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Original Paper

An Analysis of Relationship Between RAS Mutations and Prognosis of Primary Tumour Resection for Metastatic Colorectal Cancer Patients

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Key Words
Colorectal Cancer • Asymptomatic • Metastatic • Unresectable • Primary Tumour Resection • RAS Gene

Abstract
Background/Aims: Non-radical primary tumour resection (PTR) of asymptomatic metastatic colorectal cancer (mCRC) can prolong survival time of some patients. Patients with mutated RAS gene have worse survival outcome. This study aimed to investigate the impact of RAS gene mutations on the prognosis of asymptomatic unresectable mCRC patients who underwent PTR. ***Methods:*** A retrospective observational cohort study was deduced among mCRC patients who experienced PTR or had intact primary tumour (IPT). All of them had the primary tumour tissue genotyping tested for RAS (KRAS and NRAS) gene mutations. The tumour-related overall survival (OS) time and progression-free survival (PFS) time was estimated. From January 2011 to June 2014, 421 mCRC patients with asymptomatic, unresectable, metastatic disease were enrolled in this study. Among them, 282 patients underwent PTR and 139 patients had IPT. ***Results:*** The mutation rate of RAS was 53.8% (221/411). With a median followed-up time of 46.5 months, the overall survival time of mCRC patients harboring wtRAS or mtRAS was 28.0 versus 22.0 months (p = 0.043) in PTR group and was 21.6 versus 17.8 months (p=0.071) in IPT groups. A Multivariate regression analysis suggested that RAS gene (p=0.039, HR=1.288,95%CI [1.072~2.911]), metastatic organ number (p=0.033, HR=3.091,95%CI [1.090~5.755]) and systemic therapy response (p=0.019, HR=0.622,95%CI [0.525~0.811]) were independent prognostic factors in PTR population. ***Conclusion:*** We found that wild-type RAS gene was a favorable factor for the asymptomatic unresectable mCRC patients experiencing PTR.

L. Liang and J. Tian contributed equally to this work.

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Introduction

Colorectal cancer (CRC) is a common type of malignant tumour worldwide. Almost 20% – 25% of patients with CRC have metastatic disease at the time of initial diagnosis and 75% – 90% of them were unresectable [1]. The median survival time of metastatic colorectal cancer (mCRC) patients presenting with unresectable distant metastasis was about 5 months with best supportive care [2, 3].

In general, the purpose of primary tumour resection (PTR) is to prevent or treat colon or rectal primary tumour related complications, such as intestinal obstruction, acute significant bleeding, perforation and systemic chemotherapy related complications brought from new molecular target therapy drugs (e.g. anti-angiogenesis monoclonal antibody) for colorectal cancer patients who had unresectable metastatic lesions [4]. But, considerable retrospective studies have suggested that CRC patients with asymptomatic primary tumour as well as synchronous unresectable metastases who underwent PTR had significantly longer survival time compared to those who only received palliative systemic chemotherapy [5-7]. A series of perspective clinical trials have been carried out to verify the survival superiority of PTR in this setting, such as CAIRO4 [NCT01606098], SYNCHRONOUS [ISRCTN30964555] and Korean trial [NCT01978249] [8-10].

It is crucial that oncologists should make a decision for asymptomatic unresectable mCRC patients who are most likely to be benefited from the non-curative resection of primary tumour. Dorajoo SR et al. [11] reported that clinical features, such as advanced age, poorly differentiated tumour, metastasis to liver, lung and bone, carcinomatosis, hypoalbuminaemia and elevated carcinoembryonic antigen levels, could significantly shorten post-operative survival of PTR. DeMestier et al. [12] summarized that other clinic-pathological characteristics, including WHO-PS score, primary tumour site, chemotherapy regimen, liver metastasis burden and extra-hepatic metastatic disease were also the independent prognostic factors. Turner N et al. [13] suggested that systemic inflammation was also a negative factor form CRC patient who experience PTR.

The genetic features classification is regarded as basis for personalized therapy in mCRC in recent years, yet only RAS (KRAS, NRAS) and BRAF mutation have so far been wildly accepted as the biomarkers tools in clinical practice to help doctors administrate right therapy for right patients at the right time. The utility of BRAF gene as a biomarker is limited because BRAF V600E mutation rate is about 5% to 9% among colorectal cancer [14]. The RAS mutations prevalence (exon 2 and non-exon 2 of KRAS and NRAS) ranges from 50% to 60% [15] and have been taken as a predictive marker of resistance to EGFR blockage target therapy in conventional practice use [16]. RAS mutations may also have negative impact on patients' prognosis in metastatic setting. Osumi H et al. [17] evaluate the relationship between RAS mutations and clinical survival outcomes after mastectomy in mCRC patients and demonstrated that mutant-type RAS (mtRAS) was associated with shorter overall survival time. Waring P et al. [18] suggested that KRAS mutations in mCRC predispose to aggressive biology and possibly selection for therapy resistant clones. Payandeh M et al. [19] reported that there was a significant difference of KRAS codons mutations for survival.

At the present time, there are no biomarkers so far that have been identified to be a favorable predictor of PTR clinical survival outcome. Whether RAS mutation is related to a worse survival even if mCRC patients presented asymptomatic disease and undergo PTR remains uncertain. Therefore, we designed this retrospective observational study, trying to examine the impact on clinical survival among the population of mCRC patients who experienced PTR and verify the prognostic value to determine the subpopulation who would be really benefited from the non-curative surgery procedure.

Materials and Methods

Patients

A non-randomized retrospective cohort observation study was deduced among a series of consecutive metastatic colorectal patients. Some of these mCRC patients (1) presented asymptomatic primary and unresectable metastatic diseases needed no immediate surgery intervention or other treatments, (2) required positive treatment and (3) received the treatment plan made by physicians and surgeons together, then they underwent primary tumour resection. If the patients were unwilling to experience the surgical resection, they would accept palliative systemic chemotherapy with intact primary tumour (IPT) all the time. All of the patients had the primary tumour tissue genotyping tested for RAS (KRAS and NRAS gene) mutations. The protocol was approved by the ethics committee (ethics committee of Zhongshan Hospital of Fudan University, Shanghai), and written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines.

RAS mutations analysis

Tumour specimen was from formalin-fixed, paraffin-embedded resected primary tumour mass or biopsy tissue under endoscopy. DNA extracted by FFPE QIAGNE kit and RAS statues were analyzed by the amplification refractory mutation system (ARMS). KRAS mutations referred to exon 2 (codons 12, 13), exon 3 (codons 59, 176, 181), exon 4 (codons 146, 117). NRAS mutations referred to exon 2 (codons 12, 13), exon 3 (codons 59, 61), exon 4 (codons 146, 117).

