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## Research Article

## Survival Analysis and Prognostic Predictor Study of Colorectal Cancer Patients with Single-Site Metastasis

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## ARTICLE INFO

## Article history:

Received: 17 December, 2020

Accepted: 2 January, 2021

Published: 29 January, 2021

## Keywords:

Colorectal cancer  
metastatic colorectal cancer  
prognostic predictor  
metastasis  
survival rate

## ABSTRACT

Metastatic colorectal cancer (mCRC) patients have various metastasis patterns, which reflect diverse biological characteristics of different patient subgroups. We analyse the prognosis of mCRC patients according to the metastatic site and clarify the relationship between tumor or patient characteristics and the metastatic sites. The whole sequencing and clinical data of 2329 CRC patients were obtained from TCGA and a database of the MSKCC. Kruskal Wallis Tests were used to analyse measurement data. Survival was illustrated by Kaplan-Meier curves, with P value determined by Log-rank Test. Hazard's ratio was determined through the univariate and multivariate COX proportional hazards regression model. The mortality rate of CRC patients with liver-only metastasis (mCRC-liver) did not increase versus non-metastatic patients. The survival rate of patients with non-regional lymph node-only metastasis (mCRC-NRLN) was lower versus mCRC-liver. Mutations of *KRAS* and *TCF7L2* genes were associated with mortality of mCRC-liver. *APC* mutation was associated with reduced mortality in mCRC-lung and mCRC-NRLN. *BRAF* mutation was associated with increased mortality of mCRC-peritoneum. In a multivariate COX analysis, gender affected the survival rate of mCRC-liver. Age and the number of gene mutations affected the survival rate of mCRC-lung and mCRC-NRLN respectively. Receiving chemotherapy is an unfavourable factor for prognosis of mCRC-liver, but the length of chemotherapy treatment is an advantageous prognosis factor. This study depicts the long-term survival features of a group of mCRC patients. These findings promoted our understanding of the prognosis characteristics of CRC and have positive guiding significance for clinical management of CRC patients.

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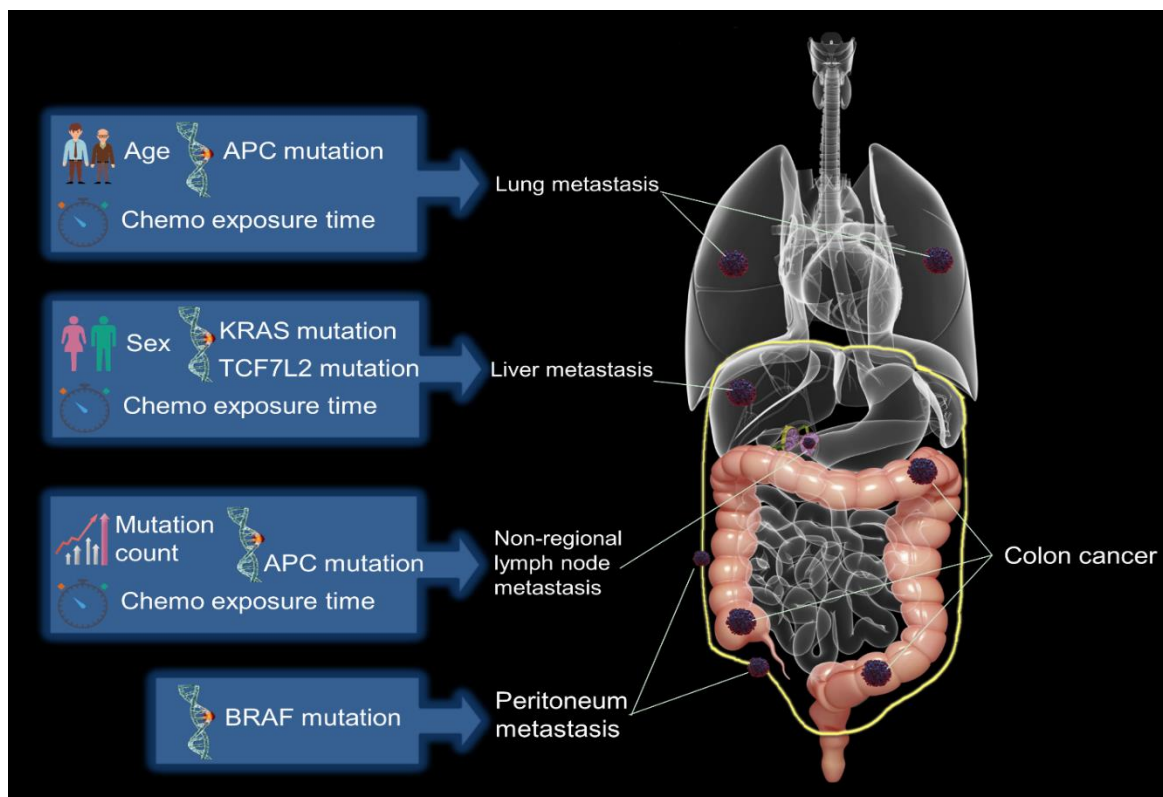
## Graphical Abstract

Schematic diagram summarizing factors closely related to the overall survival of CRC patients with different single site metastasis.

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<http://dx.doi.org/10.31487/j.COR.2021.01.04>



## Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide [1]. A variety of CRC patients undergo significant clinical events, including post-diagnostic recurrences or metastases. In spite of recent progress in early detection, systematic treatment and localized therapy, CRC is still one of the major causes of cancer-related deaths and morbidity in the world [2-5]. Approximately 694,000 deaths from CRC occurred worldwide in 2012 [6]. Untreated metastatic colorectal cancer (mCRC) patients have poor prognosis with an average survival time of 5-6 months [7]. Therefore, the assessment of the features of potential disease outcomes and the identification of their predictors are crucial for efficacious patient surveillance and for the treatment and control of this disease in both the short and long term.

A large number of CRC-related deaths are caused by metastatic disease. About 20% of patients had metastatic disease at the time of diagnosis [8]. Although liver, lungs and peritoneum are the most prominent sites of CRC metastasis, there are many other sites of metastasis, e.g. bones, brain and distant lymph nodes [9-12]. Autopsy studies investigating metastasis patterns revealed that distinctive primary cancers metastasize to different sites with different frequencies, and different CRC patients have different spread patterns [12]. Metastasis number and tumor volume are important indicators to predict prognosis [13]. As to whether the anatomical location of the metastasis and the origin of the primary lesion have any influence on the prognosis, there is no final conclusion at present [13, 14]. Elias *et al.* reported that there was no correlation between metastasis site and prognosis, while Carpizo *et al.* observed a significant correlation [13, 15]. Nevertheless, both studies are limited by the small sample size. The survival rate of CRC patients with lung-only

metastases seems to be at least equivalent to that of patients with liver only metastases, while the prognosis of patients with bone, peritoneal and brain metastases is much worse [16-18]. Two additional studies focus on the survival pattern of mCRC patients, but one covers various patients before modern chemotherapy and the other is much smaller in scale [19, 20]. It is possible that distinct genotypes of mCRC may have different patterns of progression, malignancy and prognosis. We performed the current research to investigate the survival of mCRC patients with different metastatic sites at the time of diagnosis.

Furthermore, it has increasingly been accepted that the biological behaviour and prognosis of CRC may differ depending on the location of the primary lesion and the status of specific mutations, (*KRAS*, *BRAF*, and *MMR*), although the data are not consistent [19, 21]. Several retrospective analysis shows that these somatic and germ line mutations might affect the metastasis pattern, however, most of these are limited, uncontrolled single-agency studies with various correlations [22, 23]. The second aim of the study explored the relationship between molecular markers, primary sites and metastatic sites.

Although CRC is a common disease worldwide, its long-term prognosis characteristics and predictive factors are still unclear. In the present study, we analysed 23 years of data obtained from a prospective patient population with colorectal cancer in The Cancer Genome Atlas (TCGA) and Memorial Sloan Kettering Cancer Center (MSKCC) databases. The specific aims of this study are i) to examine long-term survival characteristics of patients with mCRC; ii) to analyse and compare the clinical data of mCRC patients with different metastatic sites and patients with primary CRC and iii) to study the difference of gene mutation/expression in mCRC patients at different metastatic sites.

## Materials and Methods

### I Clinical Cohorts

The whole sequencing and clinical data of 2368 samples of colorectal cancer patients were obtained: Metastatic Colorectal Cancer (MSKCC, Cancer Cell 2018) 1134 samples, Colorectal Adenocarcinoma (TCGA, PanCancer Atlas) 594 samples, Colorectal Adenocarcinoma (TCGA, Firehouse Legacy) 640 samples (Link) [24-27]. The patient cohort included 2329 incident cases diagnosed between 1995 and 2018.

### II Study Design

We divided this study into four parts. In the first part, we compared clinical properties between Group B: metastatic colorectal cancer (mCRC) patients and Group A: non-metastatic primary colorectal cancer (pCRC) patients. In the second part, we analysed the clinical properties between mCRC patients with single-site tumor metastasis (Group C: liver, Group D: lung, Group E: lymph node, and Group F: peritoneum) and Group A: pCRC patients. We also focused on genomic changes in those with single-site tumor metastasis. In the third part, we explored and compared the gene mutations that were significantly correlated to the survival rate of CRC patients. Additional groups were as follows. Group G: mCRC-liver metastasis-KRAS mut patients; Group H: mCRC-liver metastasis-KRAS wild patients; Group I: mCRC-liver metastasis-*TCF7L2* mut patients; Group J: mCRC-liver metastasis-*TCF7L2* wild patients; Group K: mCRC-lung metastasis-*APC* mut patients; Group L: mCRC-lung metastasis-*APC* wild patients; Group M: mCRC-non-regional lymph node metastasis-*APC* mut patients; Group N: mCRC-

non-regional lymph node metastasis- *APC* wild patients; Group O: mCRC-peritoneum metastasis-*BRAF* mut patients; Group P: mCRC-peritoneum metastasis-*BRAF* wild patients. Finally, in the fourth part, we used COX proportional hazards regression model to analyse which factors significantly contribute to the survival rate of patient in Group C, Group D, Group E, and Group F individually.

