The Prognostic Impact of Primary Tumor Site Differs According to the KRAS Mutational Status

A Study By the International Genetic Consortium for Colorectal Liver Metastasis

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Objective: To examine the prognostic impact of tumor laterality in colon cancer liver metastases (CLM) after stratifying by Kirsten rat sarcoma 2 viral oncogene homolog (KRAS) mutational status.

Background: Although some studies have demonstrated that patients with CLM from a right sided (RS) primary cancer fare worse, others have found equivocal outcomes of patients with CLM with RS versus left-sided (LS) primary tumors. Importantly, recent evidence from unresectable metastatic CRC suggests that tumor laterality impacts prognosis only in those with wild-type tumors.

Methods: Patients with rectal or transverse colon tumors and those with unknown KRAS mutational status were excluded from analysis. The prognostic impact of RS versus LS primary CRC was determined after stratifying by KRAS mutational status.

Results: 277 patients had a RS (38.6%) and 441 (61.4%) had a LS tumor. Approximately one-third of tumors (28.1%) harbored KRAS mutations. In the entire cohort, RS was associated with worse 5-year overall survival (OS) compared with LS (39.4% vs 50.8%, $P = 0.03$) and remained significantly associated with worse OS in the multivariable analysis (hazard ratio 1.45, $P = 0.04$). In wild-type patients, a worse 5-year OS associated with a RS tumor was evident in univariable analysis (43.7% vs 55.5%, $P = 0.02$) and persisted in multivariable analysis (hazard ratio 1.49, $P = 0.01$). In contrast, among patients with KRAS mutated tumors, tumor laterality had no impact on 5-year OS, even in the univariable analysis (32.8% vs 34.0%, $P = 0.38$).

Conclusions: This study demonstrated, for the first time, that the prognostic impact of primary tumor side differs according to KRAS mutational status. RS tumors were associated with worse survival only in patients with wild-type tumors.

Keywords: KRAS, primary tumor location, prognosis

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In the last several years, laterality of the primary colon tumor has emerged as a new predictive and prognostic factor of colorectal cancer (CRC), particularly in the metastatic setting [metastatic colorectal cancer (mCRC)]. In fact, a recent meta-analysis of 66 relevant studies assembled a cohort of over 1 million patients with unresectable mCRC, and demonstrated that a left-sided (LS) primary tumor location (PTL) was associated with a significantly reduced risk of death.[1] A smaller but more homogenous meta-analysis of prospective clinical trials of patients with unresectable mCRC corroborated these findings and confirmed that PTL is prognostic in unresectable mCRC.[2] Therefore, there is little doubt regarding the predictive and prognostic role of PTL in patients with unresectable mCRC.

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However, the prognostic significance of primary tumor sidedness may differ when it comes to resectable colon cancer liver metastases (CLM). In contrast to studies regarding unresectable mCRC, those in resectable CLM have produced much more contradictory results.[3–6] There is currently no meta-analysis in patients with resectable CLM that could synthesize these variable results and thus conclude whether PTL is prognostic. As such, whether PTL plays a prognostic role in CLM remains largely unknown.

In an effort to explore why outcomes differ, we compared the design of the respective studies in resectable and unresectable mCRC. The main methodologic difference was that in the former, the KRAS mutational status was unknown and therefore not accounted for in the survival analysis, with the exception of 2 retrospective studies.[7,8] In contrast, the majority of the studies in unresectable mCRC were conducted in homogenous cohorts of wild-type patients treated in the context of randomized controlled trials.[9,10]

Thus, the aim of the study was to explore the hypothesis that KRAS mutational status influences the prognostic association of PTL in resectable CLM. To answer this question, we decided to use the large international, multi-institutional database of the International Genetic Consortium for Colorectal Liver Metastasis (IGCLM), which has been specifically assembled to capture patients with genetic data such as KRAS mutations.

METHODS

Study Design

All patients who underwent curative-intent surgery for colorectal cancer liver metastases (CRLM) between January 1, 2000, and December 31, 2016, and had genetic data were retrospectively identified from the patient records of 9 tertiary academic centers from the United States (The Johns Hopkins University, Baltimore, MD; Stanford University School of Medicine, Stanford, California; Digestive Disease Institute, Cleveland Clinic, Cleveland, OH), Europe (Medical University of Vienna, Vienna, Austria; Medical University of Graz, Graz, Austria; Charite — University of Berlin, Berlin, Germany; Haukeland University Hospital, Bergen, Norway) and Japan (Yokohama City University Graduate School of Medicine, Yokohama, Japan; Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan). The institutional review boards of all participating institutions approved the study. Of note, this is a unique study cohort as patient data from 2 more institutions (Yokohama and Kumamoto) have been added to the originally constructed database of the IGCLM.

