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***Retrospective Study***

**Real-world comparison of effectiveness and safety of chemotherapy plus bevacizumab with or without anti-PD-1 immunotherapy as first-line therapy in microsatellite stable, unresectable metastatic colorectal cancer: a multicenter retrospective cohort study**

Gao Z *et al.* Anti-PD-1 immunotherapy in MSS mCRC

**Abstract****BACKGROUND**

Microsatellite stable (MSS) metastatic colorectal cancer (mCRC) is characterized by an immunosuppressive tumor microenvironment, leading to limited efficacy of immunotherapy in these patients. Clinical trial data suggest that chemotherapy and anti-angiogenic therapy may have the potential to enhance the response to immunotherapy in these patients. However, whether these research findings can be “replicated” in clinical practice still requires further validation through real-world studies. This study aims to evaluate the effectiveness and safety of chemotherapy combined with bevacizumab with or without anti-programmed death 1 (PD-1) immunotherapy as the first-line regimen for MSS mCRC in the real world.

**AIM**

To evaluate the effectiveness and safety of chemotherapy combined with bevacizumab with or without anti-PD-1 immunotherapy as the first-line regimen for MSS mCRC in the real world.

## METHODS

We conducted a retrospective analysis of patients with MSS mCRC diagnosed at Peking University First Hospital and Jilin Cancer Hospital between January 2020 and December 2024. Patients were stratified into two treatment groups: (1) An experimental group receiving first-line chemotherapy combined with bevacizumab and anti-PD-1 immunotherapy, and (2) A control group receiving chemotherapy plus bevacizumab alone. Propensity score matching was employed to balance baseline characteristics, including age, gender, Eastern Cooperative Oncology Group performance status, number of metastatic sites, and primary tumor location. The primary endpoints were progression-free survival and overall survival, while secondary endpoints included disease control rate, objective response rate, and treatment-related adverse events. Survival outcomes were assessed using Kaplan-Meier analysis with log-rank testing. Additionally, inverse probability of treatment weighting was applied for sensitivity analysis to validate the robustness of our findings.

## RESULTS

The propensity score matching analysis identified 103 well-balanced patient pairs with a median follow-up of 25.5 months. The experimental group demonstrated numerically higher objective response (36.00% *vs* 23.08%,  $P = 0.309$ ) and disease control rates (96.00% *vs* 91.03%,  $P = 0.6759$ ) compared to the control group, though these differences were not statistically significant. Similarly, no significant survival benefit was observed for either progression-free survival [hazard ratio (HR) = 0.7076, 95% confidence interval (CI): 0.4069-1.23,  $P = 0.22$ ] or overall survival (HR = 1.154, 95%CI: 0.4712-2.827,  $P = 0.75$ ). Multivariate analysis identified liver metastases as an independent poor prognostic factor (HR = 3.36, 95%CI: 1.71-6.60,  $P < 0.001$ ), while subgroup analyses revealed potential benefits of the experimental regimen in male patients (HR = 0.33, 95%CI: 0.14-0.81,  $P = 0.025$ ) and those with right-sided primary tumors (HR = 0.40, 95%CI: 0.17-0.95,  $P = 0.022$ ). Safety profiles were comparable between groups, though elevated lactate

dehydrogenase emerged as an independent risk factor for poorer outcomes in the experimental group (HR = 4.11, 95%CI: 1.02-16.55,  $P = 0.046$ ).

## CONCLUSION

Chemotherapy combined with bevacizumab and anti-PD-1 immunotherapy could not demonstrate promising efficacy in treating MSS mCRC compared to the standard first-line chemotherapy regimen with bevacizumab. Male patients or those with right-sided mCRC may derive benefits from immune-based combination therapy. Further research is needed to investigate specific clinical characteristics or biomarkers to identify patients who may derive benefit from combined immunotherapy approaches.

**Key Words:** Microsatellite stable; *RAS* mutation; Metastatic colorectal cancer; Immune checkpoint inhibitors; Programmed death 1

Gao Z, Wang XY, Shen ZG, Liu JH, Wang XY, Wu SK, Jin X. Real-world comparison of effectiveness and safety of chemotherapy plus bevacizumab with or without anti-PD-1 immunotherapy as first-line therapy in microsatellite stable, unresectable metastatic colorectal cancer: a multicenter retrospective cohort study. *World J Gastroenterol* 2025; In press

**Core Tip:** Patients with microsatellite stable metastatic colorectal cancer typically exhibit an immunosuppressive tumor microenvironment and demonstrate a low response rate to immunotherapy. Clinical trial data suggest that chemotherapy and anti-angiogenic therapy may have the potential to enhance the response to immunotherapy in these patients. However, whether these research findings can be “replicated” in clinical practice still requires further validation through real-world studies. This study aims to evaluate the effectiveness and safety of chemotherapy combined with bevacizumab with or without anti-programmed death 1 immunotherapy as the first-line regimen for microsatellite stable metastatic colorectal cancer in the real world.

