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Article

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Primary Tumor Location as a Prognostic Factor in Metastatic Colorectal Cancer

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Abstract

Background: We sought to clarify the prognostic impact of primary tumor location in metastatic colorectal cancer (mCRC).

Methods: We evaluated the association between tumor location and survival parameters in patients with previously untreated mCRC receiving first-line chemotherapy ± bevacizumab in three independent cohorts: a prospective pharmacogenetic study (PROVETTA) and two randomized phase III trials, AVF2107g and NO16966. Cancers proximal or distal of the splenic flexure were classified as right-sided or left-sided, respectively. The primary end point was overall survival (OS). Data were analyzed with Cox proportional hazards and logistic regression models. All statistical tests were two-sided.

Results: Among evaluable patients in the PROVETTA (n = 200), AVF2107g (n = 559), and NO16966 (n = 1268) studies, 72.0%, 63.1%, and 73.7% had left-sided tumors, respectively. In PROVETTA, patients with left-sided tumors had superior OS (left-sided vs right-sided: hazard ratio [HR] = .44, 95% confidence interval [CI] = .28 to .70, $P < .001$) and progression-free survival (HR = .52, 95% CI = .36 to .75, $P < .001$) outcomes. Multivariable analyses confirmed right-sided location as a negative prognostic variable, independent of mucinous histology and *BRAF* mutational status. Data from the AVF2107g (HR for OS = .55, 95% CI = .43 to .70) and NO16966 trials (HR for OS = .71, 95% CI = .62 to .82 both $P < .001$) also showed favorable outcomes in patients with left-sided tumors. In both randomized studies, the efficacy of bevacizumab was independent of tumor location.

Conclusions: These data demonstrate that primary tumor location is an important prognostic factor in previously untreated mCRC. Given the consistency across an exploratory set and two confirmatory phase III studies, side of tumor origin should be considered for stratification in randomized trials.

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Tumors arising from the colorectal tract are a heterogeneous complex of diseases that result from the accumulation of distinctive genetic and epigenetic alterations (1,2). Despite increased understanding into the molecular pathways underlying colorectal cancer (CRC), relatively few biomarkers are prognostic for survival (1–3). Germline mutations in DNA mismatch repair genes, definitive of Lynch syndrome, in stage II/III disease and *BRAF*^{V600E} mutations in stage IV disease are notable exceptions (4–6).

Biological and clinical evidence supports that proximal (right-sided) and distal (left-sided) CRCs follow different molecular pathways of carcinogenesis. Right-sided tumors are more likely to be diploid and to be characterized by mucinous histology, high microsatellite instability, CpG island methylation, and *BRAF* mutations (6–10). In contrast, left-sided tumors are frequently infiltrating, constricting lesions, with a phenotype that involves chromosomal instability and aneuploidy (7–9). Microarray studies of sporadic CRC biopsies demonstrate unique gene expression profiles for right- and left-sided cancers, potentially related to distinct embryonic origins and postnatal regulation (11,12). Extensive sequencing analyses described a characteristic branching pattern of cancer evolution supporting that tumor biology is characterized simultaneously by intratumor heterogeneity and the preservation of ancestral aberrations within the primary tumor and corresponding metastatic sites (13,14).

Previous attempts to evaluate the effect of primary tumor location on outcome in metastatic CRC (mCRC) have been complicated by sample size, a high degree of heterogeneity in received treatments, and limited information on molecular and pathologic features (15–17). The objectives of the present analysis were first to assess primarily the prognostic impact and secondly the predictive effect of primary tumor location for an antiangiogenic treatment by interrogating three large independent patient cohorts. Because of the prognostic significance of *BRAF* mutations and mucinous histology (5,6,18) and the association of these characteristics with right-sided mCRC, a multivariable model and a subgroup analysis in nonmucinous and *BRAF* wild-type cancers were used to separately assess outcomes in the PROVETTA study (chosen as an exploratory set based on availability of clinical and molecular features). The prognostic effect of primary tumor location was subsequently verified and validated using data from two large phase III trials.

Methods

Data Sources

Study protocols were approved by the review boards of participating institutions and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. The analysis on side of origin was conducted on a database of 455 mCRC patients prospectively enrolled in a pharmacogenetic translational study (PROVETTA; NCT01363739) (19). Eligibility criteria included histologically confirmed CRC and measurable metastatic disease according to Response Evaluation Criteria in Solid Tumors (RECIST) and no previous exposure to systemic mCRC therapy, irinotecan, or bevacizumab. Patients received the FOLFIRI regimen—irinotecan (180 mg/m^2), bolus 5-fluorouracil (400 mg/m^2), and leucovorin (400 mg/m^2), followed by a 46-hour infusion of 5-fluorouracil (2400 mg/m^2) on day 1 of every two-week cycle. Bevacizumab (5 mg/kg) was given intravenously prior to the administration of irinotecan. Treatment with FOLFIRI and bevacizumab was continued until disease progression or prohibitive toxicity. All patients provided written informed consent prior to enrollment. The primary end point was progression-free survival (PFS) according to the angiogenic-related pharmacogenetic profile; secondary end points include overall survival (OS) and objective response rate (ORR) by RECIST. Pharmacogenetic data have been reported elsewhere (20).

