[a] (n=161)
APC	75.0%	63.6%	81.9%
TP53	54.0%	34.8%	64.6%
KRAS	42.0%	45.5%	40.3%
PIK3CA	20.1%	27.3%	14.6%
FBXW7	16.5%	22.7%	12.5%
SMAD4	11.6%	15.2%	9.7%
TGFBR2	10.3%	27.3%	1.4%
BRAF	9.4%	24.2%	2.1%
NRAS	8.9%	7.6%	9.0%

[a]Excludes 18 transverse colon and 5 unknown.

Gene Expression Profiles

Several studies have identified CRC subtypes by performing unsupervised gene expression clustering, with several consistent features across all subtyping schemes, such as MSI-high/CIMP-high subtype predominantly in right-sided CRCs and a mesenchymal subtype with poor prognosis. However, there was a lack of consistency among other subtypes delineated, and each classification scheme had between 3 and 6 distinct subtypes identified.[59–64] To unite these disparate subtyping schemes, an international CRC Subtyping Consortium applied multiple independent subtyping classifications across a unified set of 3,962 samples. The association between these disparate classification schemes was determined and 4 consensus molecular subtypes (CMSs) emerged.[65]

The CMSs: Key features of the 4 CMSs are outlined in Table 3. Notably, CMS1, which is predominantly composed of right-sided CRCs, is also enriched for MSI-high, CIMP-high, and *BRAF* mutation. Although genotypic features such as *KRAS* and *BRAF* mutation status are enriched in some subtypes (ie, *BRAF* in CMS1; *KRAS* in CMS3), their presence or absence does not specifically define any subtype, demonstrating the limited value of genotype in defining broader CRC biology. Similarly, there is heterogeneity among right-sided CRCs, such that even though right-sided CRCs are enriched in CMS1

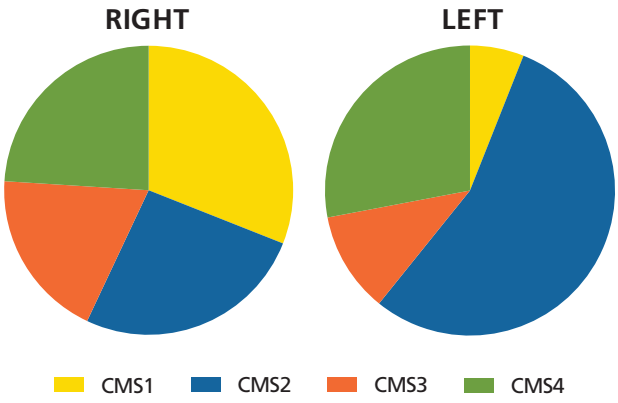

Figure 1. Distribution of CMS by right- and left-sided CRC.[65] Right-side CRC was defined as the cecum through transverse colon, and left-side CRC was defined as the splenic flexure through rectum.
Abbreviations: CMS, consensus molecular subtype; CRC, colorectal cancer.

and CMS3, all 4 CMSs are represented among right-sided CRCs[65] (Figure 1).

The CMSs are associated with prognosis, which varies based on relapse status. CMS4 has the worst overall survival (OS) among all stages and the worst relapse-free survival among those who were initially stages I–III. However, among those who experienced relapse, OS was markedly worse in CMS1 and superior in CMS2. For example, in the PETACC-3 trial, the hazard ratio (HR) for CMS1 versus CMS2 for survival after relapse was 5.00 (95% CI, 2.86–9.09).[65]

The underlying unique biology of each subtype is also reflected by differential activation of various biologic pathways (Table 3). CMS1 has increased immune infiltration signatures, which are expected given the association between mutation burden, neoantigen load, and immune infiltration in CRCs.[66] CMS2 has marked activation of WNT and MYC pathways. CMS3 has marked activation of multiple metabolic pathways. Finally, CMS4 is enriched for signatures of epithelial–mesenchymal transition (EMT), the pro-EMT transforming growth factor-β,[67] and angiogenic pathways. CMS4 is also characterized by particularly high expression of stroma-derived genes associated with cancer-associated fibroblasts.[68,69] Although this does introduce the potential for misclassifying tumors if tumor microdissection is not performed and greater stromal contamination results,[70] a similar poor prognostic mesenchymal subtype was reproducibly independently identified in several classification schemes using microdissected tumors, suggesting that CMS4 is a bona fide subtype with a more prominent stromal microenvironment that helps drive tumor biology.[71]