## **INTRODUCTION**

The standard treatment paradigm for metastatic colorectal cancer (mCRC) involves sequential fluorouracil-based chemotherapy (with oxaliplatin or irinotecan), VEGF inhibitors (primarily bevacizumab), and EGFR-targeted therapies (for RAS wild-type tumors)[1,2]. Despite these options, clinical outcomes remain suboptimal, with median progression-free survival (PFS) of 11 months for first-line therapy[3], 8.7 months for second-line chemo-antiangiogenic combinations[3], and 5.6 months for third-line trifluridine/tipiracil plus bevacizumab[4]. Targeted-immunotherapy combinations demonstrate particularly poor outcomes (median PFS: 1.8 months)[5]. These effects highlight the urgent need for new treatment strategies.

While immune checkpoint inhibitors (ICIs) have revolutionized treatment for dMMR/MSI-H colorectal cancer (CRC)[6], their efficacy remains limited in microsatellite stable (MSS) disease[7], which constitutes approximately 90% of proficient mismatch repair (pMMR) CRC cases[8]. Recent clinical trials exploring combination strategies demonstrate promising results: The AtezoTRIBE study reported improved PFS [12.9 months *vs* 11.4 months, hazard ratio (HR) = 0.78] with atezolizumab added to FOLFOXIRI/bevacizumab in pMMR patients[9]. The phase II CheckMate 9 × 8 trial investigated the efficacy of leucovorin, fluorouracil, and oxaliplatin (FOLFOX) chemotherapy combined with bevacizumab and nivolumab *vs* FOLFOX plus bevacizumab [SOC (standard of care)] as first-line treatment for unresectable CRC patients. Results demonstrated that adding atezolizumab to first-line FOLFOXIRI plus bevacizumab may prolong PFS in mCRC, particularly benefiting patients with dMMR, high tumor mutational burden, or high immune scores, without additional safety concerns. Immunoscore-IC may serve as a predictive biomarker for immunotherapy response in colorectal cancer[10]. Similarly, the METIMMOX trial showed a PFS benefit (6.6 months *vs* 5.6 months) for FLOX chemotherapy combined with nivolumab *vs* chemotherapy alone in MSS mCRC[11].

Based on the aforementioned research findings, both chemotherapy and bevacizumab can induce an immune-enriched tumor phenotype, thereby creating a more favorable immune microenvironment for immune checkpoint blockade. We have initiated a multicenter retrospective cohort study to evaluate the safety and efficacy of chemotherapy combined with bevacizumab and anti-programmed death 1 (PD-1) immunotherapy as the first-line treatment of MSS mCRC in the real world.

## **MATERIALS AND METHODS**

### ***Study design and participants***

This study employed a multicenter retrospective cohort research design. Patients with MSS mCRC who were treated at Peking University First Hospital and Jilin Cancer Hospital between January 1, 2020 and December 30, 2024 were enrolled. The experimental group consisted of patients who received first-line treatment with chemotherapy combined with bevacizumab and anti-PD-1 immunotherapy, while the control group comprised patients who received conventional treatment (chemotherapy combined with bevacizumab).

Eligible patients met all of the following criteria: (1) Histologically confirmed unresectable metastatic colorectal adenocarcinoma (AJCC 8<sup>th</sup> edition stage IV) with measurable disease per RECIST 1.1; (2) pMMR/MSS status with wild-type BRAF; (3) Receiving either chemotherapy/bevacizumab/anti-PD-1 combination or chemotherapy/bevacizumab alone; (4) No prior radiotherapy or  $\geq 4$  weeks since last radiotherapy; and (5) Eastern Cooperative Oncology Group (ECOG) performance status  $\leq 2$ . The exclusion criteria included: dMMR/MSI-H status or BRAF mutations, symptomatic brain metastases, uncontrolled infections, gastrointestinal dysfunction impairing drug absorption, or high-risk gastrointestinal complications.

