Original Article

A study of gene variation in All-*RAS* wild-type metastatic colorectal cancer and its correlation with cetuximab

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Background: This study sought to explore the biological significance of genetic variation in *RAS* wild-type metastatic colorectal cancer (mCRC) in the real world, the difference in the efficacy of cetuximab in the treatment of mCRC with different genetic variants and identify clinical features and new predictors of efficacy.

Methods: A retrospective analysis of the data of 60 patients with stage IV mCRC who received cetuximab at The First and Second Affiliated Hospital of Soochow University from 2016 to 2020 was conducted. The patients were divided into the following 3 groups according to the genetic test results: (I) group A (the all-*RAS* wild-type group); (II) group B (the all-*RAS* wild-type group with the tumor suppressor gene mutation); and (III) group C (the all-*RAS* wild-type group with the oncogenic driver gene mutation). A subgroup analysis was conducted to examine left CRC and local intervention, and the progression-free survival (PFS) and overall survival (OS) of the patients were observed.

Results: The all-*RAS* wild-type mCRC patients were divided into group A (n=10), group B (including the *TP53*, *APC*, *PTEN*, *BRCA2*, and *SMAD4* variants) (n=42), and group C (including the *ERBB2*, *BRAF*, *PIK3CA*, and *RET* variants) (n=8). The median PFS of groups A, B, and C were 15.0, 12.0, and 3.0 months, respectively (P=0.007). Fitting sex as a stratified variable to the Cox survival analysis model showed that only the PFS of groups B and C differed significantly (P=0.011). In the left-sided mCRC patients, the median PFS of groups A, B, C were 3.0, 13.0, and 3.0 months, respectively (P=0.009). Among the patients in group B, the median PFS of the metastatic local intervention subgroup was 14.0 months, and the non-local intervention subgroup was 12.0 months (P=0.55). Only the type of combined gene mutation was an independent factor affecting PFS.

Conclusions: The PFS and OS of mCRC patients with all-*RAS* wild-type and no combined mutations treated with cetuximab were not better than those of patients with combined mutations. Compared to mCRC patients with all-*RAS* wild-type and oncogenic driver gene mutations, cetuximab significantly prolonged the PFS of all-*RAS* wild-type patients with the tumor suppressor gene mutations.

Keywords: Metastatic colorectal cancer (mCRC); second-generation sequencing; cetuximab; gene alteration; prognosis

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Introduction

Anti-epidermal growth factor receptor (*EGFR*) monoclonal antibodies (mAbs) (i.e., cetuximab and panitumumab) have been approved for use in combination with cytotoxic chemotherapy (1) for the first-line treatment of metastatic colorectal cancer (mCRC), and as a monotherapy or combination therapy for later-line treatments (2). Cetuximab is a chimeric mouse-human immunoglobulin G mAb that can bind to the extracellular domain of EGFR and induces the downregulation of proto-oncogene signaling. Cetuximab also can blinding to natural killer cells may trigger an immune-mediated antitumor response, leading to antibody-dependent cell-mediated cytotoxicity (3). It is well known that the *RAS* gene (*KRAS/NRAS*) in mCRC is a standard biomarker for predicting first-line anti-*EGFR* therapy. Even in patients with all-*RAS* wild-type mCRC, the efficacy of cetuximab differs, and it is unclear whether combined variants other than the *RAS* gene affects the efficacy.

Some retrospective studies have been conducted on the survival benefits of cetuximab in mCRC patients with different genetic variants; however, most studies have examined a single-gene variant (4). In the real world, there are many kinds of gene variants in mCRC patients, and multiple genetic variants often exist simultaneously. Different from the single gene variants in previous studies, this study innovatively studied the influence of co-mutated genes on the efficacy of cetuximab by grouping tumor suppressor genes and oncogenic driver genes. To extend understandings of the efficacy and influencing factors of cetuximab in treating all-*RAS* wild-type mCRC patients with different gene variant types, this study sought to retrospectively analyze the gene variants and clinical characteristics of all-*RAS* wild-type patients with mCRC, and the different prognosis of cetuximab in all-*RAS* wild-type patients, all-*RAS* wild-type patients with tumor suppressor gene mutations, and all-*RAS* wild-type patients with oncogenic driver gene mutations. A stratified study was also conducted to examine left-sided CRC and local interventions. Additionally, we searched for prognostic-related gene variant signatures to predict the efficacy of cetuximab in treating mCRC. We present the following article in accordance with the REMARK reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1237/rc).

Highlight box

Key findings

- The PFS and OS of mCRC patients with all-RAS wild-type and no combined mutations treated with cetuximab were not better than those of patients with combined mutations.

What is known and what is new?

- It is well known that the RAS gene in mCRC is a standard biomarker for predicting first-line anti-EGFR therapy.
- We innovatively studied the influence of co-mutated genes on the efficacy of cetuximab by grouping tumor suppressor genes and oncogenic driver genes.

What is the implication, and what should change now?

- Alternative treatment strategies should be considered for mCRC patients with multiple oncogenic driver gene variants, even those genetically tested and determined to have the all-RAS wild-type, and all patients should undergo tumor-tissue based NGS testing at the baseline to determine if they would benefit from cetuximab monotherapy or combination therapy.

Methods

Study design and participants

The data of patients with mCRC treated with cetuximab at the Oncology Department of The First Affiliated Hospital of Soochow University and The Second Affiliated Hospital of Soochow University from August 2016 to December 2020 were collected. Patients were considered eligible for the trial if they met the following inclusion criteria: (I) had histologically confirmed stage IV colorectal adenocarcinoma (according to the 8th UICC/AJCC TNM Staging System); (II) had the all-*RAS* gene wild-type as detected by next-generation sequencing (NGS) technology; (III) had undergone 4 cycles of cetuximab and at least 1 radiographic evaluation; and (IV) had a World Health Organization performance status of 0–2 before the start of the trial. At the baseline, patients had to have at least 1 lesion (with a diameter of more than 10 mm in the non-lymph-node lesions, or a short axis >15 mm in the lymph-node lesions) that had not been previously irradiated, that could be measured by computed tomography (CT) or magnetic resonance imaging (MRI), and that was suitable for repeated measurement. Patients were excluded from the study if they met any of the following main exclusion criteria: (I) had 2 or more primary tumors; (II) had a pathological type of squamous cell carcinoma, adenosquamous carcinoma, or another pathological type other than adenocarcinoma; and/or (III) had incomplete case information.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics committee of The First Affiliated Hospital of Soochow University (No. 2022-482) and ethics committee of The Second Affiliated Hospital of Soochow University (No. LK-2020-071-02). Informed consent was taken from all individual participants.