Patient Selection

The database was queried to identify the study cohort. Patients who did not have data on their KRAS mutational status were excluded. Those who had a primary cancer of the rectum or the transverse colon were excluded as well. Demographic, clinicopathologic, and genetic data from the IGCLM database were used either to describe the baseline characteristics of the cohort(s) or as co-variates for the survival analysis because of their known prognostic relevance.

Definition of Right Versus Left PTL

We excluded patients with transverse colon primary tumors (with the exception of ''hepatic flexure'' tumors, which were included in the RS group) for 2 reasons. First, it would allow for comparison with the largest study to date on PTL in resectable CLM, which employed this exclusion criterion.[8] Second, it is often impossible to determine retrospectively from the pathologic report whether the primary tumor was located before or after the point that separates the first two-thirds from the final third of the transverse colon. Importantly, location compared to that point determines the embryologic origin (midgut vs hindgut) of the tumor.

KRAS Mutation Profiling

All patients underwent sequencing of the following KRAS codons: 12, 13, and 61, with the exception of the patients from Haukelund University, who only underwent sequencing of codons 12 and 13. Patients from the early 2000s were not tested for all mutations in exons 3, and none of the patients was tested for exon 4 mutations. Standard molecular biology techniques that have been previously described were employed. Either primary or metastatic tissue was used for the measurements, as a high concordance of the KRAS mutational status between primary and corresponding metastases has been reported.[11]

Sub-Analysis

We performed a sub-analysis after excluding patients who underwent concurrent resection and intraoperative ablation for 2 reasons. First, although still under debate, many reports concur that radio-frequency ablation results in inferior long-term outcomes compared to hepatic resection in patients with CLM, and is applied only when surgical resection is not feasible.[12] Second, it would allow for comparison with the largest study to date on PTL in resectable CLM, which employed this exclusion criterion.[8]

Statistical Analysis

Demographic, clinicopathologic, and perioperative features of the study population were stratified according to the primary tumor site and KRAS mutational status. Summary statistics were presented as totals and frequencies for categorical variables or as median values with interquartile ranges (IQRs) for continuous variables. Categorical variables were assessed using the chi-square or the Fisher exact test, as appropriate. Overall survival (OS) was measured from the date of hepatic resection until the date of death or last follow-up. Survival curves were generated using the Kaplan-Meier method, and differences between curves were evaluated with the log-rank test.

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Univariable and multivariable analyses to identify predictors of survival were performed by using Cox proportional hazards regression models. Variables with $P < 0.05$ in univariable analysis were entered into multivariable analysis. Last, we performed nearest neighbor propensity score matching for potential confounders using the MatchIt package for R 3.5.1. Confounders were defined as variables unevenly distributed across LS and right-sided (RS) cancers in the wild-type population ($P < 0.1$). Logistic regression was used to estimate the distance measure and the caliper was set to 0.2. All analyses were performed using STATA version 13 (StataCorp, College Station, TX) and R 3.5.1. (https://cran.r-project.org/). A P value of <0.05 (2-tailed) defined statistical significance.

RESULTS

Patient Characteristics

Overall, 1568 patients were identified from the IGCLM database. From this cohort, patients with unknown KRAS mutational status (n = 270), rectal primaries (n = 515), or transverse primaries (n = 65) were excluded (Fig. 1). The remaining 718 patients formed the study cohort with 277 (38.6%) RS and 441 (61.4%) LS primary tumors. Detailed demographic, clinicopathologic, and genetic data were stratified by PTL and are presented in Table 1. Patients with RS tumors were more likely to be older (63.9 vs 60.7 years old, $P < 0.001$) and female (47% vs 38%, $P = 0.014$). As expected, they were also more likely to have KRAS mutated tumors (39% vs 21%, $P < 0.001$). The frequency of liver bilobar disease was lower in the RS patients (29% vs 39%, $P = 0.006$), although they were more likely to receive posthepatectomy adjuvant chemotherapy (65% vs 52%, $P = 0.003$). Lastly, patients with RS tumors were less likely to receive anti-epidermal growth factor receptor (anti-EGFR) therapies (2% vs 7%, $P = 0.001$).

