Original Article

Differences in overall survival and mutation prevalence between right- and left-sided colorectal adenocarcinoma

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Background: Prior reports have demonstrated inferior outcomes for patients with right-sided colorectal cancer (CRC) compared to patients with left-sided disease, as well as differences in treatment response based on disease sidedness. Differences in prognosis remain even among patients with metastatic disease, indicating that anatomy or stage at diagnosis alone cannot explain all of these findings. While genetic differences between right- and left-sided CRC have long been described, the genetic and molecular drivers underlying differences in prognosis and treatment response remain incompletely understood.

Methods: We compared mutation prevalence between right- (cecum to splenic flexure) and left-sided (descending colon to rectum) CRC among 38 genes in a retrospective review of next-generation sequencing data of CRC samples obtained in routine clinical practice at a single academic medical center.

Results: Among 288 cases (167 left-sided, 103 right-sided, 18 synchronous or without clear primary), patients with left-sided primaries had a longer overall survival from pathologic diagnosis (median 1,823 days *vs.* 1,006 days for right-sided cases, P=0.004). Among the assessed genes, *BRAF* and *CTNNB1* mutations were more prevalent in right-sided CRC. *BRAF* was mutated in 15.5% of right-sided CRC (95% CI: 8.5–22.5%) compared to 4.8% (95% CI: 1.6–8.0%) (P=0.003). *CTNNB1* was mutated in 3.9% of right-sided CRC (95% CI: 0.2–7.6%) compared to no instances of *CTNNB1* mutations in left-sided disease (P=0.01).

Conclusions: This difference in mutation prevalence may implicate these genetic pathways in the mechanisms underlying the discrepant outcomes and treatment responses between right- and left-sided CRC described in this and prior studies.

Keywords: Colorectal cancer (CRC); sidedness; next-generation sequencing (NGS); precision oncology

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Introduction

Previous retrospective studies, including both retrospective cohort studies and post-hoc analyses of clinical trials, have demonstrated inferior outcomes for patients with right-sided colon cancer (RCC) compared to patients with left-sided colorectal cancer (LCC) (1-4). Among the most expansive of these reports is a systematic review and metanalysis including 66 prior studies and a total of over 1.4 million patients, which demonstrated a lower risk of death among patients with left-sided primaries [hazard ratio (HR) 0.82, 95% confidence interval (CI): 0.79–0.84] compared to those with right-sided primaries at a median follow-up of 65 months (5). Differences in treatment response based on disease sidedness have also been reported (6).

Intriguingly, differences in prognosis remain even among

patients with metastatic disease (6). These findings suggest that anatomy or stage at diagnosis alone cannot explain all of the observed differences in survival between left- and right-sided disease. Moreover, these differences have led to the suggestion that disease sidedness should be included among other prognostic factors when making decisions regarding treatment intensity for patients with CRC (4,5,7).

These findings are perhaps not surprising, as genetic differences between RCC and LCC have long been described (8). More recently, genetic differences have been described on a larger scale, including descriptions of disproportionate prevalence of *KRAS* and *BRAF* mutations among RCC, as well as increased rates of microsatellite instability (7). LCC, on the other hand, has been associated with higher prevalence of *p53* and *NRAS* mutations, as well as chromosomal instability. However, despite such reports, the genetic and molecular drivers underlying differences in prognosis and treatment response remain incompletely understood.

Methods

In this setting, we conducted a retrospective review of overall survival and mutation prevalence in LCC *vs.* RCC at a single academic medical center. Relevant cases were identified from the tumor mutation database maintained by the Center for Personalized Diagnostics (CPD) at the University of Pennsylvania Health System. This database contains results from next-generation sequencing (NGS) panels ordered in routine clinical practice by practitioners within the system's oncology practice. Relevant samples were identified by querying the CPD's database for gastrointestinal malignancies between 2013 and 2016.

We reviewed clinic records to confirm the nature of the patient's primary malignancy, with colorectal adenocarcinoma being our only malignancy of interest. More extensive chart review was then conducted for those patients identified as having a CRC to determine primary location (left- *vs.* right-sided disease), date of pathologic diagnosis, stage at diagnosis, date of death, and date of last contact with our medical system. We defined RCC as arising from the cecum to splenic flexure and LCC as arising from the descending colon to rectum (5).