Procedures

The eligible patients received 400 mg/m^2 of cetuximab (Erbitux, Merck KGaA, Germany) for the first week, followed by 250 mg/m^2 of cetuximab weekly or 500 mg/m^2 d1 of cetuximab fortnightly, by intravenous infusion of targeted therapy. Patients received the cetuximab in combination with the following treatment modalities: systemic cytotoxic chemotherapy (mFolfox6, or FOLFIRI), and local treatment (stereotactic body radiation therapy, stereotactic radio surgery, intensity modulated radio therapy, surgical excision, thermal ablation therapy, or transcatheter arterial chemoembolization). To determine the treatment effects, the enrolled patients were evaluated every 2–3 months during the follow-up period using CT, MRI, and positron emission tomography-CT as assessment methods and the standard response evaluation criteria in solid tumors (RECIST, version 1.1). Follow-up included obtaining survival information by telephone or at an outpatient clinic. The study had a cut-off date of January 31, 2021.

All the patients' tumor tissue deoxyribonucleic acid (DNA) was sequenced using the NGS method at a depth of 1,000 for tissue and 6,000 for circulating-tumor DNA. With reference to the AMP/ASCO/CAP guidelines and databases, such as oncoKB, the variant genes were classified into the following categories based on the level of evidence of drug sensitivity: Class I, variants with clear clinical significance; Class II, variants with potential clinical significance; Class III, variants with no corresponding recommended drug use and possibly some clinical significance; and Class IV, other variants. The patients in this study mainly had genes in categories I, II, and III. The patients were divided into 3 groups according to the presence or absence of additional gene aberrations. Group A comprised all wild-type mCRC patients, group B comprised mCRC patients with concurrent all-*RAS* wild-type and mutations in tumor-suppressor genes (i.e., *TP53*, *APC*, *PTEN*, *BRCA2*, and *SMAD4*), and group C comprised mCRC patients with multiple alterations in oncogenic drivers (i.e., *ERBB2*, *BRAF*, *PIK3CA*, and *RET*) and all-*RAS* wild-type patients, irrespective of tumor-suppressor gene aberrances.

Outcomes

The primary outcome was progression-free survival (PFS), which was defined as the time the patient started taking the study drug until either objective disease progression (as assessed by an investigator using RECIST version 1.1) or death from any cause. The secondary outcomes were overall survival (OS), the objective response rate (ORR), and the disease control rate (DCR). OS was defined as the time the patient started taking the study until death from any cause. The ORR was defined as the percentage of patients with a confirmed complete response (CR) or partial response (PR) according to RECIST version 1.1. The DCR was defined as the percentage of patients who achieved disease control (i.e., CR, PR, or stable disease according to RECIST version 1.1) at 8 weeks or more after screening.

Statistical analysis

The data were analyzed statistically using SPSS 25.0 software, and the patients included in the study were analyzed for gene mutation status, and the mutation rate of each gene was expressed as a percentage. The distributions of the respective clinical characteristics were compared among the 3 groups using the chi-square test. The Kaplan-Meier method was used for the survival analysis and to plot the survival curves for PFS and OS, and the Log-rank method was used to compare the survival differences among groups A, B, and C. A P value <0.05 was considered statistically significant. In the multifactor analysis, the Cox regression model was used to identify which of the clinical characteristics were independent factors affecting PFS.

Results

An overview of the patient's genetic variation

Group A comprised all-wild-type patients without other mutations (n=10), group B comprised all-*RAS* wild-type patients with tumor-suppressor genes (including *TP53*, *APC*, *PTEN*, *BRCA2*, and *SMAD4*) mutations (n=42), and group C comprised all-*RAS* wild-type patients with oncogenic driver genes (including *ERBB2*, *BRAF*, *PIK3CA*, and *RET*) alterations (n=8). The specific gene distributions

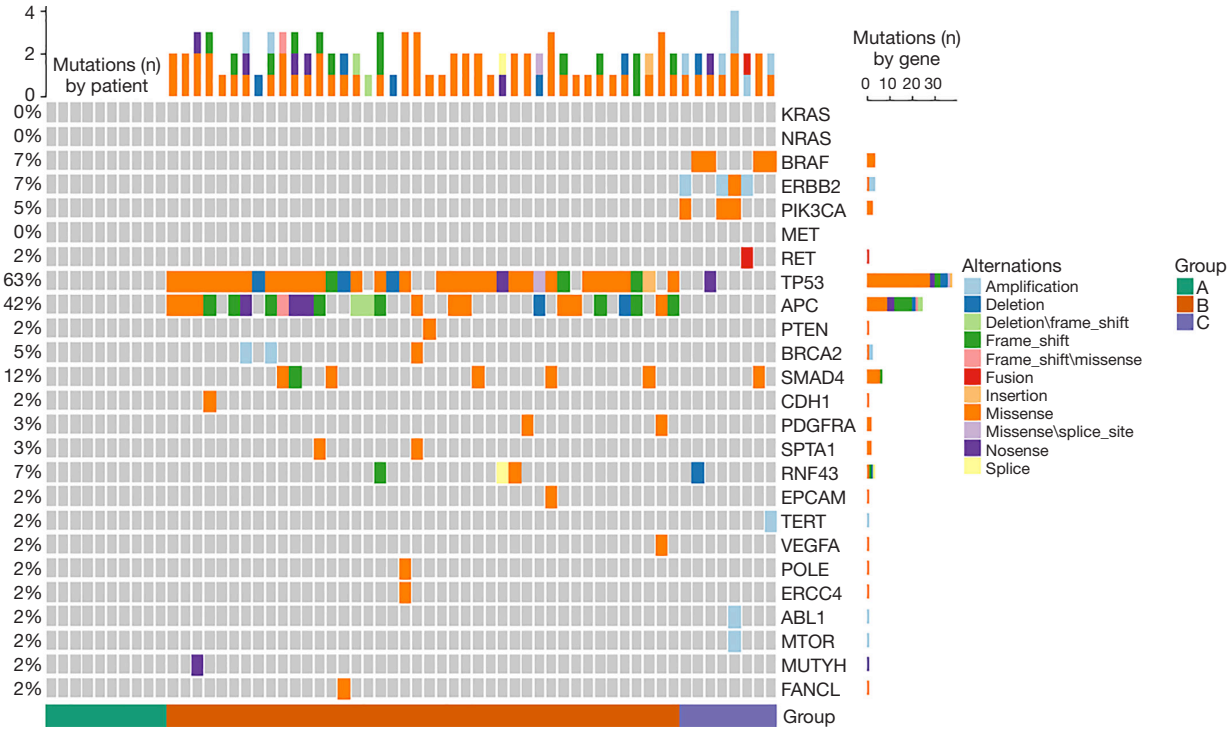

Figure 1 Distribution of aberrant genes stratified by subgroups.

