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Retrospective Study

Evaluation of efficacy and safety of targeted therapy and immune checkpoint inhibitors in metastatic colorectal cancer

Targeted therapy + ICIs in mCRC

Abstract

BACKGROUND

Colorectal cancer is among the most prevalent and deadly cancers globally, particularly in China. Treatment challenges remain in advanced and metastatic cases, especially in third- and fourth-line settings. The combination of targeted therapies with immune checkpoint inhibitors (ICIs) has shown potential in addressing the limitations of single-agent treatments.

AIM

To evaluate the efficacy and safety of targeted therapy alone and in combination with ICIs for metastatic colorectal cancer(mCRC).

METHODS

A multicenter retrospective observational study was conducted to evaluate the efficacy and safety of targeted therapy alone and in combination with ICIs for mCRC. A total of 99 patients treated with regorafenib or fruquintinib, with or without ICIs, were enrolled. Propensity score matching (PSM) and inverse probability weighting (IPW) were employed to balance baseline characteristics. The primary endpoint was progression-free survival (PFS), while overall survival (OS) and safety were secondary.

RESULTS

Patients who received combined therapy showed significantly longer median PFS rates compared to those who underwenttargeted therapy in all analyses (original: 6.0 vs. 3.4 months, $P < 0.01$; PSM: 6.15 vs. 4.25 months, $P < 0.01$; IPW: 5.6 vs. 3.3 months, $P < 0.01$). Although the median OS showed a trend toward improvement in the combination group, the difference was insignificant. Cox regression analysis revealed that combining targeted therapy with ICIs significantly reduced the risk of disease progression (HR = 0.38, $P < 0.001$). Adverse events were generally manageable with both regimens, while serious adverse events (grade 3-4) were primarily hypertension, fatigue, and reduced platelet counts. All adverse events were controlled effectively by symptomatic treatment or discontinuation of the drug, and no treatment-related deaths were observed.

CONCLUSION

The combination of targeted therapy with ICIs offers a significant advantage in terms of PFS for patients with advanced mCRC, accompanied by a favorable safety profile. These findings underscore the benefits of combination therapy in this setting, warranting further investigation in larger prospective clinical trials.

Key Words: Metastatic Colorectal cancer, Targeted therapy, Immune checkpoint inhibitors, Progression-free survival, Combination therapy

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Core Tip: This study demonstrates that combining targeted therapy with immune checkpoint inhibitors (ICIs) significantly improves progression-free survival (PFS) in

patients with metastatic colorectal cancer (mCRC). The study also highlights the manageable safety profile of the combination therapy, offering a potential new approach for advanced mCRC treatment. Further research is needed to confirm these findings in larger trials.

INTRODUCTION

In 2022, colorectal cancer (CRC) was the second most common cancer among men in China and the second leading cause of cancer-related deaths among women[1]. At diagnosis, approximately 20-30% of patients show advanced disease, and nearly 50% will acquire metastases as the condition progresses[2]. Recent global cancer statistics indicate that CRC continues to exhibit elevated incidence and fatality rates worldwide[2]. The main objectives of treatment for metastatic colorectal cancer (mCRC) are to limit tumor growth, enhance quality of life, and prolong survival as much as feasible. Advanced CRC is currently treated with chemotherapy using cytotoxic agents such as fluorouracil, oxaliplatin, and irinotecan. These are the focused treatments that specifically target the epidermal growth factor receptor (EGFR),vascular endothelial growth factor (VEGF), and its receptors[3]. Despite traditional first- and second-line therapies, the majority of mCRC patients develop disease progression. The restricted availability of third- and fourth-line therapy alternatives represents a considerable challenge. In tumors, the development of supporting blood arteries becomes essential if they exceed 1-2 mm in size[4, 5]. Three receptors (VEGFR) and six ligands constitute the VEGF system. The most essential ligand is VEGF-A. It promotes endothelial cell (EC) differentiation, migration, development, and survival and is secreted by various cell types, including cancerous cells. VEGFR-2 is the primary receptor that mediatesVEGF-A signals in ECs; VEGFR-1 may have more regulatory and inhibitory roles; and VEGFR-3 is associated with the lymphatic system and lymphangiogenesis[6, 7].