The AVF2107g and NO16966 studies were used for validation; patient populations, treatment, and outcomes have been described previously (21–23). Briefly, AVF2107g was a randomized, placebo-controlled trial of bevacizumab with irinotecan, bolus fluorouracil, leucovorin (IFL) in 813 patients with previously untreated mCRC (21). The primary end point was OS. NO16966 was a randomly assigned, noninferiority comparison of FOLFOX4 (5-fluorouracil, foiling acid, oxaliplatin) vs XELOX (capecitabine plus oxaliplatin), which was subsequently amended to a 2×2 factorial design with further random assignment to bevacizumab or placebo. The intent-to-treat population comprised 2034 patients, and the primary end point was PFS (23).

A retrospective archival series of 181 tumoral samples collected at the Unit of Medical Oncology, Azienda Ospedaliero-Universitaria Pisana was used to test intratumoral mRNA expression levels of the following candidate genes: *excision repair cross-complementation group 1 (ERCC1), vascular endothelial growth factor (VEGF) ligands A, B,* and *C,* and *VEGF receptors 1* and *2.*

Analysis Populations

The present analysis evaluated data from patients with either right-sided or left-sided primary CRC tumors. Tumor location was identified from pathologic reports (PROVETTA) and case report forms (AVF2107g and NO16966). Cancers proximal to the splenic flexure (ie, tumors occurring in the cecum, ascending colon, or transverse colon) were classified as right-sided. Left-sided cancers included those distal to the splenic flexure (ie, tumors occurring in the descending colon, sigmoid colon, or rectum). Patients with synchronous right-sided and left-sided tumors were excluded from analysis, as were patients in PROVETTA, if information regarding *BRAF* mutational status and mucinous histology was not available.

Gene Expression Analyses

Gene expression testing was performed in a College of American Pathologists/Clinical Laboratory Improvement Act–certified laboratory (Response Genetics, Inc., Los Angeles, CA) using formalin-fixed paraffin-embedded (FFPE) tumor specimens from CRC tumors. A section of all the FFPE specimens stained with hematoxylin and eosin was evaluated by a board-certified pathologist for tumor content. Adjacent sections of the tumor were sectioned at 10 microns and stained with nuclear fast red (NFR) for visualization and microdissection. After microdissection of tumor cells from the NFR-stained slides, RNA was extracted according to a proprietary procedure (US patent # 6248535) and subsequently reverse transcribed to cDNA. Real-time polymerase chain reaction (RT-PCR) was performed using primers and probes that were specifically designed for *ERCC1*, *VEGF A*, *B*, and *C,* and *VEGF receptors 1* and *2* (see Supplementary Table 1, available online). Results were obtained as a ratio of RT-PCR fluorescent signals of the genes in reference to β-actin.

Statistical Analysis

OS, defined as the time from study entry (PROVETTA) or random assignment (AVF2107g and NO16966) until any-cause death, was

the primary outcome measure of the analysis. Secondary outcomes were PFS and ORR by RECIST. In PROVETTA and NO16966, PFS was defined as the time from random assignment (start of treatment in PROVETTA) to the first documentation of progressive disease (per investigator assessment) or any-cause death (19,22). Patients undergoing curative metastasectomy were censored at the time of surgery. In AVF2107g, PFS was defined as the time from random assignment to progression or death during the study (any death occurring within 30 days after the last dose of study treatment) (21).

Median values for OS and PFS, as well as corresponding 95% CIs, were calculated using Kaplan-Meier methods. Unadjusted and multivariable Cox proportional hazards regression analyses were used to estimate the association between tumor location and outcome and to calculate corresponding hazard ratios (HRs) and 95% confidence intervals (CIs). Multivariable models included the following baseline variables: age, sex, race, Kohne score (24), prior adjuvant chemotherapy, *BRAF* mutation status, and mucinous histology (latter two variables only available in PROVETTA). An interaction test was used to determine whether primary tumor location was a bevacizumab use effect modifier on outcomes. The effect of primary location on survival outcomes was also assessed in subgroup analyses stratified by baseline factors.