are shown in *Figure 1*.

A total of 60 patients with mCRC were included in this retrospective study. Among the patients, 50 carried genetic mutations, among which, 40 (80.0%) had polygenic mutations. Notably, 33 (66%) patients had *TP53* combined with other gene variants, among which 22 patients had *APC*, making it the most common combined gene variant. For further details, see *Tables 1*,*2*.

Relationship between genetic variation and clinical features

The demographic and baseline characteristics of the 60 patients in the full analysis set are summarized in *Table 3*.

The relationship between different gene mutations and clinical features is shown in *Table 4*. Mutations in the *TP53* (86.8%), *APC* (84.0%), and *SMAD4* (85.7%) genes were the most common in left-sided mCRC.

The chi-square test was used to detect differences between groups A, B, and C at the level of each clinical characteristic; however, the P values among the groups were >0.05; thus, there were no statistically significant differences between the groups, indicating that the factors were balanced and comparable among the 3 groups (see *Table 5* for further details).

Effect of cetuximab on PFS and OS in the treatment of mCRC with different gene variants

At the time of the data cut-off date (i.e., January 31, 2021), 39 patients had progressive disease or had died; however, the OS data were not yet available. The median follow-up time was 14.5 months (range, 2.0–50.0 months).

Figure 2 shows the comparison of the PFS curves for groups A, B, and C. The median PFS time for the total sample was 12.0 months [95% confidence interval (CI): 8.95–15.05 months], and the median PFS times for groups A, B, and C were 15.0 months (95% CI: 0.00–37.72 months), 12.0 months (95% CI: 9.01–14.99 months), and 3.0 months (95% CI: 0.00–7.16 months), respectively. PFS differed significantly among the 3 groups (χ^2=9.965, P=0.007). However, the crossover in the survival curves suggested the possible existence of uncorrected confounders. Based on the univariate results, we fit a Cox proportional risk regression model with gender as a stratified variable to correct for the effect of each confounding factor and plotted the survival analysis results (see *Figures 3*,*4*). The results of the Cox

Table 1 Genetic mutations in 60 patients

Mutant genes	Number of mutations	Mutation rate (%)
TP53	38	63.3
APC	25	41.7
SMAD4	7	11.7
ERBB2	4	6.7
BRAF	4	6.7
RNF43	4	6.7
PIK3CA	3	5.0
BRCA2	3	5.0
PDGFRA	2	3.3
SPTA1	2	3.3
RET	1	1.7
PTEN	1	1.7
CDH1	1	1.7
ERCC4	1	1.7
EPCAM	1	1.7
TERT	1	1.7
VEGFA	1	1.7
POLE	1	1.7
ABL1	1	1.7
MTOR	1	1.7
MUTYH	1	1.7
FANCL	1	1.7
ERBB4	1	1.7
DPYD	1	1.7

Table 2 TP53 combined with other gene mutations

	Mutant genes	Number	%
TP53 and tumor-suppressor mutations	*TP53+APC*	14	28
	TP53+APC+BRCA2	2	4
	TP53+APC+SMAD4	2	4
	TP53+APC+CDH1	1	2
	TP53+APC+ MUTYH	1	2
	TP53+APC+SPTA1	1	2
	TP53+APC+RNF43	1	2
	TP53+ SMAD4	3	6
	TP53+ SMAD4+EPCAM	1	2
TP53 and oncogenic driver mutations	*TP53+ BRAF*	1	2
TP53 and other mutations	*TP53+RNF43*	2	4
	TP53+PDGFRA	1	2
	TP53+FANCL+YAP1	1	2
	TP53+ERBB4+FANCD2+POLE	1	2
	TP53+WT1+EPHB1+ESR1+KDR+TYR	1	2

survival analysis showed that there was no statistically significant difference in the PFS time of patients in group A compared to the PFS times of patients in groups B and C (P=0.882 and 0.071).

Figure 5 shows the comparison of the survival curves of PFS in women and men in groups B and C. The results showed that the PFS times in groups B and C differed significantly (P=0.011). In conclusion, while the results of the median PFS comparison showed that patients in group A had significantly prolonged PFS compared to groups B and C, and patients in group B had significantly prolonged PFS compared to group C, after adjusting for confounding factors and the statistical analysis, only group B had significantly prolonged PFS compared to group C.

Of the 60 patients enrolled, 18 were confirmed to have died by the follow-up cut-off date. *Figure 6* shows the OS survival curves of groups A, B, and C. There was no statistically significant difference in OS between the 3 groups (P=0.998). However, due to the short follow-up period of this study, the median number of OS events had not yet been reached.