The tumor microenvironment is typically hypoxic, resulting in elevated levels of VEGF-A, which promotes the development of highly permeable blood vessels, supplying nutrients to the tumor and aiding in early hematogenous spread. Therefore,

the inhibition of angiogenesis is essential[8]. At present, anti-angiogenic agents are primarily classified into two categories: Monoclonal antibodies (mAbs), including bevacizumab, aflibercept, and ramucirumab, and tyrosine kinase inhibitors (TKIs) such as apatinib, sorafenib, and pazopanib. Monoclonal antibodies function by directly binding to VEGF-A or obstructing the extracellular domain of VEGFR-2. Through entering the ATP "pocket" of the EGFR protein and suppressing phosphorylation and EGFR signaling activity, TKIs simultaneously disrupt angiogenic pathways by preventing VEGF from attaching to its receptor and interrupting the VEGF/VEGFR pathway, which lowers angiogenesis[9]. TKIs are useful in anti-tumor angiogenesis therapy because certain multi-target drugs, such as sorafenib, regorafenib, and pazopanib, can also inhibit the PDGFR and PDGF pathways, interfere with the recruitment and stabilization of vascular smooth muscle cells, inhibit the FGFR/FGF pathway, decrease endothelial cell proliferation, and suppress angiogenesis[10].

Anti-angiogenic targeted drugs have been used to treat metastatic CRC for over 18 years[11] as of 2025, and they are now a necessary treatment for resistant metastatic colorectal cancer. Recent examples of novel medications are fruquintinib and regorafenib. The multi-target kinase inhibitor regorafenib inhibits several proteins, including TIE2, BRAF, and VEGFR. By preventing tumor angiogenesis and changing the immune-suppressive condition of the tumor microenvironment, it can reduce the occurrence, growth, and spread of tumors[12]. Research from the CORRECT and CONCUR clinical trials showed that in mCRC patients who were not responding to traditional chemotherapy, regorafenib prolonged both OS and PFS[13, 14]. The Chinese FRESCO study and the international multicenter FRESCO-2 study demonstrated that fruquintinib, a highly selective VEGFR inhibitor (targeting VEGFR1, VEGFR2, and VEGFR3)[15], significantly extended overall survival (OS) and progression-free survival (PFS) in mCRC patients who had not responded to any conventional therapy options[16, 17]. In China, regorafenib and fruquintinib received approval in 2017 and 2018, respectively, for third-line treatment following the failure of conventional therapy

in advanced CRC. In clinical practice, the effectiveness of TKIs is frequently impeded by multiple causes, with resistance being the foremost concern.

Furthermore, the absence of strong predictive indicators hinders the precise selection of patients most likely to benefit, posing obstacles to targeted treatment regimens[18]. Moreover, while the adverse effects of TKIs are typically controllable, complications such as hypertension, proteinuria, bleeding, and thrombosis can occasionally escalate tosevere and potentially life-threatening levels. These factors pose socio-economic and medical safety challenges for the extensive application of TKIs in non-specific patient demographics.

Immune checkpoint inhibitors (ICI), particularly PD-1/PD-L1 inhibitors, have gained popularity due to their exceptional effectiveness in treating a variety of solid tumors[19]. However, their use in colorectal cancer has been restricted, mainly helping tumors with deficient mismatch repair (dMMR) or high microsatellite instability (MSI-H) phenotypes. Only 5–15% of all CRC cases are caused by these tumors, and the percentage is significantly smaller in cases of metastatic CRC[20]. The "cold" immunological microenvironment, which is defined by a low mutation burden and inadequate immune cell infiltration, is the leading cause of immunotherapy's limited efficacy in such scenarios.

Immune checkpoint inhibitors and anti-angiogenic therapy have demonstrated promise in recent years in enhancing the course of treatment for metastatic colorectal cancer (mCRC). Patients with a high tumor mutational burden (TMB-H) have been shown to benefit from the combination of immunotherapy and targeted therapy, underscoring the promise of biomarker-driven approaches to get around these restrictions[21]. Metastatic colorectal cancer usually has an immunosuppressive tumor microenvironment (TME), which is partly sustained by angiogenesis. Angiogenesis is encouraged, dendritic cell maturation is impeded, PD-L1 expression is elevated, and T-cell function is compromised by upregulated VEGF-A. Moreover, the abnormal tumor vasculature hinders the infiltration of effector T cells while facilitating the accumulation of regulatory T cells (Tregs), thereby accelerating the conversion of tumor-associated

macrophages (TAMs) into an immunosuppressive M2 phenotype, contributing to immune suppression. Tumorvasculature normalization, vascular permeability reduction, intratumoral pressure reduction, oxygen supplyenhancement, and immunosuppressive microenvironment alleviation are all possible with anti-angiogenic therapy. The immunological effects within the TME are increased, tumors become more susceptible to immune checkpoint blockade, ICI resistance is overcome, and effector T cell infiltration and effectiveness are increased due to this normalization. Through this process, "cold" tumors become "hot" tumors[22, 23].

