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RESEARCH ARTICLE

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The interplay of KRAS mutational status with tumor laterality in non-metastatic colorectal cancer: An international, multi-institutional study in patients with known KRAS, BRAF, and MSI status

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Abstract

Background: Although the prognostic relevance of KRAS status in metastatic colorectal cancer (CRC) depends on tumor laterality, this relationship is largely unknown in non-metastatic CRC.

Carsten Kamphues and Shigenori Kadowaki contributed equally to this study.

Methods: Patients who underwent resection for non-metastatic CRC between 2000 and 2018 were identified from institutional databases at six academic tertiary centers in Europe and Japan. The prognostic relevance of KRAS status in patients with right-sided (RS), left-sided (LS), and rectal cancers was assessed.

Results: Of the 1093 eligible patients, 378 had right-sided tumors and 715 had left-sided tumors. Among patients with RS tumors, the 5-year overall (OS) and recurrence-free survival (RFS) for patients with KRASmut versus wild-type tumors was not shown to differ significantly (82.2% vs. 83.2% and 72.1% vs. 76.7%, respectively, all $p > .05$). Among those with LS tumors, KRAS mutation was associated with shorter 5-year OS and RFS on both the univariable (OS: 79.4% vs. 86.1%, $p = .004$; RFS: 68.8% vs. 77.3%, $p = .005$) and multivariable analysis (OS: HR: 1.52, $p = .019$; RFS: HR: 1.32, $p = .05$).

Conclusions: KRAS mutation status was independently prognostic among patients with LS tumors, but this association failed to reach statistical significance in RS and rectal tumors. These findings confirm reports in metastatic CRC and underline the possible biologic importance of tumor location.

KEYWORDS

colorectal cancer, laterality, metastases

1 | INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy worldwide and accounts for approximately 10% of all new cancer cases yearly.[1] Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation is an early event in the carcinogenesis of CRC and occurs in around 30%–40% of colon cancers.[2] The prognostic role of KRAS mutations in non-metastatic CRC has been primarily studied using pathologic material from large clinical trials that assessed the role of chemotherapeutic agents as adjuvant treatment for resected CRC. Although a number of reports failed to demonstrate that KRAS mutation status was prognostic, larger and more adequately powered studies ultimately showed that KRAS mutation status is clearly prognostic.[3–7]

Another variable that has recently emerged as an important prognostic factor is tumor laterality (right vs. left). Earlier reports had discrepant findings; for example, for early-stage CRC cancers (stages I–II), right-sided disease was associated with superior survival, whereas for late-stage disease (stage III), left-sided disease was associated with improved survival.[8–12] In contrast, others have found that tumor laterality is not associated at all with survival in early-stage resected colon cancer.[13] Nonetheless, in the largest study to date, which included >1 million patients, Petrelli et al.[14] reported that left-sided primary tumor location was associated with a significantly reduced risk of death independent of stage.

A recent study by our group demonstrated that in metastatic CRC, the prognostic relevance of KRAS status was contingent on primary tumor laterality.[15] Specifically, KRAS was prognostic only among patients with left-sided, metastatic CRC. Importantly, this relationship is largely unknown in non-metastatic CRC. Thus, we undertook this study to investigate whether a similar interplay of KRAS mutation status and tumor laterality exists in this specific population.

2 | METHODS

2.1 | Study design, inclusion criteria, and pertinent variables

Patients with non-metastatic CRC (stages I–III) who were surgically treated between January 2000 and December 2018 and with known KRAS mutation status were retrospectively identified from institutional databases at four academic tertiary centers in Europe and two in Japan. Participating centers included Charite—University of Berlin (Berlin, Germany), Erasmus Medical Center (Rotterdam, Netherlands), Attiko Hospital (Athens, Greece), Hippokrateion Hospital (Athens, Greece), Saitama Cancer Center (Saitama, Japan), and Graduate School of Medical Sciences, Kumamoto University (Kumamoto, Japan). Patients with unknown BRAF mutation status, unknown microsatellite stability (MSI) status, double KRAS/BRAF mutations, as well as those with unknown follow-up were excluded from the study cohort.

Data on demographics and clinical features, including age at the time of diagnosis, sex, neoadjuvant systemic treatments (for those with rectal tumors), primary tumor laterality, tumor category (T),

nodal disease category, tumor grade, lymphovascular invasion (LVI), vascular invasion, BRAF status, microsatellite instability (MSI-H) status, and adjuvant systemic treatments were collected. To maintain consistency with previous studies, we defined primary tumors located in the cecum, ascending colon, and transverse colon as right-sided tumors, and tumors located in the splenic flexure, descending colon, sigmoid colon, and rectum as left-sided tumors. Data on long-term outcomes including recurrence and overall survival status at last follow-up were collected.