Cetuximab in left hemisphere mCRC with different gene variant types

There were 49 patients with mCRC whose primary tumor location was on the left side, 6 of whom were in group A, 36 of whom were in group B, and 7 of whom were in group C. The median PFS time in patients with left-sided mCRC was 12.0 months (95% CI: 9.85–14.15 months). The median PFS times were 3.0, 13.0, and 3.0 months for groups A, B, and C, respectively, in patients with left-sided mCRC, and the differences among the 3 groups were significant (see *Figure 7*, P=0.009). However, due to the small number of patients in group A included and not included in the post-

Table 3 Demographic and baseline characteristics

Clinical feature	Participants (n=60)
Age (years)	
≤60	30 (50.0%)
>60	30 (50.0%)
Gender	
Men	39 (65.0%)
Women	21 (35.0%)
Degree of tissue differentiation	
Low	4 (6.7%)
Low-medium	8 (13.3%)
Medium	33 (55.0%)
Unknown	15 (25.0%)
Primary lesion site	
Left	49 (81.7%)
Right	11 (18.3%)
Number of transferred organs	
Single	32 (53.3%)
Multiple	28 (46.7%)
Transfer type	
Simultaneous	38 (63.3%)
Heterochronous	22 (36.7%)
Local intervention of metastatic site	
No	32 (53.3%)
Yes	28 (46.7%)

statistics, the results only showed that PFS was significantly longer in group B patients compared to group C patients with left-sided mCRC. As *Figure 8* shows, there was no statistically significant difference in the OS between groups A, B, and C in patients with left-sided mCRC (P=0.945).

Effect of local intervention

There were 42 patients in group B, including 20 patients who underwent local intervention and 22 patients who did not undergo local intervention. *Figure 9* suggests that there was no statistical difference in PFS between the local intervention and non-local intervention groups in the Group B patients (P=0.55). The local intervention mCRC patients had a median PFS time of 14.0 months (95% CI: 8.64–19.36 months) and the non-local intervention mCRC patients had a median PFS time of 12.0 months (95% CI: 9.65–14.35 months). *Figure 10* suggests that there was also no statistically significant difference in the OS between the localized intervention and non-localized intervention groups in Group B patients (P=0.433).

Univariate and multivariate analysis

Based on the results of the univariate analysis (see *Table 6*), none of the clinical characteristic factors had a significant effect on PFS (P<0.05). Among the factors, different gene mutation types had the largest effect on PFS (0.05≤P<1). Given the limitations of the univariate analysis, gene mutation types was included in the multifactorial analysis in this study.

As *Tables 6*,*7* show, the final screened model included only the subgroup variables with different mutation types. Only subgroups B and C differed significantly in terms of their effects on patients PFS (P=0.004).

Discussion

Our survival analysis showed that the *RAS* wild-type mCRC patients with the tumor suppressor gene mutations who received cetuximab combined with chemotherapy had significantly longer PFS than those with all-*RAS* wild-type mCRC combined with the oncogenic driver gene variants and those with all-*RAS* wild-type mCRC no combined with the gene variants. Wild-type mCRC patients without genetic variants did not outperform the other 2 groups in terms of either PFS or OS. The BENEFIT trial examined the efficacy of gefitinib in patients with advanced non-small cell lung cancer (NSCLC) with *EGFR* mutations combined with different genetic variants in 3 groups based on the NGS results of the patients. The results showed that the median PFS time of patients with only the *EGFR* mutation treated with gefitinib was significantly longer than that of patients with the *EGFR* mutation combined with other gene variants, and the median PFS of patients with the *EGFR* mutation combined with tumor suppressor gene variants was significantly longer than that of patients with the EGFR mutation combined with the oncogenic driver gene variants (5). Thus, this study divided the all-*RAS* wild-type mCRC patients into the following 3 groups: (I) patients without combined gene variants; (II) patients with combined tumor suppressive gene variants (including *TP53*,

Table 4 Relationship between gene mutations and clinical features

Clinical feature	*TP53* (n=38)	*APC* (n=25)	*SMAD4* (n=7)	*BRAF* (n=4)	*ERBB2* (n=4)	*PIK3CA* (n=3)
Gender						
Men	26 (68.4%)	20 (80.0%)	3 (42.9%)	1 (25.0%)	2 (50.0%)	2 (66.7%)
Women	12 (31.6%)	5 (20.0%)	4 (57.1%)	3 (75.0%)	2 (50.0%)	1 (33.3%)
Primary lesion site						
Left	33(86.8%)	21 (84.0%)	6 (85.7%)	3 (75.0%)	3 (75.0%)	1 (33.3%)
Right	5 (13.2%)	4 (16.0%)	1 (14.3%)	1 (25.0%)	1 (25.0%)	2 (66.7%)
Number of transferred organs						
Single	18 (47.4%)	14 (56.0%)	2 (28.6%)	1 (25.0%)	1 (25.0%)	1 (33.3%)
Multiple	20 (52.6%)	11 (44.0%)	5 (71.4%)	3 (75.0%)	3 (75.0%)	2 (66.7%)
Transfer Type						
Simultaneous	23 (60.5%)	16 (64.0%)	3 (42.9%)	4 (100.0%)	1 (25.0%)	1 (33.3%)
Heterochronous	15 (39.5%)	9 (36.0%)	4 (57.1%)	0 (0.0%)	3 (75.0%)	2 (66.7%)
Liver metastasis						
No	18 (47.4%)	8 (32.0%)	5 (71.4%)	1 (25.0%)	4 (100.0%)	3 (100.0%)
Yes	20 (52.6%)	17 (68.0%)	2 (28.6%)	3 (75.0%)	0 (0.0%)	0 (0.0%)
Lung metastasis						
No	29 (76.3%)	20 (80.0%)	4 (57.1%)	2 (50.0%)	2 (50.0%)	2 (66.7%)
Yes	9 (23.7%)	5 (20.0%)	3 (42.9%)	2 (50.0%)	2 (50.0%)	1 (33.3%)

APC, *PTEN*, *BRCA2*, and *SMAD4*); and (III) patients with combined oncogenic driver gene variants (including *HER2/ERBB2*, *BRAF*, *PIK3CA*, and *RET*). A Kaplan-Meier analysis of the enrolled mCRC patients was performed to clarify the indication population for treatment with cetuximab. In this study, it was observed that treatment with cetuximab in patients with all-*RAS* wild-type and no combined mutation was not superior to that of patients with a combined mutation in terms of PFS and OS. Our results differ slightly to those for NSCLC.

Oncogenic driver gene

In a series of previous retrospective studies or single-arm phase-II studies, other genetic alterations in the *EGFR* signaling pathway have been found to be associated with resistance to *EGFR* mAbs, and the activation of intracellular signaling pathways downstream of *EGFR* (including the *RAS-RAF-MAPK*, *PI3K-PTEN-AKT*, and *JAK/STAT* signaling pathways) has been shown to be an important mechanism for generating resistance to *EGFR* mAbs (6-10). Changes in any of the components may lead to the constitutive activation of the *EGFR*, consequent intracellular signaling, and ultimately drug resistance (11).