Assessment measure of treatment outcomes

The patients were radiologically examined every six to eight weeks to assess treatment response according to clinical guidelines, such as the NCCN guideline, which recommend abdominopelvic computed tomography (CT) scans, liver magnetic resonance imagines (MRI), and chest X-rays. If chest X-rays indicated metastatic disease in the lungs, chest CT scans were performed to confirm the diagnosis. The assessments were performed immediately if clinical signs indicated disease progression. A complete CBC with differentials and chemistry profiles was performed prior to each cycle. Objective tumour responses were measured according to the RECIST 1.1 criterion, including complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). During treatment, hematological toxicities and non-hematological toxicities were assessed according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) scale version 3.0 [20]. Progression free survival (PFS) time was calculated from the initial of the treatment until disease first progression and overall survival (OS) time was referred to time until the date of death or last follow-up.

Statistical analysis

The statistical analyses were conducted with software of SPSS (version 12.0). Clinical characteristics were examined using the Chi squared test or Fisher's exact probability test. Time-related parameters were evaluated using the Kaplan-Meier method and were compared by Log-rank test.

Results

The prevalence of RAS gene mutation

From January 2011 to June 2014, a total of 1029 metastatic colorectal patients were first diagnosed and treated at the Department of Medical Oncology of Zhongshan Hospital affiliated to Fudan University. Finally, there were 421mCRC patients were enrolled in this study, with 282 cases underwent primary tumour resection and 139 cases had intact primary tumour. Four hundred and eleven cases successfully genotyped for RAS gene mutations. There were 176 cases and 45 cases detected mutated-type KRAS (42.8%) and NRAS (10.9%). In addition, there were 24 cases with wild-type RAS detected mutated-type BRAF of V600E (24/411, 5.8%). The date of PTR and IPT group was showed in Fig. 1, respectively.

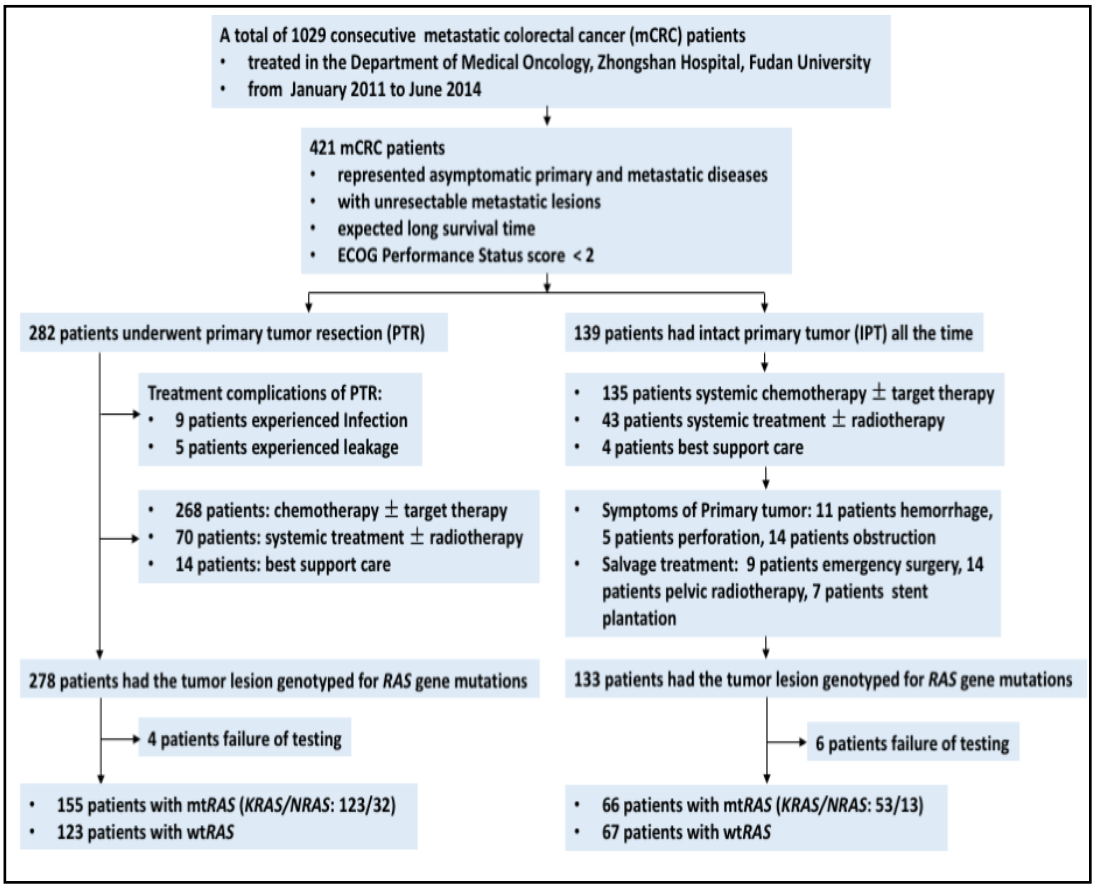

Fig. 1. The research flow chart of this study.

Clinic pathological characteristics

The clinic pathological characteristics, such as gender, age, performance status, primary tumour site, number of metastatic organs did not significantly differ between all the patients (Table 1), PTR and IPT groups (Table 2), wtRAS and mtRAS groups (Table 3).

Tumour-related Survival analysis

With a median time of 46.5 (12-51) months, three hundred and ninety (390/411 , 94%) patients died of tumour. The progression free survival time and overall survival time of mCRC patients who underwent PTR or had IPT was 9.2 versus 7.7 months (p = 0.005) and 26.0 versus 15.5 months (p = 0.001) (Fig. 2). The progression free survival time of mCRC patients who underwent PTR or had IPT with wtRAS or mtRAS were 10.8 versus 8.5 months (p = 0.059), 7.9 versus 6.8 months (p = 0.893) (Fig. 3). The overall-survival time of mCRC patients who underwent PTR or had IPT with wtRAS or mtRAS were 28.0 versus 21.6 months (p = 0.024), 22.0 versus 17.8 months (p = 0.102) (Fig. 4). Then, the progression free survival time of mCRC patients who harbored wtRAS or mtRAS experienced PTR or IPT were 10.8 versus 7.9 months (p = 0.032), 8.5 versus 6.8 months (p = 0.095) (Fig. 5). The overall-survival time of mCRC patients who harbored wtRAS or mtRAS experienced PTR or IPT were 28.0 versus 22.0 months (p = 0.043), 21.6 versus 17.8 months (p = 0.071) (Fig. 6), respectively. A statistical significance was proved by log-rank test.