Logistic regression was used to explore the associations between tumor location and ORR when adjusting for the same baseline variables as in the Cox models.

Differences in intratumoral mRNA levels of *ERCC1*, *VEGF* ligands *A*, *B*, and *C*, and *VEGF receptors 1* and *2* between right-sided and left-sided tumor sites were compared using the Wilcoxon two-sample test.

Statistical analyses were performed using SAS 9.2 software (SAS Institute, Inc., Cary, NC). All tests were performed at the two-sided .05 significance level.

Because of the exploratory nature of the analysis, adjustments for multiple comparisons were not preplanned and therefore not conducted. The findings are not conclusive and are hypothesis-generating.

Results

Patients

A total of 200, 559, and 1268 patients were included for analysis from the PROVETTA, AVF2107g, and NO16966 trials, respectively. Overall, 41.4% of patients were female, 33.3% were older than age 65 years, 36.9% had an ECOG performance status greater than or equal to 1, and 58.4% had more than 1 metastatic site. Among the evaluable populations, left-sided tumors were reported in 63.1% (AVF2107g), 72.0% (PROVETTA), and 73.7% (NO16966) of patients in each study. Baseline demographics by primary tumor location were generally similar with respect to age, race, number of metastatic sites, and exposure to adjuvant chemotherapy (Table 1). A higher percentage of patients with right-sided tumors, however, were female, had an ECOG performance status of greater than or equal to 1, and had metastatic disease at first cancer diagnosis relative to patients with left-sided tumors across the studies. Patients with left-sided tumors were more likely to have received adjuvant radiotherapy.

In PROVETTA, right-sided tumors were more frequently *BRAF*-mutated ($P < .001$); the association remained statistically significant after adjusting for mucinous histology ($P = .001$). A trend was observed in the association between mucinous histology and right-sided location ($P = .082$).

Survival Outcomes by Primary Tumor Location: PROVETTA

Unadjusted analyses showed statistically significant differences in OS and PFS, according to primary tumor location in the overall PROVETTA cohort (Table 2; Figure 1). Median OS for patients with left-sided and right-sided tumors was 42.0 and 24.8 months, respectively (left-sided vs right-sided: HR = .44, 95% CI = .28 to .70, $P < .001$). PFS was also superior in patients with left-sided CRC (median of 12.1 vs 9.9 months, respectively; HR = .52, 95% CI = .36 to .75; $P < .001$). According to multivariable analyses that adjusted for baseline variables (Table 3; Supplementary Table 2, available online), including *BRAF* mutation status and mucinous histology, patients with left-sided primary tumors had a lower risk of progression (HR = .55, 95% CI = .37 to .83, $P = .01$) and death (HR = .47, 95% CI = .28 to .80; $P = .01$).

In the subgroup of patients with nonmucinous and *BRAF* wild-type tumors (n = 155), patients with left-sided CRC had a median OS and PFS of 47.6 and 13.0 months, respectively, compared with values of 28.8 and 10.0 months in patients with right-sided CRC (OS for left-sided vs right-sided: HR = .52, 95% CI = .30 to .93, $P = .02$; PFS: HR = .54, 95% CI = .34 to .84, $P = .01$). Multivariable analyses confirmed these results (see Table 3).

Survival Outcomes by Primary Tumor Location: AVF2017 and NO16966

In univariate analyses of data from AVF2107g and NO16966, patients with left-sided tumors achieved statistically significantly superior OS vs patients with right-sided tumors (AVF2107g HR = .55, 95% CI = .43 to .70, $P < .001$; NO16966 HR =.71, 95% CI = .62 to .82, $P < .001$) (Table 2, Figure 1). Differences in OS remained statistically significant in subgroups defined by the administered treatment (ie, chemotherapy alone or chemotherapy with bevacizumab). Multivariable analyses also showed that primary tumor location and bevacizumab use were independent prognostic factors for OS (see Table 3). Moreover, the effect of primary tumor location on OS was found to be consistent across a variety of patient subgroups (Figure 2). In the unadjusted pooled analysis of both trials (n = 1827), median OS for left-sided tumors was 22.5 versus 17.0 months for right-sided tumors (HR = .66, 95% CI = .59 to .75, $P < .001$).

PFS outcomes differed by primary tumor location in AVF2017g (unadjusted analysis of left-sided vs right-sided tumors: median of 8.5 vs 7.1 months; HR = .68, 95% CI = .55 to .83, $P < .001$) (Table 2). In NO16966, the difference in PFS showed a similar trend but did not reach statistical significance (median of 8.9 vs 7.6 months; HR = .90, 95% CI = .79 to 1.03, $P = .12$). Similar results for PFS were obtained after adjusting for baseline patient characteristics (see Table 3).