As an important member of the oncogenic driver genes, *BRAF* mutations are present in 8% to 12% of mCRC cases, and every clinical trial conducted to date and real-world data have shown that the prognosis of mCRC patients with *BRAF* gene mutations is poor, especially, for those with *V600E* mutations whose mCRC prognosis is even worse (12). Whether *EGFR* is blocked or not, *BRAF V600E* mutations can still cause continuous activation of downstream signals, leading to tumor cell proliferation and survival (13,14).

Further, *BRAF* mutations only occur in tumors that do not carry *RAS* mutations. In recent years, in addition to *RAS* mutations in the tumor, *BRAF* mutation status is also important to consider before administering anti-*EGFR* therapy. *ERBB2*, an oncogenic driver gene in the *EGFR* signaling pathway, is a transmembrane glycoprotein with receptor tyrosine kinase activity. *ERBB2* amplification can bypass *EGFR* signaling and activate the *MEK-ERK*

Table 5 Clinical characteristics between different mutation groups

Clinical feature	Group A (n=10)	Group B (n=42)	Group C (n=8)	P
Gender				0.180
Women	4 (40.0%)	12 (28.6%)	5 (62.5%)	
Men	6 (60.0%)	30 (71.4%)	3 (37.5%)	
Age, mean (SD)	51.8 (12.7)	60.0 (13.0)	60.2 (10.3)	0.176
Degree of tissue differentiation				0.616
Low	1 (10.0%)	2 (4.8%)	1 (12.5%)	
Low-medium	2 (20.0%)	5 (11.9%)	1 (12.5%)	
Medium	6 (60.0%)	22 (52.4%)	5 (62.5%)	
Unknown	1 (10.0%)	13 (31.0%)	1 (12.5%)	
Primary lesion site				0.155
Left	6 (60.0%)	36 (85.7%)	7 (87.5%)	
Right	4 (40.0%)	6 (14.3%)	1 (12.5%)	
Transfer type				0.915
Simultaneous	7 (70.0%)	26 (61.9%)	5 (62.5%)	
Heterochronous	3 (30.0%)	16 (38.1%)	3 (37.5%)	
Number of transferred organs				0.185
Single	7 (70.0%)	23 (54.8%)	2 (25.0%)	
Multiple	3 (30.0%)	19 (45.2%)	6 (75.0%)	
Local intervention of metastatic site				0.851
No	5 (50.0%)	22 (52.4%)	5 (62.5%)	
Yes	5 (50.0%)	20 (47.6%)	3 (37.5%)	

Group A: all-wild-type patients without other mutations; Group B: all-*RAS* wild-type patients with tumor-suppressor genes (including *TP53*, *APC*, *PTEN*, *BRCA2*, and *SMAD4*) mutations; Group C: comprised all-*RAS* wild-type patients with oncogenic driver genes (including *ERBB2*, *BRAF*, *PIK3CA*, and *RET*) alterations.

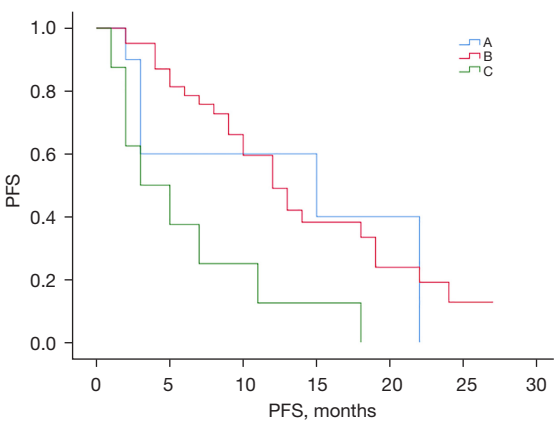

Figure 2 PFS survival curves for groups A, B, and C. PFS, progression-free survival.

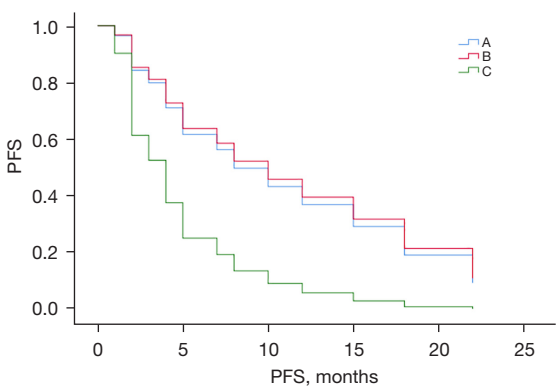

Figure 3 PFS survival curves of groups A, B, and C in female metastatic colorectal cancer patients. PFS, progression-free survival.

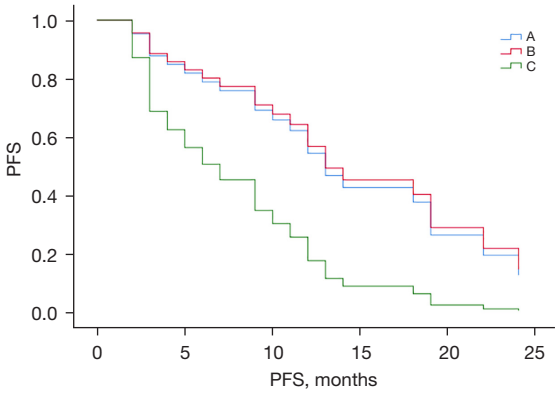

Figure 4 PFS survival curves of groups A, B, and C in male metastatic colorectal cancer patients. PFS, progression-free survival.

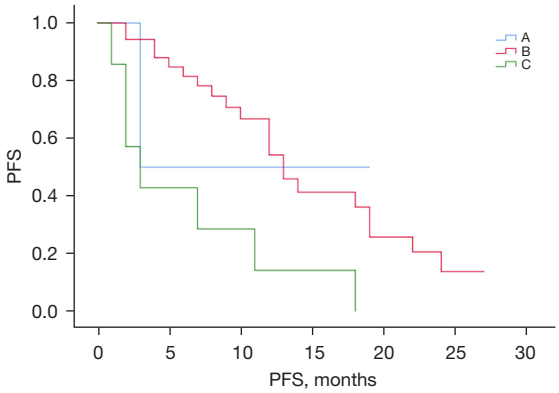

Figure 7 PFS survival curves for groups A, B, and C in left-sided metastatic colorectal cancer. PFS, progression-free survival.