A Multivariate regression analysis

The clinical and pathological factors that affect the tumor-related progress free survival time and overall survival time among asymptomatic metastatic colorectal cancer patients in this study were compared by a method of multivariate regression (Table 4). Further analysis found that RAS gene (p = 0.039, HR = 1.288, 95%CI [1.072~2.911]), metastatic organ number (p = 0.033, HR = 3.091, 95%CI [1.090~5.755]) and systemic therapy response (p = 0.019, HR = 0.622, 95%CI [0.525~0.811]) were independent prognostic factors in PTR population (Table 5). Metastatic organ number (p = 0.041, HR = 2.870, 95%CI [0.383~4.143]) and systemic therapy response (p = 0.888, HR = 0.043, 95%CI [0.059~0.931]) were independent prognostic factors in IPT population (Table 6).

Discussion

The result of this study suggested that asymptomatic unresectable mCRC patients with wtRAS gene who experienced primary tumour resection had a longer overall survival time and progression free survival time than those had mtRAS gene. Inversely, mCRC patients with mtRAS gene had a similar survival outcome, regardless of whether they underwent primary tumour resection or had intact tumour all the time.

Regardless of a retrospective observational cohort study with small sample,

Table 1. The clinic pathological characteristics and treatment data of mCRC pts with asymptomatic unresectable diseases in this research * Left-Side Colon included rectum. ^TACE referred to trans catheter chemoembolization. TAI referred to trans catheter arterial infusion

Parameter	PTR group	IPT group	p-value
Patients Number	278	133	/
Gender			0.533
Male	145 (52%)	65 (49%)	
Female	133 (48%)	68 (51%)	
Mean Age	57.2±11.9	56.0±10.1	0.713
Primary Tumor Site			0.704
Right-Side Colon	48 (17%)	25 (6%)	
Left-Side Colon*	230 (83%)	108 (94%)	
Primary Tumour Size			0.001
<5cm	121 (44%)	82 (62%)	
≥5cm	157 (56%)	51 (38%)	
Metastasis Organ			0.746
Liver	223 (80%)	102 (77%)	
Lung	195 (70%)	104 (78%)	
Peritoneal site	153 (55%)	77 (58%)	
Bone, etc	83 (30%)	36 (27%)	
Number of Metastasis Organs			0.706
Single	185(67%)	86 (65%)	
Multi	93 (33%)	47 (35%)	
Metastatic Lesion Size			0.780
<5cm	181 (65%)	86 (65%)	
≥5cm	93 (33%)	47 (35%)	
CEA Level			0.787
<5μg/ml	80 (29%)	40 (30%)	
≥5μg/ml	198 (71%)	93 (70%)	
RAS Gene Type			0.243
Wild type	123 (44%)	67 (50%)	
Mutated type	155 (56%)	66 (50%)	
First Line Chemo Regimen			0.436
FOLFOX or CAPEOX	137 (49%)	71 (53%)	
FOLFIRI	141 (51%)	62 (47%)	
Second Line Chemo Regimen			0.401
FOLFOX or CAPEOX	123 (44%)	62 (47%)	
FOLFIRI	125 (45%)	55 (41%)	
Capecitabine	20 (7%)	14 (11%)	
None	10 (4%)	2 (1%)	
Molecular Target Therapy			0.001
Cetuximab	77 (28%)	27 (20%)	
Bevacizumab	72 (26%)	66 (50%)	
None	129 (46%)	40 (30%)	
Response to First Line Therapy			0.580
Partial Response	50 (18%)	21 (16%)	
Stable Disease	173 (62%)	80 (60%)	
Progressive Disease	55 (20%)	32 (24%)	
Second Line Treatments			0.554
Ablation	140 (50%)	64 (48%)	
TACE or TAI^	181 (65%)	93 (70%)	
Toxicity of Drugs Therapy			0.204
Grade 1 to 2	228 (82%)	102 (77%)	
Over Grade 2	50 (18%)	31 (23%)	
Surgery Complications			/
Infection	15 (5%)	/	
Leakage	15 (5%)	/	
Symptoms of Primary Tumour			/
Haemorrhage	/	9 (7%)	
Perforation	/	3 (2%)	
Obstruction	/	12 (9%)	
Salvage Treatment of Primary Tumour			/
Emergency Surgery	/	7 (5%)	
Pelvic Radiotherapy	/	12 (9%)	
Stent Plantation	/	5 (4%)	

Table 2. The clinic pathological characteristics and treatment data of mCRC pts with asymptomatic unresectable diseases who underwent primary tumour resection (PTR) or had intact primary tumour (IPT)

Characteristics	PTR group			IPT group		
	wtRAS	mtRAS	p-value	wtRAS	mtRAS	p-value
Patients Number	155	123	/	67	66	/
Gender			0.213			0.663
Male	86 (56%)	59 (48%)		34 (51%)	31 (47%)	
Female	69 (44%)	64 (52%)		33 (49%)	35 (53%)	
Mean Age	56.5±11.8	58.1±12.1	0.964	55.7±11.2	56.3 ± 9.3	0.374
Primary Tumor Site			0.808			0.532
Right-Side Colon	26 (17%)	22 (18%)		14 (21%)	11 (17%)	
Left-Side Colon	129(83%)	101 (82%)		53 (79%)	55 (83%)	
Primary Tumour Size			0.102			0.337
<5cm	109(70%)	75 (61%)		44 (66%)	38 (58%)	
≥5cm	46 (30%)	48 (39%)		23 (34%)	28 (42%)	
Metastasis Organ			0.019			0.645
Liver	122(79%)	101(82%)		46 (68%)	56 (85%)	
Lung	101(65%)	94 (76%)		50 (74%)	54 (81%)	
Peritoneal site	69 (45%)	84 (68%)		33 (49%)	44 (67%)	
Bone, etc	30 (19%)	53 (43%)		13 (19%)	23 (35%)	
Number of Metastasis Organs			0.214			0.182
Single	108(70%)	77 (63%)		47 (70%)	39 (59%)	
Multi	47 (30%)	46 (37%)		20 (30%)	27 (41%)	
Metastatic Lesion Size			0.301			0.339
<5cm	105(68%)	76 (62%)		43 (64%)	37 (56%)	
≥5cm	50 (32%)	47 (38%)		24 (36%)	29 (44%)	
CEA Level			0.709			0.484
<5μg/ml	46 (30%)	34 (28%)		22 (31%)	18 (27%)	
≥5μg/ml	109(70%)	89 (72%)		45 (69%)	48 (73%)	
First Line Treatment Regimen			0.382			0.539
FOLFOX or CAPEOX 80 (52%)		57 (46%)		34 (51%)	37 (56%)	
FOLFIRI	75 (48%)	66 (54%)		33 (49%)	29 (44%)	
Second Line Chemo Regimen			0.569			0.554
FOLFOX or CAPEOX 63 (41%)		60 (49%)		30 (45%)	32 (49%)	
FOLFIRI	75 (48%)	50 (41%)		31 (46%)	24 (36%)	
Capecitabine	11 (7%)	9 (7%)		5 (8%)	9 (14%)	
None	6 (4%)	4 (3%)		1 (1%)	1 (1%)	
Molecular Target Therapy			0.001			0.001
Cetuximab	77 (50%)	0 (0%)		27 (40%)	0 (0%)	
Bevacizumab	33 (21%)	39 (32%)		22 (33%)	44 (67%)	
None	45 (29%)	84 (68%)		18 (27%)	22 (33%)	
Response to First Line Therapy				0.113		0.418
Partial Response	31 (20%)	19 (15%)		12 (18%)	9 (14%)	
Stable Disease	100(65%)	73 (59%)		42 (64%)	38 (57%)	
Progressive Disease	24 (15%)	31 (26%)		13 (18%)	19 (29%)	
Second Line Treatments			0.183			0.303
Ablation	77 (50%)	63 (51%)		27 (40%)	37 (56%)	
TACE or TAI^	86 (55%)	95 (77%)		47 (70%)	46 (70%)	
Toxicity of Drugs Therapy			0.555			0.570
Grade 1 to 2	129(83%)	99 (80%)		50 (75%)	52 (79%)	
Over Grade 2	26 (17%)	24 (20%)		17 (25%)	14 (11%)	
Surgery Complications			0.713			/
Infection	9 (6%)	6 (5%)		/	/	
Leakage	8 (5%)	7 (6%)		/	/	
Symptoms of Primary Tumour						/
Haemorrhage	/	/		3 (4%)	6 (9%)	
Perforation	/	/		1 (1%)	2 (3%)	
Obstruction	/	/		7 (10%)	5 (8%)	
Salvage Treatment of Primary Tumour						/
Emergency	/	/		4 (6%)	3 (2%)	
Pelvic Radiotherapy	/	/		6 (9%)	6 (4%)	
Stent Plantation	/	/		3 (5%)	2 (1%)	