Survival was calculated from date of pathologic diagnosis to death or last follow-up via the Kaplan-Meier method. Censorship was assumed not to affect the probability of survival. Survival curves were generated via the Real Statistics Resource Pack (v. 4.14, Real Statistics, Trento, Italy) for Excel 2016 (Microsoft, Redmond, WA, USA), with statistical significance determined via log-rank test. Comparisons between mutation prevalence were made via two-sided Z test for proportions, and 95% CIs were calculated around point estimates of mutation prevalence. Finally, we compared the mutation prevalence for individual genes in our sample with those found in the Catalogue of Somatic Mutations in Cancer (COSMIC) database as a means of external validation.

NGS was performed at the CPD, and reported results included 38 genes (*ABL1*, *AKT1*, *APC*, *ATM*, *BRAF*, *CDH1*, *CSF1R*, *CTNNB1*, *EGFR*, *ERBB2*, *ERBB4*, *FBXW7*, *FGFR1*, *FGFR2*, *FGFR3*, *FLT3*, *GNA11*, *GNAQ*, *GNAS*, *HRAS*, *IDH1*, *IDH2*, *JAK3*, *KDR*, *KIT*, *KRAS*, *MET*, *NRAS*, *PDGFRA*, *PIK3CA*, *PTEN*, *RB1*, *RET*, *SMAD4*, *SMO*, *STK11*, *TP53*, and *ZRSR2*). Prior to sequencing analysis, formalin fixed-paraffin embedded (FFPE) sections were examined for adequacy by a surgical pathologist. To be considered adequate for mutational analysis, samples were required to have a tumor volume of greater than 10%. Genomic DNA was extracted from FFPE tissue according to manufacturer's instructions (Qiagen, Inc., Hilden, Germany). Targeted analysis for mutations was performed via enrichment of specific loci using the Illumina TruSeq Amplicon (Illumina Inc., San Diego, CA, USA) or a custom Agilent Haloplex assay (Agilent Technologies, Santa Clara, CA, USA). Sequencing of enriched libraries was performed on the Illumina MiSeq and HiSeq platforms using multiplexed, paired-end reads. Analysis and interpretation utilized a customized bioinformatics process, and variant classifications were made using the hg19 genome build (9).

Mutations were classified as pathogenic, variants of uncertain significance, or benign by the CPD based on a literature review and query of publicly available databases (including dbSNP, COSMIC, ExAC, and the 1000 Genomes Project). Pathogenic variants were defined as those with known or predicted loss or gain of function of the protein products.

This retrospective study was approved by the Institutional Review Board at the University of Pennsylvania prior to the collection of data.

Results

Among 288 cases identified via the above review, there were 167 left-sided and 103 right-sided cases (*Table 1*). In addition, there were 18 cases of patients with synchronous

bilateral disease or otherwise without clear primary, which were excluded from all subsequent analyses. Selected demographic and disease characteristics are summarized in *Table 2*. Patients with RCC were disproportionately older [mean 61.0 years (95% CI: 58.5–63.6) *vs.* 55.4 years (95% CI: 53.5–57.3) for LCC]. There were more males among patients with LCC (53% *vs.* 45%), though this difference was not statistically significant. The majority of patients (n=150) had metastatic disease at time of diagnosis. Among these patients, 90 had LCC and 60 had RCC. Consequently, a higher proportion of patients with RCC had metastatic disease at the time of diagnosis [58% (95% CI: 49–68%) *vs.* 53% (95% CI: 46%–61%) for LCC], though this disparity was also not statistically significant.

In the survival analysis, patients with LCC had a longer overall survival (*Figure 1*). Median overall survival from date of pathologic diagnosis was 1,823 days for patients with LCC *vs.* 1,006 days for RCC (P=0.004). Among patients with metastatic disease at diagnosis, the survival advantage for patients with LCC persisted (*Figure 2*). Median overall survival for these patients was 1,124 days for LCC and 750 days for RCC (P=0.047).

Among the assessed genes, *BRAF* and *CTNNB1* mutations were more prevalent in RCC (*Figure 3*). BRAF was mutated in 15.5% of RCC samples (95% CI: 8.5–22.5%) compared to 4.8% (95% CI: 1.6–8.0%) (P=0.003). *CTNNB1* was mutated in 3.9% of RCC (95% CI: 0.2–7.6%) compared to no instances of *CTNNB1* mutations in LCC (P=0.01). Among RCC, there was also a trend toward more *KRAS* mutations at 57.3% (95% CI: 47.7–66.8%) *vs.* 44.9% (95% CI: 37.4–52.5%) and more *PIK3CA* mutations at 26.2% (95% CI: 17.7–34.7%) *vs.* 17.4% (95% CI: 11.6–23.1%). The prevalence of other mutations was similar between the two groups (data not shown).