The primary chemotherapy regimens were oxaliplatin-based (FOLFOX or CAPEOX) or irinotecan-based (FOLFIRI). Anti-PD-1 agents included five approved inhibitors (penpulimab, pembrolizumab, sintilimab, tislelizumab, toripalimab), combined with bevacizumab.

This retrospective study received ethical approval from Peking University First Hospital and Jilin Cancer Hospital with waived informed consent, adhering to Declaration of Helsinki principles while utilizing anonymized clinical data. The patient selection process is detailed in Figure 1.

### *Procedures*

Follow-up data were prospectively collected through multiple sources including hospital records, telephone interviews, outpatient visits, and rehospitalization records. The collected parameters encompassed: (1) Baseline characteristics (age, sex, height, weight, ECOG performance status); (2) Tumor-related factors (primary location, number of metastatic sites, differentiation grade); and (3) Treatment response metrics (percentage reduction in tumor volume, PFS, overall survival (OS), follow-up duration, and survival status).

Peripheral blood parameters were obtained within 7 days prior to initiating combination therapy (chemotherapy plus bevacizumab and anti-PD-1 immunotherapy). These included neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio, platelet-to-lymphocyte ratio, body mass index, advanced lung cancer inflammation index (= body mass index  $\times$  albumin/NLR), and systemic immune-inflammation index (= platelets  $\times$  neutrophils/lymphocytes).

The study's primary endpoint was PFS, defined as the interval from enrollment to first documented disease progression (RECIST v1.1) or death from any cause, whichever occurred first. Secondary endpoints comprised: (1) OS (time from enrollment to death, with censoring at last follow-up for surviving patients); (2) Objective response rate (ORR, proportion achieving complete or partial response per RECIST v1.1); (3) Disease control rate (DCR, proportion with complete/partial response or stable disease); and (4) Treatment-emergent adverse events (graded by CTCAE v4.0). The database was locked in January 2025 for final analysis.

### *Statistical analysis*

To minimize confounding effects between the experimental and control groups, we employed propensity score matching (PSM) with a 1:4 nearest-neighbor matching ratio, balancing key baseline characteristics including age, sex, ECOG performance status, metastasis pattern, and primary tumor location. Categorical variables were analyzed using  $\chi^2$  or Fisher's exact tests, while continuous variables were compared *via* Mann-Whitney *U* tests. Survival outcomes were evaluated using Kaplan-Meier analysis with log-rank testing, and independent prognostic factors were identified through Cox proportional hazards regression models [reporting HRs with 95% confidence intervals (CIs)].

For continuous hematological biomarkers, optimal prognostic cutoffs were determined using the survminer package's `surv_cutpoint` algorithm stratifying patients into high- and low-expression cohorts[12]. The predictive performance of hematological indicators for immunotherapy response in MSS mCRC was quantified through receiver operating characteristic curve analysis. All statistical computations were performed using R (version 4.4.2), with two-tailed *P*-values < 0.05 considered statistically significant.

### *Sensitivity analysis*

To mitigate potential biases and ensure the robustness of our findings, we conducted three complementary sensitivity analyses: (1) Comprehensive univariate and multivariate regression analyses; (2) PSM with varying matching ratios (1:1 to 1:3) to assess PFS and OS; and (3) Inverse probability of treatment weighting to adjust for baseline characteristics and evaluate treatment outcomes.

## **RESULTS**

### *Patients*

Following 1:4 PSM, the final cohort consisted of 103 patients, including 25 in the experimental group (chemotherapy plus bevacizumab and anti-PD-1 immunotherapy) and 78 in the control group (chemotherapy plus bevacizumab alone). The cohort

comprised 63 males (61.2%) and 40 females (38.8%), with 43 patients (41.7%) aged over 60 years. Clinicopathological characteristics revealed 39 cases (37.9%) of right-sided colon cancer, 88 patients (85.4%) who had undergone primary tumor resection, and 29 patients (28.2%) with metastases involving two or more organs. Molecular analysis showed that all patients exhibited pMMR, while RAS mutations were detected in 60 cases (58.3%). Detailed baseline characteristics are presented in Table 1, and covariate balance between the two groups was confirmed (Figure 1).