Table 3. The clinicopathological characteristics and treatment data of mCRC pts with asymptomatic unresectable diseases who underwent primary tumour resection (PTR) or had intact primary tumour (IPT)

Parameter	wtRAS Group			mtRAS Group		
	PTR	IPT	p-value	PTR	IPT	p-value
Patients Number	155	67	/	123	66	
Gender			0.516			0.896
Male	86 (56%)	34 (51%)		59 (48%)	31 (47%)	
Female	69 (44%)	33 (49%)		64 (52%)	35 (53%)	
Mean Age	56.5±11.8	55.7±11.2	0.875	58.1±12.1	56.3±9.3	0.521
Primary Tumor Site			0.463			0.833
Right-Side Colon	26 (17%)	14 (21%)		22 (18%)	11 (17%)	
Left-Side Colon	129(83%)	53 (79%)		101(82%)	55 (83%)	
Primary Tumour Size			0.492		0.650	
<5cm	109(70%)	44 (66%)		75 (61%)	38 (58%)	
≥5cm	46 (30%)	23 (34%)		48 (39%)	28 (42%)	
Metastasis Organ			0.701			0.816
Liver	122(79%)	46 (68%)		101(82%)	56 (85%)	
Lung	101(65%)	50 (74%)		94 (76%)	54 (81%)	
Peritoneal site	69 (45%)	33 (49%)		84 (68%)	44 (67%)	
Bone, etc	30 (19%)	13 (19%)		53 (43%)	23 (35%)	
Number of Metastasis Organs			0.944		0.496	
Single	108(70%)	47 (70%)		77 (63%)	39 (59%)	
Multi	47 (30%)	20 (30%)		46 (37%)	27 (41%)	
Metastatic Lesion Size			0.605		0.444	
< 5cm	105(68%)	43 (64%)		76 (62%)	37 (56%)	
≥ 5cm	50 (32%)	24 (36%)		47 (38%)	29 (44%)	
CEA Level			0.639			0.957
<5μg/ml	46 (30%)	22 (31%)		34 (28%)	18 (27%)	
≥5μg/ml	109(70%)	45 (69%)		89 (72%)	48 (73%)	
First Line Chemo Regimen			0.906			0.203
FOLFOX or CAPEOX 80 (52%)		34 (51%)		57 (46%)	37 (56%)	
FOLFIRI	75 (48%)	33 (49%)		66 (54%)	29 (44%)	
Second Line Chemo Regimen			0.781			0.477
FOLFOX or CAPEOX 63 (41%)		30 (45%)		60 (49%)	32 (49%)	
FOLFIRI	75 (48%)	31 (46%)		50 (41%)	24 (36%)	
Capecitabine	11 (7%)	5 (8%)		9 (7%)	9 (14%)	
None	6 (4%)	1 (1%)		4 (3%)	1 (1%)	
Molecular Target Therapy			0.176			0.001
Cetuximab	77 (50%)	27 (40%)		0 (0%)	0	
Bevacizumab	33 (21%)	22 (33%)		39 (32%)	44 (67%)	
None	45 (29%)	18 (27%)		84 (68%)	22 (33%)	
Response to First Line Therapy			0.755			0.850
Partial Response	31 (20%)	12 (18%)		19 (15%)	9 (14%)	
Stable Disease	100(65%)	42 (64%)		73 (59%)	38 (57%)	
Progressive Disease	24 (15%)	13 (18%)		31 (26%)	19 (29%)	
Second Line Treatments			0.122			0.481
Ablation	77 (50%)	27 (40%)		63 (51%)	37 (56%)	
TACE or TAI^	86 (55%)	47 (70%)		95 (77%)	46 (70%)	
Toxicity of Drugs Therapy			0.137			0.781
Grade 1 to 2	129(83%)	50 (75%)		99 (80%)	52 (79%)	
Over Grade 2	26 (17%)	17 (25%)		24 (20%)	14 (11%)	
Surgery Complications			/			/
Infection	9 (6%)	/		6 (5%)	/	
Leakage	8 (5%)	/		7 (6%)	/	
Symptoms of Primary Tumour			/			/
Haemorrhage	/	3 (4%)		/	6 (9%)	
Perforation	/	1 (1%)		/	2 (3%)	
Obstruction	/	7 (10%)		/	5 (8%)	
Salvage Treatment of Primary Tumour						/
Emergency	/	4 (6%)		/	3 (2%)	
Pelvic Radiotherapy	/	6 (9%)		/	6 (4%)	
Stent Plantation	/	3 (5%)		/	2 (1%)	