2.2 | Statistical analysis

Categorical variables were described as totals and frequencies, whereas numerical variables were presented as medians with interquartile ranges (IQR). Continuous variables were compared using the χ^2 test while numerical variables were compared using the Wilcoxon–Mann–Whitney or Kruskal–Wallis tests, as appropriate. RFS and OS were calculated from the date of surgery using the Kaplan–Meier method, and differences in RFS and OS were assessed with the Log-rank test. Cox proportional hazards regression models were used to identify potential predictors of survival. Variables that were found to have a statistically significant association with outcomes on the univariable analysis ($p < .05$) were included in the multivariable analysis. The proportional assumption of the Cox model was tested using the Schoenfeld residuals test, and the model was stratified based on variables that did not meet the proportional assumption for the Cox model. Interaction between variables was also tested and included in the final model. Statistical analysis was performed using Stata/MP version 13.1 (StataCorp).

3 | RESULTS

3.1 | Characteristics of patients with right- versus left-sided tumors

A flow chart that demonstrates cohort selection is illustrated in Figure 1. A total of 1093 patients met the inclusion criteria. Of those, 378 had right-sided (RS) tumors and 715 had left-sided (LS) tumors. Among the latter, there were 251 patients with rectal tumors. Detailed demographic, clinicopathologic, and genetic data of patients who had RS versus LS CRC and "truly left" versus rectal tumors are summarized in Tables S1 and S2, respectively.

Patients with RS tumors were more likely to be older (68 vs. 64 years old, $p < .001$) and female (50.9% vs. 39%, $p < .001$). As expected, they were also more likely to have BRAF mutated tumors (13.5% vs. 1.8%, $p < .001$) and MSI-H tumors (24.9% vs. 3.9%, $p < .001$). In addition, these patients were more likely to have high grade tumors (11.9% vs. 6.3%, $p = .02$). Lastly, patients with RS tumors were less likely to receive adjuvant chemotherapy (38.6% vs. 45.3%, $p = .03$).

3.2 | Characteristics of patients with RS KRASmut versus wild-type tumors

Of the 378 patients with RS tumors, 261 (69%) had wild-type tumors and 117 (31%) had KRASmut tumors. Detailed demographic, clinicopathologic, and genetic data of patients who had RS versus LS CRC stratified by KRAS status are summarized in Table 1.

Among patients who had RS tumors, the demographic and clinicopathologic characteristics did not differ by KRAS status. In regard to genetic characteristics, patients with wild-type tumors were more likely to have MSI-H (31.4% vs. 10.3%, $p < .001$) and BRAFmut tumors (19.5% vs. 0%, $p < .001$). For treatment variables, patients with wild-type tumors were less likely to receive adjuvant chemotherapy (34.5% vs. 47.9%, $p = .014$).

3.3 | Characteristics of patients with LS KRASmut versus wild-type tumors

Of the 715 patients with LS tumors, 488 (68.2%) had wild-type tumors and 227 (31.8%) had KRASmut tumors. Detailed demographic, clinicopathologic, and genetic data of patients who had LS versus RS CRC stratified by KRAS status are summarized in Table 1. Among patients who had LS tumors, patients with wild-type tumors were more likely to be male (64.6% vs. 53.3%, $p = .004$) and patients with KRASmut tumors were more likely to have lymphovascular invasion (58% vs. 48.4%, $p = .02$). In regard to genetic characteristics, patients with wild-type tumors were less likely to have MSI-H (2.9% vs. 6.2%, $p = .034$) but more likely to have BRAFmut tumors (2.7% vs. 0%, $p < .001$).

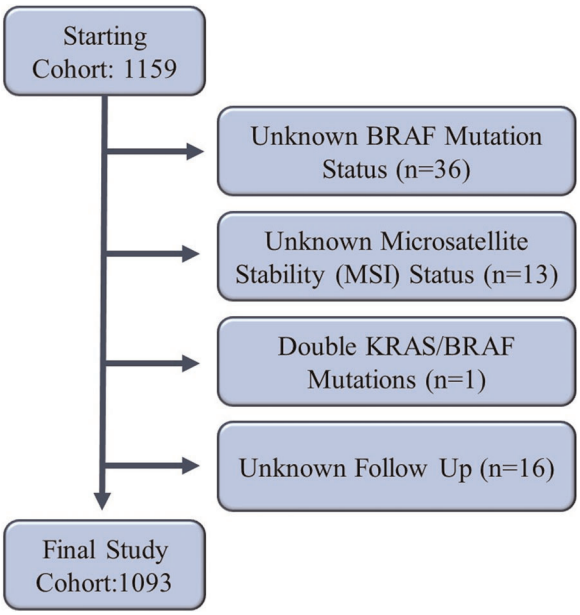

FIGURE 1 Flow chart of the study cohort [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Patient characteristics stratified by primary tumor side and KRAS mutation status