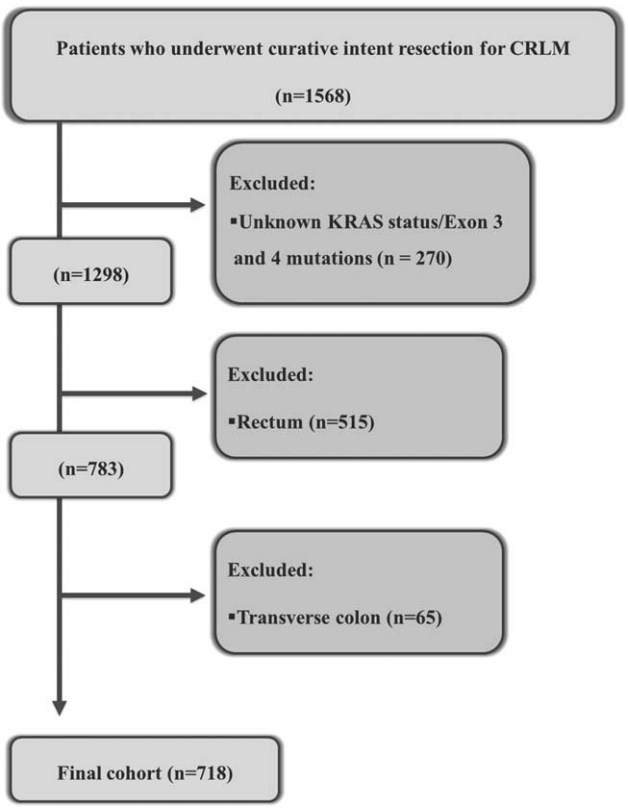

FIGURE 1. Patient selection flowchart.

Following the comparison of RS versus LS patients, we also compared demographic, clinicopathologic, and genetic data, stratified this time by both KRAS mutational status and PTL (Table 2). Among those with wild-type tumors, patients with RS tumors were more likely to be older and female. The frequency of liver bilobar disease and of the receipt of anti-EGFR therapies was also lower in those patients. In contrast, among patients with KRAS mutated tumors, age and sex were equally distributed between patients with RS versus LS tumors. The only differences were a higher rate of both nodal disease and posthepatectomy chemotherapy for those with RS tumors.

OS in the Entire Cohort

At a median follow-up of 30.4 months (IQR, 16.5–52.7 months), median survival for the entire cohort was 53.6 months and 5-year survival was 46.4%. Fig. 2A demonstrates that survival after resection of CLM was significantly different ($P = 0.035$) when stratified by PTL, with a median OS of 45.5 months for those with RS tumors versus 61.6 months for those with LS tumors. Five-year OS for RS tumors was 39.4% compared to 50.8% for LS tumors. Importantly, after controlling for known prognostic clinicopathologic and genetic factors on multivariable analysis, RS remained independently associated with a higher risk of death [hazard ratio (HR): 1.45, $P = 0.042$] (Table 3). Furthermore, KRAS mutational status, extrahepatic disease, receipt of prehepatectomy chemotherapy, concurrent intraoperative ablation, and number of metastases >3 were independently associated with a higher risk of death.

OS in the Wild-Type Patients

At a median follow-up of 30.7 months (IQR, 16.5–53.6 months), patients with wild-type tumors had a median survival of 65.9 months and a 5-year survival of 51.6%. Fig. 2B demonstrates that survival after resection of CLM was significantly different when stratified by PTL, with a median OS of 47.2 months for those with RS tumors versus 67.8 months for those with LS tumors ($P = 0.023$). Five-year OS for RS tumors was 43.7% compared to 55.5% for LS tumors. Importantly, RS remained independently associated with a higher risk of death even after controlling for other prognostic factors (HR: 1.49; 95% confidence interval: 1.09–2.04; $P = 0.01$) (Table 4). Nodal disease of the primary tumor, receipt of prehepatectomy chemotherapy, and extrahepatic disease were also independently associated with a higher risk of death.

OS in the KRAS Mutated Patients

At a median follow-up of 30.1 months (IQR, 15.8–51.5 months), median survival for patients with KRAS mutated tumors was 39.7 months and 5-year survival was 33.6%. Fig. 2C demonstrates that survival after resection of CLM was similar between patients with RS versus LS tumors, with a median OS of 39.7 months for those with RS tumors versus 40.1 months for those with LS tumors ($P = 0.383$). Five-year OS for RS tumors was 32.8% compared to 34.0% for LS tumors. Extrahepatic disease and a CEA higher than 100 ng/mL was independently associated with a higher risk of death (Table 5).

Sub-analysis of OS After Excluding Patients Who Underwent Resection and Concurrent Ablation

The findings of the main analysis were corroborated in the sub-analysis after excluding the 73 patients who underwent concurrent resection and ablation. When considering the entire cohort, RS was not associated with a higher risk of death, even in univariable analysis (HR: 1.25, $P = 0.08$). Upon stratifying patients by mutational

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TABLE 1. Patient Demographic, Clinicopathologic, and Genetic Characteristics Stratified by PTL (n = 718)