The prevalence of *CTNNB1* mutations in our cohort (1.5%, 95% CI: 0.0–2.9%) was lower than that seen among large intestine adenocarcinomas in the COSMIC database (5.0%, 95% CI: 4.5–5.6%). The overall prevalence of *BRAF* mutations in our sample (8.9%, 95% CI: 5.5–12.3%) was consistent with the prevalence of *BRAF* mutations in COSMIC (10.6%, 95% CI: 10.4–10.8%). All observed *BRAF* mutations in this cohort were previously described pathogenic mutations. The majority (4 of 8 among LCC and 11 of 16 among RCC) harbored V600E mutations. Multiple mutations were noted at the 466, 469, and 594 codons, with G466V, N581S, D594N, D594G observed in LCC cases and L597R, G466A, G469A, G469V, D594N observed in RCC samples.

Table 1 CRC cases by location

CRC cases	Number
Right-sided	103
Cecum/ascending	83
Transverse	20
Left-sided	167
Descending/sigmoid	87
Rectal/rectosigmoid	80
Excluded CRC	18
Unknown primary site	14
Synchronous lesions	4

CRC, colorectal cancer.

Discussion

The results obtained in this study for overall survival in LCC *vs.* RCC were consistent with those seen in prior

Table 2 Patient and tumor characteristics

Parameter	Left-sided primaries (n=167)		Right-sided primaries (n=103)	
	Mean	95% CI	Mean	95% CI
Mean age at diagnosis (years)	55.4	(53.5–57.3)	61.0	(58.5–63.6)
Proportion male	0.53	(0.45–0.60)	0.45	(0.35–0.54)
Proportion metastatic at diagnosis	0.53	(0.46–0.61)	0.58	(0.49–0.68)
Mean ECOG PS (best achieved, 0–4)	0.60	(0.49–0.72)	0.72	(0.58–0.87)
Proportion MSI high/MMR deficient	0.03	(0.00–0.06)	0.08	(0.02–0.15)

CI, confidence interval; PS, performance status; MSI, microsatellite instability; MMR, mismatch repair.

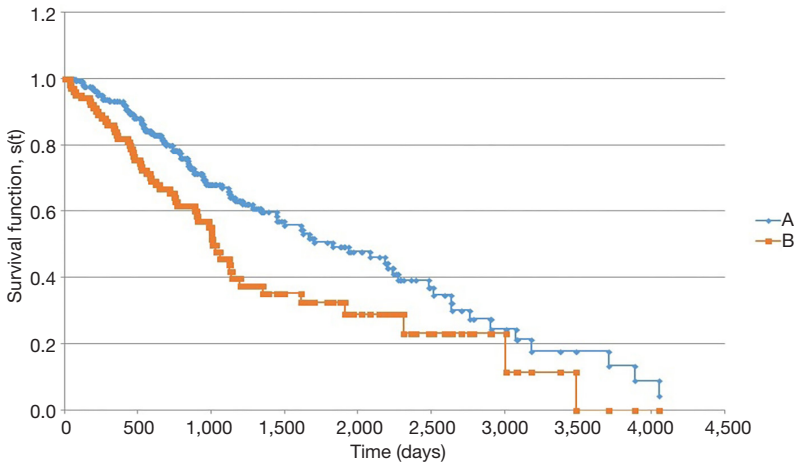

Figure 1 Overall survival for all patients. Kaplan-Meier survival function (Y-axis) versus time in days from pathologic diagnosis to death or last follow-up. (A) Patients with left-sided disease; (B) patients with right-sided disease.