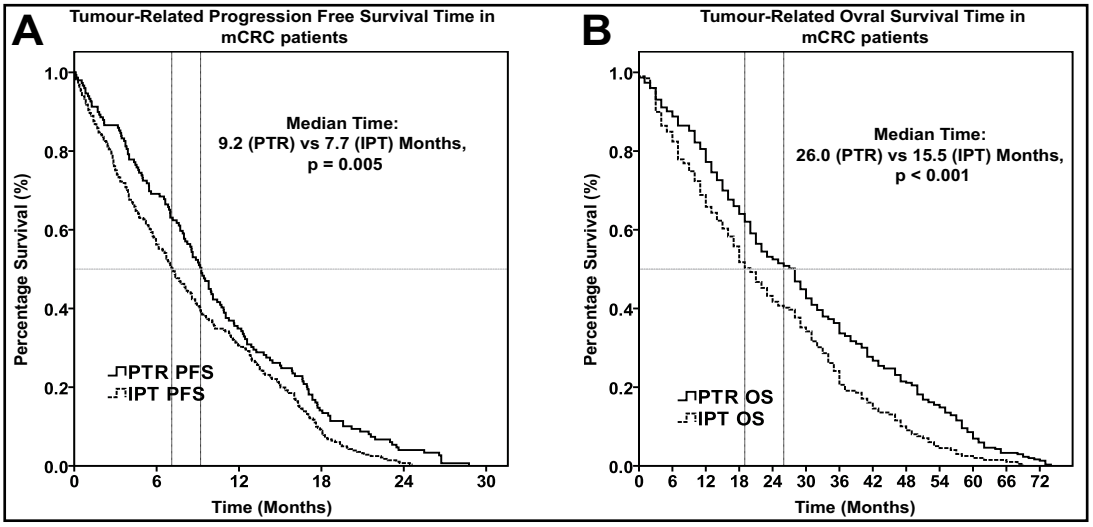

Fig. 2. Survival outcomes were analyzed for tumour-related progression free survival time (PFS) and overall survival time (OS) between the PTR group and IPT group by Kaplan – Meier method. With a median followed-up time of 46.5 months, the PFS and OS time of mCRC patients who underwent PTR or had IPT were 9.2 versus 7.7 months (p = 0.005), 26.0 versus 15.5 (p = 0.001) respectively. A statistical significance was proved by log-rank test.

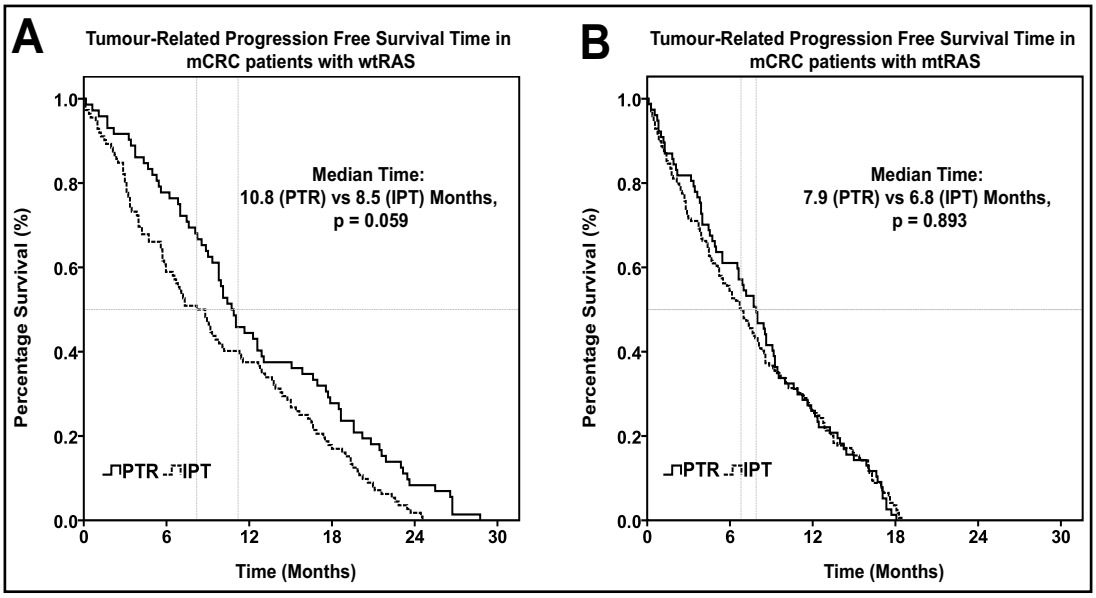

Fig. 3. Survival outcomes were analyzed for tumour-related progression free survival time (PFS) between the wtRAS group (A) and mtRAS group (B) by Kaplan – Meier method. The PFS time of mCRC patients who underwent PTR or had IPT with wtRAS or mtRAS were10.8 versus 8.5 months (p = 0.059), 7.9 versus 6.8 months (p = 0.893), respectively. A statistical significance was proved by log-rank test.

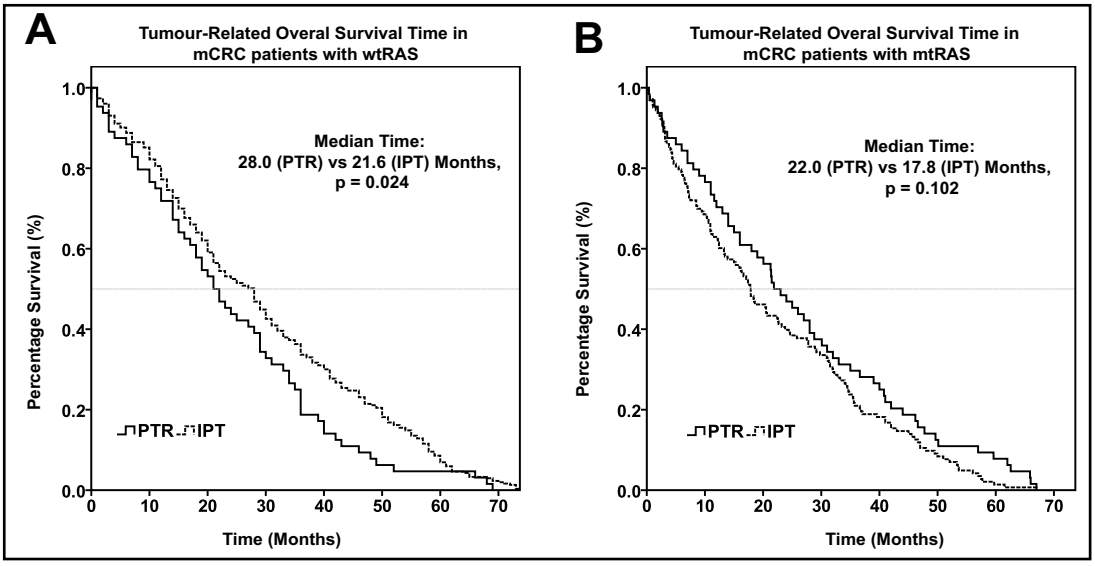

Fig. 4. Survival outcomes were analyzed for tumour-related overall survival time (OS) between the wtRAS group (A) and mtRAS group (B) by Kaplan – Meier method. The OS time of mCRC patients who underwent PTR or had IPT with wtRAS or mtRAS were 28.0 versus 21.6 months (p = 0.024), 22.0 versus 17.8 months (p = 0.102), respectively. A statistical significance was proved by log-rank test.