Characteristic	All patients	Right sided			Left-sided		
		KRAS-wt	KRAS-mut	*p*	KRAS-wt	KRAS-mut	*p*
No. of patients		261	117		488	227	
Patient characteristics							
Age (years), median (IQR)		68 (61–74)	67 (60–74)	.59	64 (58–71)	64 (57–71)	.82
Sex							
Male	621 (56.9)	125 (48.1)	60 (51.3)	.57	315 (64.6)	121 (53.3)	**.004**
Female	471 (43.1)	135 (51.9)	57 (48.7)		173 (35.4)	106 (46.7)	
Primary tumor characteristics							
T category							
T1	83 (7.6)	20 (7.7)	10 (8.6)	.14	32 (6.6)	21 (9.3)	.06
T2	230 (21.1)	55 (21.1)	13 (11.1)		123 (25.2)	39 (17.2)	
T3	677 (61.9)	163 (62.4)	82 (70.1)		291 (59.6)	141 (62.1)	
T4	103 (9.4)	23 (8.8)	12 (10.3)		42 (8.6)	26 (11.4)	
Stage combination							
T1–T2	313 (28.6)	75 (28.7)	23 (19.7)	.06	155 (31.8)	60 (26.4)	.15
T3–T4	780 (71.4)	186 (71.3)	94 (80.3)		333 (68.2)	167 (73.6)	
Primary tumor nodal metastases	411 (37.6)	86 (33.0)	49 (41.9)	.09	185 (37.9)	91 (40.1)	.58
Tumor grade ($n = 836$)							
Low	85 (10.2)	15 (10.5)	10 (9.9)	.90	36 (9.7)	24 (11.0)	.61
Intermediate	685 (81.9)	110 (76.9)	80 (79.2)		316 (84.7)	179 (81.7)	
High	66 (7.9)	18 (12.6)	11 (10.9)		21 (5.6)	16 (7.3)	
Lymphovascular invasion ($n = 838$)	447 (53.3)	84 (58.7)	55 (53.9)	.45	181 (48.4)	127 (58.0)	**.02**
Vein invasion ($n = 838$)	544 (64.9)	103 (72.0)	63 (61.8)	.09	235 (62.8)	143 (65.3)	.54
Genetic characteristics							
MSI status	122 (11.2)	82 (31.4)	12 (10.3)	**<.001**	14 (2.9)	14 (6.2)	**.034**
BRAF status	64 (5.9)	51 (19.5)	0	**<.001**	13 (2.7)	0	**.013**
Adjuvant chemotherapy	470 (43.0)	90 (34.5)	56 (47.9)	**.014**	222 (45.5)	102 (44.9)	.88
Total							
5FU-based regimen	388 (71.9)	45 (50.0)	44 (78.6)	**.002**	157 (70.7)	92 (90.2)	**<.001**
Oxaliplatin-based regimen	33 (7.0)	5 (5.6)	4 (7.1)		17 (7.7)	7 (6.9)	
Capecitabine	3 (0.7)	2 (2.2)	1 (1.8)		–	–	
Other/unknown	99 (20.4)	38 (42.2)	7 (12.5)		48 (21.6)	3 (2.9)	

Note: Bold values are statistically significant at $p < .05$.

Abbreviations: IQR, interquartile range; mut, mutant type, wt, wild type.

3.4 | Main analyses

3.4.1 | OS in the entire cohort and in RS versus LS CRC

With a median follow-up of 73.6 months, 237 patients (21.7%) died. After CRC resection, 1-, 3-, and 5-year OS of the entire cohort were 89.9%, 83.6%, and 72.7%, respectively. Factors that were independently associated with survival in the entire cohort are summarized in Table S3.

Among patients with RS CRC, KRAS mutation was not associated with OS on either univariable or multivariable analysis (Table 2). The 5-year OS for patients with RS KRASmut versus wild-type tumors was 82.2% versus 83.2% ($p = .43$), respectively (Figure 2A). The factors that were independently associated with worse survival were age (HR: 1.05; $p < .001$) and N category (HR: 1.83; $p = .03$).

Among patients with LS CRC, KRAS mutation was associated with OS on both univariable (5-year OS: KRASmut vs. wild-type; 79.4% vs. 86.1%, respectively; $p = .004$; Figure 2B) and multivariable analysis (HR: 1.52; $p = .019$; Table 2). The other factors that were independently associated with worse survival were age (HR: 1.05; $p < .001$), male sex (HR: 2.06; $p < .001$), advanced T category (HR: 1.69; $p = .045$), and lymphovascular invasion (HR: 1.72; $p = .019$).