### III Statistical Analysis

The data online were used to analyse without change. Frequency data was analysed by Chi-squared Test. Measurement Data (Quantitative) was analysed by Kruskal Wallis Test. Survival was illustrated by the Kaplan-Meier curves, with P value determined by Log-rank Test. Hazard's ratio (HR) was determined through the univariate and multivariate COX proportional hazards regression model. Data analysis were performed using SPSS20.0 (SPSS Inc. Chicago, IL).

## Results

### I Comparison of Clinical Characteristics Between Patients with Metastatic Colorectal Cancer (mCRC) and Primary Colorectal Cancer Patients Without Metastasis (pCRC)

Overall, our analysis included a total of 1133 patients diagnosed with pCRC (1137 samples) and 979 patients with mCRC (1011 samples), among which 565 (26.8%) were younger than 50-year-old, 913 (43.2%) were between 50 and 69-year-old (middle-aged), and 634 (30.0%) were older than 69-year-old (older-aged). More detailed information on the grouping is provided in (Table 1).

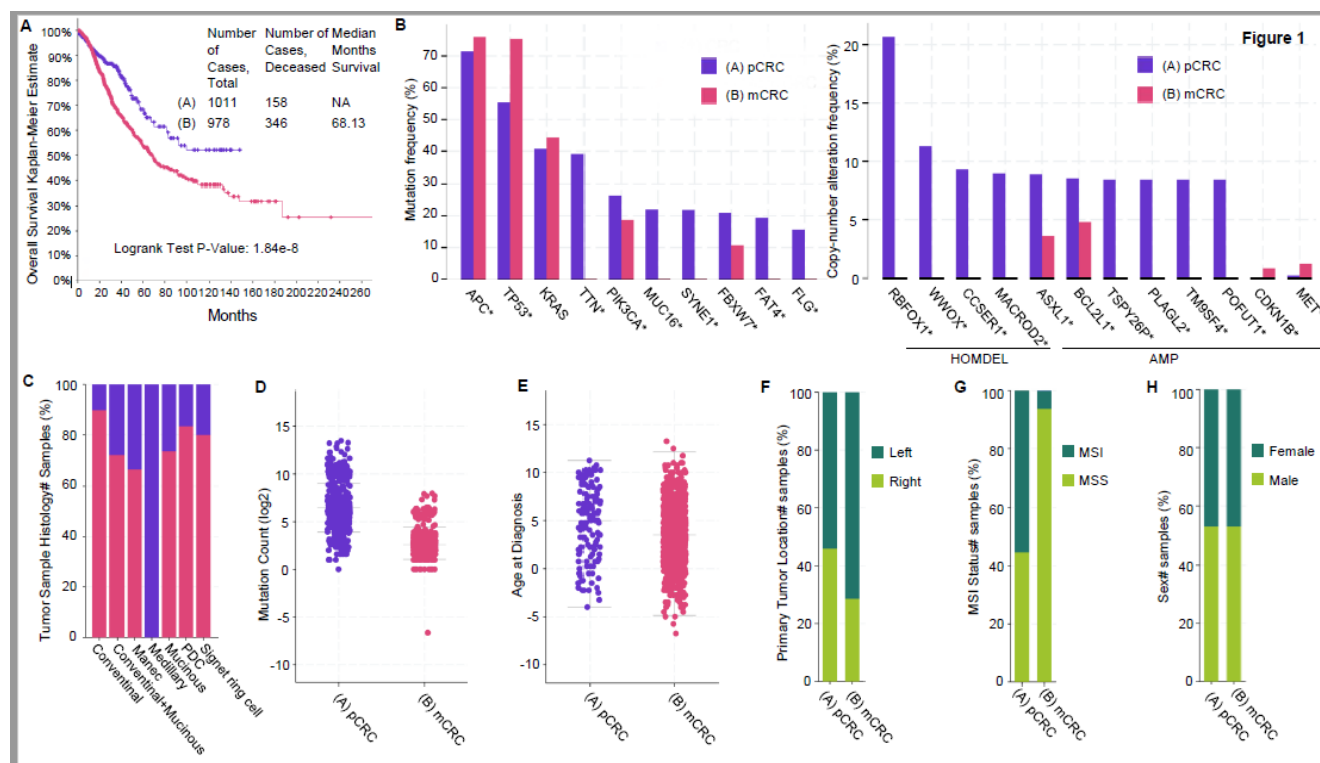
**Table 1:** Effect of gene mutation on survival rate of CRC patients with single-site tumor metastasis (liver, lung, lymph node, and peritoneum). To reduce statistical bias, patients with gene mutation rate  $\geq 10\%$  and cells with the number of patients  $\geq 7$  were selected. "----" means patients with gene mutation rate  $< 10\%$  or the number of patients  $< 7$ .

Mutation contribute to overall survival	Only liver metastasis	Only lung metastasis	Only lymph metastasis	Only peritoneum metastasis
APC mutation	No	Yes*	Yes*	No
TP53 mutation	No	No	No	No
KRAS mutation	Yes*	No	No	No
PIK3CA mutation	No	No	No	No
SMAD4 mutation	No	----	----	No
TCF7L2 mutation	Yes*	----	No	----
ATM mutation	----	No	----	----
AMER1 mutation	----	No	----	----
ERBB4 mutation	----	----	----	No
NOTCH3 mutation	----	----	----	No
BRAF mutation	----	----	----	Yes*

Yes\* Means  $P < 0.05$  mutation type gene compared with wild type gene.

First, we compared the impact of CRC metastases on the study population's overall survival (OS). The results indicated that the 10-year survival rate of patients with mCRC (group B) is significantly lower than that of patients with pCRC. There were 158 pCRC patients and 346 mCRC dead at the end of the study. The percentage of deaths was 13.9% (158/1,133) and 35.3% (346/979) in the pCRC and mCRC groups, respectively. The median survival for the mCRC group was 68.13 months. Next, we analysed the mutation frequency of the two groups. Only *APC* and *TP53* gene mutations were significantly more frequent in

patients with mCRC than in those with pCRC. However, the mutations of other genes including *TTN*, *PIK3CA*, *MUC16*, *SYNE1*, *FBXW7*, *FAT4*, and *FLG* in the metastatic foci of mCRC patients were reduced (Figure 1B, left panel). Copy number analysis indicated that only *CDKN1B* and *MET* genes were found to be amplified more frequently in metastatic lesions than in patients without metastasis and that deletion of *RBFOX1*, *WWOX*, *CCSER1*, *MACROD2* and amplification of *ASXL1*, *BCL2L1*, *TSPY26P*, *PLAGL2* were significantly reduced in metastatic foci (Figure 1B, right panel).



**Figure 1:** Comparison of clinical properties among metastatic colorectal cancer (mCRC) and non-metastatic primary colorectal cancer (pCRC) patients. **A)** overall survival; **B)** the mutation frequency (left panel) and copy number analysis (right panel); **C)** microsatellite instability (MSI) status; **D)** tumor sample histology analysis; **E)** primary tumor location analysis; **F)** Mutation Count; **G)** age at diagnosis; **H)** sex number samples. Asterisks indicate significant differences.

Tumor sample histology analysis suggests that tumors with conventional histological type are more likely to metastasize, while medullary carcinoma hardly metastasizes (Figure 1C). Mutation Count (log2) reveals that the number of gene mutations in mCRC patients is less than that in pCRC patients (Figure 1D). With regard to age at diagnosis, the average age of colon cancer patients without metastasis is 60-year-old, while that of colon cancer patients with metastasis is less than 60-year-old (Figure 1E), implying that we should pay more attention to the tumor metastasis tendency of young patients, increase examination methods in

time, or be more radical in treatment strategy. Of note, primary tumor location analysis demonstrates that cancers originating from left colon are more prone to metastasis than cancers originating from right colon (Figure 1F). An intriguing finding of this study is that microsatellite instability (MSI) status in mCRC cancer patients is less than that in pCRC patients (Figure 1G). In addition, the gender is not a factor affecting the metastasis of colorectal cancer (Figure 1H). The statistical results of (Figures 1C-1H) are shown in (Table 2).