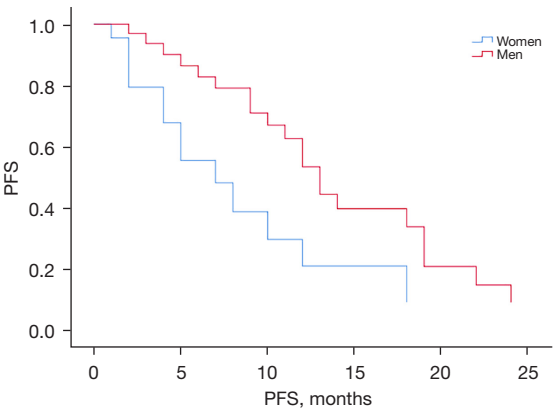

Figure 5 Survival curves of PFS in groups B and C with gender as a stratification variable. PFS, progression-free survival.

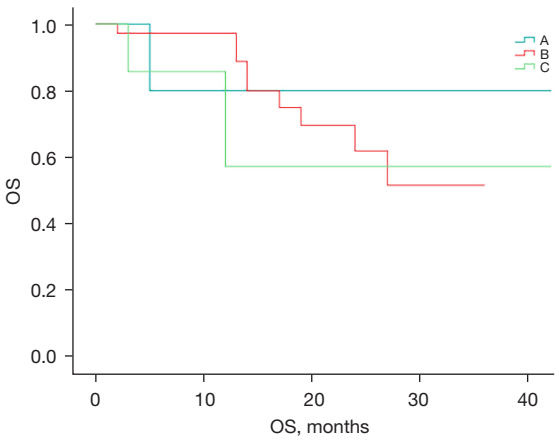

Figure 8 OS survival curves for groups A, B, and C in left-sided metastatic colorectal cancer. OS, overall survival.

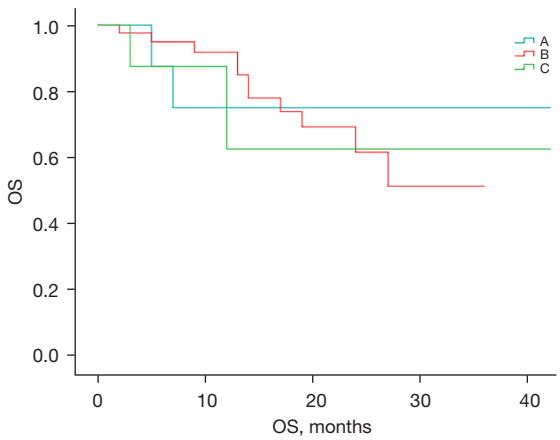

Figure 6 OS survival curves for groups A, B, and C. OS, overall survival.

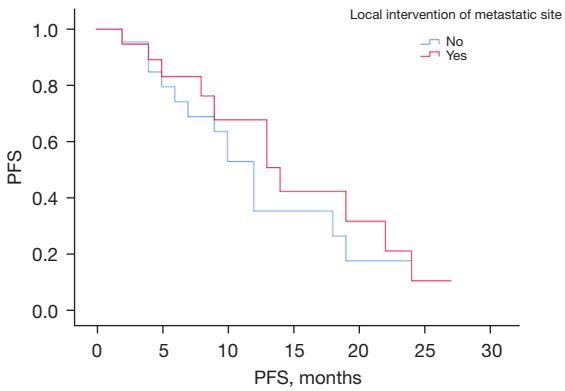

Figure 9 Survival curves of PFS with different local interventions in group B patients. PFS, progression-free survival.

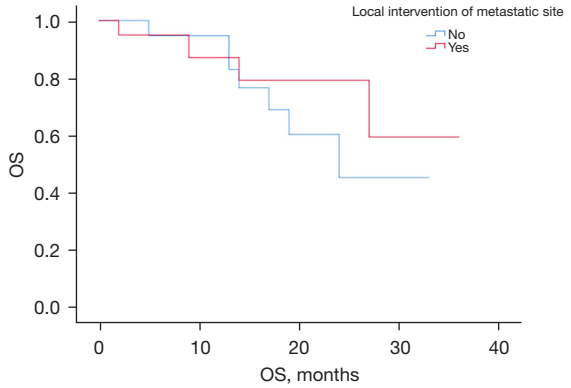

Figure 10 Survival curves of OS with different local interventions in group B patients. OS, overall survival.

cascade response. *ERBB2* amplification has been observed in some patients with all-*RAS* and *BRAF* wild-type mCRC who are insensitive to cetuximab treatment (15). Through prospective randomized trial in mice, it was finally found that genotype-response correlation showed that *HER2* was specifically amplified in cetuximab resistant and *KRAS/NRAS/BRAF/PIK3CA* wild-type cases (16). Similarly, Yonesaka *et al.* (17) observed that *ERBB2* signaling was activated in mCRC patients who showed resistance to cetuximab treatment, activation depending on *ERBB2* amplification or heregulin upregulation. Mutations in another oncogenic driver gene, *PIK3CA*, occur in approximately 10–18% of patients with CRC, mainly in

Table 6 1-way Cox regression analysis

	B	SE	Wald	P	HR
Group					
A	Ref				
B	–0.161	0.459	0.123	0.726	0.851
C	1.047	0.550	3.622	0.057	2.850
Gender					
Women	Ref				
Men	–0.459	0.329	1.945	0.163	0.632
Age	0.004	0.014	0.082	0.774	1.004
Degree of tissue differentiation					
Low	Ref				
Low-medium	0.116	1.125	0.011	0.918	1.123
Medium	0.426	1.030	0.171	0.679	1.532
Unknown	0.346	1.061	0.106	0.745	1.413
Primary lesion site					
Left	Ref				
Right	0.304	0.400	0.580	0.446	1.356
Transfer type					
Simultaneous	Ref				
Heterochronous	0.571	0.335	2.902	0.088	1.770
Number of transferred organs					
Single	Ref				
Multiple	0.526	0.328	2.570	0.109	1.693
Local intervention of metastatic site					
No	Ref				
Yes	–0.365	0.327	1.247	0.264	0.695