TABLE 2 Univariable and multivariable overall survival analysis stratified by primary tumor location

	Right sided				Left sided			
	Univariate		Multivariate		Univariate		Multivariate	
Characteristic	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
Age	1.04 (1.02–1.07)	**.001**	1.05 (1.02–1.09)	**<.001**	1.05 (1.03–1.07)	**<.001**	1.05 (1.03–1.07)	**<.001**
Male sex	1.28 (0.84–1.96)	.26	–		2.07 (1.43–3.00)	**<.001**	2.06 (1.38–3.07)	**<.001**
T category								
T1–T2	Ref				Ref		Ref	
T3–T4	1.66 (0.96–2.84)	.068	–		2.74 (1.74–4.31)	**<.001**	1.69 (1.01–2.83)	**.045**
Primary tumor nodal metastases	1.93 (1.26–2.93)	**.002**	1.83 (1.06–3.15)	**.03**	1.97 (1.43–2.72)	**<.001**	1.24 (0.85–1.80)	.26
Tumor grade								
Low	Ref				Ref			
Intermediate	1.33 (0.53–3.35)	.54	–		1.19 (0.64–2.21)	.58		
High	1.32 (0.38–4.57)	.66	–		1.81 (0.77–4.27)	.17		
Lymphovascular invasion	1.99 (1.11–3.60)	**.021**	1.78 (0.95–3.32)	.07	2.91 (1.95–4.34)	**<.001**		
Vein invasion	2.06 (1.08–3.99)	**.03**	1.88 (0.96–3.65)	.06	2.51 (1.64–3.86)	**<.001**	1.72 (1.09–2.72)	**.019**
KRAS mutation	1.19 (0.77–1.86)	.43	–		1.61 (1.16–2.22)	**.004**	1.52 (1.07–2.15)	**.019**
MSI mutation	1.15 (0.71–1.85)	.56	–		0.63 (0.23–1.71)	.37		
BRAF mutation	1.32 (0.76–2.31)	.32	–		1.85 (0.69–5.01)	.22		
Adjuvant chemotherapy	1.09 (0.70–1.68)	.71	–		0.88 (0.64–1.22)	.45		

Note: Bold values are statistically significant at $p < .05$.

Abbreviations: HR, hazard ratio; MSI, microsatellite instability.

3.4.2 | RFS in RS versus LS CRC

Similar to OS, KRAS mutation was prognostic only in LS and not RS tumors for RFS. Among those with RS tumors, 5-year RFS for KRASmut versus wild-type was 72.1% versus 76.7% ($p = .215$), respectively (Figure 3A). In contrast, among patients with LS tumors, 5-year RFS for KRASmut versus wild-type was 68.8% versus 77.3% ($p = .005$), respectively (Figure 3B).

The only factor that was independently associated with worse RFS among patients with RS tumors was N category (HR: 2.55; $p = .001$; Table 3). Among those with LS tumors, male sex (HR: 1.65; $p = .012$), advanced T category (HR: 2.49; $p = .001$), and KRAS

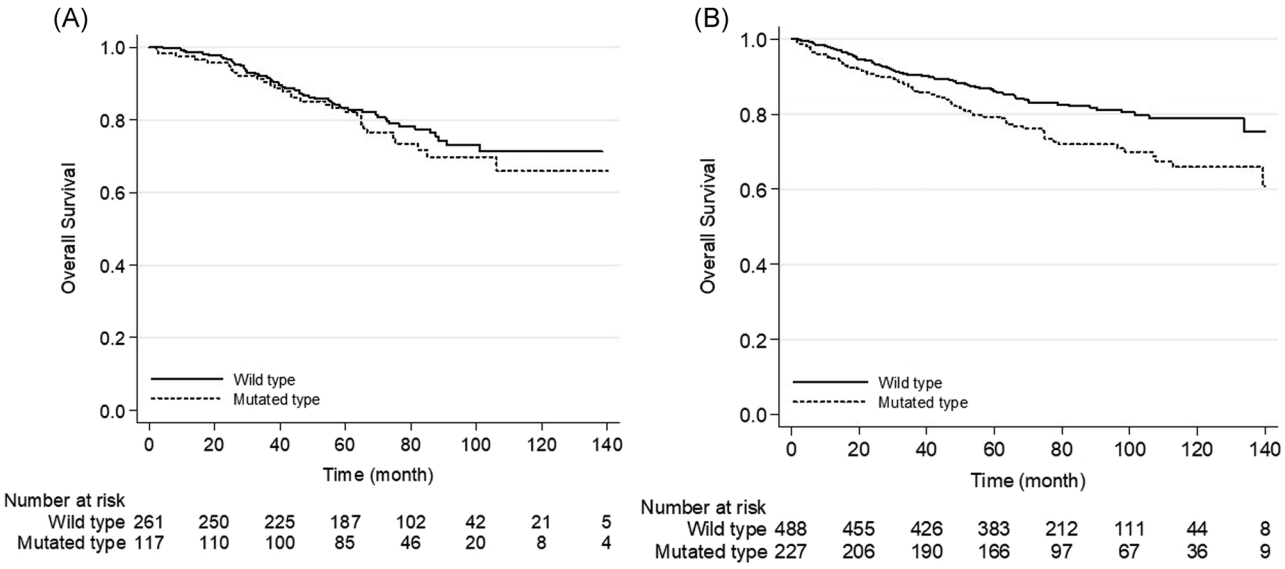

FIGURE 2 (A and B) Overall survival of patients with right- and left-sided tumors stratified by KRAS mutation status