Immunotherapy and targeted therapy have recently demonstrated great promise as backup treatments for mCRC. The effectiveness of fruquintinib in conjunction with anti-PD-1 immunotherapy in patients with advanced colorectal cancer was assessed in a retrospective research. According to the findings, combination therapy considerably increased overall survival (17.5 months vs. 11.3 months, P = 0.008) and median progression-free survival (5.9 months vs. 3.0 months, P = 0.009) when compared to monotherapy[24]. Another retrospective investigation indicated that combining fruquintinib with anti-PD-1 therapy increased anti-tumor responses in non-MSI-H/pMMR mCRC patients[25]. The REGONIVO study demonstrated that the combination of regorafenib and immunotherapy resulted in an objective response rate (ORR) of 33.3% in non-MSI-H/pMMR mCRC patients, in contrast to an ORR of less than 5% observed with PD-1 inhibitors, such as nivolumab, used alone. The median PFS of 7.9 months was significantly longer than that observed with previous monotherapy, which typically ranged from 3 to 4 months[26]. The integration of immunotherapy and targeted therapy has demonstrated encouraging outcomes in renal cell carcinoma, as evidenced by the KEYNOTE-426 and JAVELIN Renal 101 trials, which confirmed the effectiveness of pembrolizumab in conjunction with axitinib (Pembrolizumab/Axitinib) and avelumab in conjunction with axitinib (Avelumab/Axitinib) in metastatic renal cell carcinoma (mRCC)[27, 28].

Hence, we have initiated a multicenter retrospective study to examine the patterns of targeted therapy and the combination of such treatments with immune checkpoint

inhibitors in advanced mCRC. The goal was to determine whether this combined approach provides additional survival advantages. This study was designed to provide comprehensive insights to optimize treatment strategies for advanced mCRC, potentially influencing clinical practices by aiding clinicians in selecting the most effective and safest treatments for these complex cases. In conclusion, integrating anti-angiogenic therapy with immunotherapy represents a promising strategy for enhancing outcomes in mCRC patients. This integrative approach can address the shortcomings of using single agents by strengthening anti-tumor immune responses and could potentially extend survival benefits to patients. Future research should investigate the mechanisms and optimal application of this combination therapy further, opening new avenues in mCRC treatment.

MATERIALS AND METHODS

Study design and patients

This observational study focused on advanced or metastatic CRC patients who received either targeted or immunotherapy therapy, with PFS as the primary endpoint. In the real world, targeted immunotherapy combinations have demonstrated higher disease control rates and longer PFS than targeted therapy alone as a third-line treatment for microsatellite-stable (MSS) mCRC[29]. The participants were recruited from the Affiliated Hospital of North China University of Science and Technology, Tangshan People's Hospital, and Qinhuangdao People's Hospital. Eligible patients had histologically confirmed metastatic adenocarcinoma of the colon or rectum. Further inclusion criteria were as follows: Age 18–90 years; Unresectable advanced disease; Previous failure of at least one standard second-line therapy, including treatments with irinotecan, oxaliplatin, and fluoropyrimidines; Presence of at least one measurable lesion according to RECIST 1.1 criteria; Completion of at least one cycle of a treatment regimen containing fruquintinib or regorafenib. Exclusion criteria included A history of autoimmune disease; Severe bone marrow, hepatic, or renal insufficiency; Presence of malignant tumors other than CRC; Prior receipt of fruquintinib, regorafenib, or anti-

tumor immunotherapy; Incomplete patient data; Use of other anti-tumor drugs not classified as immunotherapy; Lack of complete follow-up data. This study was performed following the principles of the Declaration of Helsinki (as revised in Fortaleza, Brazil, in October 2013) and the international standard of good clinical practice (GCP) and was approved by the Ethics Committee of the North China University of Science and Technology Affiliated Hospital (Approval ID: 202409300002).

Data collection

This study divided patients into a targeted therapy group and a targeted-plus-immunotherapy group. Drug doses were administered following approved labeling and guidelines, with adjustments made for individual patient tolerance. The starting dose of furaquintinib is 5 mg, which can be reduced to 3 mg or 4 mg later in the treatment if it is not well tolerated. Regorafenib has a starting dose of 160 mg and a minimum dose of 80 mg. We collected various data on advanced CRC patients, including demographic details, clinicopathological characteristics, and laboratory results. This data encompassed age, gender, the location of the primary tumor, previous treatments, the degree of pathological differentiation, locations of metastases, whether the primary lesion had been surgically removed, mismatch repair (MMR) status, mutations in KRAS/NRAS genes, and the number of therapeutic lines received.

