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Original Article

Patients with RAS wild-type right-sided unresectable liver-confined mCRC also benefit from cetuximab plus chemotherapy in first-line treatment

Peng Zheng*, Li Ren*, Qingyang Feng*, Dexiang Zhu*, Wenju Chang, Guodong He, Meiling Ji, Mi Jian, Qi Lin, Tuo Yi, Ye Wei, Jianmin Xu

*Department of General Surgery, Zhongshan Hospital, Fudan University, Shanghai, People's Republic of China. *Equal contributors.*

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Abstract: Growing evidence indicates that primary tumor location of metastatic colorectal cancer (mCRC) can affect response to specific therapy. This study aimed to assess the impact of primary tumor location on efficacy of cetuximab in Chinese patients with mCRC. We included patients with RAS wild-type liver-limited mCRC treated with first-line cetuximab plus chemotherapy or chemotherapy alone between June 2008 and December 2016. All patients were categorized as having left-sided tumors or right-sided tumors. Progression free survival (PFS), overall survival (OS), objective response rate (ORR) and conversion rate of surgery for liver metastases was analyzed according to tumor location and treatment. Right-sided tumors were characterized with larger primary tumor, poorer differentiation, more lymph node metastases and larger and more liver metastases. For patients with left-sided tumors (N=233), addition of cetuximab to chemotherapy significantly improved ORR (68.9% vs. 30.6%, OR=5.01, P < 0.001), conversion rate of liver surgery (33.5% vs. 10.8%, OR=4.18, P < 0.001), PFS (12.1 months vs. 6.1 months, HR=0.42, P < 0.001), and OS (not evaluable vs. 23.1 months, HR=0.31, P < 0.001). Among patients with right-sided tumors (N=85), cetuximab plus chemotherapy, compared with chemotherapy alone, also significantly improved ORR (56.8% vs. 29.3%, OR=3.18, P=0.010), PFS (9.3 months vs. 5.1 months, OR=0.57, P=0.012) and OS (25.3 months vs. 16.8 months, HR=0.56, P=0.032) but conversion rate of liver surgery (20.5% vs. 9.8%, HR=2.38, P=0.171). Our results demonstrated differential effect of cetuximab on efficacy outcomes based on tumor sidedness. Also, we found that patients with right-sided tumors also benefit from cetuximab plus chemotherapy but not as great as left-sided tumors and in general, did worse. In conclusion, findings of previous studies about differential effect of anti-EGFR therapy based on tumor sidedness are applicable to an Asian population.

Keywords: Colorectal liver metastases, cetuximab, predictive marker, primary tumor location

Introduction

Cetuximab plus chemotherapy regimens are typically used in the first-line treatment of RAS wild-type (wt) metastatic colorectal cancer (mCRC) [1, 2]. Our previous trial (NCT01564810) [3] compared first-line chemotherapy plus cetuximab with chemotherapy alone in Chinese patients with initially unresectable liver-limited KRAS exon 2 wt mCRC. And our results demonstrated improved conversion rate to the radical resection of liver metastases (LM), progression free survival (PFS), overall survival (OS) and objective response rate (ORR) in cetuximab arm. Whereas other trials have shown that benefit of anti-EGFR therapy is still limited in patients without RAS mutations when testing more extensively than KRAS exon 2 mutations [2, 4, 5]. To further refine patient selection, many other markers [6-9] were investigated, but none of these was extensively applied as predictor in clinical practice. Recently, the primary tumor location emerged as a potential predictor for cetuximab.

During the past decade there has been an increased interest in the differences between left and right-sided colorectal tumors. The physiologic basis for this is that the left and right-sided tumors have different embryologic origins, microenvironments, and distinct blood supplies. Subsequently, growing evidence has indicated that left and right-sided tumors are distinct entities with regard to epidemiology,

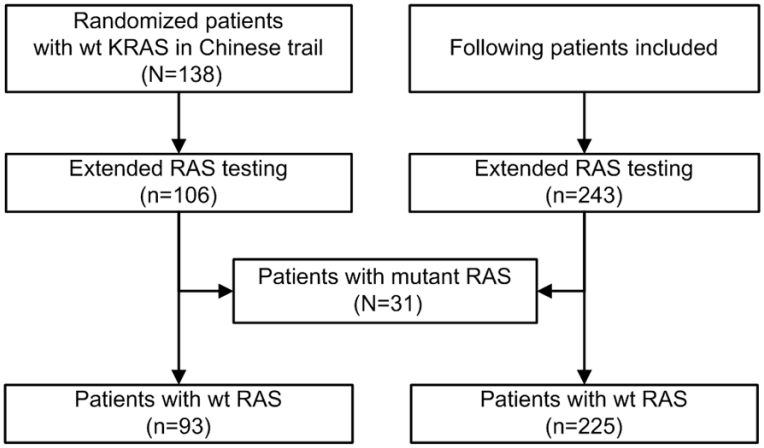

Figure 1. Consort diagram. Before December, 2013, only KRAS exon 2 mutations were tested before administration of cetuximab. Extended RAS testing was retrospectively done in this study. From December, 2013, extended RAS testing was routinely performed in clinical practice. Abbreviation: wt, wild type.

pathology and molecular biology [10-12]. These differences manifest as different clinical behavior that right-sided tumors typically displayed worse prognosis [10, 13-16]. Furthermore, the influence of tumor location on efficacy of particular therapy was recognized as correlative but incompletely understood.