EREG *and* AREG *Expression:* The EGFR ligands epiregulin (*EREG*) and amphiregulin (*AREG*) are differentially expressed between right- and left-sided CRCs. 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* wild-type metastatic CRCs.[72–74] *EREG* and *AREG* expressions are significantly higher in left-sided CRCs,[4,44,75] and are inversely correlated with promoter methylation and CIMP-high status.[44] Although the association of *EREG* and *AREG* expression and improved outcomes with anti-EGFR inhibitors has been highlighted as evidence of autocrine/juxtacrine EGFR signaling driving CRC growth, the strong association of sidedness and *EREG*/*AREG* expression suggests that part of the effect may be confounded by other differences in CRC biology.

CRC Side and Clinical and Prognostic Impact

Stage II–III Disease

There is differential prognosis by stage between patients with right- and left-sided CRCs. Tumor registries and retrospective cohorts suggest that right-sided tumors have a slightly better prognosis in stage II colon cancer, but slightly worse prognosis in stage III disease, likely associated with the higher prevalence of good-prognosis MSI-high tumors in right-sided stage II cancers.[2,9,10] Moreover, analyses of prospective clinical trials of patients with stage III CRC who received adjuvant chemotherapy also demonstrated inferior disease-free survival in those with right-sided CRCs (HR, 0.70; 95% CI, 0.61–0.81).[76]

Stage IV Disease

Patients with metastatic CRC with a right-sided primary also have inferior prognosis compared with those with a left-sided primary.[17] This is highlighted by a pooled analysis of 3 studies of 2,027 evaluable patients treated with first-line chemotherapy, in which those with left-sided CRCs had significantly better progression-free survival (PFS) and OS compared with those with right-sided CRCs, including after adjusting for *BRAF* mutation and mucinous histology.[77]

Table 3. Key Features of the 4 Consensus Molecular Subtypes

	CMS1 MSI/Immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
Proportion of total samples	14%	37%	13%	23%
% right-sided (vs left-sided)	77%	23%	51%	35%
Grade 3 (vs 1–2)	45%	5%	12%	19%
MSI-high	76%	1%	16%	8%
CIMP-high	67%	3%	16%	10%
Hypermutated (≥180 events)	94%	6%	28%	9%
Somatic copy number alteration–high	20%	92%	54%	84%
KRAS mutated	23%	28%	68%	38%
NRAS mutated	4%	7%	9%	4%
BRAF mutated	42%	1%	7%	7%
Gene set enrichment analysis	Immune infiltration Cytotoxic T cell infiltration T_H1 infiltration PD-1 activation Natural killer cell infiltration	Epithelial signature MYC activation WNT activation SRC activation	Epithelial signature Sugar/amino acid/ nucleotide metabolism Fructose/mannose metabolism Glutamine metabolism	Epithelial mesenchymal transition Matrix remodeling Stromal infiltration TGF-β activation VEGF-VEGFR activation

Abbreviations: CIMP, CpG island methylator phenotype; CMS, consensus molecular subtypes; MSI, microsatellite instability; TH1, type 1 helper T cell; PD-1, programmed death-1; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.
Data from Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. Nat Med 2015;21:1350–1356.