In univariate and multivariable models (see Table 3), left-sided tumors were associated with higher response rates. In AVF2107g, ORRs were 44.2% and 28.2% in patients with left-sided and right-sided tumors, respectively (unadjusted OR = 2.02, 95% CI = 1.40 to 2.92, $P < .001$). The association of ORR with primary tumor location was also statistically significant in NO16966 (left 51.6% vs right 42.0%; unadjusted OR = 1.47, 95% CI = 1.14 to 1.89, $P < .01$).

Differences in survival outcomes for left-sided tumors located in the descending or sigmoid colon vs the rectum are described in the Supplementary Materials (available online).

Gene Expression Analyses

The comparison of right-sided vs left-sided primary tumors within the independent set of 181 samples showed no differences

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Table 1. Baseline patient and disease characteristics of patients by primary tumor location*

Characteristic	Study, No. (%)					
	PROVETTA		AVF2107g		NO16966	
	Patients with right-sided tumors (n = 56)	Patients with left-sided tumors (n = 144)	Patients with right-sided tumors (n = 206)	Patients with left-sided tumors (n = 353)	Patients with right-sided tumors (n = 333)	Patients with left-sided tumors (n = 935)
Age						
≤50 y	5 (8.9)	25 (17.4)	40 (19.4)	90 (25.5)	57 (17.1)	162 (17.3)
51–65 y	26 (46.4)	64 (44.4)	99 (48.1)	161 (45.6)	156 (46.8)	469 (50.2)
>65 y	25 (44.6)	55 (38.2)	67 (32.5)	102 (28.9)	120 (36.0)	304 (32.5)
P	.31		.25		.48	
Sex						
Male	38 (67.9)	88 (61.1)	95 (46.1)	226 (64.0)	171 (51.4)	573 (61.3)
Female	18 (32.1)	56 (38.9)	111 (53.9)	127 (36.0)	162 (48.6)	362 (38.7)
P	.38		<.001		.002	
Race						
White	56 (100)	144 (100)	163 (79.1)	280 (79.3)	268 (80.5)	775 (82.9)
Black	-	-	29 (14.1)	35 (9.9)	11 (3.3)	9 (1.0)
Other	-	-	14 (6.8)	38 (10.8)	54 (16.2)	151 (16.1)
P	N/A		.12		.01	
ECOG performance status						
0	53 (94.6)	123 (85.4)	113 (54.9)	212 (60.1)	187 (56.2)	590 (63.1)
≥1	3 (5.4)	21 (14.6)	93 (45.1)	141 (39.9)	145 (43.5)	343 (36.7)
P	.071		.23		.03	
Stage at diagnosis						
I–III	11 (19.6)	49 (34.0)	-	-	147 (44.1)	495 (52.9)
IV	45 (80.4)	95 (66.0)	-	-	186 (55.9)	440 (47.1)
P	.046				.006	
No. of metastatic sites						
1	20 (35.7)	63 (43.8)	87 (42.2)	129 (36.5)	143 (42.9)	400 (42.8)
>1	36 (64.3)	81 (56.3)	119 (57.8)	224 (63.5)	190 (57.1)	533 (57.0)
P	.30		.18		.98	
Prior therapy						
Adjuvant chemotherapy	9 (16.1)	42 (29.2)	47 (22.8)	119 (33.7)	102 (30.6)	321 (34.3)
P	.056		.007		.22	
Mucinous histology						
Yes	14 (25.0)	21 (14.6)	–	–	–	–
No	42 (75.0)	123 (85.4)	–	–	–	–
P	.08					
BRAF mutational status						
Wild-type	46 (82.1)	140 (97.2)	–	–	–	–
Mutated	10 (17.9)	4 (2.8)	–	–	–	–
P	<.001					
Kohne Score						
Low	20 (35.7)	64 (44.4)	87 (42.2)	129 (36.5)	142 (42.6)	400 (42.8)
Intermediate	28 (50.0)	61 (42.4)	102 (49.5)	179 (50.7)	159 (47.7)	462 (49.4)
High	2 (3.6)	12 (8.3)	12 (5.8)	33 (9.3)	28 (8.4)	67 (7.2)
N/A	6 (10.7)	7 (4.9)	5 (2.4)	12 (3.4)	4 (1.2)	6 (0.6)
P	.20		.21		.73	

* *P* values were based on χ^2or Fisher's exact test whenever appropriate. ECOG = Eastern Cooperative Oncology Group; NR = not reported.

in intratumoral mRNA expression for *VEGF-A*, *VEGF-B*, and *VEGF-C*, as well as *VEGFR-1* and *VEGFR-2* (Supplementary Table 3, available online). Left-sided cancers (n = 124) expressed lower levels of *ERCC1* mRNA than right-sided tumors (n = 57) (median levels: .645 [range = .108-3.099] vs .730 [range = .007-3.322] × 10^{-3} *ERCC1*/β-actin mRNA, *P* = .04) (Supplementary Figure 1, available online).