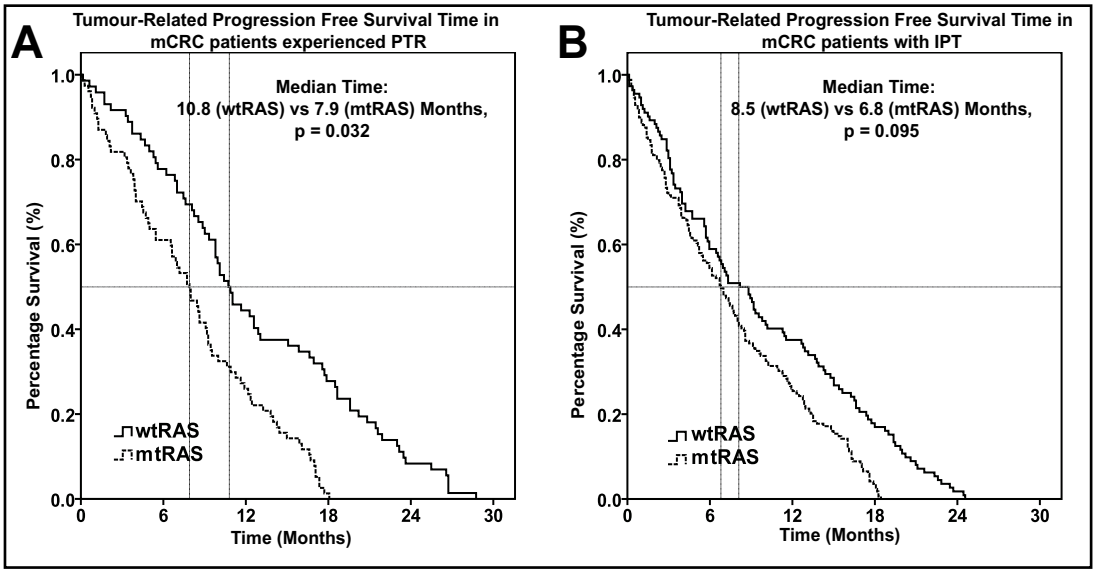

Fig. 5. Survival outcomes were analyzed for tumour-related progression free survival time (PFS) between the PTR group (A) and IPT (B) by Kaplan – Meier method. The PFS time of mCRC patients who harbored wtRAS or mtRAS experienced PTR or IPT were 10.8 versus 7.9 months (p = 0.032), 8.5 versus 6.8 months (p = 0.095), respectively. A statistical significance was proved by log-rank test.

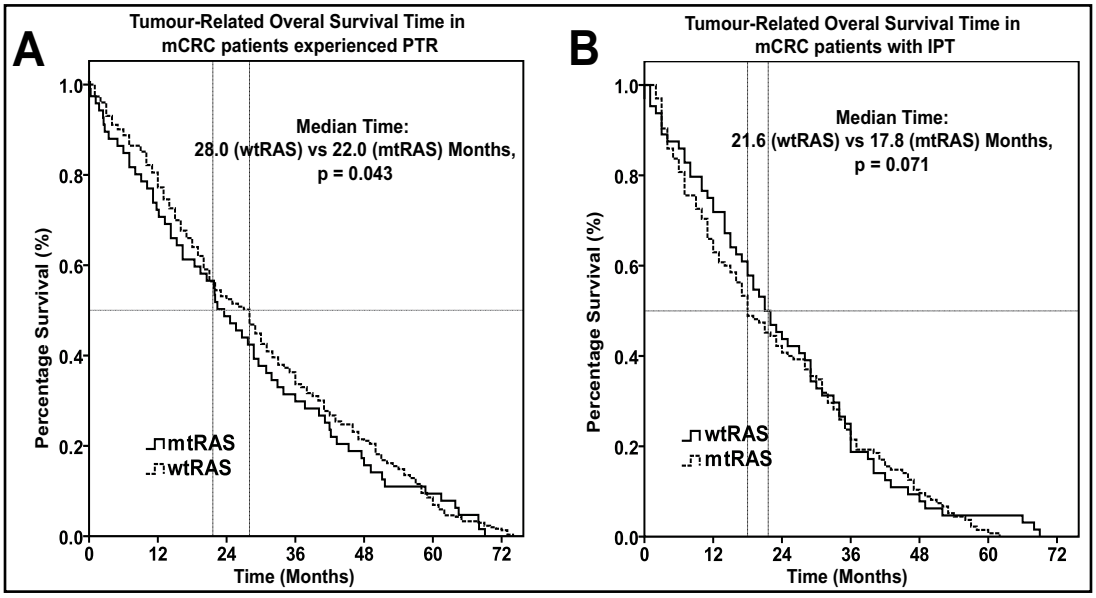

Fig. 6. Survival outcomes were analyzed for tumour-related overall survival time (OS) between the PTR group (A) and IPT (B) by Kaplan – Meier method. The OS time of mCRC patients who harbored wtRAS or mtRAS experienced PTR or IPT were 28.0 versus 22.0 months (p = 0.043), 21.6 versus 17.8 months (p = 0.071), respectively. A statistical significance was proved by log-rank test.

Table 4. A Multi-factor regression analysis was deduced on the clinical and pathological factors that affect the tumor-related progress free survival time and overall survival time among asymptomatic metastatic colorectal cancer patients in this study (All the cases)