The predominance of available evidence suggested that there is no benefit with anti-EGFR therapy in right-sided RAS wt tumors in the first-line setting. Crucial studies including CALGB40705 [17], FIRE-3 [18], CRYSTAL [18], and pooled analysis of more studies have confirmed that same finding [16, 19]. In subsequent lines of treatment, several studies also suggested probably non-benefit with anti-EGFR therapy in right-sided tumors [20-22]. But more definitive studies are needed to prove it. In NCCN guidelines [1], anti-EGFR therapy plus chemotherapy was not recommend in first-line treatment for right-sided RAS wt tumors but could be considered in subsequent lines.

The aim of this analysis was to assess the impact of primary tumor location on efficacy of cetuximab in Chinese patients with RAS wt liver-limited mCRC in first-line treatment.

Methods

Study design and patients

This study retrospectively recruited patients with KRAS exon2 wt colorectal adenocarcinoma with synchronous liver-confined metastases. All primary tumors received radical resection and liver metastases assessed as unresectable by a local multidisciplinary team (MDT) consist of more than three liver surgeons and one radiologist. Two sets of patients were included. Patients from previous study (NCT-01564810) [3] were included. The second set was captured with the same criteria as above (**Figure 1**). Only patients with wt RAS were analyzed in this study. The Chinese trial compared chemotherapy (mFOLFOX6 or FOLFIRI) plus cetuximab with chemotherapy alone as first-line treatment for patients with initially unresectable liver-limited KRAS exon 2 wt mCRC. The primary end point was the conversion rate to radical resection for liver metastases, which was assessed by MDT. The trial was approved by the local ethic committees and all patients provided written and oral informed consent, including research on tumor tissue.

Categorization of primary tumor location

Tumors were divided into two groups according to the anatomical tumor site: Primary tumors originating in the splenic flexure, descending colon, sigmoid colon and rectum were classified as left-sided tumors. Primary tumors originating in the appendix, cecum, ascending colon, hepatic flexure, and transverse colon were classified as right-sided tumors. Tumors occurring with unclear locations or multi-positions were excluded from the present analysis.

Tissue collection and examination of RAS mutations

Formalin-fixed paraffin-embedded (FFPE) tissue was obtained from the Department of Pathology of Zhongshan Hospital (Shanghai, China). An experienced pathologist reviewed each section and indicated the area of the tumor. Macro-dissection was performed using the H&E-stained slides to enrich the number of tumor cells in each sample. RAS mutations were detected analyzed using the China Food and Drug Administration (CFDA)-approved AmoyDx™ KRAS/NRAS/BRAF Mutations Detection Kit (AmoyDx, Xiamen, China),

Table 1. Baseline characters according to tumor location

	Left-sided tumors (N=233)	Right-sided tumors (N=85)	P
Age, years, Mean ± SD	56.7±11.0	57.6±11.7	0.497
Gender, n (%)			0.173
Male	164 (70.4%)	53 (62.4%)	
Female	69 (29.6%)	32 (37.6%)	
ECOG PS			0.743
0	195 (83.7%)	68 (80.0%)	
1	38 (16.3%)	17 (20.0%)	
CEA level at diagnosis, ng/mL, n (%)			0.617
≥ 5	177 (76.0%)	69 (81.2%)	
< 5	56 (24.0%)	16 (18.8%)	
Tumor diameter, cm, Mean ± SD	6.66±1.86	7.99±2.52	< 0.001
Histological grade, n (%)			< 0.001
Well (Grade 1)	4 (1.7%)	0 (0%)	
Moderate (Grade 2)	175 (75.1%)	44 (51.7%)	
Poor (Grade 3 and 4)	54 (23.2%)	41 (48.3%)	
T stage, n (%)			0.434
T1/T2	54 (23.3%)	14 (16.5%)	
T3/T4	179 (72.7%)	71 (83.5%)	
N stage, n (%)			0.042
N0	42 (18.0%)	18 (21.2%)	
N1	113 (48.5%)	28 (32.9%)	
N2	78 (33.5%)	39 (44.7%)	
Vascular invasion, n (%)			0.603
No	190 (81.5%)	65 (76.5%)	
Yes	43 (18.5%)	20 (23.5%)	
Perineural invasion, n (%)			0.997
No	190 (81.6%)	69 (81.5%)	
Yes	43 (18.4%)	16 (18.5%)	
Tumor deposits, n (%)			0.094
No	117 (50.2%)	31 (36.5%)	
Yes	116 (49.8%)	54 (63.5%)	
Distribution of LM			0.738
Unilobar	85 (36.5%)	27 (31.8%)	
Bilobar	148 (63.5%)	58 (68.2%)	
Numbers of LM			
Median (IQR)	4 (2-8)	5 (3-11)	0.037
Diameter of the largest LM, mm			
Median (IQR)	39 (25-66)	49 (31-73)	0.041

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status; LM, liver metastases.

based on Amplification Refractory Mutation System (ARMS) technology in a certified laboratory (Table S1).