Discussion

We evaluated the effect of primary tumor location on outcome in patients with mCRC in a subgroup of 200 patients enrolled in a pharmacogenetic study for which data on mucinous histology and *BRAF* mutational status were available. We used a multivariable model that accounted for pathologic features with well-known adverse prognostic effects and statistically significant associations with right-sided tumor origin. Patients with tumors distal to the splenic flexure had lower risk of death and progression relative to patients with cancers proximal to the splenic flexure. The prognostic significance of primary tumor location on survival outcomes was also confirmed in the subgroup with nonmucinous and *BRAF* wild-type cancer. These analyses demonstrated that the prognostic value of primary tumor site is independent of other variables. Initial results were

Table 2. Unadjusted analyses of survival outcomes by primary tumor location and study*

Cohort	OS events No. (%)	Median OS, months (95% CI)		HR (95% CI)	P	PFS events No. (%)	Median PFS, months (95% CI)		HR (95% CI)	P
		Right-sided tumors	Left-sided tumors				Right-sided tumors	Left-sided tumors		
PROVETTA										
All eligible patients (n = 200)	77 (38.5%)	24.8 (19.1 to 29.8)	42.0 (32.1 to 53.5)	.44 (.28 to .70)	<.001	147 (73.5%)	9.9 (8.3 to 11.7)	12.1 (11.1 to 15.8)	.52 (.36 to .75)	<.001
Nonmucinous/*BRAF* wild-type subgroup (n = 155)	54 (34.8%)	28.8 (20.1 to 33.0)	47.6 (32.1 to 53.5)	.52 (.30 to .93)	.02	112 (72.3%)	10.0 (8.8 to 13.1)	13.0 (11.5 to 15.9)	.54 (.34 to .84)	.01
AVF2107g										
Evaluable patients (n = 559)	264 (47.2)	14.6 (12.0 to 16.9)	20.4 (18.7 to 24.2)	.55 (.43 to .70)	<.001	363 (64.9)	7.1 (5.6 to 8.1)	8.5 (8.2 to 10.3)	.68 (.55 to .83)	<.001
CT arm (n = 282)	144 (51.1)	13.6 (10.6 to 16.7)	18.0 (15.7 to 21.7)	.62 (.44 to .86)	.01	206 (73.0)	5.4 (4.4 to 5.8)	8.0 (5.7 to 8.3)	.72 (.55 to .96)	.02
CT + bevacizumab arm (n = 277)	120 (43.3)	15.9 (12.7 to 19.6)	24.2 (19.5 to NE)	.49 (.34 to .70)	<.001	157 (56.7)	8.7 (8.1 to 10·.6)	11.1 (9.4 to 13.8)	.62 (.45 to .85)	.01
NO16966										
Evaluable patients (n = 1268)	994 (78.3)	18.0 (16.5 to 19.8)	23.0 (21.5 to 24.4)	.71 (.62 to .82)	<.001	1127 (88.9)	7.6 (7.0 to 8.3)	8.9 (8.5 to 9.3)	.90 (.79 to 1.03)	.12
CT arms (n = 827)	664 (80.3)	17.0 (14.7 to 18.5)	22.0 (20.5 to 23.7)	.67 (.57 to .80)	<.001	751 (90.8)	7.0 (6.0 to 7.9)	8.3 (7.9 to 8.8)	.87 (.74 to 1.03)	.10
CT + bevacizumab arms (n = 441)	330 (74.8)	20.6 (17.6 to 24.7)	24.7 (22.2 to 27.6)	.78 (.61 to .99)	.04	376 (85.3)	8.6 (7.6 to 10.2)	10.0 (9.2 to 10.8)	.95 (.76 to 1.19)	.64

* Cox proportional hazards regression analyses. All statistical tests were two-sided. CI = confidence interval; CT = chemotherapy; HR = hazard ratio; OS = overall survival; PFS = progression-free survival.