Outcomes and definitions

The primary outcome of this study was PFS, while secondary outcomes included OS and safety measures. PFS was determined as the duration from the start of treatment with regorafenib to the point of disease progression or death from any cause, whichever occurred first. OS was measured from the commencement of regorafenib treatment until death for any reason. The effectiveness of the treatment was evaluated using the World Health Organization-based RECIST 1.1 criteria, and adverse events (AEs) were categorized and graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0.

Statistical analyses

Baseline characteristics and clinical variables were reported as frequencies and percentages, with differences between groups evaluated using the Chi-square or Fisher's exact test, depending on the data. The Mann-Whitney *U* test was applied for non-normally distributed continuous variables, while ordinal variables were assessed using rank-sum tests. To reduce selection bias, PSM was conducted, employing 1: 1 nearest-neighbor matching without replacement and a caliper width set at 0.2 of the propensity score. Covariates, including gender, age, hospital, primary tumor location, surgical status, number of treatment lines, sites of metastasis, and baseline carcinoembryonic antigen (CEA) levels were integrated into the matching model. This matched set of data was then used for further analysis.

Moreover, IPW was used to adjust for initial differences between the groups, with each patient's weight calculated as the inverse of their propensity score. Weights at the extremes were capped at the 5th and 95th percentiles to reduce their effect on the results. This weighted set was then employed for sensitivity analyses to verify the stability of the findings. Survival rates were analyzed using the Kaplan-Meier method, which provided survival curves, median survival times, and 95% confidence intervals. Comparisons of survival curves across treatment groups were made using the log-rank test. To identify factors that might affect outcomes, both univariate and multivariate Cox regression analyses were conducted to derive hazard ratios (HRs) and their 95% confidence intervals (CIs). All statistical tests were performed using SPSS version 26.0 (IBM Corp.) and R version 4.4.1 (R Foundation for Statistical Computing), with a significance threshold set at a two-sided *P*-value of less than 0.05.

RESULTS

Patient baseline characteristics

Among 145 mCRC patients treated with regorafenib or fruquintinib as third- or fourth-line therapy, 10 were excluded due to incomplete clinical data and less than one

treatment cycle. Further, 15 patients were excluded for lacking comprehensive follow-up data, and 21 were excluded due to the concurrent use of other anti-tumor drugs, resulting in 99 patients being eligible for the study (Figure 1) . The median age of these patients at diagnosis was 61 years, ranging from 40 to 90, with 57 (57.6%) male. The lung was the most common site of metastasis, occurring in 55.6% of the patients. Within the subgroup that received both targeted and immunotherapy, 3 (9.7%) were treated with pembrolizumab, 21 (67.7%) with sintilimab, 3 (9.7%) with envafolimab, 1 (3.2%) with cadonilimab, 1 (3.2%) with nivolumab, and 2 (6.4%) with penpulimab. Besides baseline CEA levels before enrollment, other baseline characteristics such as age, sex, and primary tumor location were generally well-balanced. Details on the balance achieved through PSM and IPW are provided in Table 1.

Clinical efficacy

No patients achieved a complete response (CR) following treatment in both groups. In the group receiving targeted therapy alone, 10 patients achieved a partial response (PR), 33 maintained stable disease (SD), and 25 experienced progressive disease (PD). The ORR in this group was 14.7%, with a disease control rate (DCR) of 63.2%. In the group receiving combined targeted therapy and immunotherapy, 5 patients reached PR, 15 had SD, and 11 developed PD, resulting in an ORR of 16.1% and a DCR of 67.7%. There was no significant statistical difference in ORR between the groups (P= 1). Although the combined therapy group showed a higher DCR than the targeted therapy group alone, these differences were not statistically significant ($\chi 2$ = 0.189, P= 0.664). We also considered the effect of the targeted drug dose on its efficacy. While the recommended dose for continuous administration of furaquintinib is 5 mg per day, some of the patients in the study only tolerated 3 mg, and while the recommended dose of regorafenib is 160 mg, severalpatients in the study chose to receive 80 mg, the lowest effective dose, as their initial dose. Therefore, the dose may influence the clinical efficacy.

The median number of treatment cycles was 4 (range 1-18). Sixty-six patients were started on furaquintinib 5 mg, 21 were started on furaquintinib 3 mg, and 6 patients had their furaquintinib dose reduced from 5 mg to 3 mg. Twelvepatients were started on regorafenib at a dose of 80 mg, of whom 17 discontinued treatment due to adverse events or astrong patient preference for tolerability.