Statistical analysis

Differences in the baseline characteristics were calculated using a Chi-square test or Fisher's exact test for categorical variables and T-test for continuous variables. Survival curves were generated using the Kaplan-Meier method and compared using a log-rank test. Hazard ratios (HRs) and 95% confidence intervals (95% CI) were calculated using the Cox proportional hazards model. Odds ratios (ORs) and 95% CI were calculated using a logistic regression model. With multivariable regression models, covariates included primary tumor

Table 2. Efficacy results based on primary tumor location

	All (n=318)		Left-sided tumors (n=233)		Right-sided tumors (n=85)	
	Cetuximab plus chemotherapy (n=166)	Chemotherapy alone (n=152)	Cetuximab plus chemotherapy (n=122)	Chemotherapy alone (n=111)	Cetuximab plus chemotherapy (n=44)	Chemotherapy alone (n=41)
ORR, %	65.7	30.3	68.9	30.6	56.8	29.3
OR	4.41		5.01		3.18	
95% CI	2.75-7.06		2.87-8.73		1.29-7.81	
p (Chi-square or Fisher's)	< 0.001		< 0.001		0.010	
p for interaction	/		0.400			
Radical resection rate of LM, %	30.1	10.5	33.6	10.8	20.5	9.8
OR	3.66		4.18		2.38	
95% CI	1.98-6.78		2.06-8.47		0.67-8.43	
p (Chi-square or Fisher's)	< 0.001		< 0.001		0.171	
p for interaction	/		0.447			
PFS, months						
Median	11.3	5.8	12.1	6.1	9.3	5.1
HR	0.46		0.42		0.57	
95% CI	0.36-0.59		0.31-0.56		0.36-0.93	
p (log-rank)	< 0.001		< 0.001		0.012	
p for interaction	/		0.292			
OS, months						
Median	35.0	21.7	NE	23.1	25.3	16.8
HR	0.43		0.31		0.56	
95% CI	0.30-0.61		0.19-0.50		0.32-0.97	
p (log-rank)	< 0.001		< 0.001		0.032	
p for interaction	/		0.083			

Abbreviations: ORR, objective response rate; LM, liver metastases; PFS, progression free survival; OS, overall survival; HR, hazard ratio; OR, odds ratio; 95% CI, 95% confidence interval; NE, not evaluable.

location, treatment, surgery for LM and the following baseline characteristics that significantly differed between tumor location groups: diameter, differentiation, and N stage of primary tumor, number and diameter of LM. All statistical analyses were conducted using the statistical software SPSS version 18.0 (SPSS Inc., Chicago, IL). A p value < 0.05 was considered statistically significant.

Results

Patients and mutations

From June, 2008 to December, 2016, a total of 318 patients with wt RAS were included in this study: 93 from our previous clinical trial, and 225 as following according to the same criteria. 31 (12.6%) of 247 patients originally typed as KRAS exon 2 wt harbored other RAS mutations (**Figure 1**). As to BRAF, 32 (10.1%) of 318 patients with wt RAS harbored a mutation. The detected BRAF mutations more prevalent among right-sided tumors (12.9% vs. 9.0%, P=0.588) and exclusive of RAS mutations as previously reported, although not statistically significant.

Baseline characteristics

Among all 318 patients, 166 (52.2%) received chemotherapy plus cetuximab and 152 (47.8%) received chemotherapy alone in first-line treatment. The baseline characteristics were generally comparable between treatment groups (Table S2). In subgroups according to primary tumor location, no significant differences of baseline characteristics were observed (Table S3).

Differences associated with tumor location

Among all patients, 233 (73.3%) had left-sided tumors and 85 (26.7%) had right-sided tumors. Several differences in baseline characteristics were observed between subgroups according to primary tumor location (**Table 1**). Right-sided tumors were larger in size (mean: 8.0 cm vs. 6.7 cm, P < 0.001), poorer differentiated (Grade 3 and 4: 48.3% vs. 23.2%, P < 0.001) and more frequently lymph node positive (N2/N1/N0:

Table 3. Efficacy results based on treatment arm

	All (n=318)		Cetuximab plus chemotherapy (n=166)		Chemotherapy alone (n=152)	
	Left-sided tumors (n=233)	Right-sided tumors (n=85)	Left-sided tumors (n=122)	Right-sided tumors (n=44)	Left-sided tumors (n=111)	Right-sided tumors (n=41)
ORR, %	50.6%	43.5%	68.9	56.8	30.6	29.3
OR	1.33		1.68		1.07	
95% CI	0.81-2.19		0.83-3.41		0.49-2.34	
p (Chi-square or Fisher's)	0.261		0.150		0.871	
Radical resection rate of LM, %	22.7%	15.3%	33.6	20.5	10.8	9.8
OR	1.63		1.97		1.12	
95% CI	0.84-3.17		0.86-4.48		0.34-3.70	
p (Chi-square or Fisher's)	0.147		0.103		0.851	
PFS, months						
Median	9.2	7.3	12.1	9.3	6.1	5.1
HR	0.75		0.63		0.89	
95% CI	0.57-0.99		0.43-0.93		0.61-1.31	
p (log-rank)	0.028		0.012		0.524	
OS, months						
Median	29.5	21.9	NE	25.3	23.1	16.8
HR	0.50		0.33		0.62	
95% CI	0.35-0.71		0.19-0.57		0.39-0.99	
p (log-rank)	< 0.001		< 0.001		0.042	

Abbreviations: ORR, objective response rate; LM, liver metastases; PFS, progression free survival; OS, overall survival; HR, hazard ratio; OR, odds ratio; 95% CI, 95% confidence interval; NE, not evaluable.