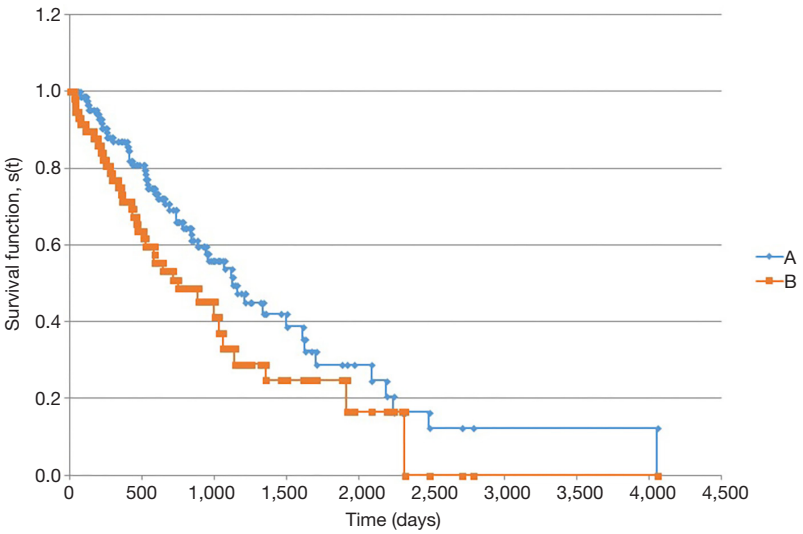

Figure 2 Overall survival for patients with metastatic disease at time of diagnosis. Kaplan-Meier survival function (Y-axis) versus time in days from pathologic diagnosis to death or last follow-up. (A) Patients with left-sided disease; (B) patients with right-sided disease.

studies, namely, that RCC carried a worse prognosis than did LCC. Also, as seen in prior studies, stage at diagnosis does not seem to explain this finding in isolation, as our cohort demonstrated a statistically significant difference in overall survival even among those patients who had metastatic disease at the time of diagnosis.

The differing mutation prevalence noted here may implicate these genetic pathways in the mechanisms underlying the discrepant outcomes and treatment responses between RCC and LCC described in this study. V600E *BRAF* mutations, the most common mutation seen in this gene, are well established as conferring a worse prognosis in CRC, with poorer results reported in BRAF-mutant CRC in multiple studies. These findings have been reported in both metastatic (10-14) and earlier-stage disease (15,16), though the impact has been particularly striking in patients with metastatic disease. In addition, RCC has been previously reported to have higher rates of *BRAF* mutations compared to LCC (7).

Of note, 5 of the 103 RCC samples in this cohort and

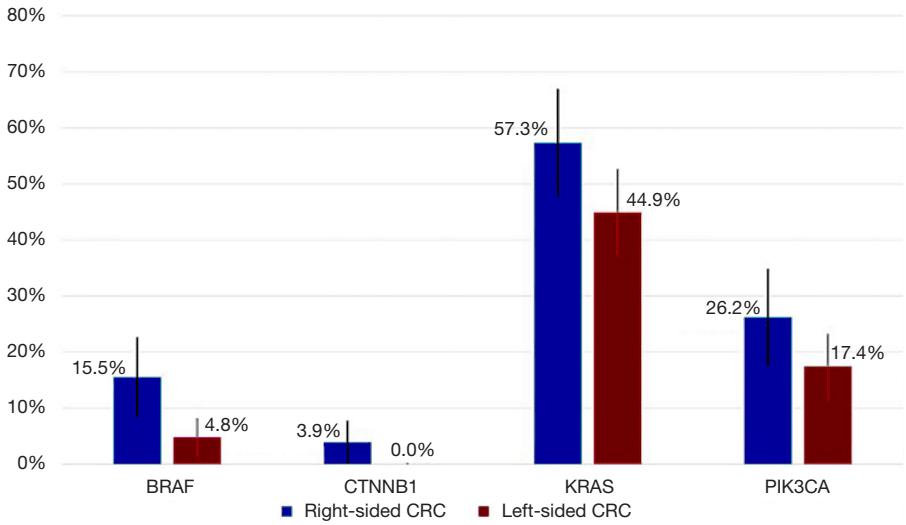

Figure 3 Mutation prevalence (Y-axis) by primary tumor location, with error bars indicating 95% confidence intervals. CRC, colorectal cancer.

4 of the 167 LCC samples harbored non-V600E *BRAF* mutations. The clinical implications of non-V600E mutations are less clear than are those of V600E mutations. Jones *et al.* recently reported the largest description of clinicopathologic characteristics of non-V600E-mutant CRC (17). In their analysis of 9,643 CRC specimens with available NGS data, non-V600E mutations were associated with a favorable overall prognosis compared to both V600E *BRAF*-mutant cases as well as compared to patients with *BRAF* wild-type disease (median overall survival 60.7 *vs.* 11.4 *vs.* 43.0 months, respectively). Moreover, non-V600E-mutant disease was less likely to be found in RCC compared to V600E-mutant disease (36% right-sided among all non-V600E-mutant samples in their study *vs.* 81% right-sided for V600E-mutant samples).