Table 7 Multifactor Cox regression analysis (stepwise regression)

Group	B	SE	Wald	P	HR (95% CI)
Group 1					
A	Ref				
B	–0.161	0.459	0.123	0.726	0.851 (0.346–2.092)
C	1.047	0.550	3.622	0.057	2.850 (0.969–8.378)
Group 2					
C	Ref				
A	–1.047	0.550	3.622	0.057	0.351 (0.119–1.032)
B	–1.208	0.417	8.382	0.004	0.299 (0.132–0.677)

exon 9 (*E542K* and *E545K*) and exon 20 (*H1047R*) (18). In 2005, *PIK3CA* mutations in the PI3K-PTEN-AKT signaling pathway were identified as possible predictors of anti-*EGFR* resistance in *RAS* wild-type mCRC (19). Since then, several systematic review studies have confirmed that *PIK3CA* mutations serve as predictors of anti-*EGFR* resistance in *RAS* wild-type mCRC (20-23). Thus, previous research has shown that mutations in oncogenic driver genes (*BRAF*, *ERBB2*, and *PIK3CA*) lead to cetuximab resistance, which is consistent with our findings that the survival benefit was reduced in the all-*RAS* wild-type group with the oncogenic driver gene mutation treated with cetuximab.

Tumor suppressor gene

The tumor suppressor gene *PTEN* is also a member of the *EGFR* signaling pathway; however, some studies have shown that the loss of *PTEN* may be related to anti-*EGFR* resistance; however, the role of *PTEN* loss in mCRC still unclear. Several studies have reported inconsistent results on the effect of *PTEN* loss against *EGFR* resistance (24). Further studies and prospective large randomized clinical trials need to be conducted to confirm the role of *PTEN* in anti-*EGFR* treatment resistance. Additionally, as only 1 patient carried the *PTEN* loss mutation in the *RAS* wild-type group with tumor-suppressor mutations in this study, the impact on the OS is minor (25,26).

Primary tumor location

In terms of PFS, the subgroup analysis showed cetuximab had a better effect on the patients with the all-*RAS* wild-type with the tumor suppressor variants in the left half of mCRC than that of patients with the all-*RAS* wild-type with oncogenic driver variants. The location of the primary tumor is very important in mCRC (27). A subgroup analysis of GALGB/SWOG 80405 showed (28) that primary tumor location was an independent prognostic factor for mCRC. The embryological origin, anatomy, and clinical manifestations of left and right CRC differ. Studies have shown that right CRC does not benefit from anti-*EGFR* therapy (in terms of both PFS and OS) in the context of first-line treatment of mCRC (29,30). Compared to left CRC, right CRC is more likely to contain downstream or bypass drivers of *EGFR* (e.g., *RAS*, *BRAF*, and *PIK3CA* mutations, hypermethylation, *HER2* overexpression, and reduced *EGFR* ligand expression), leading to anti-*EGFR* resistance. However, even after eliminating these currently known molecular events, the effect of tumor site on efficacy cannot be fully explained. The difference between the left and right CRC is significant, and comprehensive analyses of multiple randomized studies have also confirmed this difference (29,31). The 2017 update to the NCCN guidelines limits cetuximab first-line therapy to those with primary tumors on the left side. Thus, this study conducted a subgroup analysis of the efficacy of cetuximab in patients with different genetic variants in left *RAS* wild-type mCRC.

Local intervention

Several previous studies have suggested an improvement in the prognosis of mCRC patients with local interventions, especially the resection of hepatic metastasis. However, few trials have investigated the effects of local intervention on the efficacy of cetuximab. The present study found that

local intervention failed to improve the efficacy (including the PFS and OS) of cetuximab in mCRC patients with all-*RAS* wild-type combined with the tumor-suppressor variant. Further confirmations of these findings in large clinical studies are needed to inform the future clinical application of cetuximab.

In this study, the univariate and multivariate analyses showed that *RAS* wild-type mCRC combined with the tumor suppressor gene or oncogenic driver gene variant was an independent risk factor for PFS. Recent research in China has shown that the primary tumor site is an independent factor influencing the prognosis of PFS in patients with *KRAS* wild-type mCRC treated with cetuximab ($P<0.05$). We did not find any correlation between the primary tumor site and prognosis; however, this may have been due to the small number of cases with primary tumors on the right side, and the large difference to the number of cases with primary tumors on the left.

In the present study, both the overall analysis and the subgroup analysis revealed no significant difference in the efficacy of the 3 groups of patients in terms of OS ($P>0.05$). This may be related to the short follow-up period of this study in which only 18 patients died (3/10 in group A, 11/42 in group B, and 4/8 in group C) and the median number of OS events not yet reached. Thus, a longer follow-up study needs to be conducted to determine whether there is a significant difference in OS between mCRC patients with different combined genetic variant types treated with cetuximab.

Acquired resistance mechanism

In this study, 39 (65.0%) patients showed eventual progression. Acquired resistance refers to the patients who are initially effective for treatment and finally progress. Clinical data suggest that the remission duration of patients who undergo anti-*EGFR* therapy is relatively short, with most tumors becoming refractory within 3–12 months (32). Thus, numerous mechanisms may contribute to patients' acquired resistance to anti-*EGFR* therapy, including secondary changes in the *RAS-RAF* signaling pathway (33,34), the activation of the *IGF-1R* pathway (35), *MET* overexpression and amplification (36), *HER2* amplification and *HER3/4* ligand overexpression (16,17), *EGFR S492R* mutation (37,38), and altered *VEGF* signaling (39).

The first acquired resistance mechanism is the secondary alteration of the RAS-RAF signaling pathway. *RAS* mutations play a crucial role in acquired resistance. About 50% of acquired resistance cases are due to secondary *RAS* mutations (33,34,40,41). The global Phase III ASPECT study used NGS to detect *RAS* mutations in ctDNA of patients treated with anti-*EGFR* therapy. The results showed that *RAS* mutations occurred in 32% of 164 patients whose baseline ctDNA was *RAS* wild type (42). Further, research has shown that alterations in these genes are likely due to the cloning of pre-existing drug-resistant cells.