44.7%/32.9%/21.2% vs. 33.5%/48.5%/18.0%, P=0.042). As to evaluation of liver metastases, right-sided tumors had higher number (median: 5 vs. 4, P=0.037) and larger liver metastases (median: 49 mm vs. 39 mm, P=0.041).

Relevant prognostic value of tumor location

Among all patients, left-sided tumors, compared with right-sided tumors, were associated with superior PFS (9.2 months vs. 7.3 months, P=0.028) and OS (29.5 months vs. 21.9 months, P < 0.001). For patients treated with cetuximab plus chemotherapy, PFS (12.1 months vs. 9.3 months, P=0.012) and OS (Not evaluable vs. 23.1 months, P < 0.001) were significantly greater in left-sided tumors vs. right-sided tumors. In addition, OS (23.1 months vs. 16.8 months, P=0.042) was significantly superior in chemotherapy treated patients with left-sided tumors vs. patients with right-sided tumors (**Table 3**).

Potential predictive value of tumor location

Among patients with left-sided tumors, addition of cetuximab to chemotherapy significantly improved ORR, conversion rate of liver surgery, PFS, and OS. Among patients with right-sided tumors, cetuximab plus chemotherapy, compared with chemotherapy alone, also significantly improved ORR, PFS and OS (**Table 2**; **Figure 2**). Of note, the HRs and ORs were more favorable towards the addition of cetuximab to chemotherapy in patients with left-sided tumors compared with patients with right-sided tumors. For patients who achieved early tumor shrink (ETS), left-sided tumors treated with chemotherapy plus cetuximab had the longest OS. Furthermore, median OS of patients with right-sided tumors was 36.8 months in cetuximab group and 32.9 months in chemotherapy group (P=0.505) (Figure S1).

Multivariable analysis

Upon multivariable analysis for all patients, the primary tumor location remained prognostic for OS (Table S4). For patients treated with cetuximab plus chemotherapy, multivariable analysis indicated that primary tumor location was prognostic (Table S5). Nevertheless, primary tumor location was not prognostic in multivari-

