Immunotherapy-based treatment patterns and clinical outcomes across lines of therapy in microsatellite stable metastatic colorectal cancer: A real-world study

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Abstract. Microsatellite stable (MSS) metastatic colorectal cancer (mCRC) hardly benefits from immune-monotherapy. Immune checkpoint inhibitor (ICI)-based combinations have been explored with mixed results. To evaluate the treatment patterns and outcomes across lines of immunotherapy in MSS mCRC, a retrospective real-world data analysis was performed in the present study. A total of 202 patients treated at Renmin Hospital of Wuhan University from June 2019 to December 2023 were included in the analysis, encompassing 38, 41 and 123 cases that received first-(1L), second-(2L) and third-line or above (3L+) ICI-based therapies, respectively. ICI combined with chemotherapy, ICI combined with chemotherapy and anti-VEGF(R)/EGFR/HER2 monoclonal antibody, and ICI combined with tyrosine kinase inhibitor (TKI) were the main options for the 1L, 2L and 3L+ cohorts, respectively. The objective response rates of the 1L, 2L and 3L+ treatment cohorts were 63.2, 22.0 and 11.4%, respectively, and the disease control rates of these three cohorts were 94.7, 75.6 and 70.7%, respectively. The median progression-free survival of the 1L, 2L and 3L+ cohorts was 11.1, 6.5 and 4.7 months, respectively, and overall survival (OS) was not reached, 16.0 and 11.9 months, respectively. After propensity score matching, patients who received ICI cross-line therapy at least once in the 3L+ cohort showed directional OS improvement (14.3 vs. 11.3 months; hazard ratio, 0.66; 95% confidence interval, 0.39-1.14; P=0.123) compared with those who did not receive ICI cross-line therapy. Grade ≥3 adverse events (AEs) occurred in 43.6% of patients, with thrombocytopenia, leukopenia and abnormal hepatic function being the most common. The incidence of immune-related AEs (irAEs) was 38.6% and that of grade ≥3 irAEs was 15.3%. These findings suggest that chemotherapy remains the cornerstone of 1L and 2L treatment for MSS mCRC, while the combination of ICI and TKI has developed into a major trend at the 3L+ setting with improved survival and controllable safety.

Introduction

Colorectal cancer (CRC) is the third most common cancer type diagnosed worldwide and the second leading cause of cancer-related death; it also presents the highest total lifetime risk of developing and dying from gastrointestinal cancer (1,2).

The introduction of immune checkpoint inhibitors (ICIs) has reshaped treatment and outcomes in patients with a variety of solid tumors including metastatic CRC (mCRC). Notably, patients with cancer harboring the mismatch repair-deficient (dMMR)/microsatellite instability-high (MSI-H) phenotype have shown a higher response and better efficacy to ICI treatment, with a significantly prolonged 5-year survival rate (76±4 vs. 54±2%; P<0.001) (3). However, only 15% of early-stage CRC and 5% of mCRC cases are dMMR/MSI-H (4). The vast majority of patients with mCRC are in a MMR proficient (pMMR)/microsatellite stable (MSS) genomic state characterized by an immunosuppressive tumor microenvironment (TME) with a low tumor mutational burden and a lack of tumor-infiltrating lymphocytes (5). Therefore, unsurprisingly, ICI monotherapy has failed to demonstrate meaningful clinical activity in this patient population.

To overcome immunoresistance and enhance effective antitumor immune response, an increasing number of basic research and clinical trials are exploring ICI-based combination strategies to synergistically stimulate the transformation of an immune-desert TME into an immunosupportive TME. A series of exploratory studies have reported mixed results

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from ICI combined with chemotherapy (CT), radiotherapy (RT), antiangiogenic targeted agents (such as regorafenib and fruquintinib) or other cytotoxic drugs in treating mCRC (6-11).

However, to the best of our knowledge, routine clinical practice data on immune-based combination modalities by line of treatment for patients with pMMR/MSS mCRC are still lacking. Therefore, the present retrospective study was conducted to evaluate the real-world ICI-based treatment patterns and outcomes in patients with pMMR/MSS mCRC across all treatment lines, especially those in third-line or above (3L+) settings who have limited treatment options under current guidelines and are in urgent need of effective treatment strategies.

Patients and methods

Study design and population. This retrospective study was conducted at the Renmin Hospital of Wuhan University (Wuhan, China). Patients who were treated at the Department of Oncology of Renmin Hospital of Wuhan University from June 10, 2019 to December 20, 2023 were screened and enrolled. Patients were selected according to the following inclusion criteria: i) Patients who were ≥18 years of age; ii) had histologically or cytologically confirmed metastatic pMMR/MSS colorectal adenocarcinoma; iii) had received at least one dose of ICI at the first-line (1L), second-line (2L) or 3L+ setting; iv) and had available follow-up data. The exclusion criteria included: i) Patients with other malignancies (except skin basal cell carcinoma and cervical carcinoma *in situ*); ii) a history of or active autoimmune disease or being treated with immunosuppressive therapy; and iii) with incomplete medical data.

Follow-up for survival analysis continued until July 20, 2024, which served as the final cut-off date for the present study. The patients typically returned to Renmin Hospital of Wuhan University for regular re-examination, including in outpatient or inpatient clinics. For patients who did not attend clinic visits, follow-up was conducted via telephone. The clinical data from June 10, 2019 to July 20, 2024 were collected and accessed retrospectively through the medical electronic system at the hospital, which included age, sex, histological type, primary tumor location, type of metastasis, metastatic site, RAS/BRAF mutation status, MSI/MMR status, treatment information, adverse events (AEs) and survival status. MSI or MMR status was tested using immunohistochemistry, polymerase chain reaction or next-generation sequencing.

In addition, there are currently diverse definitions and similar terms for 'ICI reuse', represented by 'ICI rechallenge', 'ICI retreatment', 'ICI re-administration', 'ICI restart', 'ICI re-exposure', 'ICI reinitiation', 'ICI reintroduction' and 'ICI resumption', which mainly vary based on the cause of interruption, with or without in-between therapy and the subsequent treatments. In the present study, to achieve terminological uniformity, 'ICI cross-line therapy' was used as a surrogate for a series of similar concepts. ICI cross-line therapy was defined as the reuse of ICI in the same patient across different lines of treatment after prior interruption, regardless of the reason for discontinuation (such as disease progression, toxicity, completion of planned treatment or other clinical decisions).

Outcome variables. The primary outcomes of interest were median progression-free survival (PFS), overall survival (OS), objective response rate (ORR), disease control rate (DCR) and safety. PFS was defined as the time from 1L, 2L or 3L+ treatment initiation to disease progression or death from any cause (whichever occurred first) or last visit (for censored patients). OS was measured from the treatment initiation to death or date of last follow-up. The last follow-up date was recorded as the censored data for survival analysis when death or progression time could not be confirmed or if the patient was still alive.

Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors v1.1 (12) and classified as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD). ORR was determined as the rate of a best response of CR or PR. DCR included CR, PR and SD. Data on treatment-related AEs (TRAEs) and immune-related AEs (irAEs) were collected and graded for severity according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (13).