Primary CRC site is prognostic but not predictive of outcome with bevacizumab-based therapy. An analysis of 2 prospective randomized controlled trials of chemotherapy with or without bevacizumab found that a statistical interaction test between side and bevacizumab use was nonsignificant, indicating that side is not a predictive biomarker for or against benefit of bevacizumab.[77]

However, primary CRC site is both prognostic and predictive of benefit with anti-EGFR therapy among patients with *KRAS* wild-type, refractory, metastatic CRC. In the CO.17 randomized trial, among those with *KRAS* codon 12/13 wild-type disease, there was a significant improvement in PFS with cetuximab in patients with a left-sided primary (HR, 0.28; 95% CI, 0.18–0.45), whereas there was no difference in PFS among those with a right-sided primary (HR, 0.73; 95% CI, 0.42–1.27; interaction *P*=.002).[15] Additional retrospective studies also showed that patients with left-sided CRCs had better PFS with anti-EGFR therapy compared with those with right-sided CRCs, even among patients with *KRAS*/*BRAF* wild-type[4] and extended *RAS* and *BRAF* wild-type mutations.[78]

Similar results have been found with first-line cetuximab combined with chemotherapy among patients with CRC wild-type in *KRAS* and *NRAS* exons 2 to 4 (pan-RAS wild-type). In the CALGB/SWOG 80405 study randomizing patients with *KRAS* wild-type metastatic CRC to receive first-line chemotherapy with either cetuximab or bevacizumab, 474 patients were known to be pan-RAS wild-type. In this study, a right-sided primary was associated with inferior OS among the cetuximab group (HR, 1.81 vs left-sided; 95% CI, 1.27–2.56). PFS was also significantly worse among those with a right-sided primary who received cetuximab, but was not significantly worse among those who received bevacizumab,[16] thus indicating that a right-sided primary is a predictive biomarker of inferior survival with cetuximab-based chemoimmunotherapy (interaction *P*=.0009 for OS).[16] These results are corroborated by an analysis of the 394 patients with pan-RAS wild-type metastatic CRC randomized to receive first-line FOLFIRI with either cetuximab or bevacizumab in the FIRE-3 study. In this trial, there was again inferior OS with a right-sided primary, markedly among patients who received cetuximab (median OS, 18.3 vs 38.3 months; *P*<.001), but also among