mCRC Patients with Asymptomatic Unresectable Diseases	Multi-Factors Cox Regression Analysis					
Clinical-pathological Characteristics	Tumour-Related Progresssion Free Survival			Tumour-Related Overall Survival		
	Hazards Rates	95% Confidence Interval	p-value	Hazards Rates	95% Confidence Interval	p-value
Gender (Male/Female)	0.552	0.984 - 1.730	0.442	0.498	0.893 - 1.902	0.587
Mean Age	1.298	0.734 - 2.269	0.321	1.300	0.634 - 2.667	0.474
PS Score (0/ 1 - 2)	0.732	0.381 - 1.874	0.373	0.633	0.282 - 2.074	0.438
RAS Status (Wild/ Mutated)	1.592	0.221 - 3.021	0.411	1.219	0.093 - 1.987	0.328
Primary Tumor Size (<5cm / ≥5cm)	1.231	1.007 - 2.189	0.035	1.309	0.782 - 2.013	0.413
Primary Tumor Site (right/left)	0.732	0.480 - 1.538	0.612	0.876	0.801 - 2.182	0.711
Metastasis Organ	1.897	0.258 - 2.981	0.098	2.134	0.981 - 3.214	0.104
Number of Metastasis Organs (single-/multi-)	2.294	1.532 - 3.012	0.029	2.189	1.318 - 3.134	0.024
Metastatic Lesion Size (<5cm / ≥5cm)	3.271	0.624 - 4.174	0.851	3.037	0.671 - 5.371	0.531
PTR / IPT	0.712	0.451 - 1.321	0.081	0.821	0.698 - 0.909	0.032
First Line Chemotherapy Regimen	0.987	0.109 - 3.012	0.752	0.827	0.201 - 2.125	0.289
Anti-EGFR Target Therapy (Yes/No)	0.873	0.501 - 2.917	0.082	0.782	0.491 - 1.864	0.067
Anti-VGFR Target Therapy (Yes/No)	0.791	0.201 - 1.982	0.109	0.821	0.581 - 2.182	0.069
First-Line Therapy Response (CR/SD/PD)	3.912	1.891 - 5.098	0.038	2.039	1.082 - 2.983	0.023
Any Adverse Events from Systemic Therapy	1.253	0.672 - 2.129	0.528	1.513	0.589 - 3.862	0.677
Second Line Treatments	0.954	0.561 - 1.842	0.657	0.814	0.421 - 1.928	0.824
Complications of Surgery	1.021	0.251 - 2.021	0.133	1.902	0.581 - 2.873	0.431

Table 5. A Multi-factor regression analysis was deduced on the clinical and pathological factors that affect the tumor-related progress free survival time and overall survival time among asymptomatic metastatic colorectal cancer patients who experienced primary tumor resection (PTR group)

mCRC Patients with Asymptomatic Diseases	Multi-Factors Cox Regression Analysis					
Experienced Primary Tumor Resection	Tumour-Related Progresssion Free Survival			Tumour-Related Overall Survival		
	Hazards Rates	95% Confidence Interval	p-value	Hazards Rates	95% Confidence Interval	p-value
Gender (Male/Female)	0.673	0.724 - 1.530	0.342	0.921	0.801 - 4.902	0.744
Mean Age	1.441	0.186 - 1.548	0.164	1.927	0.476 - 3.808	0.358
PS Score (0/ 1 - 2)	0.815	0.716 - 2.141	0.731	0.790	0.529 - 3.074	0.689
RAS Status (Wild/ Mutated)	1.828	1.209 - 3.001	0.045	1.288	1.072 - 2.911	0.039
Primary Tumor Size (<5cm / ≥5cm)	2.313	1.102 - 4.902	0.101	1.991	1.812 - 5.328	0.277
Primary Tumor Site (right/left)	0.932	0.167 - 2.580	0.322	0.894	0.301 - 3.021	0.591
Metastasis Organ	2.981	0.812 - 3.616	0.569	2.521	0.716 - 3.164	0.422
Number of Metastasis (single-/multi-)	2.523	1.022 - 4.072	0.091	3.091	1.089 - 5.755	0.033
Metastatic Lesion Size (<5cm / ≥5cm)	2.021	0.784 - 3.936	0.085	1.988	0.751 - 3.331	0.105
First Line Chemotherapy Regimen	0.987	0.109 - 3.299	0.896	0.899	0.488 - 2.789	0.891
Anti-EGFR Target Therapy (Yes/No)	0.744	0.288 - 1.917	0.112	0.682	0.333 - 2.314	0.081
Anti-VGFR Target Therapy (Yes/No)	0.612	0.301 - 2.863	0.210	0.533	0.281 - 1.903	0.209
First-Line Therapy Response (CR/SD/PD)	0.588	0.191 - 0.985	0.025	0.622	0.525 - 0.811	0.019
Any Adverse Events from Systemic Therapy	2.513	0.992 - 4.219	0.877	2.883	0.819 - 4.612	0.711
Second Line Treatments	0.852	0.368 - 2.164	0.236	0.799	0.617 - 2.394	0.236
Complications of Surgery	3.214	0.593 - 5.891	0.323	2.919	0.875 - 4.093	0.531

Table 6. A Multi-factor regression analysis was deduced on the clinical and pathological factors that affect the tumor-related progress free survival time and overall survival time among asymptomatic metastatic colorectal cancer patients who had intact primary tumor all the time (IPT group)

mCRC Patients with Asymptomatic Diseases and Intact Primary Tumor All the Time	Multi-Factors Cox Regression Analysis					
	Tumour-Related Progresssion Free Survival			Tumour-Related Overall Survival		
	Hazards Rates	95% Confidence Interval	p value	Hazards Rates	95% Confidence Interval	p value
Gender (Male/Female)	0.494	0.684 - 2.302	0.550	0.401	0.793 - 3.902	0.609
Mean Age	1.217	0.861 - 2.226	0.229	1.007	0.536 - 2.417	0.816
PS Score (0/ 1 - 2)	0.392	0.199 - 2.174	0.637	0.794	0.182 - 2.342	0.490
RAS Status (Wild/ Mutated)	1.791	1.013 - 4.231	0.211	1.691	1.023 - 3.287	0.108
Primary Tumor Size (<5cm / ≥5cm)	2.312	1.807 - 3.189	0.501	2.091	1.801 - 3.013	0.233
Primary Tumor Site (right/left)	0.772	0.180 - 1.989	0.289	0.543	0.201 - 2.822	0.288
Metastasis Organ	2.310	0.814 - 4.326	0.701	2.066	0.912 - 3.199	0.622
Number of Metastasis (single-/multi-)	3.143	1.328 - 6.842	0.062	2.870	0.383 - 4.143	0.041
Metastatic Lesion Size (<5cm / ≥5cm)	3.306	0.922 - 5.654	0.566	2.944	0.841 - 4.299	0.633
First Line Chemotherapy Regimen	0.572	0.231 - 2.102	0.578	0.732	0.144 - 3.217	0.592
Anti-EGFR Target Therapy (Yes/No)	0.633	0.083 - 0.890	0.078	0.710	0.190 - 1.064	0.059
Anti-VGFR Target Therapy (Yes/No)	0.702	0.101 - 1.332	0.093	0.821	0.181 - 2.182	0.107
First-Line Therapy Response (CR/SD/PD)	0.601	0.081 - 0.082	0.039	0.888	0.059 - 0.931	0.043
Second Line Treatments	0.801	0.266 - 2.365	0.091	0.784	0.311 - 3.317	0.522
Any Adverse Events from Systemic Therapy	2.899	0.988 - 6.223	0.799	3.003	0.792 - 5.122	0.880

the results of this study suggested a significant difference and condition, which lacked reports.