Statistical analysis. All statistical analyses in the present study were performed using SPSS statistical software (version 26.0; IBM Corp.) and R (version 4.2.3; R Project for Statistical Computing; http://www.r-project.org/). Categorical variables are summarized by percentages and are compared by the χ^2 test or Fisher's exact test. Continuous variables are described as the median and interquartile range (IQR), and are compared using the Kruskal-Wallis rank-sum test. The median follow-up time was determined using the reverse Kaplan-Meier estimator. PFS and OS were plotted and compared using Kaplan-Meier curves and log-rank tests under the assumption of proportional hazard rates. The two-stage method was used for the correct comparison of two crossing survival curves. A Cox proportional hazards regression model analysis was performed to calculate the hazard ratio (HR) and bilateral 95% confidence interval (CI). Propensity score matching (PSM) was used to control confounding and balance baseline characteristics between groups with a 1:1 matching ratio and a caliper of 0.02. Demographic characteristics, AEs and other clinical data are summarized descriptively. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. In total, 202 patients with mCRC were included in the present study (Fig. 1), of whom 38 (18.8%) had a 1L ICI-based treatment initiation (1L cohort), 41 (20.3%) had an initiated 2L ICI-based treatment (2L cohort) and 123 (60.9%) received initiated ICI-based treatment at 3L+ settings (3L+ cohort). Of the entire cohort, the median age of the patients was 60 years (IQR, 51-68 years), 137 (67.8%) patients were male, 156 (77.2%) had a primary tumor located in left-side colon and rectum, 150 (74.3%) had ≥2 metastatic organs and 134 (66.3%) had liver metastasis. The incidence of lung metastasis increased with the progression of treatment lines (28.9 vs. 43.9 vs. 57.7%). Gene mutation data were available for 132 patients (65.3%) and 67 (33.2%) harbored RAS/BRAF mutations. All patients had pMMR and/or MSS phenotypes. Notably, 80.7% of patients underwent surgical resection of the primary lesion. None of the patients in the 1L

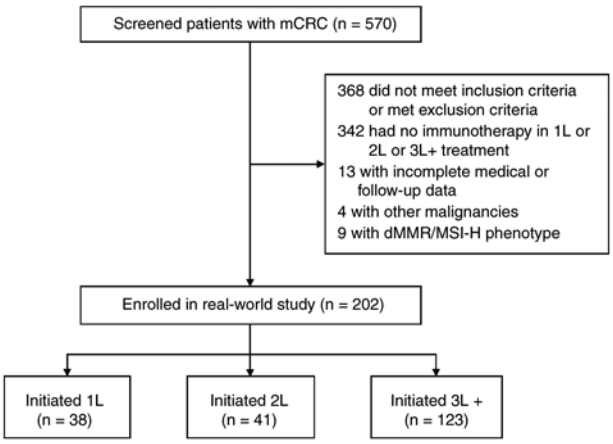

Figure 1. Flowchart of the present study. 1L, first-line treatment; 2L, second-line treatment; 3L+, third-line or above treatment; dMMR, mismatch repair-deficient; mCRC, metastatic colorectal cancer; MSI-H, microsatellite instability-high.

cohort had received previous targeted therapy, while in the 2L and 3L+ cohorts, 73.2 and 91.9% of the patients, respectively, had a history of targeted therapy. Patient baseline characteristics, stratified according to the line of therapy, are presented in Table I.

Treatment patterns. ICI-based treatment patterns are described in Table II and Fig. 2. The most commonly used regimens in 1L therapy were ICI combined with CT alone (31.6%) and ICI combined with CT and anti-VEGF(R)/EGFR/HER2 targeted agent (28.9%) (Table II). The 2L therapy was still dominated by CT, among which ICI plus CT and anti-VEGF(R)/EGFR/HER2 monoclonal antibody accounted for 30.5%. However, in the 3L+ treatment of pMMR/MSS mCRC patients, the predominant regimen has shifted to the combination of ICI and TKI, accounting for 77.7% (115/148). Among them, the ICI+TKI±RT regimen accounted for 60.1% (89/148), and the ICI+TKI+CT±RT regimen accounted for 17.6% (26/148) (Fig. 2). The proportion of patients receiving ICI combined with TKI alone (1L: 2.6%; 2L: 11.9%; 3L: 56.1%; P<0.0001) increased gradually with each advancing line of therapy (Table II). However, CT lost its dominance in the 3L+ setting, and the proportion of patients receiving ICI combined with CT alone (1L: 31.6%; 2L: 13.6%; 3L: 12.8%; P=0.016) decreased gradually with each advancing line of therapy (Table II).

Given the long-lasting immunological memory properties of immunotherapy, increasing evidence supports the survival benefit of ICI cross-line therapy in clinical practice (14-18). In the present study, among the 148 patients receiving 3L+ ICI-based treatments, 25 patients had immunotherapy initiation at 1L and/or 2L, known as ICI cross-line therapy. Of the 123 patients who received initial ICI at the 3L+ setting, 29 received subsequent ICI cross-line therapy.

Treatment response. The ORR of the 1L, 2L and 3L+ cohorts was 63.2, 22.0 and 11.4%, respectively, and the DCR was 94.7, 75.6 and 70.7%, respectively (Fig. 3A). In total, there was 1 (2.6%) case of confirmed CR and 23 (60.5%) cases of PR in the 1L cohort and 9 (22.0%) cases of PR and 22 (53.7%) cases of SD in the 2L cohort. As expected, the ORR and DCR in the 3L+ cohort was lower than that in 2L and 1L cohorts. However, the antitumor response in the 14 (11.4%) PR cases and 73 (59.3%) SD cases in the 3L+ cohort was acceptable compared with previous reports (19,20). In the efficacy-evaluable population, a reduction in the size of target lesions was achieved in 29/38 (76.3%), 27/40 (67.5%) and 52/121 (43.0%) patients in the IL, 2L and 3L+ cohorts, respectively (Fig. 3B).

Survival and prognosis. As of the data cut-off in July 20, 2024, the median follow-up was 19.8 (range, 0.4-57.6) months, the median PFS of the 1L, 2L and 3L+ cohorts was 11.1 (95% CI, 8.0-14.1), 6.5 (95% CI, 4.5-8.6) and 4.7 (95% CI, 4.3-5.2) months, respectively, and the median OS time was not reached, 16.0 (95% CI, 13.3-18.7) and 11.9 (95% CI, 10.1-13.6) months, respectively (Fig. 4A and B). In the 3L+ cohort, 54 (26.7%) patients received ICI cross-line therapy at least once, while the remaining 94 patients did not. After PSM, 43 pairs of patients with balanced baseline features were matched (Tables SI-SIII). Compared with patients in the non-ICI cross-line group, no significant difference was found in the PFS rate before and after PSM (Fig. 5A and C). However, for OS, although the difference was statistically significant before PSM (Fig. 5B), only directional OS improvement (HR, 0.66; 95% CI, 0.39-1.14; P=0.123) was observed after PSM (Fig. 5D), with a median OS time of 14.3 months in the ICI cross-line group and 11.3 months in the non-ICI cross-line group.

Univariate analysis showed that patients with liver metastasis (4.0 vs. 8.2 months; HR, 1.97; 95% CI: 1.35-2.87; P=0.001), RAS/BRAF mutations (3.2 vs. 6.3 months; HR, 1.62; 95% CI, 1.03-2.54; P=0.031) and previous anti-VEGF/EGFR therapy (4.6 vs. 10.1 months; HR, 1.97; 95% CI, 1.21-3.21; P=0.028) had poorer median PFS times in the 3L+ cohort (Fig. 6A and Table III). Multivariate analysis indicated that liver metastasis (HR, 2.08; 95% CI, 1.24-3.50; P=0.006), RAS/BRAF mutation (HR, 1.83; 95% CI, 1.11-3.02; P=0.018) and age >60 years (HR, 0.58; 95% CI, 0.35-0.98; P=0.041) were independent prognostic factors for PFS in patients with pMMR/MSS mCRC who received ICI at the 3L+ setting (Table III). As for OS, Eastern Cooperative Oncology Group performance status (ECOG PS) and RAS/BRAF mutation were two independent prognostic factors in the 3L+ cohort (Fig. 6B and Table III). Univariate and multivariate analyses were also conducted for the 1L and 2L cohorts (Figs. S1 and S2 and Tables SIV-SVII). Specifically, ECOG PS of 0-1 (HR, 0.15; 95% CI, 0.04-0.56; P=0.005) was an independent protective factor for PFS in the 1L cohort, while liver metastasis (HR, 3.99; 95% CI, 1.59-10.00; P=0.003) was an independent risk factor for PFS in this cohort (Table SIV and SV). In the 2L cohort, male sex (HR, 0.41; 95% CI, 0.19-0.87; P=0.020) was an independent protective factor for PFS (Tables SVI and SVII).