Survival Analysis

The 95% confidence interval for the median follow-up time ranged from 4.1 to 14.1 months. The median duration of treatment was 3.4 months in the targeted therapy group (ways = 0) and 6.0 months in the combined targeted immunotherapy group (ways = 1).

Before matching, the median PFS was 3.4 months in the targeted therapy group and 6.0 months in the combination group, showing a statistically significant difference (P< 0.01), suggesting that adding immunotherapy to targeted therapy significantly extends PFS. The median OS was 13 months for the targeted therapy group and 20.9 months for the combination group. Although this difference was not statistically significant (P= 0.23), it suggests a potential trend toward improved OS with combined therapy. PSM Analysis: After adjusting with propensity score matching, the median PFS for the targeted therapy group was slightly increased to 4.25 months, while it was 6.15 months for the combination group, maintaining a significant difference (P< 0.01). This suggests that combined targeted immunotherapy (TT+ICI) continues to offer a PFS benefit even when accounting for baseline characteristics. After the same adjustment, the median OS for the targeted therapy group was reduced to 11.35 months compared to 16.45 months for the combination group. The difference remained non-significant (P= 0.93), indicating a diminished apparent advantage in OS with combined therapy after adjusting for baseline factors. IPW Analysis: IPW analysis echoed the initial results, with the median PFS at 3.3 months for the targeted therapy group and 5.6 months for the combination group, again showing a significant difference (P< 0.01). This underscores the PFS benefit of the combined therapy approach. The median OS from the IPW analysis was

10.8 months in the targeted therapy group and 20.9 months in the combination group, mirroring the original data with no significant difference (P= 0.23), suggesting some improvement in OS without statistical significance. Summary: PFS Consistency: Across all three analytical methods (original data, PSM, and IPW), targeted therapy and immunotherapy consistently demonstrated a significant extension in PFS. This finding confirms the robustness of the combination therapy in controlling disease progression, irrespective of potential imbalances in baseline characteristics.

OS Non-Significance: In all three analyses, OS did not reach statistical significance, though the combination of targeted therapy and immunotherapy did show a trend toward longer median OS. This pattern suggests that an extended PFS might not directly lead to a significant improvement in OS in patients undergoing multiple previous treatments. This observation could indicate that a longer follow-up or larger cohort may be necessary to observe meaningful differences in OS outcomes.

Univariate and multivariate analyses across the overall study population identified the treatment regimen and primary tumor location as significant determinants of PFS. Patients receiving combined TT+ICI experienced a notable extension in PFS compared to those receiving TT alone (median PFS: 6.0 vs. 3.4 months, HR = 0.48, P< 0.001). Specifically, patients with tumors originating in the rectum had a better prognosis than those with tumors in the colon (median PFS: 4.8 vs. 3.4 months, HR = 0.55, P< 0.001). Other factors such as age, gender, radical surgery, line of treatment, metastatic status, CEA levels, and genetic mutations did not significantly impact PFS. However, liver and peritoneal metastases were associated with a significantly increased risk (HR = 18.67, P< 0.001), suggesting the need for further study given the small number of patients in this subgroup.

Cox univariate and multivariate subgroup analyses indicated that combined TT+ICI significantly extended PFS compared to TT alone across the general study population. Univariate analysis showed distinct survival advantages with TT+ICI in various subgroups, including patients aged 60 years and older (HR = 0.47, P= 0.006), those with CEA levels over 25 ng/mL (HR = 0.38, P< 0.001), individuals with colon cancer (HR =

0.52, *P*= 0.043), patients with rectal cancer (HR = 0.43, *P*= 0.021), third-line therapy recipients (HR = 0.51, *P*= 0.008), males (HR = 0.51, *P*= 0.034), KRAS/NRAS wild-type cases (KRAS: HR = 0.43, *P*= 0.002; NRAS: HR = 0.52, *P*= 0.009), and patients with liver metastases alone (HR = 0.30, *P*= 0.017) or both liver and lung metastases (HR = 0.18, *P*= 0.031). However, no significant PFS benefits were observed in patients under 60 years, those with KRAS/NRAS mutations, or certain metastatic profiles, possibly due to the small size of these subgroups.