The implications of *CTNNB1* mutations are even less clear. *CTNNB1* mutations are relatively rare in CRC. In our cohort, 1.5% of samples harbored a *CTNNB1* mutation, which is lower than the 5.0% rate reported in COSMIC, but similar to rates described in other sources in the literature (18). The clinical relevance of these mutations is uncertain, particularly as *CTNNB1* mutations are rarely found in isolation. Malapelle *et al.* found that mutations in *CTNNB1* in CRC are associated with constitutive *RAF/MEK/ERK* pathway signaling, typically via association with mutations in other cancer-related genes (18). However, studies that have assessed correlations between *CTNNB1* mutational status and clinical outcomes have not reported consistent trends (19).

The present study, like most of the previously reported results regarding survival and CRC sidedness, is a retrospective cohort study and therefore subject to the biases associated with retrospective studies. Moving forward, the implementation of broader NGS approaches in a prospective fashion may help to identify other gene variants related to the survival differences noted in this and other studies. Most prior prospective studies have been limited to *RAS* testing. In addition, the impact of broader measures, such as tumor mutational burden requires assessment in a prospective fashion.

The present study was conducted at a single referral center and consequently may not be generalizable to other centers. In addition, while our study demonstrated worse outcomes for patients with right-sided disease as demonstrated in their worse overall survival from time of diagnosis compared to patients with LCC, the patients with RCC were significantly older than patients with LCC, as noted above. Consequently, the worse outcomes of the patients with RCC may have been partially driven by factors other than disease biology. In addition, the impact of other possible prognostic markers including immunohistochemistry for *CDX2* and microsatellite instability markers was not assessed in this study, in part due to limited availability of this data.

The relative timing of NGS data collection with respect to prior therapy was also not assessed in this study, though

most NGS assays were performed on patients' initial pathology specimens. Previous studies have demonstrated an impact of systemic therapy on clonal evolution of CRC, which may have implications for treatment outcomes and survival (20). For instance, hypothesis-generating data from the REVERCE study suggest the sequencing of cetuximab and regorafenib in *RAS* wild-type CRC may impact survival due to earlier generation of *RAS* mutations when EGFR inhibitors are utilized (21).

This study could benefit from additional statistical power, and the preceding analysis could be repeated as additional data is generated at our institution. Alternatively, a similar analysis could be conducted using larger pooled databases, similar to the methods utilized by Jones *et al.* (17). The overall prevalence of *BRAF* mutations in our sample (8.9%, 95% CI: 5.5–12.3%) was consistent with the prevalence of *BRAF* mutations among large intestine adenocarcinomas in the COSMIC database, as well as rates described elsewhere in the literature (22), lending some support to the external validity of these data. Unfortunately, the reference database we used (COSMIC) does not readily contain location data (left- *vs.* right-sided) for most of its documented cases of colorectal cancer, making leveraging this database for a similar analysis problematic.

Overall, the present study re-demonstrates the discrepant prognosis of left- *vs.* right-sided CRC reported in prior studies. Also seen are signals of differing prevalence of mutations in multiple genes between LCC and RCC, most notably *BRAF* and *CTNNB1*. These genetic pathways may have relevance to prognosis in other CRC cohorts. For instance, the association between *BRAF* mutational status and primary sidedness described by Jones *et al.* may have some explanatory power for the discrepancies in prognosis between LCC and RCC seen in prior studies (17). However, other reports that have stratified their results by *BRAF* mutational status have found persistent differences in outcomes based on CRC sidedness (6). Further work is therefore needed to more clearly elucidate genetic differences between RCC and LCC and mechanistic relationship between these mutations and differences in prognosis. Further research is also needed regarding non-genetic biologic differences between LCC and RCC that may have prognostic significance. For instance, tumor burden at the time of diagnosis, which was not captured in this study, may have some explanatory power beyond stage alone. A better understanding of such factors is needed to guide both discussions of prognosis as well as treatment decisions.

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Footnote

Conflicts of Interest: Disclosures for A Loaiza-Bonilla include: Speakers Bureau: Celgene, Bristol Myers-Squibb, Guardant, Caris Life Science, Eisai; Advisory Board: Bayer, Astra Zeneca; Consulting: Massive Bio; Research: Ipsen. CE Jensen and JY Villanueva have no conflicts of interest to declare.

Ethical Statement: The study was approved by the Institutional Review Board at the University Pennsylvania prior to the collection of data (approval number 827031). Data was collected retrospectively, and the study was deemed to represent minimal risk to patients, so informed consent was not obtained; however, patients' data has been secured according to requirements of IRB protocol. Findings did not affect any treatment decisions.

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