Safety. All patients received at least one dose of ICI and were included in the safety analysis. Most patients (94.6%) experienced at least one TRAE (Table IV). However, no unexpected safety concerns were reported. The most common TRAEs leading to dose reduction and/or treatment interruption were thrombocytopenia (67/202, 33.2%), leukopenia (65/202, 32.2%), abnormal liver function (63/202, 31.2%), electrolyte disturbance (60/202, 29.7%), anemia (59/202, 29.2%) and

Table I. Patient demographics and baseline characteristics.

Variables	Total (n=202)	1L cohort (n=38)	2L cohort (n=41)	3L+ cohort (n=123)	P-value[a]
Median age (IQR), years	60 (51-68)	60 (50-70)	62 (52-69)	59 (51-66)	0.260
>60	93 (46.0)	18 (47.4)	24 (58.5)	51 (41.5)	0.162
≤60	109 (54.0)	20 (52.6)	17 (41.5)	72 (58.5)	
Sex					0.349
Male	137 (67.8)	26 (68.4)	24 (58.5)	87 (70.7)	
Female	65 (32.2)	12 (31.6)	17 (41.5)	36 (29.3)	
ECOG PS score					0.865
0-1	187 (92.1)	35 (92.1)	39 (95.1)	113 (91.9)	
2	15 (7.4)	3 (7.9)	2 (4.9)	10 (8.1)	
Primary site					0.771
Left	156 (77.2)	28 (73.7)	33 (80.5)	95 (77.2)	
Right	46 (22.8)	10 (26.3)	8 (19.5)	28 (22.8)	
Metastatic organs					0.217
1	52 (25.7)	14 (36.8)	9 (22.0)	29 (23.6)	
≥2	150 (74.3)	24 (63.2)	32 (78.0)	94 (76.4)	
Type of metastasis					
With liver metastasis	134 (66.3)	26 (68.4)	29 (70.7)	79 (64.2)	0.714
With lung metastasis	100 (49.5)	11 (28.9)	18 (43.9)	71 (57.7)	0.006
RAS/BRAF status					0.050
RAS/BRAF MT	67 (33.2)	8 (21.1)	11 (26.8)	48 (39.0)	
RAS/BRAF WT	65 (32.2)	10 (26.3)	14 (34.1)	41 (33.3)	
Unknown	70 (34.7)	20 (52.6)	16 (39.0)	34 (27.6)	
MMR/MSI status					>0.999
pMMR/MSS	202 (100.0)	38 (100.0)	41 (100.0)	123 (100.0)	
Prior treatment					
Surgery of primary lesion	163 (80.7)	27 (71.1)	36 (87.8)	100 (81.3)	0.163
Chemotherapy	174 (86.1)	10 (26.3)	41 (100.0)	123 (100.0)	<0.001
Radiotherapy	73 (36.1)	3 (7.9)	16 (39.0)	54 (43.9)	<0.001
Targeted drugs[b]	143 (70.8)	0	30 (73.2)	113 (91.9)	<0.001
Anti-EGFR mAb	30 (14.9)	0	4 (9.8)	26 (21.1)	0.003
Anti-VEGF(R) mAb	119 (58.9)	0	22 (53.7)	97 (78.9)	<0.001
TKIs	45 (22.3)	0	4 (9.8)	41 (33.3)	<0.001

[a]Kruskal-Wallis rank sum test, Pearson's χ^2 test and Fisher's exact test. [b]Targeted drugs included anti-EGFR mAb (cetuximab and nimotuzumab), anti-VEGF(R) mAb (bevacizumab and ramucirumab) and TKIs (inhibitors against VEGFR, such as regorafenib, fruquintinib, apatinib and anlotinib, inhibitors against BRAF, such as dabrafenib and vemurafenib, and inhibitors against MEK, such as trametinib). Values are expressed as n (%) unless otherwise specified. 1L, first-line treatment; 2L, second-line treatment; 3L+, third-line or above treatment; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR, epidermal growth factor receptor; IQR, interquartile range; mCRC, metastatic colorectal cancer; mAb, monoclonal antibody; MMR, mismatch repair; MSI, microsatellite instability; MSS, microsatellite stable; MT, mutated type; pMMR, mismatch repair proficient; TKIs, tyrosine kinase inhibitors; VEGF(R), vascular endothelial growth factor (receptor); WT, wild type.

thyroid dysfunction (54/202, 26.7%). Grade ≥3 AEs were reported in 88 (43.6%) patients, including thrombocytopenia (19/202, 9.4%), leukopenia (14/202, 6.9%), neutropenia (11/202, 5.4%), abnormal hepatic function (10/202, 5.0%) and thyroid dysfunction (8/202, 4.0%). For the incidence of TRAEs in specific ICI-based combinations, no significant differences were observed among the 1L, 2L and 3L+ cohorts (Table SVIII). The AEs of the combined ICI and CT regimen were still mainly bone marrow hematopoietic function inhibition and gastrointestinal reactions, while the AEs of the ICI-TKI combination were mainly hand-foot skin reactions, edema, proteinuria and abnormal thyroid function. The addition of TKI and/or CT to ICI does not seem to significantly increase the occurrence and severity of irAEs in each cohort (Table SVIII). But we acknowledge that this finding is specific to our observation and does not constitute a direct comparison

Table II. Treatment patterns in the patient cohort.

Variables	1L	2L	3L+	P-value[a]
Total patients	38 (18.8)	59 (29.2)	148 (73.3)[b]	<0.0001
Initiated line of ICI[c]	38 (18.8)	41 (20.3)	123 (60.9)	<0.0001
ICI cross-line therapy	0 (0.0)	18 (8.9)	54 (26.7)[d]	<0.0001
Treatment regimens				
ICI ± CT ± RT	17 (44.7)	14 (23.7)	32 (21.6)	0.013
ICI alone	1 (2.6)	1 (1.7)	7 (4.7)	0.539
ICI + CT	12 (31.6)	8 (13.6)	19 (12.8)	0.016
ICI + RT	0 (0.0)	1 (1.7)	2 (1.4)	0.741
ICI + CT + RT	4 (10.5)	4 (6.8)	4 (2.7)	0.102
ICI + anti-VEGF(R)/EGFR/HER2 ± CT ± RT	20 (52.6)	24 (40.7)	44 (29.7)	0.022
ICI + anti-VEGF(R)/EGFR/HER2[e]	1 (2.6)	2 (3.4)	9 (6.1)	0.562
ICI + anti-VEGF(R)/EGFR/HER2 + CT	11 (28.9)	18 (30.5)	26 (17.6)	0.076
ICI + anti-VEGF(R)/EGFR/HER2 + RT	1 (2.6)	1 (1.7)	1 (0.7)	0.577
ICI + anti-VEGF(R)/EGFR/HER2 + CT + RT	7 (18.4)	3 (5.1)	8 (5.4)	0.017
ICI + TKI ± CT ± RT	1 (2.6)	20 (33.9)	115 (77.7)	<0.0001
ICI + TKI[f]	1 (2.6)	7 (11.9)	83 (56.1)	<0.0001
ICI + TKI + CT	0 (0.0)	10 (16.9)	21 (14.2)	0.033
ICI + TKI + RT	0 (0.0)	2 (3.4)	6 (4.1)	0.454
ICI + TKI + CT + RT	0 (0.0)	1 (1.7)	5 (3.4)	0.443
ICI + anti-VEGF(R)/EGFR/HER2 + TKI ± CT ± RT	0 (0.0)	1 (1.7)	5 (3.4)	0.443
ICI + anti-VEGF(R)/EGFR/HER2 + TKI	0 (0.0)	0 (0.0)	2 (1.4)	0.516
ICI + anti-VEGF(R)/EGFR/HER2 + TKI + CT	0 (0.0)	0 (0.0)	2 (1.4)	0.516
ICI + anti-VEGF(R)/EGFR/HER2 + TKI + RT	0 (0.0)	0 (0.0)	1 (0.7)	0.720
ICI + anti-VEGF(R)/EGFR/HER2 + TKI + CT + RT	0 (0.0)	1 (1.7)	0 (0.0)	0.205