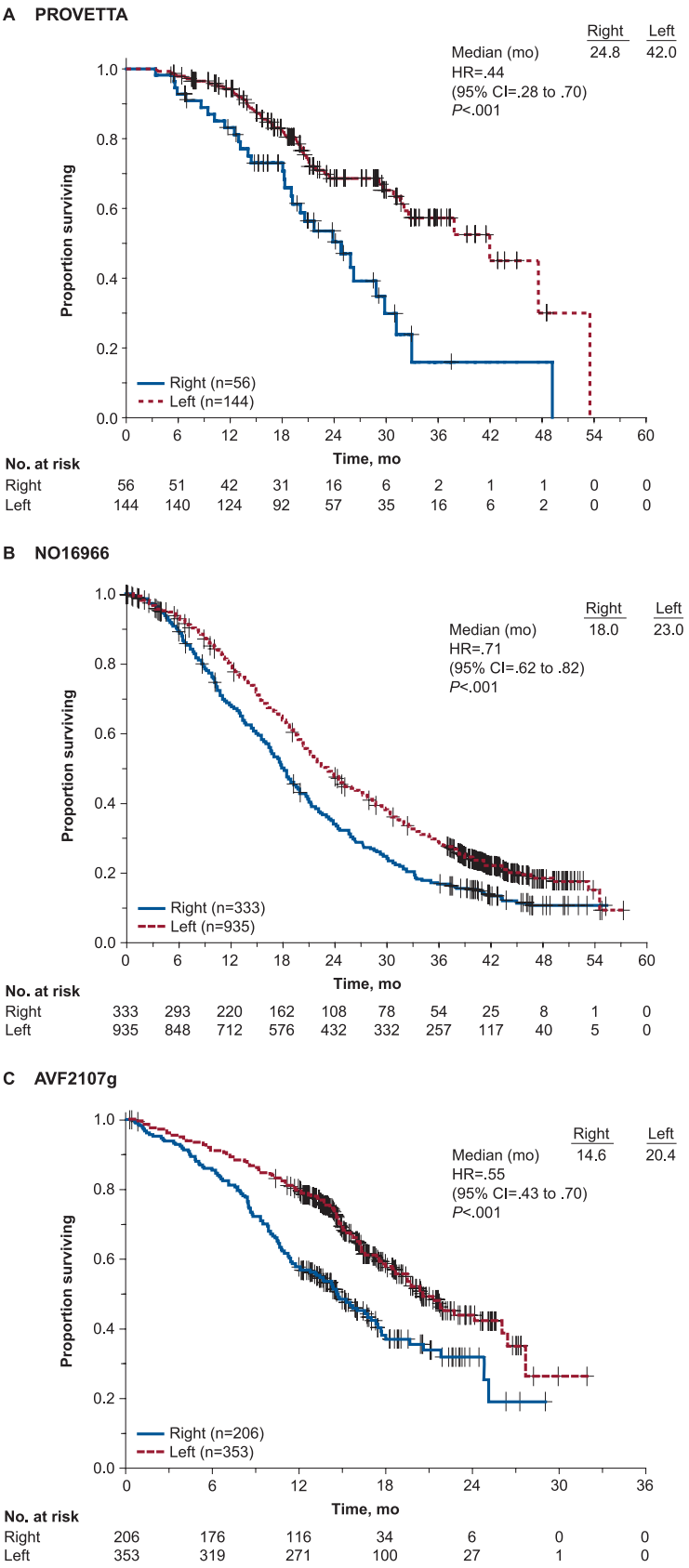

Figure 1. Analyses of overall survival by primary tumor location. Panels A, B, and C show Kaplan-Meier estimates of the probability of overall survival by primary tumor location in the PROVETTA (A), NO16966 (B), and AVF2107g (C) studies, respectively. Patients with left-sided tumors (**red**) had statistically significantly increased OS compared with patients with right-sided tumors (**blue**) within each study. All statistical tests were two-sided. BV = bevacizumab; CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; FOLFOX4 = 5-fluorouracil and folinic acid plus oxaliplatin; HR = hazard ratio; IFL = irinotecan, bolus fluorouracil, and leucovorin; P = placebo; XELOX = capecitabine plus oxaliplatin.

Table 3. Multivariable analyses of survival outcomes and ORR by primary tumor location and treatment*

Cohort	Comparison	OS		PFS		ORR	
		HR (95% CI)	P	HR (95% CI)	P	OR (95% CI)	P
PROVETTA							
All eligible patients (n = 200)	Left vs right	.47 (.28 to .80)	.01	.55 (.37 to .83)	.01	1.23 (.59 to 2.57)	.59
Nonmucinous/*BRAF* wild-type subgroup (n = 155)	Left vs right	.47 (.25 to .88)	.02	.57 (.35 to .91)	.02	1.31 (.53 to 3.27)	.56
AVF2017g (n = 559)							
Cancer location†	Left vs right	.52 (.40 to .67)	<.001	.69 (.56 to .86)	.001	2.48 (1.66 to 3.69)	<.001
Bevacizumab use†	Yes vs no	.71 (.55 to .91)	.01	.53 (.42 to .65)	<.001	1.51 (1.05 to 2.16)	.03
NO16966 (n = 1268)							
Cancer location‡	Left vs right	.72 (.63 to .83)	<.001	.90 (.78 to 1.02)	.10	1.49 (1.15 to 1.92)	.01
Bevacizumab use‡	Yes vs no	.82 (.72 to .94)	.01	.79 (.70 to .90)	<.001	.98 (.77 to 1.24)	.84