Patient Characteristics		Left-sided Primary	Right-sided Primary	*P*
n		441	277	
Age at the time of surgery [median (IQR)]		60.7 (50.9, 67.1)	63.9 (55.4, 71.4)	**<0.001**
Sex (%)	Male	275 (62)	147 (53)	**0.014**
	Female	166 (38)	130 (47)	
Primary T stage (%)	T1-T2	44 (11)	31 (12)	0.564
	T3-T4	372 (89)	227 (88)	
Primary lymph node (%)	No	156 (36)	96 (35)	0.711
	Yes	274 (64)	179 (65)	
Synchronous CRLM (%)	No	216 (50)	152 (55)	0.136
	Yes	220 (50)	123 (45)	
CEA >100 ng/mL (%)	No	323 (86)	198 (89)	0.247
	Yes	53 (14)	24 (11)	
CLM number ≥3 (%)	No	280 (64)	176 (64)	0.947
	Yes	159 (36)	101 (36)	
CLM size >3 cm (%)	No	186 (51)	141 (58)	0.085
	Yes	181 (49)	103 (42)	
Bilobar liver disease (%)	No	260 (61)	189 (71)	**0.006**
	Yes	166 (39)	76 (29)	
Extrahepatic disease (%)	No	394 (90)	244 (89)	0.483
	Yes	42 (10)	31 (11)	
KRAS-mutated (%)	No	348 (79)	168 (61)	**<0.001**
	Yes	93 (21)	109 (39)	
R0 resection (%)	No	297 (81)	197 (81)	0.926
	Yes	68 (19)	46 (19)	
Bevacizumab (%)	No	229 (65)	145 (65)	0.973
	Yes	124 (35)	79 (35)	
Prehepatic resection chemotherapy (%)	No	147 (34)	100 (36)	0.489
	Yes	286 (66)	174 (64)	
Posthepatic resection chemotherapy (%)	No	192 (48)	88 (35)	**0.003**
	Yes	212 (52)	160 (65)	
Anti-EGFR (%)	Yes	31 (7)	5 (2)	**0.001**

Bold fond indicates statistical significance ($P < 0.05$).
CLM indicates colon cancer liver metastases; IQR, interquartile range; PTL, primary tumor location.

status; however, 2 distinct trends were uncovered. In patients with wild-type tumors, RS remained independently associated with a higher risk of death even after controlling for all other prognostic factors (HR: 1.45; 95% confidence interval: 1.01–2.06; $P = 0.04$). In contrast, in patients with KRAS mutated tumors, RS was not associated with a higher risk of death, even in univariable analysis (HR: 0.76, $P = 0.20$).

Sensitivity Analyses

Sensitivity analyses were performed after excluding patients with extrahepatic metastases (Supplemental Tables 1–3, http://links.lww.com/SLA/B721), those who received anti-EGFR agents (Supplemental Tables 4 and 5, http://links.lww.com/SLA/B721), those who underwent operations when modern chemotherapeutics (ie, oxaliplatin-based, irinotecan-based, anti-EGFR agents, and anti-vascular endothelial growth factor agents) were available (Supplemental Tables 6–8, http://links.lww.com/SLA/B721), or after including patients with KRAS exon 3 or 4 mutations (Supplemental Tables 9–11, http://links.lww.com/SLA/B721). Importantly, the 2 major findings of the study did not change in these additional analyses. Specifically, among patients with wild-type tumors, those with RS colon cancer had a shorter survival, whereas among those with KRAS mutated tumors, tumor laterality was not prognostic. Furthermore, a propensity score analysis to adjust for possible confounders confirmed that prognostic survival difference between right and left primary colon cancer was still detected only in patients harboring KRAS wild-type tumors (Supplemental Table 12, http://links.lww.com/SLA/B721 and Supplemental Figure 1, http://links.lww.com/SLA/B721).

DISCUSSION

In this international, multi-institutional analysis of patients with resected CLM, we found that among patients with wild-type tumors, those with RS colon cancer had a shorter survival (Fig. 2B). This result persisted in the multivariable analysis. In contrast, among those with KRAS mutated tumors, tumor laterality was not prognostic even in the univariable analysis (Fig. 2C). Importantly, these findings were corroborated in a sub-analysis after excluding patients who underwent concurrent resection and ablation. Furthermore, these findings were corroborated in a second sub-analysis which included only patients who underwent operations when all modern chemotherapeutics were available (ie, oxaliplatin-based, irinotecan-based, anti-EGFR agents, and anti-vascular endothelial growth factor agents).