### *Effectiveness*

With a median follow-up duration of 25.5 months (as of December 2024), the overall cohort demonstrated a median PFS of 10.5 months and OS of 37.2 months. In subgroup analysis, the experimental group achieved a median PFS of 11.9 months compared to 9.7 months in the control group. However, this difference in PFS did not reach statistical significance (HR = 0.7076, 95%CI: 0.4069-1.23,  $P = 0.22$ ) (Figure 2). Similarly, no significant difference was observed in OS between groups (HR = 1.154, 95%CI: 0.4712-2.827,  $P = 0.75$ ) (Figure 3). Regarding response rates, the experimental group demonstrated an ORR of 36.00% vs 23.08% in the control group ( $P = 0.309$ ), while the DCR reached 96.00% and 91.03%, respectively ( $P = 0.6759$ ) (Table 2). Collectively, these results suggest comparable efficacy between the two treatment approaches without statistically significant differences in survival outcomes or response rates.

### *Sensitivity analysis*

In the unadjusted original cohort analysis, the experimental group showed significantly superior PFS compared to controls (HR = 0.414, 95%CI: 0.2462-0.6972,  $P = 0.037$ ; Figure 4), while OS remained comparable between groups (HR = 1.268, 95%CI: 0.7562-2.125,  $P = 0.61$ ; Figure 5). Cox regression analyses confirmed the proportional hazards assumption (all  $P > 0.05$ ) and identified absence of liver metastases as an independent predictor for improved PFS ( $P < 0.001$ ; Table 3). Sensitivity analyses using inverse probability of treatment weighting demonstrated consistent results for both PFS ( $P =$

0.2398; Figure 6) and OS ( $P = 0.5632$ ; Figure 7), with maintained balance in baseline characteristics after weighting (Figure 8). Notably, varying the PSM ratios (1:1 to 1:4) did not significantly alter the PFS (Figs. 8-10) or OS (Figs. 11-13) outcomes, reinforcing the robustness of primary findings.

### *Safety*

The safety evaluation revealed comparable toxicity profiles between treatment groups, with no statistically significant differences in adverse event incidence. While the experimental group exhibited numerically higher rates of grade 1-2 hypothyroidism (7.7% vs 0.0%,  $P = 0.057$ ) and rash (7.7% vs 0.0%,  $P = 0.057$ ) compared to controls, these differences did not reach statistical significance. Importantly, the incidence of grade 3-4 adverse events was similar between groups, demonstrating comparable treatment tolerability (Table 4).

### *Subgroup analysis*

Male patients demonstrated significantly improved outcomes with chemotherapy combined with bevacizumab and anti-PD-1 immunotherapy (HR = 0.33, 95%CI: 0.14-0.81,  $P = 0.015$ ). Similarly, patients with right-sided primary tumors showed enhanced treatment response (HR = 0.40, 95%CI: 0.17-0.95,  $P = 0.039$ ) (Figure 14).

### *Exploratory biomarker analysis*

Our investigation of baseline hematological parameters identified several factors associated with improved PFS in patients receiving chemotherapy combined with bevacizumab and anti-PD-1 immunotherapy. Univariate analysis demonstrated significant benefits for patients with right-sided colorectal cancer, normal carbohydrate antigen 199 levels, low NLR, high advanced lung cancer inflammation index, low systemic immune-inflammation index, low absolute leukocyte count, high red blood cell distribution width, and low lactate dehydrogenase (LDH) levels. Multivariate

analysis established LDH as an independent prognostic risk factor (HR = 4.11, 95%CI: 1.02-16.55,  $P = 0.046$ ) (Table 5).

The predictive value of LDH was further substantiated by receiver operating characteristic analysis, showing area under the curve values of 0.81, 0.71, and 0.79 for predicting treatment response at 9 months, 12 months, and 15 months respectively (Table 6, Figure 17). When stratifying patients by the optimal LDH cutoff value, survival analysis revealed significantly prolonged PFS in the low-LDH group compared to the high-LDH group ( $P = 0.013$ ).

## **DISCUSSION**

For patients with MSS CRC, who account for nearly 90% of the population, the overall efficacy of immunotherapy is poor[13]. Currently, strategies to overcome this challenge mainly include exploring biomarkers that can more accurately predict therapeutic efficacy or attempting to convert “cold tumors” into “hot tumors” through combination therapies[14]. For instance, studies have demonstrated that Immunoscore-IC is currently the most promising biomarker for predicting therapeutic benefits from immune combination therapy[15]. Notably, responders to immune combination therapy exhibited significantly higher densities of CD8<sup>+</sup> T cells, regulatory T cells, and M2 macrophages compared to non-responders[16]. Furthermore, extensive research has identified tertiary lymphoid structures (TLS) as key prognostic biomarkers in colorectal cancer[17]. A notable study revealed that the presence of mature TLS can independently predict the efficacy of ICIs in solid tumors, irrespective of programmed death-ligand 1 expression levels[18]. Given these findings, TLS holds significant potential in overcoming current challenges in immunotherapy response prediction. Among these, combined chemotherapy and targeted therapy are popular areas of exploration[19,20]. ICIs combined with chemotherapy has become the first-line standard treatment for many solid tumors[21-23]. Immunotherapy and chemotherapy complement each other, with potential mechanisms including synergistic effects, reduced inhibition, increased immune cells, and the formation of immune memory[24].