The second acquired resistance mechanism is due to the activation of other growth factor receptor signaling pathways. For example, *IGF-1R*, *MET* (15), and *HER2* (43) can bypass *EGFR* to activate *EGFR* downstream effectors and trigger subsequent intracellular signaling pathways, thereby inducing tumor cell proliferation and resistance to apoptosis. *IGF-1R* belongs to the transmembrane tyrosine kinase family and is activated upon binding to *IGF-1* or *IGF-2*. Binding leads to activation downstream of the *RAS-RAF-MAPK* and *PI3K-AKT* pathways. Additional pre-clinical studies have shown that signaling via *IGF-1R* activation also leads to an increase in *EGFR* activation (44), resulting in acquired resistance to *EGFR*-targeted therapies (44,45). The *MET* gene leads to cell proliferation and survival via the activation of intracellular signaling cascades, including the *PI3K-AKT*, *RAC1-CDC42*, *RAP1*, and *RAS-MAPK* pathways (46). The interaction of *EGFR-MET* with *MET* pathway activation induced by *TGF-α* overexpression has been suggested as a possible mechanism for the acquired resistance to cetuximab in CRC (47). This was demonstrated in a study by Liska *et al.* (36) in 2011. Interestingly, further analysis showed that cetuximab also restored the effect through the pharmacological inhibition and silencing of *MET*. Further, both mechanisms (i.e., *HER2* gene amplification and *HER3/4* ligand heregulin overexpression) may lead to the sustained activation of ERK signaling, thus leading to secondary resistance to *EGFR*-targeted therapy (16,17). Several studies have shown that previously uncommon *HER2* amplifying clones may amplify under the pressure of anti-*EGFR* therapy, leading to disease progression due to acquired drug resistance.

The *EGFR S492R* mutation is also a possible reason for the development of acquired resistance to *EGFR*-targeted therapy (37). The mutation reduces the affinity of the receptor for the ligand and interferes with the binding of cetuximab. It has not been detected in untreated patients in several studies (38). In contrast, the *S492R* mutation does not affect the action of panitumumab. Thus, panitumumab treatment appears to be a reasonable strategy for patients with *S492R* mutations who develop disease progression

after treatment with cetuximab.

In addition, alterations in VEGF signaling may also lead to acquired resistance to EGFR-targeted therapies. Ciardiello *et al.* (48) showed that the high expression of VEGF in CRC cells is correlated with resistance to EGFR inhibitors. Bianco *et al.* (49) found higher levels of VEGF and VEGFR1 secretion in cetuximab-resistant cells compared to cetuximab-sensitive cells. Additionally, EGFR monoclonal resistant cells could be inhibited by VEGFR1 silencing or Vandetanib. These results suggest that combined VEGFR and EGFR inhibition restores patients' sensitivity to anti-EGFR drugs and provides further evidence of the association between increased VEGF/VEGFR1 expression with resistance to anti-EGFR therapy.

Primary resistance mainly including changes of *EGFR* and *EGFR* ligands, *RAS* mutation, *BRAF* mutation, *PTEN* loss, activation of the *PIK3CA/PTEN* or *JAK/STAT* signaling pathways. These therapies could be used to reverse the resistance: new *EGFR*-targeted inhibitors (eg. GC1118, MM-151), a combination of multitargeted inhibitors, metabolic regulators, new cytotoxic drugs, modification or activation of immune cells, suppression of cancer-associated fibroblasts and anti-*VEGFR* agents (39).

Limitations

This study had a number of limitations: First, this study was retrospective, and unlike prospective studies, it could not control for various types of confounding factors; thus, the data may be biased. Second, while this study was conducted as a multicenter clinical study, the number of cases was small, the sample size of some of the subgroups after grouping was small, and the number of cases varied widely among subgroups; thus, the analysis of the results should be interpreted with caution, and the accuracy of the conclusions needs to be validated by large samples of evidence-based medicine. Third, due to objective constraints, the follow-up period of this study was short; thus, the OS endpoint was not met in most cases, and the study results may partially change with the extension of the follow-up period. PFS is the primary study endpoint of this study due to its ability to provide earlier results for analysis, which can be more accurately detected and attributed to the effect of the investigational treatment without being influenced by any subsequent treatment (50). However, for large clinical trials of advanced tumors, OS is the gold standard endpoint because it is easy to measure and accurate. Conversely, PFS lacks accepted consensus criteria, and there may be other measures that limit the survival benefits, which may affect the results.

Conclusions

In the real world, where there are multiple lines of treatment options for mCRC, the advent of NGS offers new possibilities for determining the prognosis of tumor patients, evaluating hyper-indicated targeted therapies for refractory cancers, and accelerating research on matched targeted therapies (51,52). The staging of CRC was correlated with the depth of tumor invasion, the number of lymph node metastasis and the presence of distant metastasis. Existing studies have shown that the prognosis of mCRC is related to the status of *RAS* and *BRAF*, lymph node metastasis or not, the time of metastasis, the number and size of metastasis, the general status of patients, complications and so on. In this study, patients with all-*RAS* wild-type mCRC were selected as the research objects, and the efficacy of cetuximab treatment in patients with all-*RAS* wild-type mCRC, all-*RAS* wild-type mCRC with tumor suppressor gene variant and all-*RAS* wild-type mCRC with oncogene driver gene variant were compared after excluding common prognostic factors. In the balance of other related factors, cetuximab treatment was shown to have a greater benefit in mCRC all-*RAS* wild-type patients with the tumor suppressive gene variant. Our findings provide a certain basis for the selection of treatment strategies for patients with mCRC in clinical practice. Notably, in the cetuximab treatment of all-*RAS* wild-type mCRC patients with tumor suppressor gene variants, the local intervention did not provide any survival benefits. Thus, local treatments should only be carefully administered to *RAS* wild-type patients with the tumor suppressor gene variant treated with cetuximab. Alternative treatment strategies should be considered for mCRC patients with multiple oncogenic driver gene variants, even those genetically tested and determined to have the all-*RAS* wild-type, and all patients should undergo tumor-tissue based NGS testing at the baseline to determine if they would benefit from cetuximab monotherapy or combination therapy.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1237/rc

Data Sharing Statement: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1237/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1237/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics committee of The First Affiliated Hospital of Soochow University (No. 2022-482) and ethics committee of The Second Affiliated Hospital of Soochow University (No. LK-2020-071-02). Informed consent was taken from all individual participants.

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