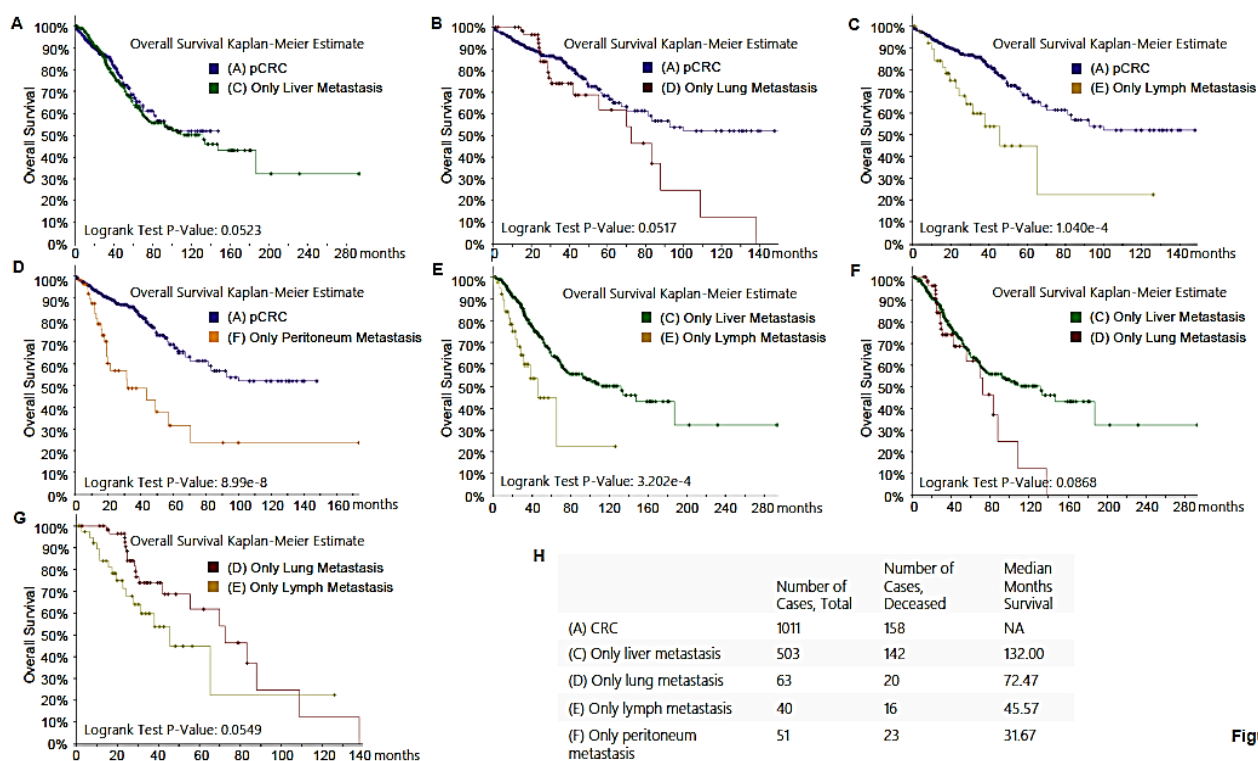
**Table 2:** The statistical results of (Figures 1C-1H and Figures 3A-3E).

	Statistical Test	p-Value
Figure 1C Tumor Sample Histology	Chi-squared Test	P<0.01
Figure 1D Mutation Count	Kruskal Wallis Test	P<0.01
Figure 1E Age at Diagnosis	Kruskal Wallis Test	P<0.01
Figure 1F Primary Tumor Location	Chi-squared Test	P<0.01
Figure 1G MSI Status	Chi-squared Test	P<0.01
Figure 1H Sex	Chi-squared Test	P=0.969
Figure 3A MSI Status	Chi-squared Test	P<0.01
Figure 3B Tumor Sample Histology	Chi-squared Test	P<0.01
Figure 3C Primary Tumor Location	Chi-squared Test	P<0.01
Figure 3D Sex	Chi-squared Test	P=0.413
Figure 3E Age at Diagnosis	Kruskal Wallis Test	P<0.01

## II Survival Rate Comparison Between mCRC Patients with Single-Site Tumor Metastasis and pCRC Patients, As Well as The Survival Rate Comparison Among mCRC Patients with Different Metastatic Foci

We next analysed survival discrepancies between mCRC patients with single-site tumor metastasis (liver, lung, lymph node, or peritoneum) and pCRC patients, thus focusing on the role of metastatic sites in mCRC. As shown in (Figures 2A & 2B), there is no statistical difference between the survival rates of mCRC patients with liver-only (mCRC-liver) or lung-only metastasis (mCRC-lung) and those of pCRC patients, suggesting that liver or lung metastasis does not reduce the survival rate of colon cancer patients. Strikingly, the survival rate of mCRC patients with lymph node metastasis is statistically different from that of pCRC patients (Figure 2C). It is worth noting that the lymph node metastasis here refers not to regional lymph node metastasis, but to non-regional

lymph node metastasis. These results demonstrate that non-regional lymph node (NRLN) metastasis can significantly reduce the survival rate and is an unfavourable factor for mCRC patients. The clinical significance of this finding lies in that the resection scope should include non-regional lymph nodes during surgical treatment, and that detection of resected non-regional lymph nodes will help predict the risk of patients' death, i.e., if the resected non-regional lymph nodes are positive, it indicates that the mortality rate of the patient will be increased compared with the patients with negative non-regional lymph nodes. In addition, we also demonstrated that peritoneal metastasis can significantly decrease the survival rate of mCRC patients and is another unfavourable factor (Figure 2D). This result suggests that we should pay more attention to the exclusion of peritoneal metastasis during preoperative examination and postoperative follow-up.



**Figure 2**

**Figure 2:** Comparison of survival rates between mCRC patients with single-site tumor metastasis and patients with pCRC, and comparison of survival rates among mCRC patients with different metastatic foci. **A)** mCRC patients with liver-only metastasis versus pCRC patients, **B)** mCRC patients with lung-only metastasis versus pCRC patients, **C)** mCRC patients with lymph node metastasis versus pCRC patients, **D)** mCRC patients with peritoneal metastasis versus pCRC patients, **E)** mCRC patients with non-regional lymph node only metastasis versus patients with liver only metastasis, **F)** mCRC patients with lung metastasis versus liver metastasis, **G)** non-regional lymph node metastasis versus lung metastasis, **H)** comparison and summary of median survival between each group.

Next, we examined the effect of different metastatic sites on survival rate of mCRC patients. The survival rate of mCRC patients with non-regional lymph node-only metastasis (mCRC-NRLN) (Figure 2E) or peritoneal-only metastasis (mCRC-peritoneum) (Figure S1) is lower than that of patients with mCRC-liver. Moreover, the survival rate of patients with mCRC-peritoneum is lower than that of patients with mCRC-lung (Figure S2). There was no statistical difference in survival rate between mCRC patients with lung metastasis and with liver metastasis (Figure 2F), between NRLN metastasis and lung metastasis (Figure 2G) or

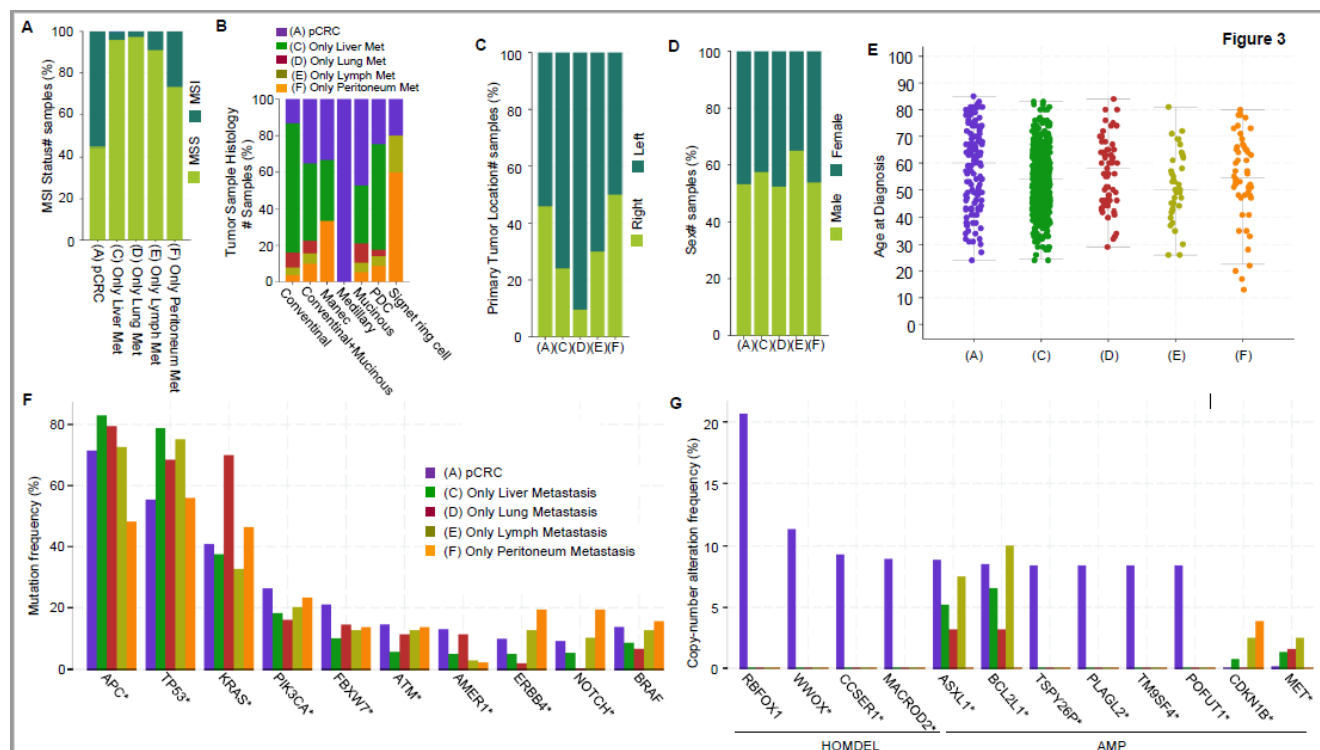
between NRLN metastasis and peritoneal metastasis (Figure S3). Taken together, one of the novel findings of this study is that liver or lung metastasis of mCRC patients does not affect the overall survival rate (Median Survival: 132 and 72.47 months, respectively), while peritoneal metastasis has the greatest impact on survival rate (Median Survival: 31.67 months) (Figure 2H).