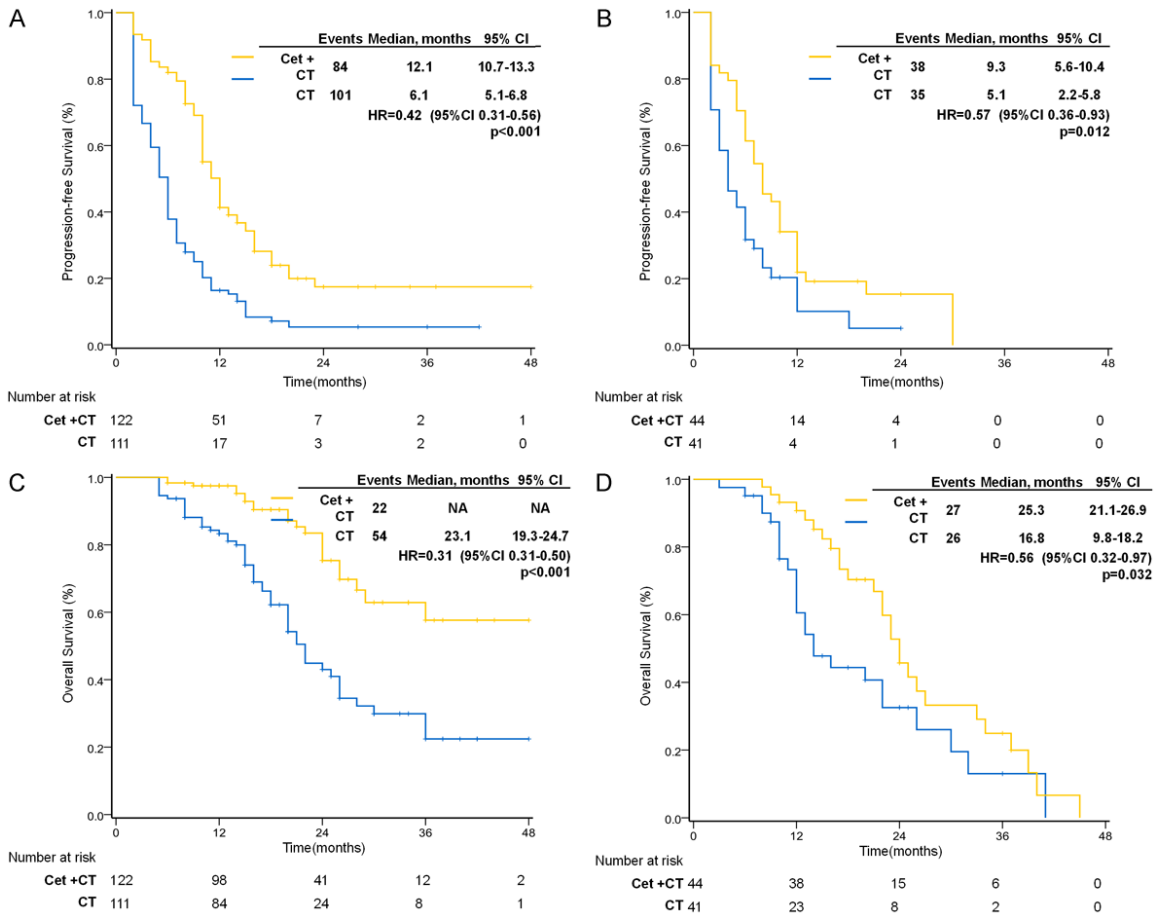

Figure 2. Survival curves based primary tumor location. A. PFS for left-sided tumors. B. PFS for right-sided tumors. C. OS for left-sided tumors. D. OS for right-sided tumors. PFS, progression free survival; OS, overall survival; HR, hazard ratio; 95% CI, 95% confidence interval.

able analysis for patients treated with chemotherapy alone (Table S6).

Discussion

In this retrospective analysis, we assessed the potential predictive and prognostic value of primary tumor location in Chinese patients with RAS wild-type preliminarily non-resectable liver-confined mCRC treated with first-line chemotherapy alone or with cetuximab.

According to our results, both left-sided and right-sided tumors significantly benefit from cetuximab in addition to chemotherapy compared with chemotherapy alone. Furthermore, cetuximab plus chemotherapy had significantly better PFS and OS and numerically higher ORR in left-sided tumors vs. right-sided tumors. The predictive value of primary tumor location for anti-EGFR therapy was observed in most previous studies and accepted in updated NCCN guidelines. However, the underlying mechanism of observed side-specific therapy response was still largely unknown. Missiaglia E et al [10] reported that an EGFR inhibitor-sensitive phenotype appears to be more prevalent in left-sided tumors. According to the consensus molecular subtypes (CMS) [12, 23], right-sided tumors with higher prevalence of CMS1 subgroup characterized by BRAF mutation and hyper-mutation, and CMS3 subgroup characterized by KRAS mutation and microsatellite instability. CRC is heterogeneous and primary tumor location may help divide CRC into relevant differences at a molecular level. Nevertheless, these differences could not completely explain side-specific response to cetuximab. Thus, it is critical to improve our understanding of the biology of tumor location, which may help to better choose agents and develop more effective therapeutic strategies.

Our results applying to an Asian population were in general consistence with previous studies predominantly North American or European. Crucial studies including CALGB40705 [17], FIRE-3 [18], CRYSTAL [18], and pooled analysis of more studies [16, 19] have confirmed that there is no benefit with anti-EGFR therapy in right-sided RAS wt tumors in the first-line setting. Differently, our results further indicated that right-sided tumors may also significantly benefit from cetuximab in addition to chemotherapy but with a limited extent compared with left-sided tumors. Possible explanations may lie in the confined selection of patients and high percentage of liver surgery in this study. As reported previously, metastatic pattern was different between tumor location groups [24, 25]. As a means to define a more homogeneous population by exclusion of patients with extrahepatic metastases, we analyzed patients with liver-limited metastases. Additionally, the combination of systemic therapy and surgery for metastases have further improved prognosis for patients with mCRC [26-28]. In our results, about 20% of patients with right-sided tumors treated with cetuximab plus chemotherapy received liver surgery. Higher conversion rate of liver surgery may amplify the treatment benefit of cetuximab therapy.

For patients who received liver surgery, efficacy outcomes indicated that patients with right-sided tumors had inferior PFS and OS compared those with right-sided tumors. Of note, median PFS and OS for patients with right-sided tumors reached 13.4 months and 37.2 months (Figure S2). This indicated that a subset of patients with righted-sided tumors achieved long survival upon conversion therapy followed by liver surgery, leading to the hypothesis that cetuximab was still optional for patients with right-sided tumors in first-line treatment, especially for those intent to surgery after conversion.

Upon multivariable analysis of all patients, the primary tumor location was independent prognostic factor for mCRC, which was consistent with previous reports [16, 29]. Of note, N stage, numbers of LM and diameter of the largest LM were also prognostic, and correlated with primary tumor location. This indicated that known clinical and pathological characteristics only accounted for part of survival differences between right- and left-sided tumors. Furthermore, primary tumor location was prognostic in multivariable analysis of cetuximab arm but not in that of chemotherapy arm. Survival of left-sided tumors, compared with right-sided tumors, was prolonged in chemotherapy arm (HR=0.68, P=0.151) and further improved (HR=0.44, P=0.010) by addition of cetuximab. Possible explanation may lie in the correlation between primary tumor location and efficacy of cetuximab. Significantly improved survival in cetuximab arm result from better response to cetuximab in left-sided tumors compared with right-sided tumors.

As a retrospective analysis, limitation of our study included potential imbalances of baseline characteristics between treatment arms and relatively small sample size of some subgroups. It should also be noted that, this study included and analyzed only patients with liver-limited mCRC. We designed and analyzed in this way to induce heterogeneity and provide results of specific subset, but results should be interpreted and extended to full-spectrum mCRC with great caution.