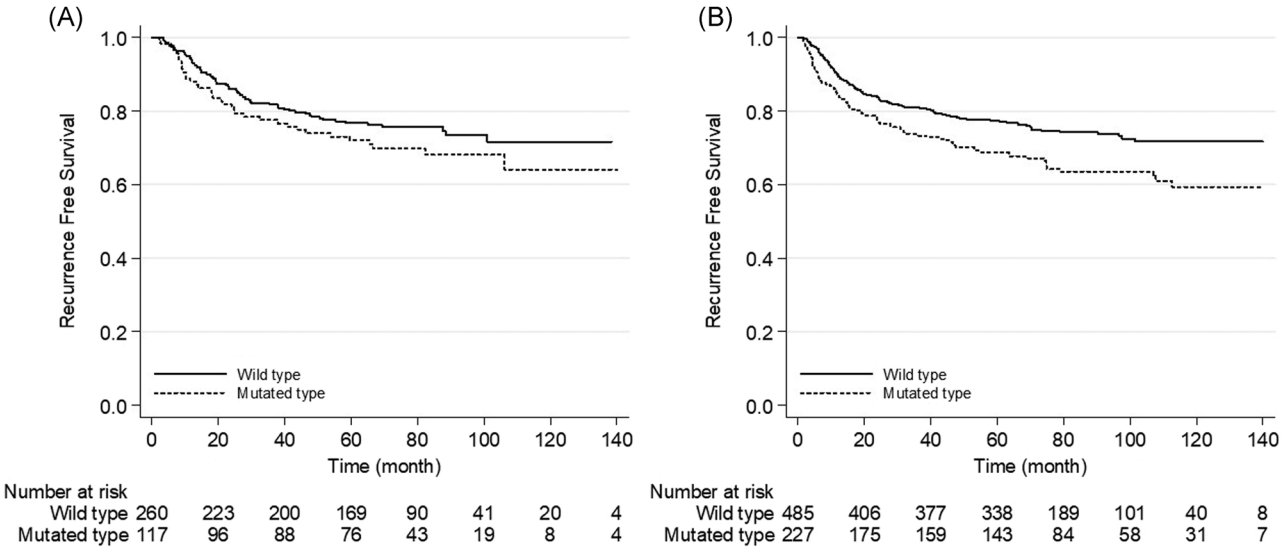

FIGURE 3 (A and B) Recurrence-free survival of patients with right- and left-sided tumors stratified by KRAS mutation status

mutation (HR: 1.43; $p = .05$) were independently associated with worse RFS (Table 3).

3.4.3 | Subgroup analyses

Given that KRAS mutation status is prognostic in left- but not right-sided non-metastatic CRC, we explored this finding by conducting a series of sensitivity analyses.

1. *Subanalysis of OS and RFS in patients with RS versus LS tumors after excluding patients with BRAFmut tumors*
   Similar to the main analysis, among patients with RS CRC, KRAS mutation was not associated with OS on both univariable and multivariable analysis. The 5-year OS for patients with RS KRASmut versus wild-type tumors was 82.2% versus 83.7% ($p = .256$), respectively. In contrast, among patients with LS CRC, KRAS mutation was associated with OS on both univariable (5-year OS: KRASmut vs. wild-type; 79.4% vs. 86.4%, respectively; $p = .003$) and multivariable analyses (HR: 1.57; $p = .01$). In addition, KRAS mutation was an independent prognostic factor of RFS only for patients with LS tumors (HR: 1.38; $p = .06$), whereas for patients with RS tumors, KRAS mutation was not prognostic even on univariable analysis (HR: 1.34; $p = .18$).
2. *Sub-analysis of OS and RFS in patients with RS versus LS tumors after excluding patients with MSI-H tumors*
   Similar to the main analysis, among patients with RS CRC, KRAS mutation was not associated with OS on both univariable and multivariable analysis. The 5-year OS for patients with RS KRASmut versus wild-type tumors was 84.3% versus 82.6%, respectively. Among patients with LS CRC, KRAS mutation was associated with OS on both univariable (5-year OS: KRASmut vs. wild-type; 79.4% vs. 85.7%, respectively; $p = .005$) and multivariable analyses (HR: 1.51; $p = .02$). In addition, KRAS mutation was an independent prognostic factor of RFS only for patients with LS tumors (HR: 1.51; $p = .03$), whereas for patients with RS tumors, KRAS mutation was not prognostic even on univariable analysis (HR: 1.06; $p = .78$).
3. *Sub-analysis of OS and RFS in patients with RS versus LS tumors after excluding patients who underwent surgery before 2005*
   Similar to the main analysis, among patients with RS CRC, KRAS mutation was not associated with OS on both univariable and multivariable analyses. The 5-year OS for patients with RS KRASmut versus wild-type tumors was 80.3% versus 81.9% ($p = .553$), respectively. In contrast, although KRAS mutation was not an independent factor of OS for patients with LS tumors (HR: 1.46; $p = .24$), it was significantly associated with worse OS on univariable analysis (78.8% vs. 87.1% for KRASmut and wild-type, respectively; $p = .045$). Among patients with RS tumors, KRAS mutation was not prognostic even on univariable analysis (HR: 1.21; $p = .55$). Similarly, KRAS mutation was not an independent prognostic factor of RFS for patients with LS tumors (HR: 1.48; $p = .078$).
4. *Subanalysis of OS and RFS in patients with "true" left-sided versus rectal tumors*