Multivariate analysis further validated the significant benefit of TT+ICI, demonstrating a 62% reduction in the risk of disease progression or death (HR = 0.38, *P*< 0.001). Additional subgroup analysis revealed pronounced benefits for patients with CEA levels ≤ 5 ng/mL (HR = 0.00, *P*< 0.001), those who had undergone surgery (HR = 0.33, *P*< 0.001), patients receiving third-line therapy (HR = 0.39, *P*= 0.003), and females (HR = 0.25, *P*= 0.004), with females showing particularly favorable outcomes.

In conclusion, TT+ICI showed significant survival improvements in older individuals, those with higher CEA levels, surgical patients, third-line therapy patients, KRAS/NRAS wild-type patients, pMMR patients, and those with liver metastases. These results provide crucial insights for prioritizing treatment in these specific subgroups. However, due to the small number of patients, more research is needed to ascertain the clinical significance in subgroups with KRAS/NRAS mutations, dMMR status, and other metastatic configurations. These findings underscore the potential of TT+ICI to tailor personalized treatment approaches effectively.

Safety profile

In terms of safety, the most common adverse events reported in both groups were hypertension, proteinuria, rash, cardiac enzyme abnormalities, liver function abnormalities, gastrointestinal bleeding, hand-foot syndrome, oral mucositis, and fatigue. The reported Grade 3–4 adverse events included hypertension, fatigue, and reduced platelet counts. There was no statistically significant difference in the incidence of overall adverse events between the two groups. All adverse events (including Grades

3 to 4) experienced by patients were controlled and resolved by symptomatic treatment or discontinuation of therapy. In addition, there were no deaths resulting from serious adverse events. A previous meta-analysis showed that while reduced safety margins due to toxicity can occur when using combinations of targeted agents and immunotherapies, the treatment's overall effects remain safe and manageable. In many cases, these combination therapies are associated with improved efficacy and significantly prolonged survival, making them the treatment strategy of choice[30].

Retrospective studies often face challenges in matching the completeness and accuracy of their data with the real-life, systematically collected information from prospective studies. This is primarily due to the reliance on medical records and patient recall of adverse reactions. While retrospective studies can reflect real-world clinical situations and offer valuable insights, it is essential to consider potential biases and limitations associated with the information collection process when interpreting and applying data on adverse reactions. This helps to avoid excessive extrapolation of study conclusions.

DISCUSSION

This study indicates that combination therapy involving targeted therapy (TT) and ICIs may provide a more effective treatment option for patients with advanced mCRC. The significant improvement in PFS demonstrated in the study underscores the effectiveness of this combination therapy in managing disease progression, particularly in patients with a history of extensive previous treatments. However, the lack of a significant improvement in OS raises the possibility that PFS's benefits may not always translate into extended living. This highlights the need for more research to optimize the delivery of TT+ICI therapy and increase its long-term benefits. Comparing the findings of this study to those of the previous REGONIVO and LEAP-017 studies reveals similar PFS outcomes[26, 31]. The objective response rates (ORR) and disease control rates (DCR) observed across various trials differ, probably due to variations in study design, patient demographics, and treatment protocols. The findings highlight

the complexity inherent in real-world studies, which, while encompassing a broader clinical context, also present challenges associated with standardization protocols and managing confounding factors. The combination therapy demonstrated manageable safety profiles, with adverse events predominantly occurring in the initial treatment cycles and effectively addressed through supportive care and dose modifications. The observations indicate the practicality of incorporating TT+ICI treatment regimens into standardclinical practice.

In the context of personalized treatment strategies and recurrence risk prediction, a comprehensive animal study demonstrated that a high-methionine diet worsened colitis, epithelial damage, and dysbiosis in a mouse model of colorectal cancer induced by AOM/DSS. This resulted in an elevation of secondary bile acids (*e.g.*, LCA and DCA), activation of bile acid receptors (TGR5), and the associated JAK2-STAT3/YAP signaling pathways, consequently facilitating tumor initiation and progression[32]. A separate animal study indicated that a low-methionine diet significantly enhanced the effectiveness of low-dose 5-fluorouracil chemotherapy, especially in mouse models exhibiting resistance to treatment[33]. This further substantiates the hypothesis that increased methionine consumptionmay adversely affect communities and patients at high risk for colorectal cancer. Future research may investigate integrating low-methionine dietary alterations into TT+ICI therapy protocols to augment anti-tumor immunity.