Another interesting finding of the study was that prehepatectomy chemotherapy was independently associated with worse OS. This result merits further attention, particularly because publications from Memorial Sloan Kettering Cancer Center and MD Anderson have reported similar findings.[13,14] Interestingly, Andreou et al from MD Anderson reported that patients treated with FOLFOX before CRLM resection had a higher rate of KRAS mutation, thus suggesting a mechanism for the selection of more aggressive disease.[15] Nonetheless, the retrospective design of the study, the heterogeneity of the employed chemotherapy protocols, and the lack of

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TABLE 2. Patient Demographic and Clinicopathologic Characteristics Stratified by the KRAS Mutational Status and PTL (n = 718)

		Wild-Type			KRAS-Mutated		
Patient Characteristics		**Left-sided Primary**	**Right-sided Primary**	***P***	**Left-sided Primary**	**Right-sided Primary**	***P***
n		348	168		93	109	
Age at the time of surgery [median (IQR)]		61.2 (51.3, 67.5)	64.2 (56.2, 71.5)	**0.001**	58.4 (50.4, 66.7)	63.0 (54.8, 70.7)	0.089
Sex (%)	Male	221 (64)	89 (53)	**0.022**	54 (58)	58 (53)	0.489
	Female	127 (36)	79 (47)		39 (42)	51 (47)	
Primary T stage (%)	T1-T2	38 (12)	17 (11)	0.814	6 (7)	14 (14)	0.156
	T3-T4	291 (88)	140 (89)		81 (93)	87 (86)	
Primary nodal disease (%)	No	114 (34)	64 (39)	0.297	42 (45)	32 (29)	**0.02**
	Yes	223 (66)	102 (61)		51 (55)	77 (71)	
Synchronous CRLM (%)	No	167 (49)	92 (55)	0.154	49 (53)	60 (55)	0.738
	Yes	176 (51)	74 (45)		44 (47)	49 (45)	
CEA >100 ng/mL (%)	No	254 (86)	115 (89)	0.313	69 (87)	83 (89)	0.698
	Yes	43 (14)	14 (11)		10 (13)	10 (11)	
CLM number ≥3 (%)	No	223 (64)	113 (67)	0.53	57 (61)	63 (58)	0.614
	Yes	123 (36)	55 (33)		36 (39)	46 (42)	
CLM size >3 cm (%)	No	146 (49)	88 (59)	0.05	40 (59)	53 (56)	0.757
	Yes	153 (51)	62 (41)		28 (41)	41 (44)	
Bilobar liver disease (%)	No	201 (60)	121 (76)	**0.001**	59 (65)	68 (65)	0.991
	Yes	134 (40)	39 (24)		32 (35)	37 (35)	
Extrahepatic disease (%)	No	311 (91)	147 (88)	0.354	83 (89)	97 (90)	0.896
	Yes	32 (9)	20 (12)		10 (11)	11 (10)	
KRAS-mutated (%)	No	348 (100)	168 (100)	NA	0 (0)	0 (0)	NA
	Yes	0 (0)	0 (0)		93 (100)	109 (100)	
R0 resection (%)	No	239 (81)	121 (81)	0.906	58 (84)	76 (81)	0.597
	Yes	57 (19)	28 (19)		11 (16)	18 (19)	
Bevacizumab (%)	No	178 (65)	83 (63)	0.613	51 (63)	62 (67)	0.541
	Yes	94 (35)	49 (37)		30 (37)	30 (33)	
Prehepatic resection chemotherapy (%)	No	112 (33)	60 (36)	0.447	35 (38)	40 (37)	0.891
	Yes	228 (67)	105 (64)		58 (62)	69 (63)	
Posthepatic resection chemotherapy (%)	No	148 (47)	54 (38)	0.074	44 (51)	34 (32)	**0.011**
	Yes	169 (53)	89 (62)		43 (49)	71 (68)	
Anti-EGFR (%)	Yes	31 (9)	4 (2)	**0.005**	0 (0)	1 (1)	0.35

Bold fond indicates statistical significance ($P < 0.05$).
CLM indicates colon cancer liver metastases; IQR, interquartile range; PTL, primary tumor location.

randomization preclude any reliable interpretation. Randomized controlled trials should further explore the optimal role and timing of chemotherapy in patients with CRLM.

The results from the previous studies regarding PTL in resectable CLM are contradicting. Some studies showed that PTL is prognostic for both OS and recurrence-free survival (RFS), others that it is prognostic for either OS or RFS, and some that it is not prognostic for either outcome. Interestingly, the common limiting factor across these contradicting studies has been the lack of KRAS data. For example, a study by the Memorial Sloan Kettering Cancer Center group found that although an LS primary was independently associated with an improved median OS, it was not associated with a difference in RFS and long-term survival.[3] The authors acknowledged the lack of data on KRAS mutational status as one of the major limitations of the study. Similarly, the