KRAS mutation was prognostic only for patients with "true" LS but not rectal tumors. Specifically, among the former, 5-year OS for KRASmut versus wild-type was 79.7% versus 87.6% ($p = .005$), respectively (Figure 4A). Among the latter, 5-year OS for KRASmut versus wild-type was 78.9% versus 83.2%, respectively, but failed to reach statistical significance ($p = .342$; Figure 4B). On multivariable analysis, KRAS mutation was an independent prognostic factor for patients with "true" LS tumors (HR: 1.91; $p = .006$), whereas for

TABLE 3 Univariable and multivariable recurrence free survival analysis stratified by primary tumor location

	Right sided				Left sided			
	Univariate		Multivariate		Univariate		Multivariate	
Characteristic	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
Age	1.01 (0.99–1.03)	.31			1.04 (1.02–1.05)	**<.001**	–[a]	
Male sex	1.19 (0.80–1.77)	.38			1.66 (1.23–2.25)	**<.001**	1.65 (1.11–2.45)	**.012**
T category								
T1–T2	Ref				Ref			
T3–T4	1.91 (1.13–3.22)	**.015**	1.68 (0.87–3.22)	0.12	3.35 (2.24–5.03)	**<.001**	2.49 (1.42–4.37)	**.001**
Primary tumor nodal metastases	2.62 (1.77–3.89)	**<.001**	2.55 (1.45–4.47)	**0.001**	2.19 (1.66–2.88)	**<.001**	1.47 (0.99–2.16)	.05
Tumor grade								
Low	Ref				Ref			
Intermediate	0.83 (0.41–1.67)	.60			1.25 (0.73–2.12)	.41	–	
High	1.31 (0.54–3.15)	.55			2.39 (1.18–4.83)	.016	–[a]	
Lymphovascular invasion	1.78 (1.09–2.92)	**.02**	1.15 (0.67–1.97)	0.61	2.74 (1.97–3.81)	**<.001**	1.90 (1.24–2.91)	.003
Vein invasion	1.62 (0.96–2.76)	.07			2.63 (1.83–3.80)	**<.001**	1.50 (0.96–2.36)	.08
KRAS mutation	1.29 (0.86–1.95)	.22			1.49 (1.13–1.98)	**.005**	1.43 (0.99–2.07)	**.05**
MSI mutation	0.48 (0.27–0.84)	**.01**	0.99 (0.53–1.87)	0.99	0.55 (0.23–1.34)	.19		
BRAF mutation	1.04 (0.59–1.82)	.90			1.98 (0.87–4.46)	.09		
Adjuvant chemotherapy	1.79 (1.21–2.66)	**.004**	0.84 (0.50–1.43)	0.52	1.25 (0.95–1.64)	.11		

Note: Bold values are statistically significant at $p < .05$.

[a]Did not meet the proportional assumption for Cox model stratified based on the variable.

patients with rectal tumors, KRAS mutation was not prognostic even on univariable analysis (HR: 1.29; $p = .34$).

Similarly, KRAS mutation was an independent prognostic factor of RFS for patients with "true" LS tumors (HR: 1.68; $p = .02$; Figure 5A), whereas for patients with rectal tumors, KRAS mutation was not prognostic even on univariable analysis (HR: 1.25; $p = .32$; Figure 5B).

4 | DISCUSSION

In this study, we utilized a double PTL and KRAS mutation status stratification to analyze survival outcomes of 1093 patients from six international academic centers who underwent resection for non-metastatic CRC. To our knowledge, this is the first study to examine the interplay of these factors in patients with non-metastatic CRC.