### III Comparison of Clinical Characteristics and Mutation between mCRC Patients with Specific Single-Site Tumor Metastasis and pCRC

Next, we further analysed the clinical characteristics of specific metastatic sites and their relationship with gene mutations. As shown in (Figure 3A), MSI status analysis suggests that patients with colorectal cancer accompanied by metastases have fewer microsatellite instable foci than patients with pCRC, of which mCRC-lung patients have the least MSI, followed by patients with mCRC-liver, patients with mCRC-RNLN and mCRC-peritoneum. Tumor sample histology analysis indicates that liver metastasis of colorectal cancer is more likely to occur in conventional histological types of tumors, peritoneal metastasis is more likely to occur in signet ring cell carcinoma, and medullary

carcinoma hardly ever metastasizes (Figure 3B). Intriguingly, primary tumor location analysis shows that compared with cancers originating from the right colon, cancers originating from the left colon are most likely to metastasize to lungs, followed by liver and non-regional lymph nodes respectively. However, the metastatic rate of cancers originating from the right colon is relatively low (Figure 3C). Gender analysis indicates that there is no gender difference in tumor metastases of colorectal cancer patients (Figure 3D). Age at Diagnosis analysis showed that the average age of colon cancer patients is lowest, about 50-year-old, in those with lymph node metastasis is, followed by liver metastasis and peritoneal metastasis, and lastly lung metastasis, however the average age for all patients with metastasis was less than 60-year-old (Figure 3E). The statistical results of (Figures 3A-3E) shows in (Table 2).



**Figure 3:** Comparison of clinical features and mutation between mCRC patients with single-site tumor metastasis and pCRC patients. **A)** MSI status, **B)** tumor sample histology analysis, **C)** primary tumor location analysis, **D)** sex number samples, **E)** age at diagnosis, **F)** the mutation frequency analysis, **G)** copy number alteration frequency analysis.

These novel findings suggest that clinical oncologists should pay more attention to the tendency of lymph node metastasis in young CRC patients, increase examination methods in time, or pay closer attention to the dissection of non-regional lymph nodes during surgical treatment. In addition, the average age of mCRC patients with lung metastases is relatively older, which also suggests that we should pay more attention to the screening of lung metastases for older patients. In order to find additional predictive biomarkers or molecular signatures, we performed mutation frequency and copy-number analysis. We selected the genes with the top 10 mutation rates in single-site tumor metastases (liver, lung, lymph node, peritoneum) for analysis. As indicated in (Figure 3F), the *APC* mutation rate in liver and lung metastasis is higher than that in patients with pCRC. The mutation rate of *TP53* in almost all metastatic foci is higher than that in patients without tumor metastasis. *KRAS*

mutation rate increases significantly in mCRC patients with lung metastasis, and the proportion of *ERBB4*, *NOTCH3* and *BRAF* mutations increases in patients with peritoneal metastasis. The trend of gene copy-number is the same as that of (Figure 1).

As shown in (Figure 3G), the frequency of *CDKN1B* gene amplification in liver, lymph node and peritoneal metastases is higher than that in non-metastatic patients, and the frequency of *MET* gene amplification in liver, lung and lymph node metastases is higher than that in non-metastatic patients. The deletions of *RBFOX1*, *WWOX*, *CCSE1*, and *MACROD2* in all metastatic foci are significantly reduced. The amplification of *BCL2L1* in lymph node metastasis is higher than that in tumor samples of patients with pCRC without metastasis, but the number of patients (4) is too small to draw strong conclusions, only 4. Mutation

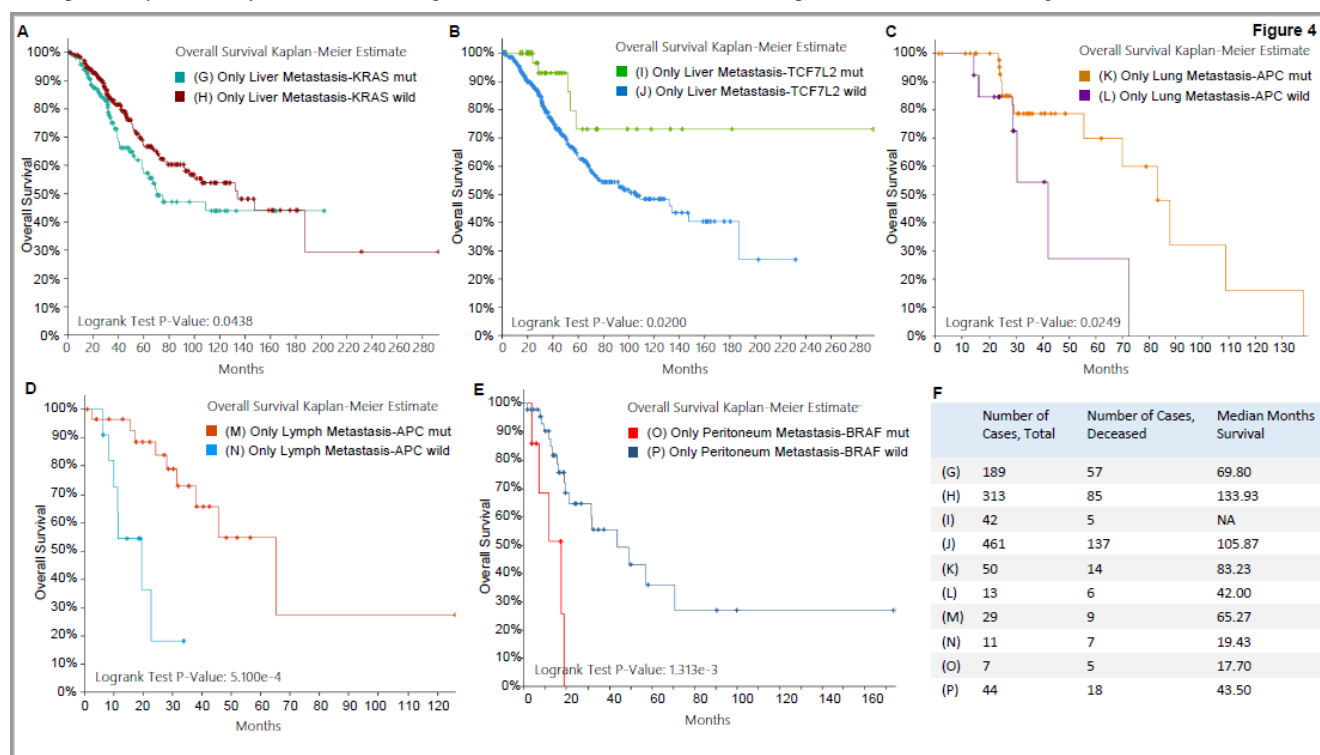
Count (log2) analysis reveals that the number of gene mutations in patients with mCRC is less than that in pCRC patients. However, for different types of metastases, there is no difference in the number of mutations between liver, lung, lymph node and peritoneal metastases (Figure S4). Previous studies have shown that for mCRC patients, the mutation genes in the primary foci and metastatic foci are exactly the same [28]. Based on our study and this previous evidence, there are likely more mutations in the primary foci of pCRC patients and compared to the primary foci of mCRC patients, suggesting that mutations in the primary foci could be used to predict and evaluate the possibility of CRC metastasis.

#### IV Gene Mutations That Significantly Affect the Survival Rate of CRC Patients with Different Single-Site Metastases

In order to further determine the role of gene mutation in CRC metastasis, we analysed the effect of gene mutation on survival rate of CRC patients with single-site tumor metastasis. All metastatic foci of CRC patients have roughly the same range of mutated genes. However, the same gene mutation in different single-site tumor metastases (liver, lung, lymph nodes, and peritoneum) has different effects on survival rate. Specifically, as shown in (Table 1), the *KRAS* and *TCF7L2* gene mutation have a significant impact on the survival rate of mCRC patients accompanied by liver only metastasis, *APC* gene mutation has a

significant effect on the survival rate of patients with mCRC-lung or mCRC-NRLN, and in mCRC patients accompanied by peritoneal only metastasis, *BRAF* gene mutation has a significant impact on their survival rates. More details are shown in (Table 1), \* means  $P < 0.05$  compared with wild type gene. The above results also suggest that different mutant genes have distinct effects on the survival rate of mCRC patients with different single-site tumor metastases, implying that gene mutations have relative specificity for the metastatic site of CRC.

In order to further understand the specific impact of these gene mutations on the survival rate of CRC patients with single-site metastasis, we compared survival rates between patients with gene mutations and those with wild-type genes. In patients with mCRC-liver, the survival rate of patients with the *KRAS* gene mutation is lower than that of patients with the wild-type *KRAS* gene, suggesting that *KRAS* mutation may be associated with reduced survival rate of patients with mCRC-liver and be used as an indicator for predicting survival rate of such patients (Figure 4A). Further analysis shows that *TCF7L2* gene mutation may be associated with increased survival rate of patients with mCRC-liver (Figure 4B), *APC* gene mutation is associated with increased survival rate of patients with mCRC-lung (Figure 4C) and mCRC-NRLN (Figure 4D), and *BRAF* gene mutation is related to reduced survival rate of patients with mCRC-peritoneum (Figure 4E). The median survival for all these patients is summarized in (Figure 4F).



**Figure 4:** Gene mutations that dramatically affect the survival rate of CRC patients with distinct single-site metastases. **A)** mCRC-liver patients with *KRAS* mutation versus those with *KRAS* WT, **B)** mCRC-liver patients with *TCF7L2* mutation versus *TCF7L2* WT, **C)** mCRC-lung patients with *APC* mutation versus *APC* WT, **D)** mCRC-NRLN patients with *APC* mutation versus *APC* WT, **E)** mCRC-peritoneum patients with *BRAF* mutation versus *BRAF* WT, **F)** Comparison of median months survival.