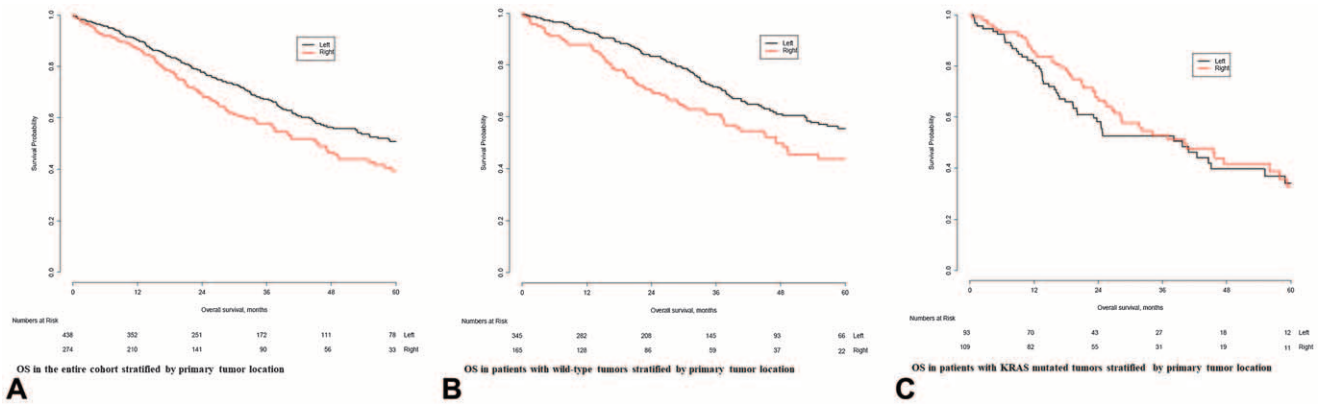

FIGURE 2. Kaplan-Meier curve of overall survival. A, In the entire cohort stratified by primary tumor location. B, In wild-type patients stratified by primary tumor location. C, In KRAS mutated patients stratified by primary tumor location.

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TABLE 3. Univariate and Multivariate Survival Analysis in the Entire Cohort (n = 456)

	Univariate		Multivariate	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
KRAS mutated tumors				
Wild-type tumors	Ref.		Ref.	
Mutated	1.62 (1.28–2.06)	<0.001	1.99 (1.30–3.06)	**0.002**
Left-sided primary	Ref		Ref	
Right-sided primary	1.28 (1.02–1.61)	0.03	1.45 (1.01–2.07)	**0.042**
Interaction between laterality and KRAS mutation variable	0.61 (0.37–0.99)	0.045	0.63 (0.34–1.17)	0.141
Patient age at the time of surgery	1.002 (0.99–1.01)	0.76	—	
Female gender	1.00 (0.79–1.25)	0.98	—	
Primary tumor stage				
T3 and T4 versus T1 and T2 stage	1.31 (0.89–1.93)	0.17	—	
Nodal disease of the primary	1.37 (1.08–1.75)	0.010	1.36 (1.00–1.85)	0.05
CEA >100 ng/dL	1.50 (1.06–2.11)	0.023	1.45 (0.95–2.21)	0.083
Prehepatectomy chemotherapy	1.3 7 (1.06–1.77)	0.016	1.90 (1.34–2.70)	**<0.001**
Synchronous liver metastases	1.09 (0.86–1.36)	0.47	—	
Extrahepatic disease	2.20 (1.57–3.10)	<0.001	1.94 (1.24–3.03)	**0.004**
Liver tumor diameter >3 cm	1.11 (0.86–1.42)	0.41	—	
Liver tumor number >3	1.48 (1.18–1.86)	<0.001	1.38 (1.20–1.88)	**0.037**
Bilobar liver disease	1.20 (0.95–1.52)	0.12	—	
R1 resection	1.54 (1.14–2.08)	0.005	1.27 (0.90–1.79)	0.166
Posthepatectomy chemotherapy	0.73 (0.58–0.93)	0.009	0.84 (0.63–1.13)	0.248
Intraoperative ablation	1.47 (1.08–2.00)	0.015	1.49 (1.03–2.15)	**0.035**
Anti-EGFR	1.26 (0.72–2.20)	0.42		

Bold fond indicates statistical significance ($P < 0.05$).
CI indicates confidence interval; HR, hazard ratio.

authors of several studies on the prognostic value of PTL in CLM have acknowledged the lack of data on KRAS mutational status as one of the major limitations of their studies.[4–6,16]

Importantly, only 2 studies on PTL in resectable CLM completely accounted for RAS mutations in their analysis. The first study came from our group at Johns Hopkins Hospital, and the other study from Yamashita et al followed soon after.[7,8] Both studies concluded that patients with RS colon cancer had a shorter survival. Neither study stratified patients by their KRAS mutational status before assessing the impact of PTL on survival. Instead, they controlled for RAS mutations in the multivariable analysis, and PTL remained independently associated with survival. Although controlling for confounders using multivariable analysis is a valid statistical method, it can be misleading in the case of collinearity of two variables.[17] Collinearity occurs when 2 prognostic factors correlate not only with prognosis, but also with each other. In this case, it is likely that right sidedness and RAS mutations are collinear variables, and thus controlling for RAS mutations in the multivariable analysis may not necessarily mean that PTL is an independent prognostic factor. This is because RAS mutations are more common in RS tumors, and thus positive mutational status can be predicted from tumor laterality. In turn, the higher risk of death for patients with RS tumors may have been falsely attributed to laterality instead of the associated RAS mutations.