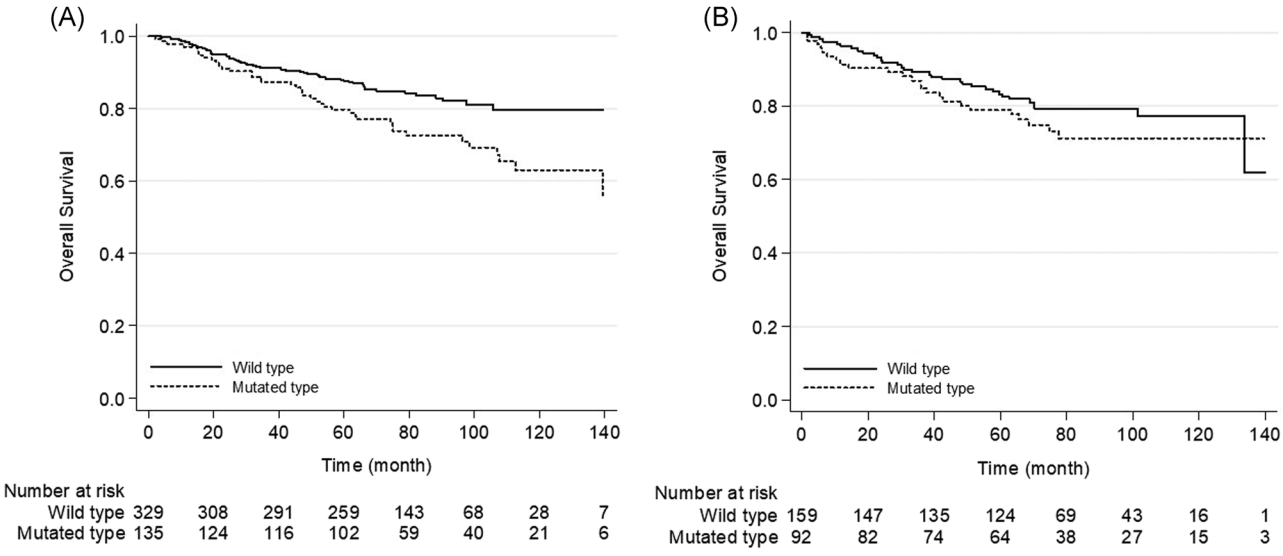

FIGURE 4 (A and B) Overall survival of patients with "true" left and rectal tumors stratified by KRAS mutation status

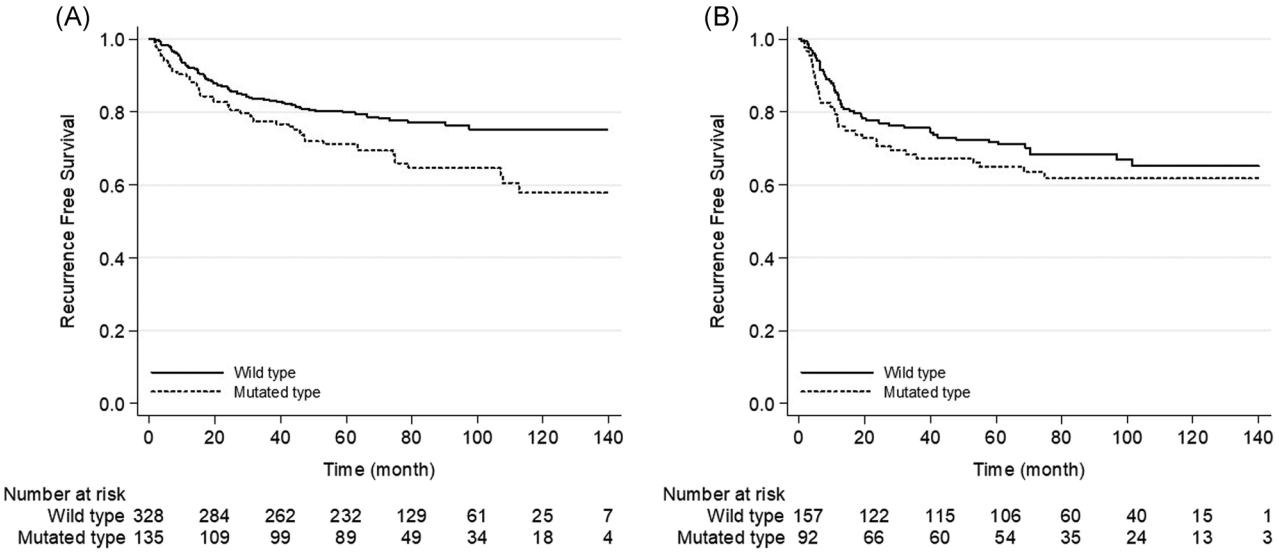

FIGURE 5 (A and B) Recurrence-free survival of patients with "true" left and rectal tumors stratified by KRAS mutation status

The main finding of this study is that KRAS mutation status was independently prognostic among patients with LS tumors, but this association was far less pronounced and failed to reach statistical significance among patients with RS tumors. Several sensitivity analyses confirmed this finding. The second finding of the study is that when left-sided tumors were divided into "truly" left and rectal tumors, the prognostic relevance of KRAS status was even more pronounced in those with "truly" left tumors, whereas the prognostic relevance of KRAS failed to reach statistical significance among patients with rectal tumors.