Our research has positive guiding significance for clinical management of patients with colorectal cancer. For mCRC patients with liver metastases, the prognosis can be predicted by mutation of *KRAS* and

*TCF7L2* genes. *KRAS* mutation may predict increased mortality of mCRC-liver patients, while *TCF7L2* mutation predicts reduced mortality. The mutation of *APC* gene can be used to predict the mortality

rate of patients with mCRC-lung or mCRC-NRLN, i.e., *APC* mutation indicates reduced mortality of these patients. In addition, for mCRC patients with peritoneal metastasis, the mortality can be predicted by mutation of *BRAF* gene. If these patients have *BRAF* mutations, we can predict the prognosis will be poor.

### V Cox Proportional Hazards Regression Model Analysis on Survival Rate of mCRC Patients with Liver-Only Metastasis

We further comprehensively analysed the factors affecting the survival rate of patients with mCRC-liver. Univariate COX Risk Model Analysis (Tables 3 & 4) suggests these factors include *KRAS*\_status, Fraction\_Genome\_Altered, Chemo\_Exposure\_of\_sequenced\_specimen

(Whether receive chemotherapy), Patient\_Tumor\_Grade, and Time\_from\_Met\_Dx\_to\_Sequencing. Specifically, *KRAS* gene mutation is an unfavourable factor for prognosis of mCRC-liver patients. Compared with *KRAS* wild type (WT) mCRC-liver patients, mCRC-liver patients with *KRAS* gene mutation have 1.308 times higher risk of death. With the increase of the fraction genome altered, the risk of death in mCRC-liver patients decreased. Compared with patients who do not receive chemotherapy, mCRC-liver patients receiving chemotherapy have a lower risk of death. The risk of death for mCRC-liver patients with intermediate differentiation and intermediate-low differentiation is lower than that of mCRC-liver patients with low differentiation. mCRC-liver patients receiving longer-term chemotherapy have a lower risk of death than receiving shorter-term chemotherapy.

**Table 3:** Baseline characteristics of mCRC patients with liver only metastasis.

Variable	Only liver metastasis(n=520)
<i>KRAS</i> _status (%)	
Mut	326(62.7%)
Wild	194(37.3%)
<i>TCF7L2</i> _status (%)	
Mut	476(91.5%)
Wild	44(8.5%)
Age_at_Diagnosis,median(range),years	53.94(24-83)
Sex, n(%)	
Male	299(57.5%)
Female	221(42.5%)
Fraction_Genome_Altered	0.21(0-0.78)
Chemo_Exposure_of_sequenced_specimen (%)	
No	204(39.2%)
Yes	316(60.8%)
Molecular_Subtype(%)	
MSS	507(97.5%)
MSI	11(2.1%)
Unknow	2(0.4%)
<i>MSI</i> _Score	1.59(0-47.7)
Mutation_Count	8.85(0-249)
Patient_Tumor_Grade, n(%)	
Poorly-Diff	34(6.5%)
Mod-Poorly-Diff	36(6.9%)
Mod_Diff	326(62.7%)
Unknow	124(23.8%)
Primary_Tumor_Site, n(%)	
Ascending Colon	40(7.7%)
Cecum	51(9.8%)
Descending Colon	35(6.7%)
Hepatic Flexure	13(2.5%)
Rectosigmoid	61(11.7%)
Rectum	111(21.3%)
Sigmoid Colon	162(31.2%)
Splenic Flexure	11(2.1%)
Transverse Colon	28(5.4%)
Unknow	8(1.5%)
Primary_Tumor_Location, n(%)	
Left	390(75%)
Right	124(23.8%)
Unknow	6(1.2%)



Sample_Type, n(%)	
Primary	209(40.2%)
Metastasis	311(59.8%)
Time_from_Met_Dx_to_Sequencing, median(range),month	32.39(-7.53-270.53)
Time_to_Metastasis, median (range),month	4.69(0-120.00)
Tumor_Sample_Histology(%)	
Conventional	326(62.7%)
Conventional_With_Mucinous_Component	30(5.8%)
Mucinous	6(1.2%)
PDC	33(6.3%)
Unknow	125(24%)
Overall_Survival_Months, median(range),month	47.26(0.57-292.93)
Overall_Survival_Status(%)	
Deceased	151(29%)
Living	369(71%)

**Table 4:** COX hazard analysis of overall survival for mCRC patients with liver only metastasis.

Parameter	Univariate analysis				Multivariate analysis			
	P	HR	95%CI		P	HR	95%CI	
			down	upper			down	upper
KRAS_status	0.014	1.308	1.056	1.620	0.863	0.973	0.716	1.323
TCF7L2_status	0.951	0.989	0.705	1.388	0.326	1.239	0.808	1.901
Age_at_Diagnosis	0.873	0.999	0.989	1.009	0.779	1.002	0.989	1.014
Sex	0.200	0.872	0.708	1.075	0.037	1.334	1.018	1.749
Fraction_Genome_Altered	0.002	0.348	0.179	0.676	0.343	0.594	0.203	1.743
Chemo_Exposure_of_sequenced_specimen	≤0.001	0.444	0.358	0.550	0.024	1.454	1.050	2.015
Molecular_Subtype	0.607	1.180	0.628	2.217	0.892	0.641	0.001	389.262
MSI_Score	0.728	0.996	0.977	1.017	0.944	1.006	0.851	1.189
Mutation_Count	0.183	1.003	0.998	1.008	0.875	1.004	0.950	1.062
Patient_Tumor_Grade	0.046	0.802	0.645	0.997	0.426	0.846	0.560	1.278
Primary_Tumor_Site	0.447	0.981	0.934	1.030	0.137	1.064	0.981	1.154
Primary_Tumor_Location	0.041	1.300	1.011	1.673	0.914	1.025	0.656	1.601
Sample_Type	≤0.001	0.446	0.357	0.555	0.548	0.903	0.647	1.260
Time_from_Met_Dx_to_Sequencing	≤0.001	0.822	0.806	0.838	≤0.001	0.811	0.792	0.831
Time_to_Metastasis	0.907	1.000	0.992	1.007	0.732	1.002	0.992	1.012
Tumor_Sample_Histology	0.179	1.111	0.953	1.294	0.891	1.020	0.768	1.355

Interestingly, multivariate cox risk model analysis yielded unexpected results. Compared with male mCRC-liver patients, female mCRC-liver patients have a higher risk of death (approximately 1.334 times). The death risk of mCRC-liver patients receiving chemotherapy is 1.454 times higher than that of mCRC-liver patients without chemotherapy. It is worth noting that this multivariate COX analysis conclusion is contrary to the previous univariate COX analysis conclusion, nonetheless the multivariate COX analysis conclusion is more important. Although chemotherapy itself is an unfavourable factor, the time of receiving chemotherapy is a favourable prognostic factor for mCRC-liver patients. mCRC-liver patients receiving longer-term chemotherapy have a lower risk of death.

#### VI Cox Proportional Hazards Regression Model Analysis on Survival Rate of mCRC Patients with Other Single-Site Metastases

Univariate COX risk model analysis indicates that factors affecting survival rate of colorectal cancer patients with mCRC-lung are Fraction Genome Altered and Time from metastasis diagnosis Met Dx to

Sequencing (Tables 5 & 6). Specifically, mCRC-lung patients with higher fraction genome altered or receiving longer-term chemotherapy have a lower risk of death. Multivariate COX risk model analysis demonstrates that factors affecting survival rate of mCRC-lung patients include age at diagnosis, primary tumor site and time from met dx to sequencing. Age at diagnosis is an unfavourable factor affecting the survival rate of patients with mCRC-lung. The older the patients at the time of diagnosis, the higher the risk of death. Intriguingly, compared with mCRC-lung patients whose primary tumor site is ascending colon, mCRC-lung patients whose primary tumor site is transverse colon or splenic flexure are less at risk of death. However, because there are so many groups of primary tumor sites that the number of patients in each group is very small, we think this result is not reliable and is a false positive result. Lastly, the time of receiving chemotherapy is a favourable factor for prognosis of mCRC-lung patients. mCRC-lung patients receiving longer-term chemotherapy have a lower risk of death.

**Table 5:** Baseline characteristics of mCRC patients with lung-only metastasis.

Variable	Only lung metastasis(n=63)
APC_status (%)	
Mut	50(79.4%)
Wild	13(20.6%)
Age_at_Diagnosis,median(range),years	56.69(29-84)
Sex, n(%)	
Male	33(52.4%)
Female	30(47.6%)
Fraction_Genome_Altered	0.1948(0-0.57)
Chemo_Exposure_of_sequenced_specimen (%)	
No	24(38.1%)
Yes	39(61.9%)
Molecular_Subtype(%)	
MSS	62(98.4%)
MSI	1(1.6%)
MSI_Score	1.59(0-47.7)
Mutation_Count	7.11(1-44)
Patient_Tumor_Grade, n(%)	
Poorly-Diff	2(3.2%)
Mod-Poorly-Diff	4(6.3%)
Mod_Diff	41(65.1%)
Unknow	16(25.4%)
Primary_Tumor_Site, n(%)	
Ascending Colon	1(1.6%)
Cecum	5(7.9%)
Descending Colon	3(4.8%)
Rectosigmoid	10(15.9%)
Rectum	34(54.0%)
Sigmoid Colon	9(14.3%)
Transverse Colon	1(1.6%)
Primary_Tumor_Location, n(%)	
Left	57(90.5%)
Right	6(9.5%)
Sample_Type, n(%)	
Primary	26(41.3%)
Metastasis	37(58.7%)
Time_from_Met_Dx_to_Sequencing, median(range),month	21.25(0.90-130.67)
Time_to_Metastasis, median (range),month	18.61(0-68.20)
Tumor_Sample_Histology(%)	
Conventional	38(60.3%)
Conventional_With_Mucinous_Component	5(7.9%)
Mucinous	2(3.2%)
PDC	2(3.2%)
Unknow	16(25.4%)
Overall_Survival_Months, median(range),month	36.16(1.3-138.03)
Overall_Survival_Status (%)	
Deceased	20(31.7%)
Living	43(68.3%)