In contrast, we chose to account for confounders by not only using multivariable analysis, but also by stratifying patients by their KRAS mutational status before assessing the prognostic impact of PTL. This minimizes the chance of residual confounding. Among patients with wild-type tumors, we found that those with RS colon cancer had a shorter survival. In contrast, among those with KRAS mutated tumors, PTL was not prognostic even in the univariable analysis. Although no other study in resectable CLM has stratified patients by their KRAS mutational status, similar stratifications have been performed in 2 studies on unresectable mCRC. The first study utilized data from a randomized, multicenter phase II trial (AIO KRK-0104) and had similar findings to our study.[18] Specifically, LS tumors were associated with significantly longer OS and PFS as compared to RS tumors, but only in patients with KRAS codon 12/13 wild-type tumors (HR OS: 0.42; HR PFS: 0.54). In contrast, PTL was not prognostic in those with KRAS codon 12/13 mutant tumors (HR OS: 1.3; HR PFS: 1.01). The authors concluded that there existed a significant interaction between KRAS status and PTL, which in turn impacted OS and PFS.[18] In the second study, Loupakis et al explored a cohort of 546 patients with unresectable KRAS mutated mCRC, and found that PTL was not prognostic in KRAS mutants (HR = 0.99, $P = 0.964$).[19] Perhaps reflecting those studies, Stinzing et al stated in a recent review that "Currently, data on RAS-MT LCC versus RCC are limited; therefore, the prognostic and predictive value of the primary tumor site within the RAS MT population still requires evaluation."[20]

BRAF mutation is the only somatic mutation that is prognostic in CLM and not accounted for in our analysis, which was a limitation of this study.[21] However, studies in unresectable mCRC suggest that the prognostic value of PTL is independent of the BRAF mutational status.[22,23] Nevertheless, given that BRAF mutations occur mostly in KRAS wild-type tumors and on the RS, it is possible that unaccounted BRAF mutations in KRAS wild-type tumors may be responsible for the worse survival in patients with RS versus LS tumors. Future studies will need to account for other genetic mutations (eg, BRAF mutation) in KRAS wild-type patients and address whether PTL remains prognostic in wild-type patients after accounting for them. Another limitation of the current study was the lack of data on histology and specifically on mucinous histology, which is more commonly observed in RS tumors and has been previously shown to negatively impact prognosis in CLM.[24] However, evidence in the mCRC setting also suggests that the prognostic value of PTL is independent of histology.[23,25] As a retrospective analysis, the study is subject to inherent bias regarding patient selection and follow-up. For example, we cannot rule out that RS patients with "worse" cancers (poor differentiation, more nodal disease, etc) never make it to

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TABLE 4. Univariate and Multivariate Survival Analysis in Wild-type Patients (n = 422)

	Univariate		Multivariate	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
Primary tumor laterality				
Left-sided primary	Ref.			
Right-sided primary	1.40 (1.05–1.86)	0.023	1.49 (1.09–2.04)	**0.013**
Patient age at the time of surgery	1.00 (0.99–1.02)	0.73	—	
Female gender	0.96 (0.72–1.27)	0.77	—	
Primary tumor stage				
T3 and T4 versus T1 and T2	1.32 (0.83–2.10)	0.24	—	
Nodal disease of the primary	1.41 (1.04–1.92)	0.02	1.42 (1.02–1.97)	**0.038**
CEA >100 ng/dL	1.30 (0.85–1.99)	0.23	—	
Prehepatectomy chemotherapy	1.39 (1.01–1.92)	0.04	1.79 (1.21–2.64)	**0.003**
Synchronous liver metastases	1.04 (0.78–1.37)	0.80	—	
Extrahepatic disease	2.03 (1.32–3.10)	0.001	1.79 (1.11–2.87)	**0.016**
Liver tumor diameter >3 cm	1.30 (0.96–1.74)	0.09	—	
Liver tumor number >3	1.43 (1.08–1.89)	0.01	1.21 (0.87–1.69)	0.26
Bilobar liver disease	1.08 (0.81–1.45)	0.59	—	
R1 resection	1.47 (1.02–2.10)	0.04	1.33 (0.91–1.94)	0.14
Posthepatectomy chemotherapy	0.76 (0.57–1.00)	0.06	—	
Intraoperative ablation	1.51 (1.04–2.19)	0.03	1.43 (0.94–2.17)	0.09
Anti-EGFR	1.44 (0.80–2.59)	0.23		