[a]Pearson's χ^2 test and Fisher's exact test. [b]Due to the existence of ICI cross-line therapy, a patient could have multiple ICI-based treatments in the 3L+ setting in the statistical analysis. Therefore, the number of treatment regimens (196 times) is numerically greater than the total number of patients receiving treatment (148 cases). [c]ICI includes anti-PD-1 mAb (pembrolizumab, nivolumab, camrelizumab, toripalimab, sintilimab, tislelizumab and penpulimab), anti-PD-L1 mAb (atezolizumab, durvalumab, envafolimab and adebrelimab) and anti-PD-1/CTLA4 mAb (cadonilimab). [d]Among the 54 patients with ICI cross-line therapy in the 3L+ setting, 29 patients had immunotherapy initiation at 3L+ and the remaining 25 patients had immunotherapy initiation at 1L and/or 2L, among which 7 patients received 1L and 2L and 3L ICI cross-line therapy without interruption. [e]Anti-VEGF mAb (bevacizumab); anti-VEGFR mAb (ramucirumab); anti-EGFR mAb (cetuximab and nimotuzumab); anti-HER2 mAb (trastuzumab and pertuzumab); anti-HER2 ADC (disitamab vedotin and trastuzumab emtansine). [f]TKI: VEGFRi (regorafenib, fruquintinib, sulfatinib, apatinib and anlotinib), BRAFi (dabrafenib and vemurafenib), MEKi (trametinib), HER2i (pyrotinib, lapatinib and tucatinib), ALKi (lorlatinib). Values are expressed as n (%). 1L, first-line treatment; 2L, second-line treatment; 3L+, third-line or above treatment; CT, chemotherapy; EGFR, epidermal growth factor receptor; ICI, immune checkpoint inhibitor; RT, radiotherapy; TKIs, tyrosine kinase inhibitors; VEGF(R), vascular endothelial growth factor (receptor); HER2, human epidermal growth factor receptor 2.

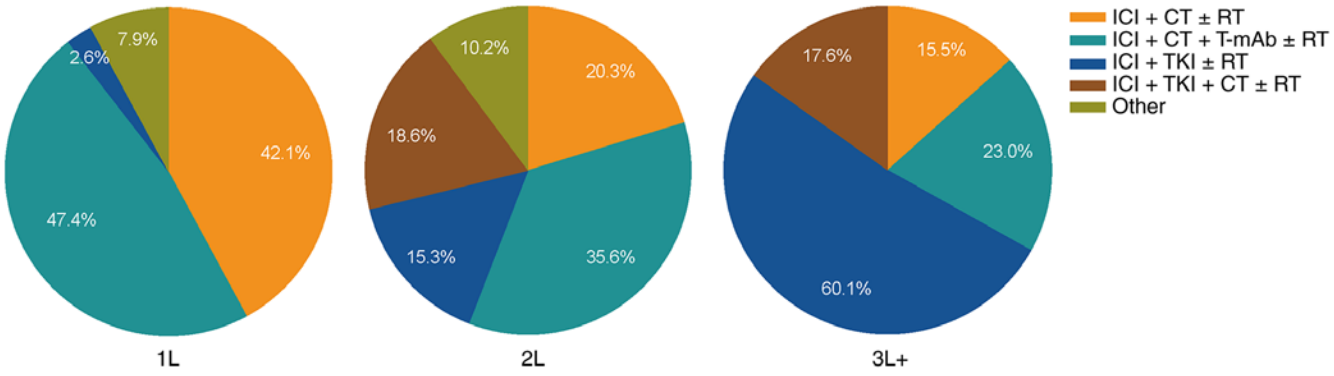

Figure 2. Major treatment regimens and proportions of patients in each cohort. 1L, first-line treatment; 2L, second-line treatment; 3L+, third-line or above treatment; CT, chemotherapy; ICI, immune checkpoint inhibitor; RT, radiotherapy; T-mAb, targeted monoclonal antibody against VEGF(R), EGFR or HER2; TKI, tyrosine kinase inhibitor.

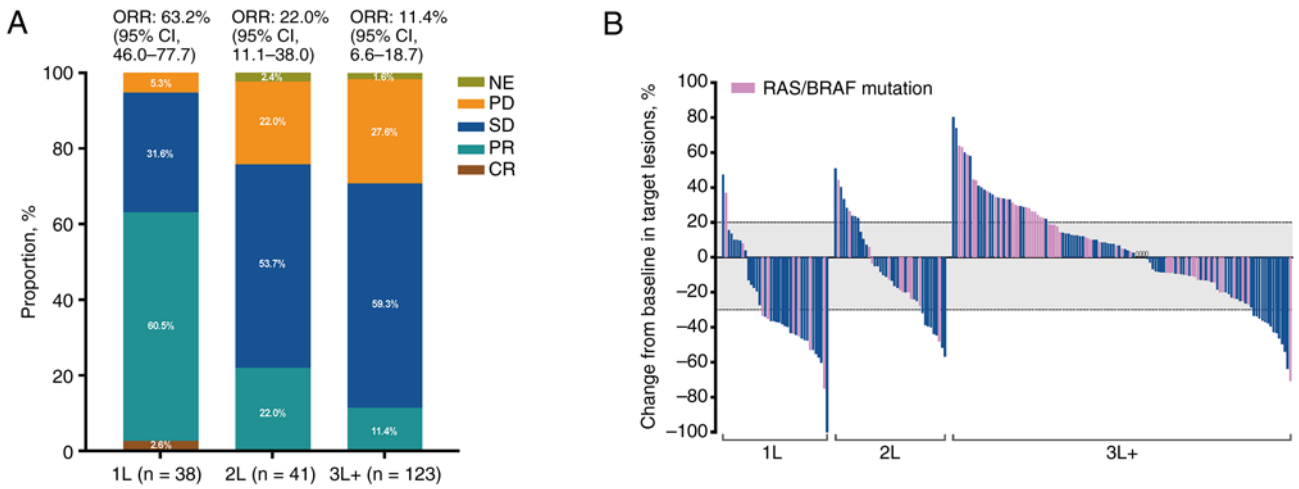

Figure 3. Tumor response. (A) Treatment efficacy in each cohort. (B) Waterfall plot showing the best percentage change in the size of the target lesions from the baseline in the efficacy-evaluable population in each cohort. The dashed lines at +20 and -30% indicate thresholds for progressive disease and partial response, respectively, according to RECIST v1.1. 1L, first-line treatment; 2L, second-line treatment; 3L+, third-line or above treatment; CI, confidence interval; CR, complete response; CT, chemotherapy; NE, not evaluable; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease.