**Table 6:** COX hazard analysis of overall survival for mCRC patients with lung only metastasis.

Parameter	Univariate analysis				Multivariate analysis			
	P	HR	95%CI		P	HR	95%CI	
			down	upper			down	upper
APC_status	0.885	0.941	0.415	2.134	0.065	0.255	0.059	1.090
Age_at_Diagnosis	0.420	1.010	0.985	1.036	0.011	1.055	1.012	1.099
Sex	0.398	0.772	0.423	1.408	0.669	1.283	0.409	4.019
Fraction_Genome_Altered	0.027	0.103	0.014	0.769	0.583	3.272	0.047	226.317
Chemo_Exposure_of_sequenced_specimen	0.394	0.767	0.416	1.413	0.154	2.305	0.732	7.258
Molecular_Subtype	0.945	1.072	0.146	7.886	0.861	0.080	0.000	1.598e11
MSI_Score	0.608	0.975	0.884	1.075	0.718	1.186	0.471	2.984
Mutation_Count	0.897	0.996	0.945	1.051	0.598	0.935	0.729	1.199
Patient_Tumor_Grade	0.613	1.388	0.389	4.952	0.720	0.762	0.173	3.358
Primary_Tumor_Site	0.262	0.898	0.745	1.083	0.021	0.412	0.194	0.875
Primary_Tumor_Location	0.756	1.179	0.417	3.327	0.071	0.045	0.002	1.305
Sample_Type	0.173	0.648	0.347	1.210	0.117	2.391	0.805	7.107
Time_from_Met_Dx_to_Sequencing	≤0.001	0.866	0.821	0.914	≤0.001	0.829	0.767	0.896
Time_to_Metastasis	0.966	1.000	0.982	1.020	0.698	0.991	0.944	1.039
Tumor_Sample_Histology	0.641	1.149	0.642	2.056	0.900	0.948	0.414	2.174

**Table 7:** Baseline characteristics of mCRC patients with lymph-only metastasis.

Variable	Only lymph metastasis (n=40)
APC_status (%)	
Mut	29(72.5%)
Wild	11(27.5%)
Age_at_Diagnosis,median(range),years	51.73(26-81)
Sex, n(%)	
Male	26(65%)
Female	14(35%)
Fraction_Genome_Altered	0.198(0-0.96)
Chemo_Exposure_of_sequenced_specimen (%)	
No	27(67.5%)
Yes	13(32.5%)
Molecular_Subtype(%)	
MSS	35(87.5%)
MSI	4(10%)
Unknow	1(2.5%)
MSI_Score	3.70(0-37.38)
Mutation_Count	16.08(1-159)
Patient_Tumor_Grade, n(%)	
Poorly-Diff	4(10%)
Mod-Poorly-Diff	6(15%)
Mod_Diff	18(45%)
Unknow	12(30%)
Primary_Tumor_Site, n(%)	
Ascending Colon	3(7.5%)
Cecum	8(20.0%)
Descending Colon	2(5%)
Rectosigmoid	4(10%)
Rectum	12(30%)
Sigmoid Colon	9(22.5%)
Primary_Tumor_Location, n(%)	
Left	28(70%)

Right	12(30%)
Sample_Type, n(%)	
Primary	32(80%)
Metastasis	8(20%)
Time_from_Met_Dx_to_Sequencing, median(range),month	15.5(-19.63-100.8)
Time_to_Metastasis, median (range),month	14.90(0-125.8)
Tumor_Sample_Histology(%)	
Conventional	19(47.5%)
Conventional_With_Mucinous_Component	4(10%)
Mucinous	1(2.5%)
PDC	3(7.5%)
Signet_Ring_Cell	1(2.5%)
Unknow	12(30%)
Overall_Survival_Months, median(range),month	28.02(0.93-126)
Overall_Survival_Status(%)	
Deceased	16(40%)
Living	24(60%)

Because isolated non-regional lymph node metastasis (NRLN) in CRC patients is uncommon, little is known about the factors that affect the survival rate of such patients. Here, our univariate COX risk model analysis shows that factors affecting survival rate of patients with mCRC-NRLN are chemo exposure of sequenced specimen and time from Met Dx to sequencing (Tables 7 & 8). Specifically, chemotherapy

and the time of receiving chemotherapy are favourable factors for prognosis of mCRC-NRLN patients. Interestingly, multivariate COX risk model analysis indicates that gene mutation count and the time of receiving chemotherapy are favourable factors for prognosis of mCRC-NRLN patients (Tables 7 & 8). In other words, mCRC-NRLN patients with higher gene mutation count have lower risk of death.

**Table 8:** COX hazard analysis of overall survival for mCRC patients with lymph-only metastasis.

Parameter	Univariate analysis				Multivariate analysis			
	P	HR	95%CI		P	HR	95%CI	
			down	upper			down	upper
APC_status	0.205	0.462	0.14	1.523	0.79	0.665	0.033	13.407
Age_at_Diagnosis	0.67	1.01	0.966	1.055	0.331	0.943	0.838	1.062
Sex	0.388	1.488	0.604	3.665	0.7	1.715	0.11	26.722
Fraction_Genome_Altered	0.105	0.171	0.020	1.446	0.982	0.863	0	2.68*10 <sup>5</sup>
Chemo_Exposure_of_sequenced_specimen	0.023	0.297	0.104	0.847	0.588	0.472	0.031	7.113
Molecular_Subtype	0.161	2.47	0.697	8.75	0.969	2.7e22	0	--
MSI_Score	0.173	1.037	0.984	1.094	0.737	0.686	0.076	6.173
Mutation_Count	0.592	0.997	0.986	1.008	0.038	0.565	0.329	0.969
Patient_Tumor_Grade	0.367	0.703	0.326	1.513	0.956	1.055	0.156	7.14
Primary_Tumor_Site	0.982	0.998	0.823	1.210	0.973	1.02	0.332	3.134
Primary_Tumor_Location	0.886	1.068	0.435	2.618	0.546	5.469	0.022	1356.578
Sample_Type	0.091	0.382	0.125	1.165	0.854	0	0	1.16E+36
Time_from_Met_Dx_to_Sequencing	≤0.001	0.867	0.814	0.925	0.002	0.757	0.637	0.901
Time_to_Metastasis	0.263	0.991	0.976	1.007	0.689	1.01	0.964	1.058
Tumor_Sample_Histology	0.501	1.180	0.729	1.911	0.963	1.037	0.221	4.854

Lastly, we performed COX analysis to determine the factors affecting the survival of patients with mCRC-peritoneum. Univariate COX risk model analysis shows that there are no factors affecting the survival rate of mCRC-peritoneum patients. However, multivariate COX risk model analysis shows that Age at Diagnosis, Chemo Exposure of sequenced

specimen, Molecular Subtype, Mutation Count, Primary Tumor Site and Sample Type all affect the survival rate of mCRC-peritoneum patients (Tables 9 & 10). Due to the small sample of mCRC-peritoneum patients, we consider these results unreliable.

**Table 9:** Baseline characteristics of colorectal cancer patients with peritoneum-only metastasis.

Variable	Only peritoneum metastasis(n=52)
BRAF_status (%)	
Mut	8(15.4%)
Wild	44(84.6%)
Age_at_Diagnosis,median(range),years	54.52(13-80)
Sex, n(%)	
Male	28(53.8%)
Female	24(46.2%)
Fraction_Genome_Altered	0.133(0-0.63)
Chemo_Exposure_of_sequenced_specimen (%)	
No	32(61.5%)
Yes	20(38.5%)
Molecular_Subtype(%)	
MSS	41(78.8%)
MSI	11(21.2%)
MSI_Score	6.39(0-42.22)
Mutation_Count	15.75(2-90)
Patient_Tumor_Grade, n(%)	
Poorly-Diff	9(17.3%)
Mod-Poorly-Diff	7(13.5%)
Mod_Diff	18(34.6%)
Unknow	18(34.6%)
Primary_Tumor_Site, n(%)	
Ascending Colon	6(11.5%)
Cecum	13(25%)
Descending Colon	4(7.7%)
Rectosigmoid	1(1.9%)
Rectum	5(9.6%)
Sigmoid Colon	13(25%)
Hepatic Flexure	4(7.7%)
Splenic Flexure	2(3.8%)
Transverse Colon	3(5.8%)
Primary_Tumor_Location, n(%)	
Left	26(50%)
Right	26(50%)
Sample_Type, n(%)	
Primary	30(57.7%)
Metastasis	22(42.3%)
Time_from_Met_Dx_to_Sequencing, median(range),month	-10.59(-1365.80-159.37)
Time_to_Metastasis, median (range),month	4.49(0-62.9)
Tumor_Sample_Histology(%)	
Conventional	17(32.7%)
Conventional_With_Mucinous_Component	7(13.5%)
Mucinous	1(1.9%)
PDC	5(9.6%)
Signet_Ring_Cell	3(5.8%)
Overall_Survival_Months, median(range),month	26.8(1.87-173.57)
Overall_Survival_Status(%)	
Deceased	24(46.2%)
Living	28(53.8%)