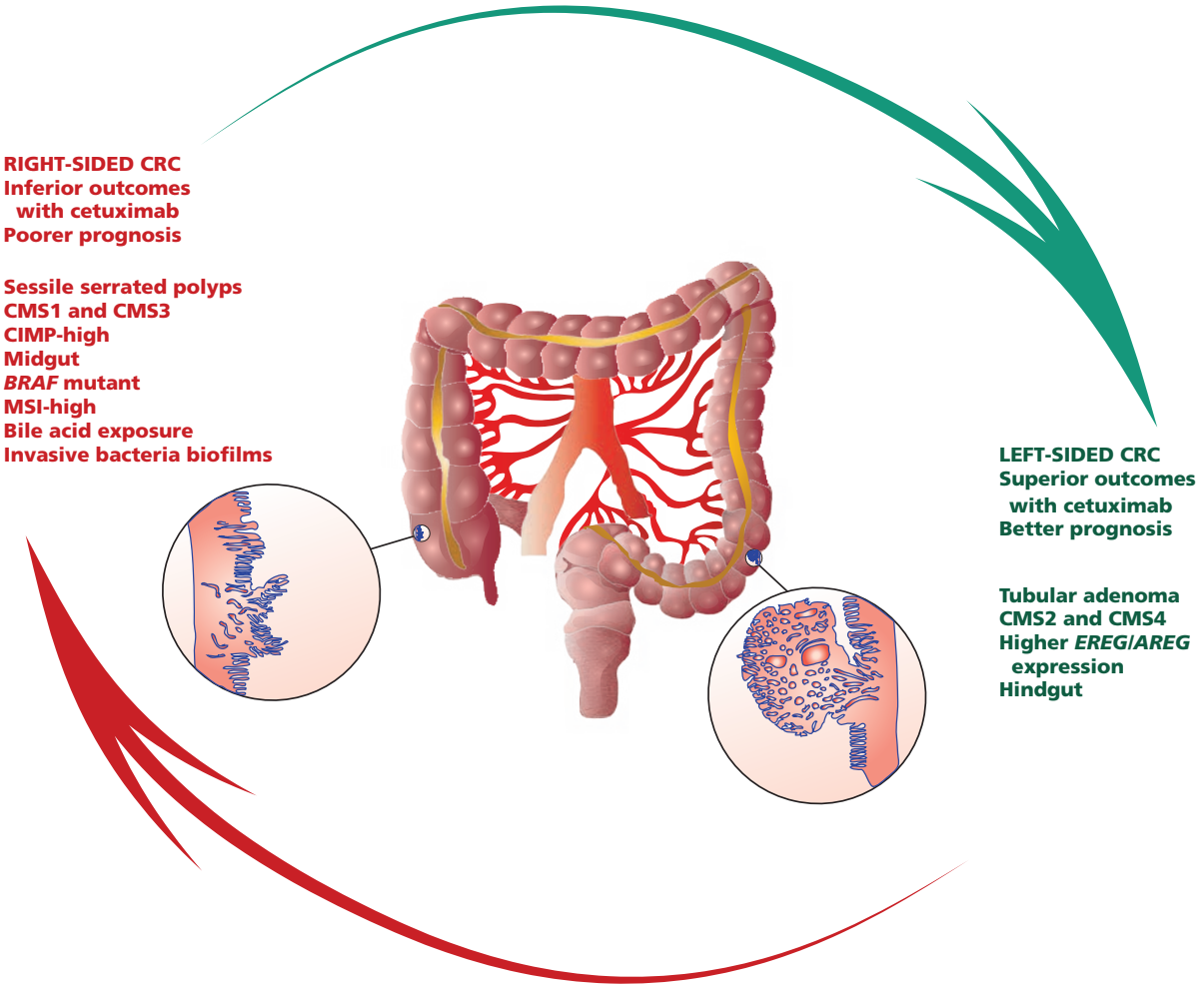

Figure 2. Summary of key biologic differences between right- and left-sided CRCs.
Abbreviations: AREG, amphiregulin; CIMP, CpG island methylator phenotype; CMS, consensus molecular subtype; CRCs, colorectal cancers; EREG, epiregulin; MSI, microsatellite instability.

those who received bevacizumab (median OS, 23.0 vs 28.0 months; *P*=.04).[16,79] On multivariate analysis, including *BRAF*-mutant status, a significant difference was seen in OS between treatment and tumor location[79] (interaction *P*=.0015). Finally, in the CRYSTAL study, 364 patients with pan-RAS wild-type metastatic CRC were randomized to receive first-line FOLFIRI with or without cetuximab, and OS was again significantly inferior in patients with right-sided tumors compared with left-sided tumors who received cetuximab and FOLFIRI (median OS, 18.5 vs 28.7 months; HR, 1.93; 95% CI, 1.24–2.99).[79] On multivariate analysis, including *BRAF*-mutant status, there was again a significant interaction *P* value of 0.0241 for OS between treatment and tumor location.[79] However, information on other molecular features, including characteristics of the *BRAF*-mutant cohorts, was not available for these studies, and there may well be enrichment for *BRAF*-mutant status among the population of *KRAS* wild-type right-sided CRCs that may confound the data. Additional data including subsequent lines of therapy by side may also provide insight into reasons for differential survival observed in these trials. Given the strikingly limited OS observed among patients with *RAS* wild-type right-sided CRC treated with first-line anti-EGFR therapy, consensus has been emerging that patients with right-sided CRC should not receive first-line anti-EGFR–based chemoimmunotherapy.

Conclusions

Right- and left-sided CRCs have markedly different underlying biologic characteristics (Figure 2), with enrichment for MSI-high, CIMP-high, and *BRAF*-mutant cancers among right-sided CRCs. These characteristics are most notably included among the CMS1 genomic subtype, which is also enriched among right-sided CRCs, although CMS3 also skews toward right-sided CRCs. Differential distribution of these genomic CRC subtypes and other biologic features among right- and left-sided CRCs may contribute to the inferior prognosis of advanced-stage right-sided CRCs and an inferior outcome with anti-EGFR therapy in right-sided CRC. Future clinical trials in CRC will need to consider important side-associated variables and side itself when considering outcomes, and hopefully will allow for personalized therapy based on underlying biologic vulnerabilities.

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