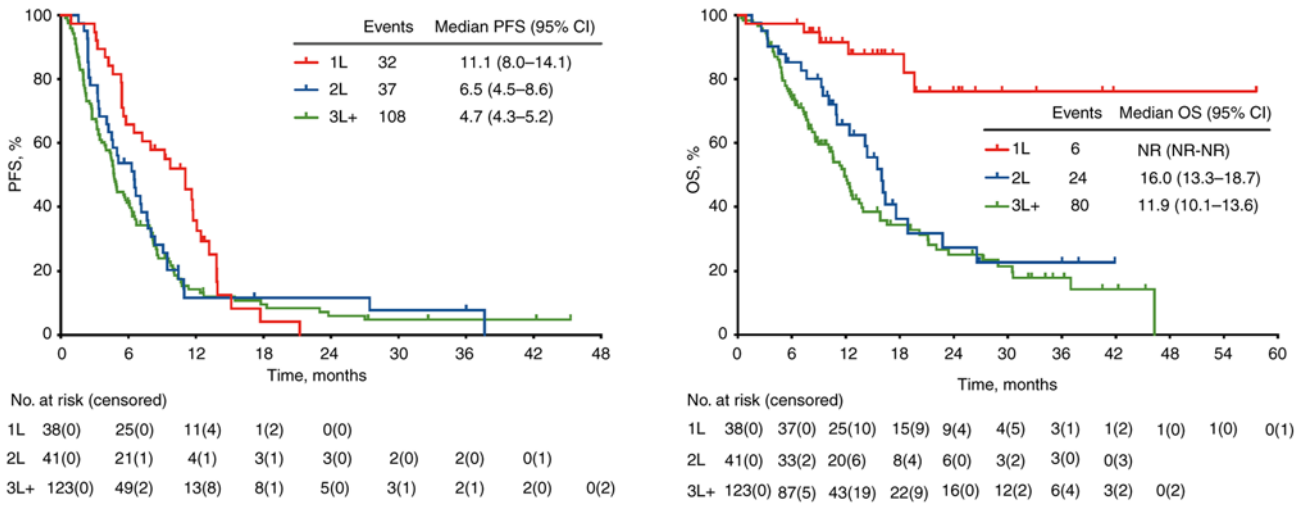

Figure 4. Kaplan-Meier curves of patients in each cohort. (A) PFS. (B) OS. 1L, first-line treatment; 2L, second-line treatment; 3L+, third-line or above treatment; CI, confidence interval; NR, not reached; OS, overall survival; PFS, progression-free survival.

with ICI monotherapy. The safety profile of each line of therapy was generally manageable and well-tolerated and no treatment-related deaths occurred.

The incidence of irAEs of any grade in the entire cohort was 38.6% (78/202). Most of the irAEs were graded 1-2. The incidence of grade ≥3 irAEs was 15.3% (31/202), including rash, abnormal liver function and hypothyroidism. All irAEs were controllable after appropriate symptomatic therapy.

Discussion

To the best of our knowledge, the present study is the first to explore the real-world ICI-based treatment patterns and clinical outcomes at different lines of therapy for patients with pMMR/MSS mCRC. The findings indicated that, unlike immuno-monotherapy, ICI-based combinations resulted in mixed results in patients with pMMR/MSS mCRC. An improved survival outcome can be realized by rational combination of ICI and chemotherapy and/or targeted agents. Notably, ICI in combination with TKI and ICI cross-line therapy may be clinically valuable options for heavily pretreated patients with mCRC at the 3L+ setting.

As is well-known, chemotherapy remains the cornerstone of mCRC treatment. Fluorouracil based doublet or triplet chemotherapy combined with anti-VEGF(R) or anti-EGFR targeted therapy is the standard 1L and 2L therapy for patients with mCRC, as recommended by the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology for Colorectal Cancer (21,22). The median PFS of 1L and 2L therapy is reported to be ~12 months and 6 months, respectively (23). Current clinical practice has shown that the breakthrough of further survival improvement is difficult. A number of efforts are being explored by researchers, including screening of benefit population and combining drugs with different mechanisms of action.

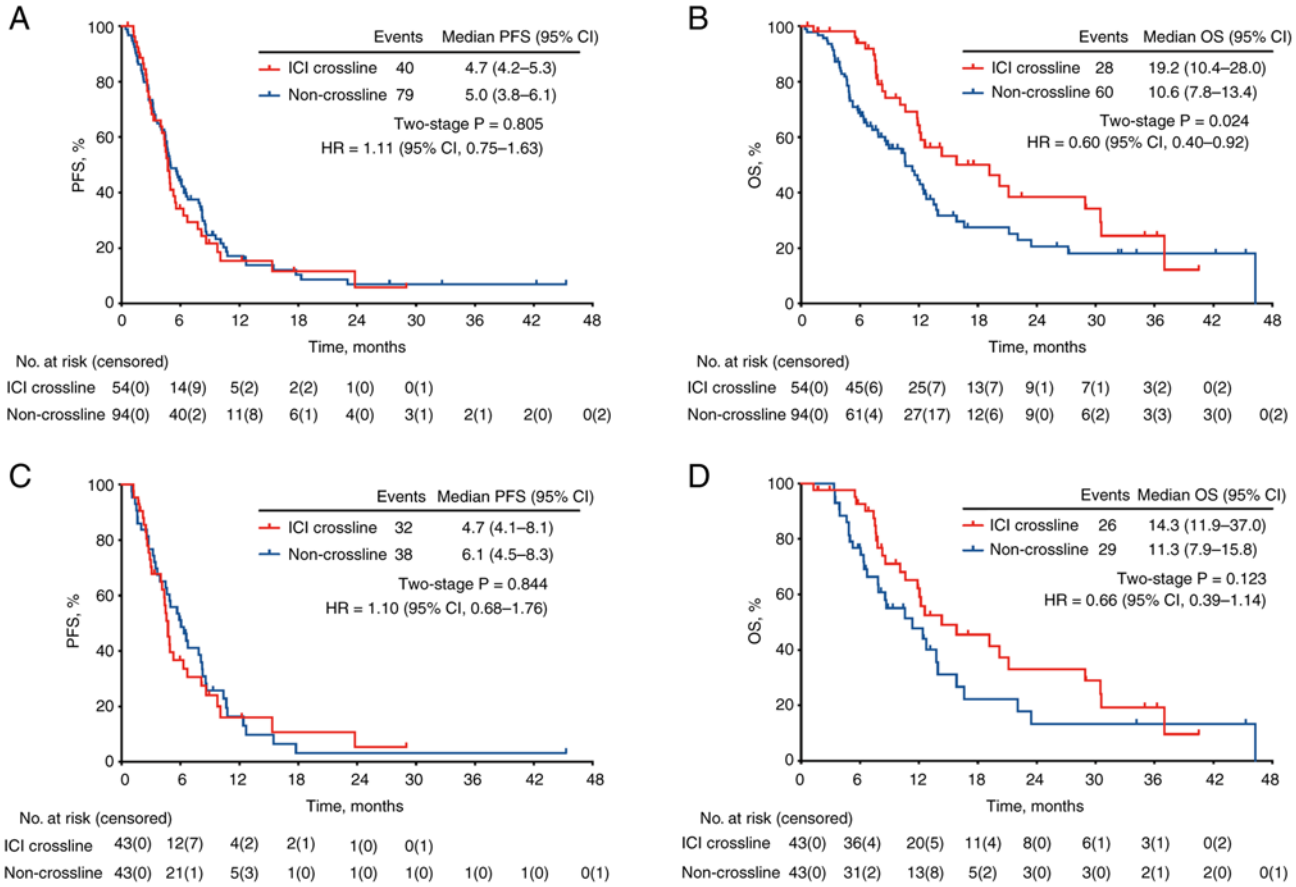

Figure 5. Kaplan-Meier curves of patients with or without ICI cross-line therapy in the 3L+ cohort before and after PSM. (A) PFS before PSM. (B) OS before PSM. (C) PFS after PSM. (D) OS after PSM. 3L+, third-line or above treatment; CI, confidence interval; HR, hazard ratio; ICI, immune checkpoint inhibitor; OS, overall survival; PFS, progression-free survival; PSM, propensity score matching.