In this study, we assessed the potential predictive and prognostic value of primary tumor location in first-line treatment for patients with RAS wild-type liver-limited mCRC in an Asian population. For left-sided tumors, a clinically meaningful benefit was observed and it is further improved than that before splitting patients by tumor location. Right-sided tumors also significantly benefit from addition of cetuxiamb but to a limited extent compared with left-sided tumors. Findings of previous studies about differential effect of anti-EGFR therapy based on tumor location are also applicable to an Asian population. Additional research is needed to identify the subset of patients with RAS wild-type right-sided mCRC who may derive benefit from cetuximab.

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Disclosure of conflict of interest

None.

Address correspondence to: Jianmin Xu, Department of General Surgery, Zhongshan Hospital, Fudan University, 180 Fenglin Road, Shanghai

200032, People's Republic of China. Tel: 86-021-64041990-3449; E-mail: xujmin@aliyun.com

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Table S1. Summary of RAS and BRAF mutations tested by ARMS

Gene	Exon	Mutation loci*
KRAS	3	Q61H, Q61L, Q61R
	4	K117N, A146T, A146V, A146P
NRAS	2	G12S, G12D
	2	G13D
	2	G12A, G12V, G12C, G13R, G13V
	3	Q61K, Q61H, Q61L, Q61R
	4	A146T
BRAF	15	V600E

*Mutations in the same table cell were tested in one PCR tube and are not distinguished respectively.

Table S2. Baseline characters according to treatment

	Cetuximab plus chemotherapy (N=166)	Chemotherapy alone (N=152)	P
Age, years, Mean ± SD	56.2±10.3	57.7±12.0	0.222
Gender, n (%)			0.947
Male	113 (68.1%)	104 (68.4%)	
Female	53 (31.9%)	48 (31.6%)	
ECOG PS			0.731
0	136 (81.9%)	127 (83.5%)	
1	30 (18.1%)	25 (16.5%)	
CEA level at diagnosis, ng/mL, n (%)			0.913
≥ 5	130 (78.3%)	116 (76.3%)	
< 5	36 (21.7%)	36 (23.7%)	
Tumor diameter, cm, Mean ± SD	7.03±2.12	6.99±2.15	0.884
Histological grade, n (%)			0.434
Well (Grade 1)	3 (1.8%)	1 (0.7%)	
Moderate (Grade 2)	110 (66.3%)	109 (71.7%)	
Poor (Grade 3 and 4)	53 (31.9%)	42 (27.6%)	
T stage, n (%)			0.791
T1/T2	38 (22.9%)	30 (19.7%)	
T3/T4	128 (77.1%)	122 (80.2%)	
N stage, n (%)			0.682
N0	30 (18.1%)	30 (19.7%)	
N1	78 (47.0%)	64 (42.1%)	
N2	58 (34.9%)	58 (38.2%)	
Vascular invasion, n (%)			0.912
No	130 (80.2%)	122 (80.3%)	
Yes	33 (19.8%)	30 (19.7%)	
Perineural invasion, n (%)			0.588
No	130 (80.2%)	126 (82.9%)	
Yes	33 (19.8%)	26 (17.1%)	
Tumor deposits, n (%)			0.219
No	85 (51.2%)	63 (44.7%)	
Yes	81 (48.8%)	89 (55.3%)	
Distribution of LM			0.708

Unilobar	62 (37.3%)	50 (32.9%)	
Bilobar	104 (62.7%)	102 (67.1%)	
Numbers of LM			
Median (IQR)	5 (3-9)	4 (2-8)	0.266
Diameter of the largest LM, cm			
Median (IQR)	38 (25.8-66.3)	44 (30-69.5)	0.259

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status; LM, liver metastases.