* Multivariable analyses adjusted for age, sex, race, Kohne score, and prior adjuvant chemotherapy. Additionally, *BRAF* mutation status and mucinous histology were adjusted in the PROVETTA study. Abbreviations: CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; HR = hazard ratio; OR = odds ratio; ORR = objective response rate; OS = overall survival; PFS = progression-free survival.

† Statistical test for cancer location and bevacizumab use interaction, *P* = .38 for OS, *P* = .59 for PFS; *P* = .27 for ORR.

‡ Statistical test for cancer location and bevacizumab use interaction, *P* = .29 for OS, *P* = .62 for PFS; *P* = .54 for ORR.

subsequently validated using data sets from two large phase III studies of first-line chemotherapy with or without bevacizumab, which confirmed the independent prognostic effect of tumor location in multivariable models, irrespective of exposure to bevacizumab.

A recent retrospective analysis using two independent and nonrandomized cohorts of patients treated with capecitabine and oxaliplatin with (n = 667) or without (n = 213) bevacizumab suggested that the addition of bevacizumab may primarily benefit patients with left-sided primary tumors. Our data do not validate those findings and reject the hypothesis of an interaction of primary tumor location with the efficacy of bevacizumab (25). Exploratory subgroup analyses of AVF2107g and NO16966 examining the impact of adjuvant treatment on benefit from bevacizumab after relapse did not show a relevant effect independently from primary tumor location (data not shown).

Another important finding of our analysis was the association of right-sided cancers with chemoresistance. In both validation studies, response rates and PFS were statistically significantly higher in patients with left-sided tumors. These data emphasize that right-sided and left-sided CRC have potentially important biological differences. Despite a trend toward a greater likelihood of achieving response for left-sided CRC in PROVETTA, the association did not reach statistical significance, perhaps because of the limited sample size.

Data derived from retrospective analyses suggest that different *ERCC1* expression could affect response to chemotherapy (26). This was recently observed also by Yang et al., who reported that *ERCC1* expression could have an impact on the benefit from actual treatment regimens (27). In our exploratory analysis on 181 CRC samples, statistically significantly higher levels of *ERCC1* mRNA was observed in right-sided tumors, and this could partly explain their chemoresistance (28).

These data are consistent with the growing evidence from large translational studies that showed distinct and specific biomolecular profiles by primary tumor location (29), with a potential impact on prognosis and benefit from chemotherapy and targeted agents. Popovici and colleagues identified a characteristic pattern of gene expression for colon cancers associated with poor prognosis, termed "BRAF mutant–like," based on a genomic profile that was similar to the profile of *BRAF* mutant tumors (30). The authors noted that BRAF mutant–like samples were statistically significantly enriched in right-sided tumors. Similarly, the Cancer Genome Atlas Network conducted a large genome-scale analysis of CRC samples, which revealed significant biological differences between right-sided tumors and tumors originating from other sites; right-sided cancers were more frequently hypermethylated and hypermutated (31). Furthermore, Adams and colleagues recently reported that gene expression levels of epiregulin and amphiregulin were highly prognostic among patients with *KRAS* wild-type advanced CRC (32). Higher levels of epiregulin and amphiregulin were associated with a left site of origin, presence of liver metastases, and high carcinoembryonic antigen, as well as with a better outcome (32). Recently, Brulé and colleagues observed that left-sided primary tumor location was a predictor of greater PFS benefit from cetuximab monotherapy in refractory *KRAS* wild-type mCRC patients (33). Other data from a large cohort of 2838 CRC patients clearly show that right-sided and left-sided CRCs are characterized by specific clinical, pathological, and molecular features; microarray profiling identified 997 genes differentially expressed between the two anatomical sites (34).

In our analysis, the splenic flexure was adopted as the landmark for distinguishing right-sided vs left-sided cancers because classification was based on standard pathologic reports. In the embryologic development of the distal intestine, the cecum, the ascending colon, the hepatic flexure of colon, and proximal two-thirds of the transverse colon originate from the midgut, whereas the distal third of the transverse and the splenic flexure, the descending colon, the sigmoid colon, and rectum derive from the hindgut (35). The inclusion of the distal third of the transverse among right-sided cancers could have partially affected the results. Given the small proportion of such cases, the impact is expected to be minimal. It should be noted that new studies are challenging the concept of a rough distinction between right-sided and left-sided CRCs, because the incidence of specific molecular features commonly associated with right-side origin seem to decrease gradually from right to left (36).