The KEYNOTE-016 study explored the response of patients with metastatic MSI-H and MSS tumors to pembrolizumab and the results showed that the ORR of MSI-H patients was as high as 71%, while the ORR of MSS patients was 0%, with a median PFS time of 2.2 months and OS of 5.0 months (24). Patients with cancer harboring the dMMR/MSI-H phenotype are considered to be the right population for ICI therapy. However, the vast majority of patients with mCRC are pMMR/MSS type with low tumor mutation load and an immune-suppressed TME (known as 'cold tumor') and show a low response to ICIs (5). However, a series of clinical studies based on immunotherapy for pMMR/MSS mCRC have also been widely explored (25-30). The single-arm phase II NIVACOR trial evaluated the efficacy of nivolumab combined with FOLFOXIRI and bevacizumab as the 1L treatment for patients with RAS/BRAF-mutated mCRC (26). The subgroup analysis of 52 MSS patients showed an ORR of 78.9% and a median PFS of 9.82 months. The BBCAPX study evaluated sintilimab plus CPAEOX and bevacizumab in the 1L treatment of patients with RAS mutant and MSS mCRC, noting an ORR of 84% and a median PFS of 18.2 months. In the study, some CR/PR patients converted into the 'immune-hot' subtype after therapy (27). The AtezoTRIBE study assessed the efficacy of atezolizumab plus FOLFOXIRI and bevacizumab vs. FOLFOXIRI plus bevacizumab as the 1L treatment for mCRC (28). The median PFS time increased by 1.6 months in the ICI group (13.1 vs. 11.5 months; P=0.012) in the whole population, but there was no statistical difference in the PFS time of the pMMR/MSS sub-group (12.9 vs. 11.4 months; P=0.071). The updated data published in the 2023 ASCO meeting suggested that patients with a high immune score may benefit from this regimen (29). The Checkmate 9X8 trial did not show a significant difference in either the median PFS (11.9 vs. 11.9 months) or OS (29.2 months vs. not reached) times regarding nivolumab plus standard-of-care (SOC; FOLFOX and bevacizumab) vs. SOC in the 1L treatment of mCRC; however, higher PFS rates after 12 months (28 vs. 9%), ORR (60 vs. 46%) and durable responses (12.9 vs. 9.3 months) were observed in the ICI group (30).

In the present study, the results showed that the overall ORR of the 1L ICI cohort was 63.2% (95% CI, 46.0-77.7), the median PFS time was 11.1 months (95% CI, 8.0-14.1) and the OS was not reached. Compared with the aforementioned prospective studies that evaluated ICI combined with chemotherapy and anti-angiogenic targeted therapy as 1L treatment, the PFS time of patients receiving this triple regimen in the present study was 9.2 months and the ORR was 63.6%. The data were generally consistent, but also exhibited certain differences, which were related to multiple factors such as the nature of each study, sample size, genotype and ethnicity of the enrolled patients. Additionally, in comparison to historical data on 1L SOC, the incorporation of ICIs did not result in a statistically significant extension of PFS time in the pMMR/MSS subpopulation. This observation is most clearly exemplified

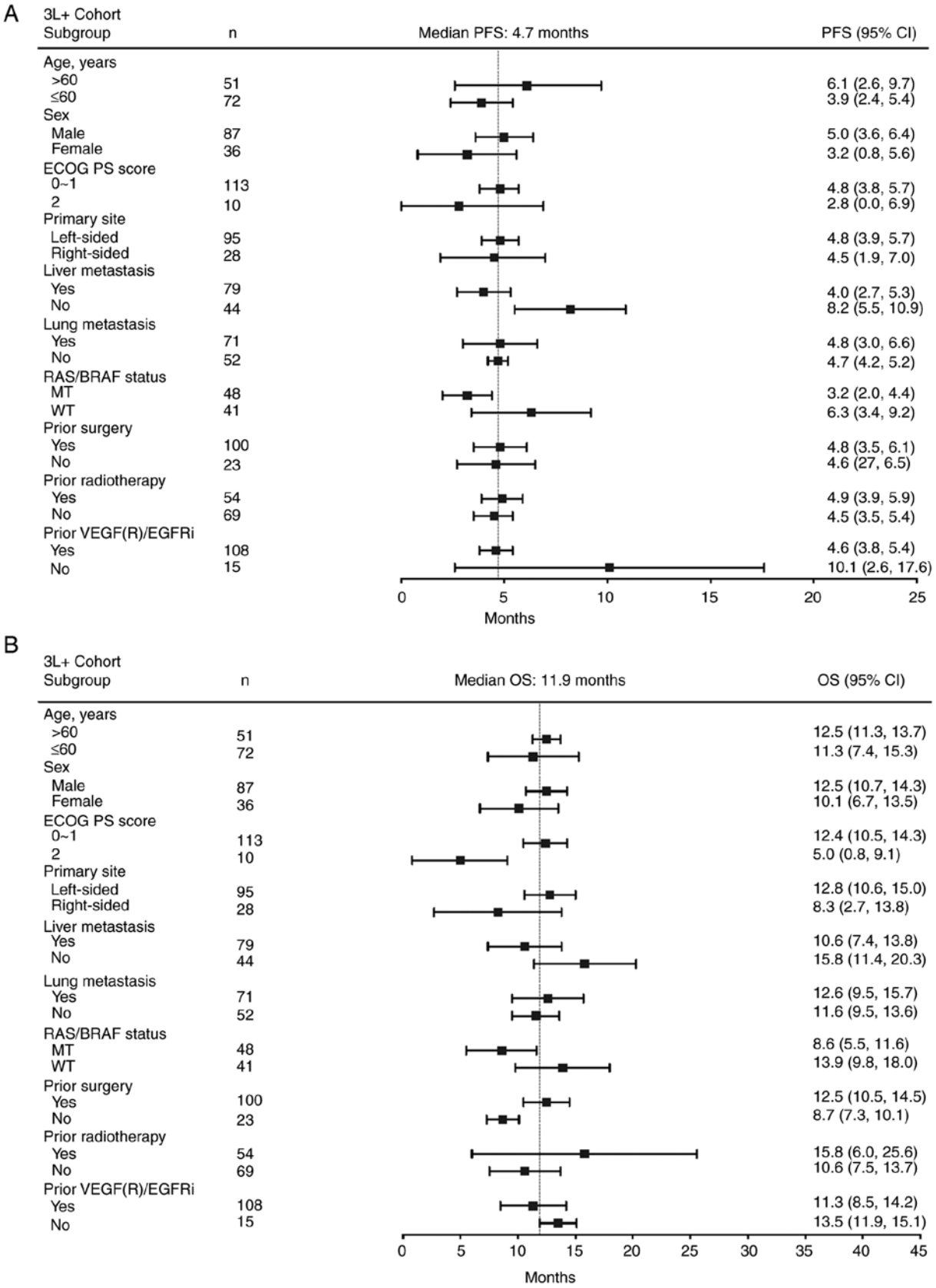

Figure 6. Univariate analysis and forest plots for PFS and OS in the 3L+ cohort. (A) PFS. (B) OS. 3L+, third-line or above treatment; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; MT, mutated type; OS, overall survival; PFS, progression-free survival; WT, wild type.

by the subgroup analysis of the AtezoTRIBE study, which reported a median PFS time of 12.9 months vs. 11.4 months (P>0.05) (28). Further investigation and biomarker exploration to identify subgroups of patients with mCRC that may benefit from ICI-based combinations is warranted. However, it has recently been shown that a small subset of patients with MSS

Table III. Univariate and multivariate analyses of the risk factors for PFS and OS in the 3L+ cohort.

	PFS				OS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
Variables	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, >/≤60 years	0.61 (0.42-0.88)	0.010	0.58 (0.35-0.98)	0.041	0.97 (0.62-1.52)	0.890	-	-
Sex, male/female	0.54 (0.34-0.86)	0.002	0.67 (0.39-1.14)	0.141	0.69 (0.42-1.14)	0.111	-	-
ECOG PS, 0-1/2	0.55 (0.23-1.27)	0.061	-	-	0.35 (0.13-0.98)	0.001	0.24 (0.11-0.53)	<0.001
Primary site, left/right	0.92 (0.60-1.46)	0.729	-	-	0.65 (0.37-1.16)	0.093	-	-
Liver metastasis, yes/no	1.97 (1.35-2.87)	0.001	2.08 (1.24-3.50)	0.006	1.50 (0.96-2.33)	0.076	-	-
Lung metastasis, yes/no	1.02 (0.69-1.49)	0.934	-	-	0.87 (0.56-1.37)	0.547	-	-
RAS/BRAF status, MT/WT	1.62 (1.03-2.54)	0.031	1.83 (1.11-3.02)	0.018	1.68 (0.99-2.87)	0.048	1.75 (1.01-3.03)	0.046
Prior surgery, yes/no	0.70 (0.41-1.19)	0.130	-	-	0.61 (0.32-1.16)	0.069	-	-
Prior radiotherapy, yes/no	0.77 (0.53-1.12)	0.171	-	-	0.76 (0.49-1.18)	0.222	-	-
Prior VEGF(R)/EGFRi, yes/no	1.97 (1.21-3.21)	0.028	2.23 (0.79-6.33)	0.131	1.33 (0.73-2.42)	0.391	-	-

3L+, third-line or above treatment; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFRi, epidermal growth factor receptor inhibitor; HR, hazard ratio; MT, mutated type; OS, overall survival; PFS, progression-free survival; VEGF(R)i, vascular endothelial growth factor (receptor) inhibitor; WT, wild type.