Table S3. Baseline characters according to treatment and tumor location

	Left-sided tumors (N=233)			Right-sided tumors (N=85)		
	Cet + CT (N=122)	CT (N=111)	P	Cet + CT (N=44)	CT (N=41)	P
Age, years, Mean ± SD	56.1±10.0	57.3±12.0	0.395	56.5±11.5	58.9±12.0	0.357
Gender, n (%)			0.970			
Male	86 (70.5%)	78 (70.3%)		27 (61.4%)	26 (63.4%)	0.845
Female	36 (29.5%)	33 (29.7%)		17 (38.6%)	15 (36.6%)	
ECOG PS			0.756			0.910
0	100 (81.9%)	95 (85.5%)		36 (81.8%)	32 (78.0%)	
1	22 (18.1%)	16 (14.5%)		8 (18.2%)	9 (22.0%)	
CEA level at diagnosis, ng/mL, n (%)			0.913			0.776
≥ 5	93 (76.2%)	84 (78.4%)		37 (84.1%)	32 (78.0%)	
< 5	29 (23.8%)	27 (23.7%)		7 (15.9%)	9 (22.0%)	
Tumor diameter, cm, Mean ± SD	6.78±1.94	6.53±1.76	0.318	7.76±2.45	8.23±2.60	0.395
Histological grade, n (%)			0.432			0.944
Well (Grade 1)	3 (1.8%)	1 (0.7%)		0(0%)	0(0%)	
Moderate (Grade 2)	88 (72.1%)	87 (78.4%)		22 (50.0%)	22 (53.7%)	
Poor (Grade 3 and 4)	31 (25.4%)	23 (20.7%)		22 (50.0%)	19 (46.3%)	
T stage, n (%)			0.511			0.766
T1/T2	32 (26.2%)	22 (19.8%)		6 (23.9%)	8 (19.5%)	
T3/T4	90 (73.7%)	89 (80.2%)		38 (86.1%)	33 (80.5%)	
N stage, n (%)			0.830			0.782
N0	21 (17.2%)	21 (18.9%)		9 (20.4%)	9 (20.0%)	
N1	62 (50.8%)	52 (46.8%)		16 (36.4%)	12 (29.3%)	
N2	39 (32.0%)	38 (34.2%)		19 (43.1%)	20 (48.7%)	
Vascular invasion, n (%)			0.270			0.229
No	93 (76.2%)	94 (84.7%)		37 (84.1%)	28 (68.3%)	
Yes	24 (23.8%)	19 (15.3%)		7 (15.9%)	13 (31.7%)	
Perineural invasion, n (%)			0.270			0.776
No	93 (76.2%)	94 (84.7%)		37 (84.1%)	32 (78.0%)	
Yes	24 (23.8%)	19 (15.3%)		7 (15.9%)	9 (22.0%)	
Tumor deposits, n (%)			0.322			0.678
No	67 (54.9%)	50 (45.0%)		18 (40.9%)	13 (31.7%)	
Yes	55 (45.1%)	61 (55.0%)		26 (59.1%)	28 (56.1%)	
Distribution of LM			0.472			0.901
Unilobar	49 (40.2%)	36 (32.4%)		13 (29.5%)	14 (35.1%)	
Bilobar	73 (59.8%)	75 (67.6%)		31 (70.5%)	27 (65.9%)	
Numbers of LM						
Median (IQR)	5 (2-8)	4 (2-8)	0.195	6 (3-10)	5 (3-14)	0.961
Diameter of the largest LM, cm						
Median (IQR)	37 (24-65)	42 (26-70)	0.138	49 (30-85)	49 (31-65)	0.799

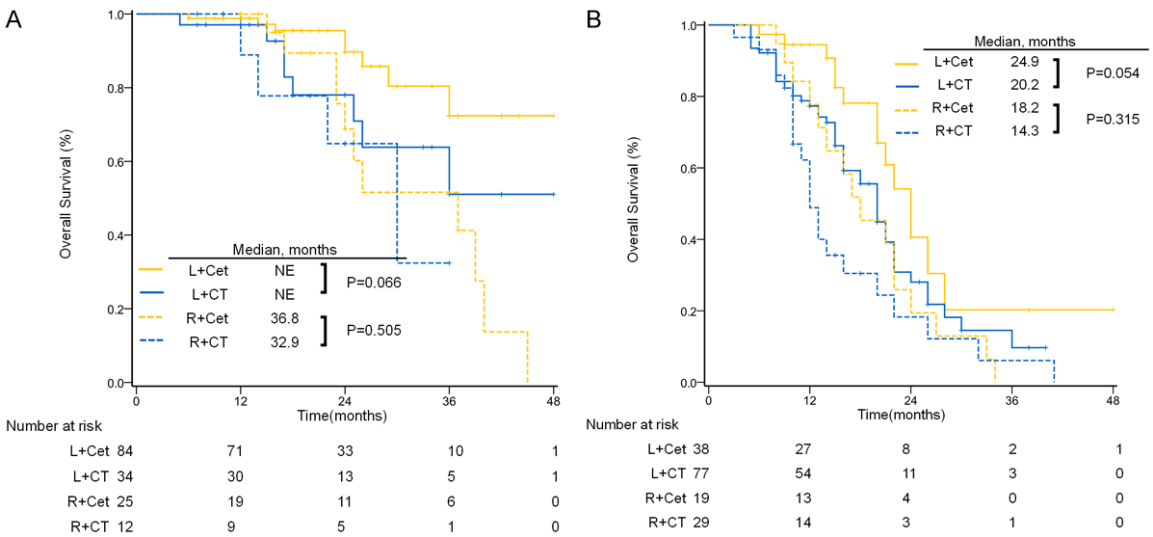

Figure S1. Survival curves of patients according to ETS. A. Patients who achieved ETS. B. Patients who did not achieved ETS. ETS, early tumor shrink, was defined as a ≥ 20% reduction of the longest diameters of measurable liver metastases in eight weeks compared with baseline at the first evaluation. L, Left-sided tumors; R, right-sided tumors; Cet, cetuximab; CT, chemotherapy; NE, not evaluable.

Table S4. Multivariable analysis investigating prognostic value of tumor location

	Univariable			Multivariable		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Tumor position						
Left vs. Right	0.502	0.354-0.714	< 0.001	0.535	0.366-0.782	0.001
Treatment						
Cet + CT vs. CT	0.428	0.299-0.611	< 0.001	0.429	0.295-0.624	< 0.001
Surgery for LM						
Yes vs. No	0.214	0.117-0.394	< 0.001	0.325	0.175-0.603	< 0.001
Tumor diameter, cm						
≤ 7 vs. > 7	0.995	0.688-1.438	0.977	0.845	0.568-1.258	0.407
Histological grade						
1 + 2 vs. 3 + 4	0.613	0.524-0.883	0.009	0.832	0.557-1.244	0.370
N stage						
N0	1.000			1.000		
N1	0.648	0.438-0.959	0.030	0.657	0.430-1.005	0.053
N2	0.401	0.238-0.674	0.001	0.471	0.273-0.813	0.007
Numbers of LM						
1-2	1.000					
3-5	0.618	0.414-0.922	0.019	0.571	0.378-0.861	0.008
≥ 6	0.343	0.214-0.549	< 0.001	0.360	0.216-0.601	< 0.001
Diameter of the largest LM, cm						
≤ 5 vs. > 5	0.660	0.458-0.951	0.026	0.637	0.423-0.958	0.030

Abbreviations: LM, liver metastases; HR, hazard ratio; Cet, cetuximab; CT, chemotherapy.