The main finding of this study has been previously corroborated by Sasaki et al.,[15] albeit in patients with metastatic CRC. Specifically, they found that KRAS was prognostic only in patients with left-sided primary tumors but not in those with right-sided tumors. Similar to the study by Sasaki et al., the difference in survival between LS/KRASmut versus LS/KRASwt (79.4% vs. 86.1%, respectively) stemmed from both a decrease in survival of patients with left-sided KRASmut versus right-sided KRASmut tumors (79.4% vs. 82.2%, respectively), as well as improved survival of patients with left-sided wild-type versus right-sided wild-type tumors (86.1% vs. 83.2%, respectively). Of note, although the magnitude of the differences between LS and RS KRASmut (2.8%) and RS and LS KRASwt (2.9%) was too small to be significant despite the numerical trend, the sum of the two (5.7%) was significant. Regarding the decreased survival of patients with LS/KRASmut compared to RS/KRASmut, it is possible that a higher rate of LS TP53 mutations, as noted by Loree et al.,[13] may accentuate the effect of LS KRAS mutations. This speculation stems from a recent study by Datta et al.[17] who demonstrated that in metastatic CRC, KRAS is not prognostic without a co-existing TP53 mutation.

To confirm the primary findings, we performed several sensitivity analyses. First, we analyzed outcomes after excluding patients with BRAF mutations, as it is a strong negative prognostic factor that is more frequently encountered in RS disease. Theoretically, decreased survival of KRASwt patients with BRAF mutations may narrow the survival gap between these patients and those with KRASmut tumors. As expected, patients with right-sided KRASwt tumors were more likely to have BRAF mutations compared to their left-sided counterparts (19.5% vs. 2.7%). We excluded these patients from the pool and re-analyzed the data. The main finding remained unchanged; KRAS was still not prognostic among patients with RS tumors.

Next, we analyzed outcomes after excluding patients with MSI-H tumors, which is a strong favorable prognostic factor that is more frequent in RS disease and can affect survival of RS wild-type and KRASmut patients.[18] Indeed, MSI-H was more prevalent in RS tumors (24.9 vs. 3.9%). After excluding these patients from analysis, the 5-year OS for patients with RS KRASmut improved while that of patients with wild-type tumors worsened. Importantly, the main finding remained unchanged; KRAS was not prognostic among patients with RS tumors.

Given that regimens containing oxaliplatin and irinotecan became popular after 2005, we also sought to validate the primary finding of the study in the subcohort of patients who were treated in the era of modern chemotherapy. When we excluded patients who underwent surgery before 2005, the strong association with OS deteriorated to a borderline significant association present only in univariable analysis and disappeared completely for RFS. This may relate to lower power, as well as the fact that modern chemotherapy improved outcomes in stage III CRC, making it even more challenging to detect a difference.

Although the binary stratification of tumor laterality as right versus left seems straightforward, it may in reality be an oversimplification. Almost all studies to date on the prognostic role of tumor laterality have included transverse colon in the right side and rectum in the left side. However, in a seminal study, Loree et al.[16]

demonstrated that the sigmoid-rectal region is distinct from other left-sided locations. The division of left-sided tumors to "truly" left and rectal tumors not only confirmed the main result of the study, but also revealed a new finding. Specifically, although a numerical trend was apparent (5-year OS for KRASmut vs. wild-type was 78.9% vs. 83.2%), the prognostic relevance of KRAS mutation status failed to reach statistical significance among patients with rectal tumors. Even if decreased statistical power partially contributed to this finding, it was obvious that the prognostic relevance of KRAS status was more pronounced among patients with "truly" left-sided tumors (5-year OS for KRASmut vs. wild-type was 79.7% vs. 87.6%). Our findings are in line with a recent report by Amini et al.[19] regarding patients with metastatic CRC, which demonstrated that mutKRAS status was independently associated with worse outcomes in patients with CRLM arising from colon but not rectal cancer.

The results of the study should be interpreted with caution given its retrospective design. Moreover, the study was limited by the lack of pertinent information on other somatic mutations aside from KRAS, BRAF, and MSI status. The inclusion of several institutions may have added heterogeneity to the study but in turn adds to the generalizability of our findings. Although the cohort was relatively large, some comparisons, in particular that for rectal tumors, may have limited statistical power. Lastly, detailed data on the systematic treatments (i.e., chemotherapy and radiotherapy) were lacking.

5 | CONCLUSIONS

Ultimately, it appears that there is a continuum of prognostic relevance of KRAS mutation status in non-metastatic and metastatic CRC; the prognostic relevance of KRAS status appears to be contingent on primary tumor laterality in both settings. In LS tumors, the presence of KRAS mutation resulted in a greater proportional decrease in survival from a better baseline prognosis, ultimately leading to a larger discrepancy in outcomes and a clear prognostic role for KRAS mutation status. The prognostic impact of KRAS mutation status was far less pronounced and failed to reach statistical significance among patients with RS and rectal tumors. The latter suggests that rectal tumors should not be grouped with left-sided tumors, contrary to current practice. The p53 mutation, which is more prevalent in LS tumors, may have contributed to the greater proportional decrease in survival of patients with LS KRASmut tumors. Future studies, preferably employing NGS techniques, may validate this hypothesis.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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