CRC harboring POLE and POLD1 proofreading deficiency respond well to ICI therapy (31). CRC is no longer considered a single unique disease and has complex molecular subtypes, each of which have distinct genetic and epigenetic signatures (such as chromosomal instability and CpG island methylator phenotype) (32). Incorporating genomic profiling (such as POLE/POLD1 mutations) into the routine clinical practice of CRC is technically feasible with existing sequencing platforms. However, its clinical utility remains limited. These mutations are rare in MSS mCRC (<2%), making routine screening inefficient (33). Although they may predict optimal immunotherapy responses in a small subset, robust prospective validation is lacking. At present, this approach is best reserved for clinical trials or comprehensive molecular profiling in refractory cases. Prioritizing standard biomarkers (such as programmed death-ligand 1 (PD-L1), tumor mutational burden and MSI/dMMR) remains the evidence-based standard.

For the 2L treatment of pMMR/MSS mCRC, chemotherapy consisting of fluorouracil, irinotecan and/or oxaliplatin remains an important component. In addition, continuing anti-angiogenesis therapy (such as bevacizumab) beyond PD has been strongly supported by the data from a series of phase III trials (34-37). However, the median PFS is still only 5-6 months with this method. At present, studies on ICI therapy for pMMR/MSS mCRC at the 2L setting are limited. In the REGOMUNE trial, 5/47 (11%) patients with MSS mCRC received the regorafenib-avelumab combination as ≥2L treatment. The PFS of the 43 efficacy-evaluable patients was 3.6 months (95% CI, 1.8-5.4) and the 6-month ORR was 0% (7). In addition, regorafenib monotherapy as the 2L treatment for RAS-mutant mCRC was explored in the STREAM study, with an ORR of 10.9% and a PFS of 3.6 months (95% CI, 1.9-6.7) observed (38). In the present study, the 2L ICI combined with TKI showed a PFS of 6.3 months and an ORR of 14.3%. Therefore, it seems that the 2L treatment of ICI plus TKI exerts certain advantages in terms of both median PFS and ORR. However, the survival data for the 2L setting was not available separately in the REGOMUNE trial. Additionally, the MSI status of the patients in the STREAM study was unclear and the treatment was a chemotherapy-free, single-agent TKI. Therefore, these data cannot be directly compared.

We previously evaluated the efficacy of TKI (fruquintinib) combined with chemotherapy as a 2L treatment for patients with pMMR/MSS mCRC. The updated results presented at the ASCO meeting in 2024 showed a median PFS of 6.9 months (95% CI, 5.2-8.6) and an ORR of 26.0% (95%

Table IV. Safety of the treatments.

Variables	Any grade AE				Grade ≥3 AE			
	1L, n=38	2L, n=41	3L+, n=123	P-value[a]	1L, n=38	2L, n=41	3L+, n=123	P-value[a]
All AEs	37 (97.4)	38 (92.7)	116 (94.3)	0.645	17 (44.7)	23 (56.1)	48 (39.0)	0.160
irAEs	13 (34.2)	13 (31.7)	52 (42.3)	0.400	6 (15.8)	7 (17.1)	18 (14.6)	0.929
Leukopenia	19 (50.0)	10 (24.4)	36 (29.3)	0.028	3 (7.9)	3 (7.3)	8 (6.5)	0.952
Neutropenia	12 (31.6)	13 (31.7)	22 (17.9)	0.078	3 (7.9)	2 (4.9)	6 (4.9)	0.761
Thrombocytopenia	20 (52.6)	18 (43.9)	29 (23.6)	0.001	6 (15.8)	3 (7.3)	10 (8.1)	0.323
Anemia	17 (44.7)	16 (39.0)	26 (21.1)	0.006	1 (2.6)	2 (4.9)	3 (2.4)	0.721
Nausea and/or vomiting	10 (26.3)	8 (19.5)	21 (17.1)	0.451	0 (0.0)	1 (2.4)	0 (0.0)	0.139
Diarrhea	4 (10.5)	7 (17.1)	18 (14.6)	0.702	0 (0.0)	1 (2.4)	5 (4.1)	0.424
Electrolyte disturbance	15 (39.5)	8 (19.5)	37 (30.1)	0.151	1 (2.6)	1 (2.4)	6 (4.9)	0.705
Abnormal liver function	17 (44.7)	14 (34.1)	32 (26.0)	0.084	1 (2.6)	3 (7.3)	6 (4.9)	0.630
Ileus	0 (0.0)	2 (4.9)	12 (9.8)	0.099	0 (0.0)	0 (0.0)	3 (2.4)	0.376
Thromboembolic event	1 (2.6)	1 (2.4)	3 (2.4)	0.998	0 (0.0)	1 (2.4)	1 (0.8)	0.523
Infusion-related reaction	1 (2.6)	2 (4.9)	4 (3.3)	0.844	0 (0.0)	0 (0.0)	2 (1.6)	0.523
Neurotoxicity	8 (21.1)	6 (14.6)	10 (8.1)	0.082	0 (0.0)	0 (0.0)	0 (0.0)	>0.999
Proteinuria	6 (15.8)	6 (14.6)	19 (15.4)	0.989	0 (0.0)	2 (4.9)	4 (3.3)	0.424
Urine occult blood	6 (15.8)	7 (17.1)	22 (17.9)	0.955	1 (2.6)	2 (4.9)	3 (2.4)	0.721
Hypoalbuminemia	5 (13.2)	6 (14.6)	19 (15.4)	0.941	0 (0.0)	0 (0.0)	1 (0.8)	0.724
Hypertension	2 (5.3)	5 (12.2)	23 (18.7)	0.109	0 (0.0)	0 (0.0)	1 (0.8)	0.724
Hyperglycemia	2 (5.3)	4 (9.8)	11 (8.9)	0.730	1 (2.6)	1 (2.4)	3 (2.4)	0.998
Mucositis[b]	3 (7.9)	4 (9.8)	16 (13.0)	0.642	0 (0.0)	1 (2.4)	1 (0.8)	0.523
Hoarseness	6 (15.8)	5 (12.2)	27 (22.0)	0.334	0 (0.0)	1 (2.4)	0 (0.0)	0.139
Hand-foot skin reaction	3 (7.9)	8 (19.5)	39 (31.7)	0.008	1 (2.6)	2 (4.9)	4 (3.3)	0.844
RCCEP	2 (5.3)	2 (4.9)	8 (6.5)	0.912	0 (0.0)	1 (2.4)	1 (0.8)	0.523
Rash	2 (5.3)	5 (12.2)	14 (11.4)	0.510	0 (0.0)	0 (0.0)	1 (0.8)	0.724
Myasthenia gravis	0 (0.0)	0 (0.0)	2 (1.6)	0.523	0 (0.0)	0 (0.0)	1 (0.8)	0.724
Immune myositis	1 (2.6)	0 (0.0)	5 (4.1)	0.410	0 (0.0)	0 (0.0)	0 (0.0)	>0.999
Cardiac dysfunction	3 (7.9)	5 (12.2)	11 (8.9)	0.776	1 (2.6)	0 (0.0)	2 (1.6)	0.614
Thyroid dysfunction	10 (26.3)	12 (29.3)	32 (26.0)	0.918	2 (5.3)	2 (4.9)	4 (3.3)	0.810
Pneumonitis[c]	7 (18.4)	6 (14.6)	15 (12.2)	0.616	1 (2.6)	1 (2.4)	2 (1.6)	0.902

[a]Pearson's χ^2 test or Fisher's exact test. [b]Mucositis includes events of stomatitis, oral mucositis and oral inflammation. [c]Pneumonitis includes events of lung infection, interstitial pneumonia and viral pneumonia. Values are expressed as n (%). 1L, first-line (treatment); 2L, second-line (treatment); 3L+, third-line or above (treatment); AEs, adverse events; irAEs, immune-related adverse events; RCCEP, reactive cutaneous capillary endothelial proliferation.