Bold fond indicates statistical significance ($P < 0.05$).
CI indicates confidence interval; HR, hazard ratio.

surgery. In turn, this selection bias may to a certain extent account for the equivocal worse survival of patients with RS KRAS mutated tumors. Of note, different independent prognostic factors were observed between the KRAS-wild and KRAS-mutant sub-cohorts. This is probably a reflection of the nonlinearity in prognostic models. Specifically, mathematical realities suggest that the interactions among different prognostic factors are far from linear, and that some variables gain or lose significance due to the absence or presence of other variables — in this case in the presence or absence of KRAS mutation.[26] Last, as mentioned in the methods section, patients from the early 2000s were not tested for all mutations in exons 3, and none of the patients were tested for exon 4 mutations. As such, some of these mutations may not have been captured. Given that a recent study reported on a variable prognostic impact of those mutations, future studies should account for them.[27]

The IGCLM is by definition an international, multi-institutional database that includes patients with resectable stage IV CRC, and in particular, liver metastases (with or without concurrent extrahepatic disease). As such, it was not feasible to analyze all different stages of CRC. Because the study findings concern only resectable CRLM (with or without concurrent resectable extrahepatic disease), they should not be extrapolated to nonmetastatic resectable CRC (stage I-III) or unresectable metastatic CRC. Interestingly, although our findings are in line with those in studies on

TABLE 5. Univariate and Multivariate Survival Analysis in KRAS Mutated Patients (n = 129)

	Univariate		Multivariate	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
Primary tumor laterality				
Left-sided primary	Ref			
Right-sided primary	0.84 (0.57–1.24)	0.38	—	
Patient age at the time of surgery	1.003 (0.98–1.02)	0.88	—	
Female gender	0.99 (0.67–1.46)	0.96	—	
Primary tumor stage				
T3 and T4 versus T1 and T2	1.24 (0.62–2.47)	0.54	—	
Nodal disease of the primary	1.36 (0.90–2.05)	0.15	—	
CEA >100 ng/dL	2.95 (1.61–5.42)	<0.001	3.16 (1.26–7.96)	**0.014**
Prehepatectomy chemotherapy	1.42 (0.93–2.17)	0.10	—	
Synchronous liver metastases	1.23 (0.84–1.81)	0.29	—	
Extrahepatic metastases	2.97 (1.65–5.34)	<0.001	2.92 (1.29–6.58)	**0.01**
Liver tumor diameter >3 cm	0.82 (0.51–1.31)	0.41	—	
Liver tumor number >3	1.50 (1.02–2.21)	0.04	1.68 (0.87–3.23)	0.12
Bilobar liver disease	1.51 (1.01–2.23)	0.042	1.04 (0.54–2.01)	0.90
R1 resection	1.84 (1.06–3.19)	0.029	1.43 (0.77–2.66)	0.25
Posthepatectomy chemotherapy	0.62 (0.42–0.93)	0.021	0.99 (0.54–1.82)	0.98
Intraoperative ablation	1.40 (0.79–2.47)	0.237		

Bold fond indicates statistical significance ($P < 0.05$).
CI indicates confidence interval; HR, hazard ratio.

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unresectable metastatic CRC, current literature on nonmetastatic resectable CRC is controversial. Most studies reported on a poorer survival of RS tumors, whereas others like Weiss et al and Warschkow et al found no significant difference in survival or even a better prognosis in those with RS tumors, respectively.[28,29] Future studies should include patients with non-metastatic resectable CRC (stage I-III) and metastatic resectable CRC, and assess whether our findings apply to all stages after stratifying by KRAS mutational status. As is always the case with retrospective research, these results should be validated by larger, ideally prospective, studies.

This study is important for three reasons. First, for the first time in a surgical cohort of CLM, our study shows that KRAS mutational status influences the prognostic impact of PTL, which confirms findings from prior studies in unresectable mCRC. As Boeckx et al noted, given that data on the interplay between PTL and RAS mutated disease is inconclusive, this study adds to the current literature.[30] Second, the double PTL-KRAS stratification that we introduced may explain the disparate results of the previous studies on the prognostic impact of PTL in resectable CLM, which did not account for KRAS mutational status. Thus, our method may be used to better inform prognosis compared to the simple RS versus LS stratification. Third, if our findings are confirmed by others, their clinical importance extends beyond informing prognosis, and can potentially be used to tailor surgical technique and/or surgical margin width, as our group previously reported for KRAS mutation status alone.[31,32] The combination of PTL and KRAS mutational status may allow for even more tailored multimodality treatments. Importantly, a recent study using NCDB data indirectly corroborates our findings.[33] Though this study was different in its scope from the index study, a sub-analysis (Goffredo et al, 2018, Fig. 3) shows that the difference in OS between RS and LS cancers was of much greater magnitude for wild-type versus KRAS mutated patients.

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