Recent studies indicate that ctDNA analysis holds considerable promise for predicting recurrence risk[34]. TheGALAXY study demonstrated that molecular residual disease (MRD) identified in circulating tumor DNA (ctDNA) four weeks after surgery serves as the most significant prognostic indicator for disease-free survival (DFS)[35]. Jiang *et al*. developed a deep learning model (attMIL) employing pathological slides to predict survival in colorectal cancer patients. The HR for OS and disease-specific survival (DSS) were 4.50 (95%CI 3.33–6.09) and 8.35 (95%CI 5.06–13.78), respectively, indicating strong predictive efficacy[36]. Integrating these tools with TT+ICI therapy may enhancepatient selection and optimize treatment results.

Conducting more large-scale prospective clinical trials with definedprotocols to validate these findings and furnish robust evidence for integrating TT+ICI into standard therapy regimens for mCRC is essential.

Research on combination therapy involving targeted therapy and ICI demonstrates significant efficacy; however, challenges such as drug resistance and the complexity of the immune microenvironment persist. Researchers are investigating novel targeted therapeutic strategies and biomarkers to tackle these challenges. The most prevalentresistance mechanisms that restrict the effectiveness of TKIs are as follows: (1) Upregulation of alternative angiogenesis pathways: To avoid immunological and medication inhibition, tumor cells may use alternate signaling pathways, such as IL-6/STAT3, IL-8, Ang1/2, c-Met/HGF, and FGF, to increase angiogenesis once VEGF signaling is suppressed. (2) Epithelial-mesenchymal transition (EMT): EMT brought on by hypoxia allows tumor cells to become more resistant and invasive. (3) Decreased intracellular drug concentration: The effective intracellular concentration of TKIs is brought down by lysosomal sequestration and efflux proteins (such as P-gp), which reduces efficacy. (4) Modifications to the tumor microenvironment: Tumor-associated fibroblasts (TAFs) and bone marrow-derived cells (BMDCs) are recruited to support extracellular matrix remodeling, immunological suppression, and tumor adaptive survival. (5) Genetic and epigenetic factors: EZH2-mediated methylation of tumor suppressor genes may lessensensitivity to TKIs, while single nucleotide polymorphisms (SNPs) affect TKI metabolism and resistance likelihood[37]. According to recent research, increased ASCT2 expression and improved glutamine metabolism may also affect the effectiveness of VEGFR TKIs, which would further encourage resistance[38]. A new IgG1 monoclonal antibody called vanucizumab targets angiopoietin-2 (Ang2) and VEGF-A. A critical contributing reason to resistance to VEGF-targeted therapy is the compensatory promotion of angiogenesis by VEGF-A and angiopoietin-2. Vanucizumab may improve therapeutic efficacy and increase the efficiency of VEGF-targeted therapy by partially reducing the effectsof other hypoxia-induced pro-angiogenic factors. In early clinical trials, Vanucizumab has shown good biological

safety[39]. Jahangiri *et al*. discovered that, following bevacizumab treatment in glioma patients, tumor tissues may confer resistance to VEGF-targeted therapy via the upregulation of c-Met[40]. Preclinical investigations employing c-Met inhibitors have demonstrated its efficacy in mitigating resistance to VEGF therapy in malignant tumors. However, the clinical evidence substantiating the effectiveness of c-Met inhibitors in conjunction with VEGF-targeted treatment is insufficient and necessitates more investigation. Famitinib is a novel tyrosine kinase inhibitor that targets VEGFR2 and PDGFRβ, demonstrating encouraging therapeutic outcomes in a clinical trial with chemotherapy-resistant metastatic colorectal cancer patients. Compared to the control group, the Famitinib group showed an increase in median PFS (2.8 months vs 1.5 months) and DCR (59.8% vs 31.4%). Combining Famitinib with conventional VEGF-targeted drugs (*e.g.*, bevacizumab) may produce enhanced targeted therapeutic results[41, 42].

TET1-MUT is closely related to improved ORR, durable clinical benefit (DCB), longer PFS, and improved OS in patients treated with ICIs. This suggests that TET1-MUT could be a new predictive biomarker for immune checkpoint inhibition[43]. Another study found that pro-inflammatory monocytes (IL1B+ monocytes), Tregs, and CD8+ Trm-mitotic cells were elevated in samples from non-pCR patients receiving PD-1 inhibitor treatment. On the other hand,IL1B+ monocytes, CCL2+ fibroblasts, and other pro-inflammatory factors (such as IL1A and IL1B) were significantly reduced in patients with pathological complete response (pCR). This led to the conversion of T and B cells to an anti-inflammatory phenotype, which in turn lessened the inhibitory effects of the inflammatory environment on CD8+ cells. *In vivo* research indicated that diminishing the pro-inflammatory cytokine IL1β augmented the quantity of CD8+ T cells and CD40+ B cells associated with complete remission while also elevating the number of PD-1+ CD8 T cells rendered exhausted by Tregs in individuals not achieving complete remission. This study indicates that pro-inflammatory monocytes (IL1B+ monocytes), IL1β, and Tregs could represent novel therapeutic targets for immunotherapy, offering

new avenues for investigating more effective combination strategies in cancer immunotherapy[44].