The data for the current analysis were derived from prospective or large randomized controlled trials; inclusion criteria and patient characteristics were well defined, and treatments were homogenous. These features strengthen the reliability of the results, which give a clinical perspective to recent findings from translational studies in CRC, confirming the concept that proximal and distal tumors are distinct clinical and biological entities.

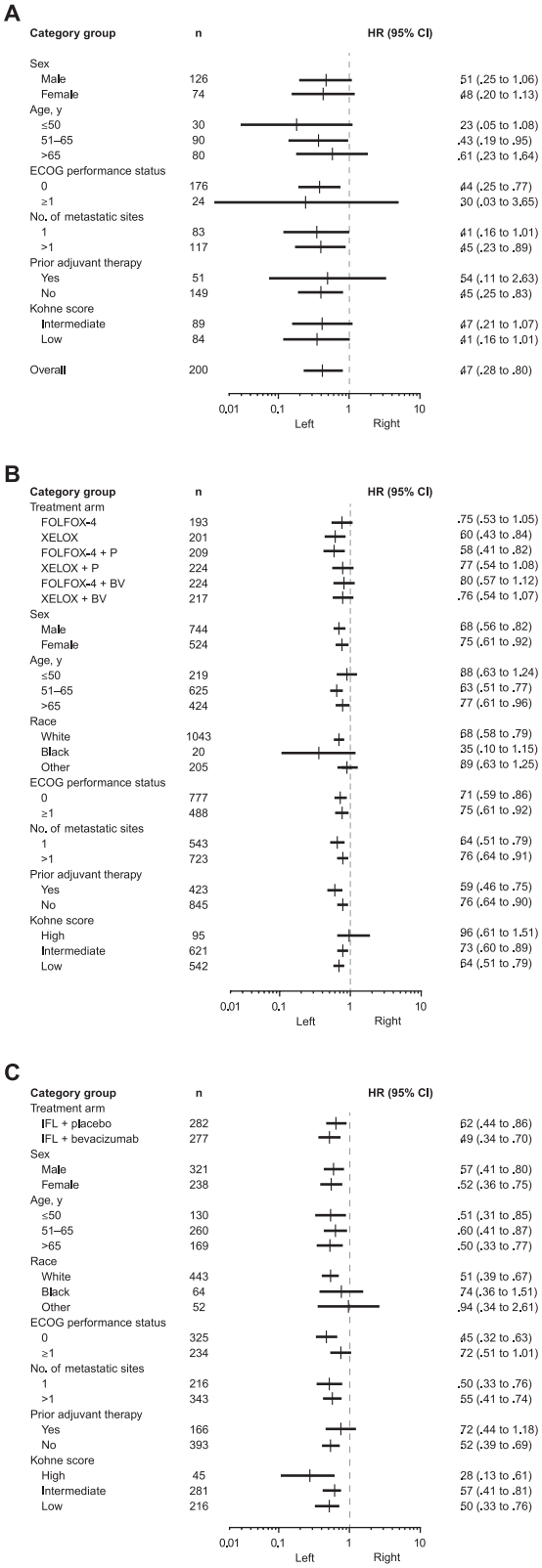

Figure 2. Subgroup analyses of overall survival by primary tumor location. Panel A shows the effect of primary tumor location on overall survival in the PROVETTA trial, stratified by baseline variables. Panels **(B)** and **(C)** show similar stratified analyses for the NO16966 and AVF2107g trials, respectively. The effect of primary tumor location was found to be statistically significant and consistent across the majority of the strata evaluated. Logistic regression analysis. All statistical tests were two-sided. CI = confidence interval; HR = hazard ratio.

This study also had some limitations: Data on BRAF mutational status and mucinous histology were not available from AVF2107g and NO16966. Those data would have allowed additional evaluation of the prognostic role of primary tumor location in specific subgroups. Moreover in the two randomized studies, primary tumor location was derived from case report forms, which limited the possibility of exploring the prognostic impact of subsegmental location or of looking at the distance from the anal verge as a continuous variable.

Our analyses demonstrate that primary tumor location has a strong prognostic effect on patients with mCRC and that the effect of bevacizumab is independent of location. This easy-to-collect dichotomous information on side of origin could be of added value in clinical decision-making, and should be considered an important stratification factor for future randomized trials. Validation of these results in adjuvant and additional metastatic studies of CRC is warranted.

As suggested by recent translational reports, the present study emphasizes the challenge of elucidating the biological and molecular basis of the influence of primary location on clinical outcome.

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