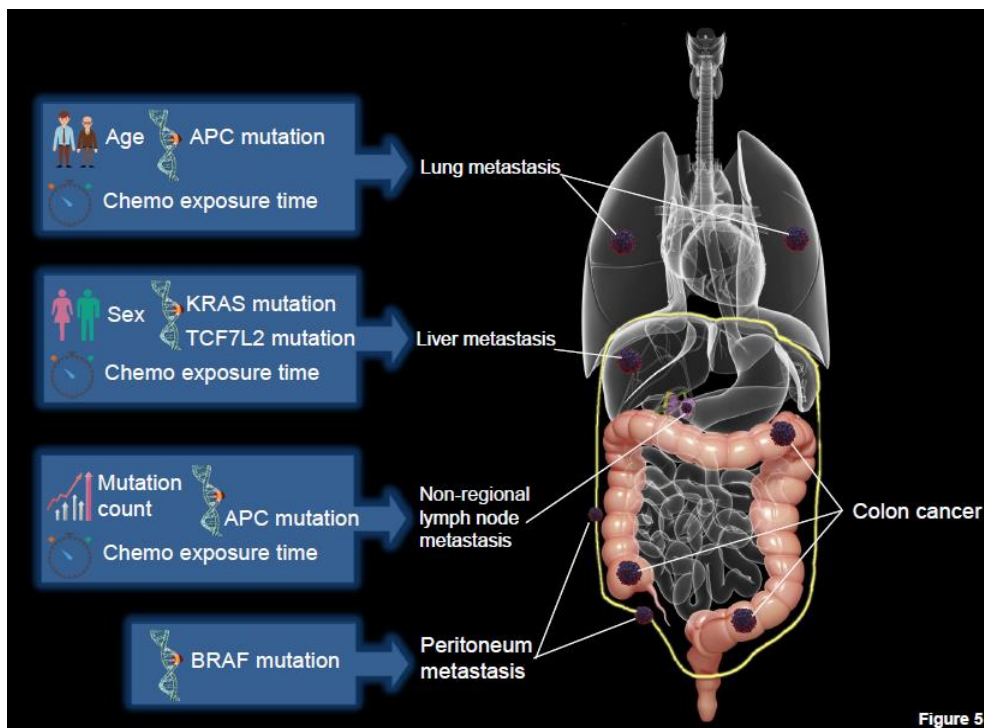
**Table 10:** COX hazard analysis of overall survival for mCRC patients with peritoneum-only metastasis.

Parameter	Univariate analysis				Multivariate analysis			
	P	HR	95% CI		P	HR	95% CI	
			down	upper			down	upper
BRAF_status	0.909	0.918	0.211	3.998	0.616	1.94	0.146	25.854
Age_at_Diagnosis	0.414	0.989	0.963	1.016	0.001	0.884	0.821	0.951
Sex	0.486	0.754	0.340	1.670	0.427	1.78	0.429	7.384
Fraction_Genome_Altered	0.845	1.281	.108	15.265	0.365	7.989	0.089	713.333
Chemo_Exposure_of_sequenced_specimen	0.225	0.597	0.259	1.374	0.015	22.192	1.815	271.313
Molecular_Subtype	0.615	0.784	0.304	2.023	0.011	0	0	0.003
MSI_Score	0.490	0.989	0.959	1.020	0.686	0.945	0.717	1.245
Mutation_Count	0.321	0.992	0.975	1.008	0.004	1.668	1.177	2.364
Patient_Tumor_Grade	0.192	0.699	0.408	1.197	0.305	2.408	0.45	12.885
Primary_Tumor_Site	0.338	0.931	0.803	1.078	0.008	0.42	0.222	0.795
Primary_Tumor_Location	0.557	1.257	.586	2.694	0.089	0.09	0.006	1.441
Sample_Type	0.131	0.538	0.241	1.202	0.001	0.009	0	0.151
Time_from_Met_Dx_to_Sequencing	0.081	0.999	0.998	1.000	0.239	0.997	0.993	1.002
Time_to_Metastasis	0.593	1.008	0.978	1.039	0.915	0.994	0.896	1.103
Tumor_Sample_Histology	0.188	1.227	0.905	1.663	0.303	1.721	0.613	4.832

## Discussion

Recent advances in understanding metastasis at molecular and gene levels have aroused increasing interest in the epidemiology of metastatic colorectal cancer (mCRC). In the present study, we assessed the relationship between metastatic patterns and survival outcomes of CRC patients, and sought to identify some prognostic predictors using the whole sequencing and clinical data of 2368 samples (2329 CRC patients)

obtained from (TCGA and MSKCC databases). Such a long follow-up period makes it a brilliant resource for studying short-term and long-term prognosis characteristics. Our results showed an important prognostic value of the distant metastasis sites at diagnosis and effect of specific gene mutation on mortality of mCRC patients with single site metastasis (Figure 5). Additionally, we have determined the independent prognostic factors of patients with liver-only metastasis and unanimously found that a longer period of chemotherapy is associated with survival of patients.

**Figure 5:** Schematic diagram summarizing factors closely related to the overall survival of CRC patients with different single site metastasis.

## I Prognostic Value of Tumor Metastasis Sites on Survival Rate

Several studies have compared the survival rates of CRC patients with different metastatic sites, however, most research is based on single institutional experience with a fairly limited sample size or concentrated in a particular metastasis site [15, 18, 29, 30]. The analysis reported here makes use of a large number of patient groups, and thoroughly examines the influence of metastatic sites on survival outcomes by adjusting demographic and clinical variables that may affect survival rates. We report the following novel findings. i) Compared with non-metastatic CRC patients, the mortality rate of CRC patients with liver-only metastasis (mCRC-liver) did not increase. ii) The mortality rate of CRC patients with non-regional lymph node-only metastasis (mCRC-NRLN) was significantly higher than that of patients without metastasis. This finding suggests that during surgical treatment, the scope of resection should include non-regional lymph nodes, and their pathological examination results can be used to predict the risk of death. iii) The mortality rate of CRC patients with peritoneum-only metastasis (mCRC-peritoneum) was significantly higher than that of patients without metastasis, suggesting that we should pay more attention to the exclusion of peritoneal metastasis during preoperative examination and postoperative follow-up. iv) It has been reported in the literatures in small patient populations that even after multivariate adjustment, patients with lung-only metastasis (mCRC-lung) have a survival advantage over mCRC-liver [20, 31-33]. Inconsistent with this conclusion, we found that there is no significant difference in survival rate between patients with mCRC-lung and those with mCRC-liver. However, from (Figure 2), we can see that the median survival of patients with mCRC-liver is 132 months, while the median survival of patients with mCRC-lung is 72.47 months, with obvious difference in value. More research is needed in a larger patient population to elucidate this issue. v) The survival rate of mCRC-NRLN patients is not statistically different from that of mCRC-lung patients, but it is lower than that of mCRC-liver patients. This difference in statistical significance may again be related to differences in group sizes. The next step is to further increase the sample size of patients with mCRC-lung for further analysis. In addition, we did not include CRC patients with two or more metastatic sites at the same time in this study, due to the insufficient number of cases.

## II Prognostic Significance of Genetic Mutations in CRC patients

It is now generally believed that sporadic CRC is usually caused by precancerous lesions via activation of oncogenes (*KRAS* and *BRAF*), inactivation of tumor suppressor genes (such as *APC*, *p16*, *p53* and *DCC*) and mismatch repair genes (*MLH1* and *MSH2*), as well as *PMS2* and *hMSH6* to a lesser extent [34]. In addition, previous studies have clearly indicated that *APC*, *TP53*, *KRAS* and other gene mutations play a role in the occurrence, development and metastasis of CRC [35-40]. The genetic alterations might influence the survival of the patients with CRC. Next, we sought to determine the correlation between mutation of specific genes and mortality of CRC patients to find more accurate indicators for predicting CRC mortality, so as to provide guidance for clinical practice. We demonstrated that for CRC patients who already have liver metastasis, mortality can be predicted by mutations of *KRAS* and *TCF7L2* genes. *KRAS* mutation may indicate increased while *TCF7L2* mutation indicates reduced mortality of patients with mCRC-

liver. For patients with mCRC-lung and mCRC-NRLN, chance of mortality can be predicted by mutation of *APC* gene. *APC* mutation reduces likelihood of mortality in these patients. Furthermore, the mutation of *BRAF* gene can be used to predict the chance of mortality of mCRC-peritoneum patients. *BRAF* mutation increases the likelihood of mortality in these patients.

In the COX univariate analysis (Tables 4, 6, 8 & 10), we only observed that *KRAS* gene mutation is associated with the survival rate of mCRC-liver patients. No gene mutation other than *KRAS* has been observed to be correlated with the survival rate of mCRC patients with single-site metastasis. Notably, the effect of *KRAS* gene mutation on survival rate of mCRC-liver patients was not significant in COX multivariate analysis. Surprisingly, we found in COX multivariate analysis that male and female sex does affect the survival rate of mCRC-liver patients. Why is this happening? The reason may be that males and females have different gene mutations, so the correlation between *KRAS* gene mutation and survival rate is no longer important compared with the influence of gender on survival rate. The specific mechanism needs further study.

## III The Influence of Other Factors on Survival Rate

As mentioned above, through multivariate COX risk model analysis, we found for the first time that gender could affect the survival rate of mCRC-liver patients. Female gender is an unfavourable factor for prognosis of mCRC-liver patients. Compared with male patients with mCRC-liver, female patients with mCRC-liver have a higher risk of death, which is 1.334 times that of male mCRC-liver patients. Moreover, we demonstrated that age would affect the survival rate of patients with mCRC-lung and that the number of tumor gene mutations affect the survival rate of mCRC-NRLN patients. Lastly, our multivariate COX risk model analysis indicates that chemo exposure of specimens can also affect the survival rate of mCRC-liver patients. Receiving chemotherapy is an unfavourable factor for prognosis of mCRC-liver patients. Compared with patients who do not receive chemotherapy, mCRC-liver patients who receive chemotherapy have a higher risk of death (1.454 times,  $p < 0.05$ ). Intriguingly, although receiving chemotherapy is an unfavourable factor for the prognosis of mCRC-liver patients, the length of chemotherapy treatment is an advantageous prognosis factor (Table 4). These results may seem contradictory, but they are not. There is more or less damage to liver function in mCRC-liver patients and the side effects of the chemotherapy drugs with conventional dosages are relatively large for them. Side effects of chemotherapy drugs may be one of the risk factors leading to death of these patients. Therefore, we suggest that for patients with mCRC-liver, it is necessary to reduce the side effects of chemotherapy drugs, reduce the use of chemotherapy drugs with greater liver toxicity, and appropriately extend the chemotherapy time for these patients, so as to obtain a better survival expectation.