Recent years have witnessed numerous promising clinical trials investigating ICI combination therapies for MSS-type mCRC patients[25-27]. Among these, the combination of ICIs with anti-angiogenic agents and chemotherapy has demonstrated particularly remarkable efficacy, emerging as the most promising therapeutic strategy for MSS-type mCRC. The CheckMate 9 × 8 trial evaluated nivolumab plus mFOLFOX6 and bevacizumab as first-line treatment for mCRC. While the primary PFS endpoint wasn't met, the combination achieved a higher 12-month PFS rate[10]. Similarly, the NIVACOR trial assessed nivolumab with FOLFOXIRI/bevacizumab in *RAS/BRAF*-mutant mCRC, showing promising results in MSS subgroup analysis (ORR: 78.9%; DCR: 96.2%; median PFS: 9.8 months, 95%CI: 8.18-15.24)[28], suggesting potential benefits for MSS CRC patients from immune-chemotherapy combinations. The BBCAP study demonstrated outstanding efficacy of sintilimab plus bevacizumab and CapeOX in *RAS*-mutant/MSS mCRC (ORR: 84%; DCR: 100%; mPFS: 17.9 months in full analysis set), with manageable safety profiles[29]. The Capability-01 trial explored chidamide plus sintilimab ± bevacizumab in chemotherapy-refractory MSS/pMMR mCRC, achieving median PFS of 7.3 months (ORR: 44%; DCR: 72%) with favorable tolerability[30], providing a viable later-line option for traditionally ICI-resistant MSS/pMMR mCRC.

However, it must be acknowledged that clinical trials are highly selective in terms of patient enrollment, limiting their generalizability, and treatment effects may be overestimated. Moreover, in clinical practice, the treatment of MSS metastatic CRC is more complex due to drug availability and patient tolerance, including decisions on whether to use immune-chemotherapy combinations with bevacizumab, and the choice of immune drugs. In this study, we aimed to assess whether immune-chemotherapy combinations with bevacizumab for advanced MSS CRC in the real world are superior to traditional first-line chemotherapy regimens. Our results showed no significant differences in PFS ( $P = 0.22$ ) or OS ( $P = 0.75$ ) between the two groups.

Studies have found gender differences in immune characteristics among different cancer types[31]. For example, males with melanoma tend to have high levels of

immune response-related features[31], while females <sup>7</sup> with non-small cell lung cancer tend to have high levels of immune response-related features[32]. Our subgroup analysis showed that male patients are more likely to benefit from immune-combination therapy. For left- and right-sided CRC, single-cell transcriptome analysis revealed significant differences in immune suppression patterns of left- and right-sided CRC in the tumor microenvironment, with ICIs potentially being more effective for right-sided CRC[33]. Similarly, our subgroup analysis similarly found that patients with right-sided CRC may <sup>5</sup> benefit from immune-combination therapy.

Our multicenter <sup>5</sup> retrospective cohort study has several limitations that warrant consideration. Firstly, the retrospective design with a small sample size inherently limits data robustness. Although the experimental group showed a trend toward improved PFS with combination therapy, it failed to demonstrate statistical significance, likely due to insufficient statistical power. These results should therefore be interpreted with caution and require validation in larger prospective studies. Secondly, a critical gap in our analysis was the inability to assess programmed death-ligand 1 expression levels, precluding any evaluation of their potential correlation with anti-PD-1 immunotherapy efficacy. This biomarker information could have provided valuable insights into treatment response variability. Third, therapeutic heterogeneity may have impacted our findings. The study incorporated multiple chemotherapy regimens (FOLFOX, FOLFIRI, and CAPEOX) and five different anti-PD-1 agents, creating substantial variability that could obscure true efficacy comparisons between treatment approaches.

## **CONCLUSION**

Chemotherapy combined with bevacizumab and anti-PD-1 immunotherapy could not provide benefits for MSS mCRC patients in first-line therapy. The subgroup analysis indicates that male patients or those with right-sided mCRC may derive benefits from the combination therapy. LDH is an indicator for predicting the efficacy of combined immunotherapy.

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