Table S5. Multivariable analysis in patient treated with cetuximab plus chemotherapy

	Univariable			Multivariable		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Tumor position						
Left vs. Right	0.325	0.185-0.572	< 0.001	0.436	0.232-0.819	0.010
Surgery for LM						
Yes vs. No	0.215	0.093-0.497	< 0.001	0.242	0.098-0.596	0.002
Tumor diameter, cm						
≤ 7 vs. > 7	0.835	0.472-1.478	0.536	0.846	0.426-1.679	0.632
Histological grade						
1 + 2 vs. 3 + 4	0.529	0.289-0.968	0.039	0.929	0.455-1.899	0.840
N stage						
N0	1.000			1.000		
N1	3.450	1.312-9.069	0.012	3.496	1.224-9.985	0.019
N2	5.841	2.081-16.39	0.001	4.950	1.597-15.341	0.006
Numbers of LM						
1-2	1.000			1.000		
3-5	1.920	0.882-4.176	0.100	1.300	0.565-2.987	0.537
≥ 6	3.223	1.472-7.057	0.003	2.253	0.958-5.301	0.063
Diameter of the largest LM, cm						
≤ 5 vs. > 5	1.504	0.808-2.797	0.198	0.417	0.200-0.869	0.020

Abbreviations: LM, liver metastases; HR, hazard ratio; Cet, cetuximab; CT, chemotherapy.

Table S6. Multivariable analysis in patient treated with chemotherapy alone

	Univariable			Multivariable		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Tumor position						
Left vs. Right	0.624	0.390-0.998	0.049	0.684	0.407-1.149	0.151
Surgery for LM						
Yes vs. No	0.278	0.110-0.704	0.007	0.367	0.145-0.930	0.035
Tumor diameter, cm						
≤ 7 vs. > 7	0.944	0.577-1.545	0.820	0.766	0.451-1.301	0.324
Histological grade						
1 + 2 vs. 3 + 4	0.741	0.469-1.173	0.201	0.796	0.481-1.317	0.374
N stage						
N0	1.000			1.000		
N1	1.154	0.652-2.043	0.623	0.885	0.482-1.627	0.694
N2	1.761	0.956-3.242	0.069	1.404	0.715-2.756	0.325
Numbers of LM						
1-2	1.000			1.000		
3-5	1.747	0.963-3.172	0.067	1.760	0.921-3.365	0.087
≥ 6	3.483	1.922-6.309	< 0.001	3.614	1.876-6.964	< 0.001
Diameter of the largest LM, cm						
≤ 5 vs. > 5	0.670	0.425-1.056	0.085	0.603	0.358-1.015	0.057

Abbreviations: LM, liver metastases; HR, hazard ratio; Cet, cetuximab; CT, chemotherapy.

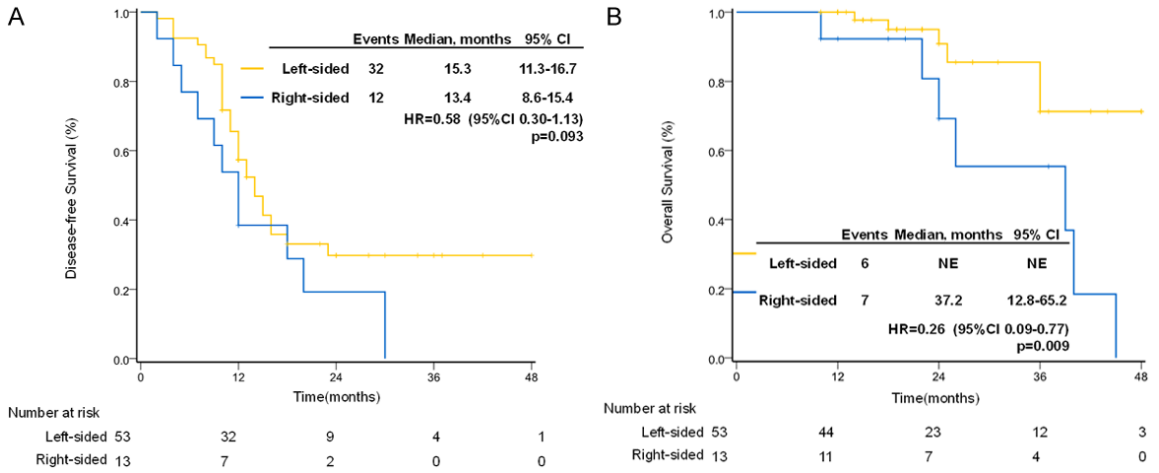

Figure S2. Survival curves of patients who received liver surgery. A. Disease-free survival. B. Overall survival. HR, hazard ratio; 95% CI, 95% confdence interval. NE, not evaluable.