CI, 17.9-36.2) (39). In the present study, 41 patients received ICI-based combinations (mainly ICI plus chemotherapy and targeted therapy) as the 2L treatment and exhibited a PFS time of 6.5 months (95% CI, 4.5-8.6), an OS time of 16.0 months (95% CI, 13.3-18.7) and an ORR of 22.0% (95% CI, 11.1-38.0). Although the vast majority of the 2L cohort patients also received chemotherapy and/or targeted agents, the results were no better than the data we previously reported for 2L therapy without ICIs. It is worth noting that there may be certain differences between the present real-world study and the previously published clinical trials. Baseline characteristics such as the patient's ethnicity, gene mutation status, comorbidities, different ICIs (programmed cell death protein 1 or PD-L1 inhibitors), chemotherapies (triplet, doublet or a single-agent), targeted agents (bevacizumab, regorafenib or fruquintinib) and various combination strategies are all factors that need to be taken into account when interpreting these results.

Clinical trials of ICI-based combinations in the 3L+ treatment for MSS mCRC have been widely developed and have reported mixed outcomes (6-11). Nivolumab plus regorafenib provided an ORR of 33% and median PFS time of 7.9 month in 25 patients with MSS mCRC (6). Pembrolizumab in combination with lenvatinib showed an ORR of 22% and a median PFS time of 2.3 months (40). Durvalumab plus cabozantinib revealed an ORR of 27.6% and a median PFS time of 4.4 months in 29 heavily treated patients with pMMR/MSS mCRC (41). Tislelizumab plus fruquintinib and stereotatic body radiotherapy exhibited an ORR of 26% and a PFS

time of 8.5 months (9). Treatment with atezolizumab plus capecitabine and bevacizumab has shown a median PFS time of 4.4 months in patients with refractory MSS mCRC (42). Tislelizumab combined with fruquintinib and fecal microbiota transplantation have demonstrated an ORR of 20% and a median PFS time of 9.6 months (10). In the present study, the results showed that the ORR of ICI-based strategies for pMMR/MSS mCRC at the 3L+ setting was 11.4%, the median PFS time was 4.7 months (95% CI, 4.3-5.2) and the OS was 11.9 months (95% CI, 10.1-13.6). The ICI-TKI combination accounted for 77.7%, including 60.1% of ICI+TKI±RT and 17.6% of ICI+TKI+CT±RT. Although there is still a gap compared with the survival outcomes reported in some of the above-mentioned clinical trials, as real-world study data, it seems to be better than the results of 3L treatment drugs such as regorafenib, fruquintinib and TAS102 recommended by the current NCCN guidelines for patients with mCRC. The scientific rationality of TKI combined with ICI is mainly reflected in the following aspects. On the one hand, small molecule TKIs, particularly anti-angiogenic TKIs (such as those targeting VEGFR), could normalize tumor vasculature, improve blood perfusion, decrease hypoxia, reduce the formation of new blood vessels, induce immunogenic cell death, activate dendritic cells, enhance T-cell response and infiltration, directly or indirectly affect checkpoint expression, reverse immunosuppressive signals that favor ICI resistance and alter glucose and amino acid metabolism in the TME (43). Ultimately, TKIs synergistically enhance the antitumor effect of ICIs by reshaping the TME into an immune-supportive type. On the other hand, the positive feedback loop between ICI-induced immune reprogramming and TKI-mediated normalization of tumor vascular further promotes the killing and clearance of tumor cells mediated by antitumor immunity.

In the present study, a directional OS improvement (14.3 vs. 11.3 months; HR, 0.66; 95% CI, 0.39-1.14; P=0.123) was observed among patients who received ICI cross-line therapy at the 3+ setting, especially during the initial 3-year follow-up period. Due to the limited variety of 3L+ drugs available, ICI reinduction or sequential use is relatively common in 3L+ treated patients with mCRC without rare mutations. These preliminary results may provide valuable support for further studies on ICI cross-line therapy in patients with pMMR/MSS CRC. However, specific biomarkers and molecular events should be investigated to optimize patient identification for this ICI-based cross-line strategy.

Patients with colorectal liver metastasis (CRLM) are historically characterized by low immune responses and poor survival outcomes (44). Similar results were observed in the present study. Patients with CRLM in the 3L+ cohort had a significantly poorer median PFS time (4.0 vs. 8.2 months; P=0.001) than those without liver metastasis. CRLM was also the independent risk factor most significantly associated with PFS (HR, 2.08; 95% CI, 1.24-3.50; P=0.006). This may be closely related to the immunosuppressive TME in patients with CRLM (45). Animal studies have found that CRLM can recruit and siphon peripheral circulating $CD8^+$ T cells to hepatocytes and then interact with $FasL^+$ monocyte-derived macrophages to drive antigen-specific Fas^+ $CD8^+$ T cell apoptosis, thereby shaping an immune-excluded TME and mediating ICI resistance (46). Rational liver metastases-oriented approaches have been designed to attenuate immune resistance and stimulate immune response, such as ICI-based combinations with radiotherapy, radiofrequency ablation, antiangiogenic agents, cytotoxic drugs, oncolytic viruses, vaccines and bispecific antibodies.

The safety data of ICI-based combinations in the present study were consistent with those reported in other clinical studies (7-10). Most of the TRAEs were graded 1-2 and all the AEs were manageable. Later-line ICI therapy was not reported to have a higher incidence of irAEs than front-line ICI therapy in the present study. Moreover, the occurrence of grade ≥3 irAEs at front-line settings is not the only determining factor for the subsequent ICI cross-line therapy. This also requires a comprehensive consideration of the survival benefits and physical condition of the patient. In addition, improved clinical outcomes of ICI cross-line therapy were seen in patients with discontinuation of front-line ICI treatment caused by irAEs other than by disease progression. Exploration of potential mechanisms underlying the differences in AEs among various ICI-based combinations is needed for individualized treatment of clinical patients.

Several limitations exist in the present study. First, it was an observational study, which may have introduced some selection bias. Second, the lack of a control group makes it challenging to compare the results with other treatment options directly. Third, due to the retrospective nature of the present study, recall bias may exist and some results, including AEs, may have been missed during data collection. Fourth, the PD-L1 and genomic profiling of most patients were unavailable. Fifth, the limited sample size reduced the statistical power. Moreover, the follow-up time was short and the OS of the 1L cohort was not reached. Last, real-world study outcomes may be affected by a variety of confounding factors in routine clinical practice, such as the baseline characteristics and comorbidities of the patients. Therefore, real-world findings need to be interpreted with caution. Despite these limitations, the present results provide valuable information for ICI-based treatment in patients with pMMR/MSS mCRC. A large-sample prospective study with dynamic biopsy collection as well as genomic and immunologic profiling should be performed to validate the present results and identify valuable biomarkers.

In conclusion, the results of the present study suggest that chemotherapy still dominates 1L and 2L treatment for pMMR/MSS mCRC and that the addition of ICI does not seem to significantly extend the median PFS. The 3L+ treatment for pMMR/MSS mCRC patients mainly consists of ICI plus TKI, demonstrating manageable safety and improved survival.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

DDC and TZ conceived and designed the present study. WSZ and SJM acquired, analyzed and interpreted the data. WSZ and SJM performed the statistical analyses and drafted the manuscript. WSZ obtained funding for the present study. DDC and TZ revised the manuscript. DDC and TZ confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of Renmin Hospital of Wuhan University (approval no. 2023K-K067). The need for informed consent was waived by the ethics committee due to the retrospective nature of the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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