In addition, we also found that some pathological factors are related to the metastatic tendency of CRC patients. For example, tumors with conventional histological type are more likely to metastasize, while medullary carcinoma hardly metastasizes (Figure 1C), and cancers originating from left colon are more prone to metastasis than cancers originating from right colon (Figure 1F). Therefore, in addition to well-

established clinico-pathological factors, histological classification and localization of primary tumor can be used to predict metastasis, disease recurrence, and clinical outcome of CRC patients.

## Conclusion

In summary, this study depicts the long-term survival features of a group of CRC patients and the comprehensive relationship between baseline variables and long-term patient outcomes. Overall, our findings expand the scope of information about patient outcomes and long-term risk markers, offer a new perspective that may be helpful for future research, and have positive guiding significance for clinical management of CRC patients. A staging system that comprehensively considers factors such as metastatic sites, gender of patients, selection of chemotherapy drugs, length of chemotherapy treatment, specific gene mutation, etc., can better stratify the treatment risks of CRC patients and more accurately predict their survival.

## Availability of Data and Materials

The datasets used for the current study are available from the corresponding author on reasonable request.

## Conflicts of Interest

None.

## Author Contributions

WD and YW designed the work. WD, KS, MS and YW analysed and interpreted the patient data. YW, KS and JV were major contributors in writing the manuscript. All authors read and approved the final manuscript.

## Acknowledgment

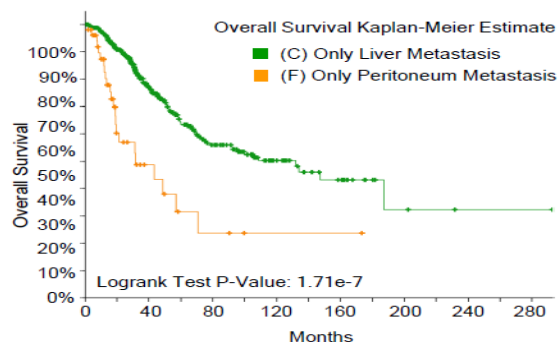
This work was supported in part by NIH-NIMHD U54MD007598 and U54CA143931, NIH/NCI U54CA14393, U56 CA101599-01; Department-of-Defense Breast Cancer Research Programme grant BC043180, NIH/NCATS CTSI UL1TR000124 to J.V. Vadgama; Accelerating Excellence in Translational Science Pilot Grants G0812D05, NIH/NCI SC1CA200517 and 9 SC1 GM135050-05 to Y. Wu; This research was partially supported by NIH National Center for Advancing Translational Science (NCATS) UCLA CTSI Grant Number UL1TR001881, NIH-NIMHD U54MD007598, and NIMHD S21 MD000103. NIH-NIMHD, NIH/NCATS and NIH/NCI have had no involvement in the study design, collection, analysis and interpretation of data, the writing of the report, or the decision to submit the article for publication.

## Consent for Publication

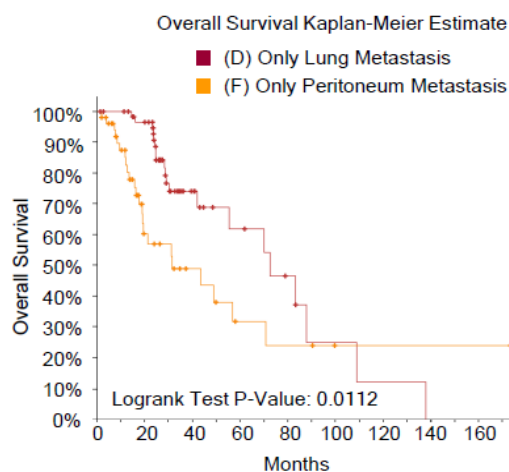
The content of this manuscript has not been previously published and is not under consideration for publication elsewhere.

## Abbreviations

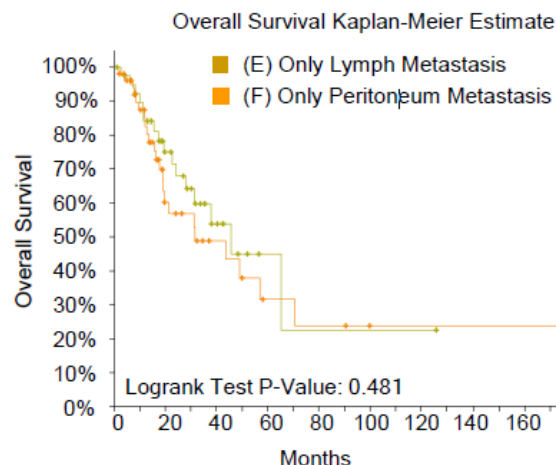
**APC:** Adenomatous Polyposis Coli  
**ASXL1:** Additional Sex Combs Like 1  
**BCL2L1:** Bcl-2-Like Protein 1  
**BRAF:** B-Raf Proto-Oncogene  
**CCSER1:** Coiled-Coil Serine Rich Protein 1  
**CDKN1B:** Cyclin Dependent Kinase Inhibitor 1B  
**CRC:** Colorectal Cancer  
**DCC:** Deleted In Colorectal Carcinoma  
**ERBB4:** Erb-B2 Receptor Tyrosine Kinase 4  
**FAT4:** FAT Atypical Cadherin 4  
**FBXW7:** F-Box/WD Repeat-Containing Protein 7  
**FLG:** Filaggrin  
**HR:** Hazard's Ratio  
**KRAS:** Kirsten Ras Oncogene  
**MACROD2:** Mono-ADP Ribosylhydrolase 2  
**mCRC:** Metastatic Colorectal Cancer  
**mCRC-liver:** CRC with Liver-Only Metastasis  
**mCRC-lung:** CRC with Lung-Only Metastasis  
**mCRC-NRLN:** CRC with Non-Regional Lymph Node-Only Metastasis  
**mCRC-peritoneum:** CRC with Peritoneum-Only Metastasis  
**MET:** MET Proto-Oncogene, Receptor Tyrosine Kinase  
**MLH1:** DNA Mismatch Repair Protein1  
**MLH2:** DNA Mismatch Repair Protein2  
**MMR:** Mismatch Repair  
**MSH6:** MutS Protein Homolog 6  
**MSI:** Microsatellite Instability  
**MUC16:** Mucin 16  
**NOTCH3:** Notch Receptor 3  
**NRLN:** Non-Regional Lymph Node  
**pCRC:** Primary Colorectal Cancer  
**PIK3CA:** Phosphoinositide 3 Kinase, Catalytic, Alpha Polypeptide  
**PLAGL2:** Pleiomorphic Adenoma Gene-Like 2  
**PMS2:** MSI Homolog 2, Mismatch Repair System Component  
**RBFOX1:** RNA Binding Fox-1  
**SYNE1:** Synaptic Nuclei Expressed Gene 1  
**TCF7L2:** Transcription Factor 7 Like 2  
**TCGA:** The Cancer Genome Atlas  
**TP53:** Tumor Protein P53  
**TSPY26P:** Testis Specific Protein Y-Linked 26, Pseudogene  
**TTN:** Titin  
**WWOX:** WW Domain Containing Oxidoreductase



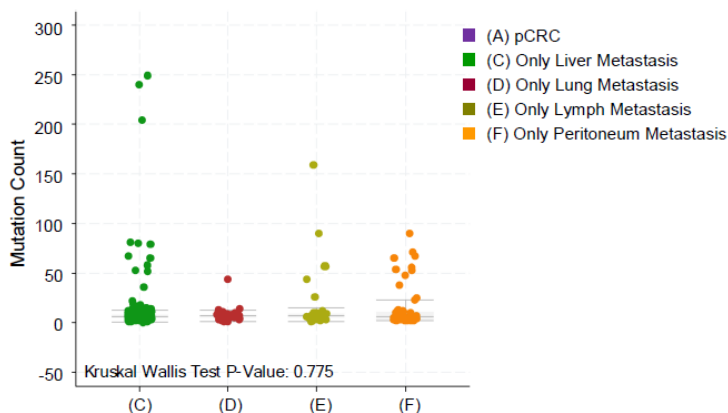
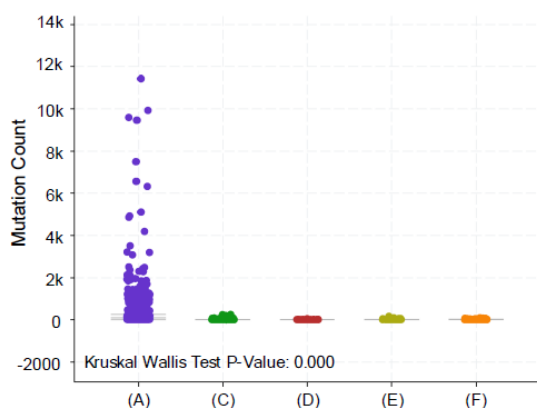
**Figure S1:** The survival rate of mCRC patients with only peritoneal metastasis is lower than that of patients with only liver metastasis.



**Figure S2:** The survival rate of mCRC patients with peritoneal only metastasis is lower than that of patients with lung only metastasis.



**Figure S3:** There was no statistical difference in survival rate between non-regional lymph node metastasis and peritoneal metastasis.



**Figure S4:** Mutation Count (log2) indicates that the number of gene mutations in patients with mCRC is less than that in patients with primary CRC.

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