There were no significant differences in baseline characteristics between the PTR and IPT group, such as clinic pathological features of primary and metastastic lesions, chemo- or molecular therapy regimens and response to systemic treatments. The similar RAS mutation prevalence and survival outcomes were found by this research group according to the literature reported in metastatic setting.

A recent pooled analysis suggested that RAS mutation (including KRAS and NRAS) prevalence in mCRC patients was about 55.9%, with a distribution as: 42.6% KRAS exon 2, 3.8% KRAS exon 3, 6.2% KRAS exon 4, 2.9% NRAS exon 2, 4.2% NRAS exon 3, 0.3% NRAS exon 4 [21]. In our study, there were 221 cases detected of RAS mutation among 435 valid results (50.8%), followed by KRAS exon 2 (163, 37.5%), KRAS exon 3 (7, 1.6%), KRAS exon 4 (6, 1.4%), NRAS exon 2 (19, 4.3%), NRAS exon 3 (14, 3.2%) and NRAS exon 4 (12, 2.7%).

The presence of KRAS mutation indicated an increasing risk of recurrence and death in RASCAL I and RASCAL II study of patients with Duke's stage C disease [22, 23]. In fact, the prognostic impact of KRAS mutation on survival of colorectal cancer patients among advanced disease population was controversial, because many non-EGFR (Epidermal Growth Factor Receptor) containing regimen of systemic treatment failed in exhibiting a difference of outcome between wtKRAS and mtKRAS CRC in some studies [24-27]. On the other hand, RAS mutation may present a negative factor for the surgery treatment of advanced patient [28, 29]. Kodaz et al. [30] reported that KRAS mutation had a worse prognostic impact on the metastatic CRC patients who underwent curative resection of liver metastasis. Vauthey et al. [31] found out that RAS mutations predicted early lung recurrence among those who had curative resection of colorectal liver metastases and suggested that surgical treatment contributed to prolongation of survival in the population of stage IV colorectal cancer patients and the outcome could be affected by the RAS mutation. In fact, RAS mutation arose early in the process of CRC by polyp–adenoma–neoplasia sequence. A more recent study deduced by Galanopoulos M and his colleagues and revealed that patients with CRC, polyps and healthy individuals could be discriminated by blood circulation free DNA when they underwent screening colonoscopy, because of the mutations in single nucleotide polymorphism (SNP) of KRAS gene [32]. Currently, preclinical studies have already demonstrated that mtRAS is the potential reason of EGFR target therapy resistance. Results from our study have also suggested that mCRC patients harboring mtRAS molecular characteristics may be a class of population who cannot benefit from active surgery treatment.

Some retrospective studies suggested a trend of survival advantage of PTR. Clancy C et al. [33] deduced a meta-analysis of 21 studies and revealed that PTR for mCRC patients was associated with a lower mortality risk (OR 0.28; 95 % CI 0.165-0.474; P < 0.001) and translated into a difference mean survival of 6.4 months in favor of resection (95 % CI 5.025-

7.858, P < 0.001). Ahmed S et al. [34] found that median survival was 15.2 months (range: 10-30.7 months) in the resection group and 11.4 months (range: 3-22 months) in the non-resection group among mCRC patients from 15 retrospective observational studies. Given that inevitable basic flaws lied in selection bias of these studies, such as good performance status, younger age, oligo metastasis and so on , which suggested that those who experienced PTR could be a better prognosis population among advanced mCRCs patients, the systemic therapy was taken as an canonical initial treatment for the advanced unresectable diseases by the major clinical guidelines from the National Comprehensive Cancer Network (NCCN), the European Society for Medical Oncology (ESMO) and the American Society for Medical Oncology (ASCRS). The non-radical surgery of the primary tumor was not recommended by current clinical guidelines, even if the patients had a good performance and presented no severe tumour related symptoms. Although we compared the prognosis survival of PRT to IPT (Fig. 2, Fig. 3, Table 4), this was not the main research conclusion in our study.

In fact, it is difficult to ethically initiate a prospective randomized controlled clinical trial for asymptomatic unresectable mCRC patients to verify palliative resection or palliative systemic chemotherapy which is an optimal treatment choice for them.

However, Chinese colorectal cancer patients who are initially diagnosed as metastatic diseases are more willing to have a non-radical resection rather than receive palliative systemic chemotherapy until disease progression, which gave us a favorable opportunity to launch this retrospective observational cohort study [35]. In our center, the decision of receiving PTR treatment was made according to patients' intention, surgeons' assessment of surgery treatment risk as well as some other patients' condition and clinical features, such as abdominal pain, bloating and other mild symptoms which suggested probable aggravation.

Although a Consensus Molecular Subtypes (CMS) Consortium analyzed CRC expression profiling data from multiple studies and classified CRC into four CMS groups with distinguishing features: CMS1 (microsatellite instability immune, 14%), hyper mutated, microsatellite unstable and strong immune activation; CMS2 (canonical, 37%), epithelial, marked WNT and MYC signaling activation; CMS3 (metabolic, 13%), epithelial and evident metabolic dysregulation; and CMS4 (mesenchymal, 23%), prominent transforming growth factor-β activation, stromal invasion and angiogenesis [36], the attempts to find out molecular markers that can predict tumour disease outcomes regardless of treatment therapy (prognostic) or that give information about the effect from a specific treatment (predictive) have had limited advance in mCRC. Due to low mutation rate of BRAF about 5% to 13%, which appeared 5.9% (24/401) in this study, all RAS gene including KRAS and NRAS examination represents a more valuable tool for predictive and prognostic biomarker than BRAF. Gene mutations provide information beyond that provided by the clinic-pathologic characteristics, such as primary tumour size, site (right colon or left colon), multi-/oligo-metastasis, CEA and CA-199 level, related to the primary tumour as well as metastases, for example, tumor site was not a significant prognostic factors by a multivariate analysis between PTR and IPT.

In addition, the case enrolled in our study was treated much earlier. Patients with asymptomatic unresectable metastases of CRC mainly received systemic chemotherapy and primary tumor resection in our center at that time. Local treatment of liver metastases was not fully in accordance with the current MDT (multidisciplinary team treatment) strategy. Loco regional therapies of the liver metastasis were not commonly used in this study population. Patients with resectable, potentially resectable, potentially topical treatable metastasis were excluded in this study. However, we believed that these did not affect the basic observation of the relationship between RAS gene and survival outcomes in this study. Patients who received PRT were likely to have a better prognosis inherently. It was the purpose of this retrospective cohort study that if they harbored wtRAS gene, they could further benefit from the PTR.

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Disclosure Statement

The authors have declared no conflicts of interest.

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