OS has been regarded as the gold standard for proving clinical benefit in cancer treatment. The difficulties in converting PFS benefit into OS benefit were highlighted by a thorough analysis of 260 phase III clinical trials involving metastatic solid tumors, which showed that the success rate of converting favorable PFS data into positive OS data was only 38%[45]. Performance status, tumor size, number and location of metastases, molecular features of the tumor, previoustreatment regimens, drug dose adjustments, treatment cycles, subsequent treatment choices, drug side effects, and follow-up duration are some of the clinical and biological factors that contribute to the fact that PFS benefit does not always translate into OS benefit. The dilution effect of survival post-progression (SPP) influences these factors, decreasing the true impact of extended PFS on OS benefit. The primary reason identified in our study is that the PFS benefit only represents the efficacy of third- and fourth-line therapies[46].

In comparison, OS shows the overall effectiveness of several medications following progression. As stated otherwise, OS is the result of many PFS advantages accumulating. Another important factor is some patients have a protractedSPP, which calls for bigger patient cohorts and more extended follow-up times. Larger patient groups and longer follow-ups to overcome random fluctuations related to SPP should be part of future studies to provide a more accurate evaluation of therapy effects.

Furthermore, depression and anxiety affect almost 50% of Chinese cancer patients[47]. Chronic or recurring emotional distress can affect immunological function, create an immunosuppressive tumor microenvironment, and stimulate the sympathetic nervous system and hypothalamic-pituitary-adrenal axis. Studies show that patients experiencingemotional distress have poorer 2-year overall survival rates (46.5% vs. 64.9%), lower objective response rates (46.8% vs. 62.1%), and shorter PFS (7.9 months vs. 15.5 months) than patients not experiencing emotional distress[48]. Future research may use standardized instruments like the Patient Health Questionnaire-9 (PHQ-9) and the Generalized Anxiety Disorder 7-item scale (GAD-7) to measure emotional distress and

perform stratified analysis to compare OS and PFS differences between patients with and without emotional distress, which could help direct better management of patients' emotional health. This emotional distress may also contribute to this study's lack of observed OS differences.

The present study has several limitations. First, as a retrospective multicenter study, there is potential for selection and information biases. Despite using PSM and IPW to adjust for initial differences, not all confounding variables may be accounted for. Second, the modest cohort size (99 patients) might limit the statistical power to detect significant differences in OS. Third, the median follow-up duration ranged from 4.1 to 14.1 months, which might be too brief to evaluate long-term survival outcomes adequately. Fourth, variability in treatment regimens, especially the use of different PD-1 inhibitors in the combination immunotherapy group, could compromise the consistency of the results. Fifth, the participants included in this study were exclusively from three hospitals in North China, which may limit the findings' broader applicability and external validity.

The study design might not account for all unknown confounding factors. More comprehensive, prospective, randomized controlled trials are necessary to corroborate these results. Sixth, while the statuses of KRAS, NRAS, and dMMR mutations were considered, other crucial molecular markers like MSI status and BRAF mutations were not thoroughly examined, limiting the molecular biological insights into the treatment responses. Seventh, while the study mentions the evaluation of adverse events, it lacks detailed reporting on the grading of adverse events for each treatment regimen and their impacts on patients' quality of life. The absence of systematic adverse event documentation may undermine the thoroughness of the safety analysis. The paucity of data on PD-1/L1 expression in our study precluded a comprehensive assessment of the potential of PD-1/L1 as a biomarker for patients with mCRC. Furthermore, in this retrospective study, the majority of the medical records lacked complete physical strength scores, preventing a precise determination of the physical strength of the patients. This limitation precluded an objective assessment of the treatment efficacy and

adverse reactions, potentially compromising the study's reliability and the persuasiveness of its conclusions.

CONCLUSION

The results of this study show that for patients with advanced or mCRC who have not responded to standard therapies, TT+ICI significantly extends PFS. This combination also maintains a favorable safety profile and manageable side effects compared to TT alone. Although there was no statistically significant improvement in OS between the two treatment groups, the combination therapy notably improved control of disease progression. This suggests that integrating TT+ICI could represent a more effective approach for treating patients with advanced mCRC.

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