Original Article

Genomic profiles and their associations with microsatellite instability status, tumor mutational burden, and programmed death ligand 1 expression in Chinese patients with colorectal cancer

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Background: Colorectal cancer (CRC) is among the most prevalent malignancies globally, with a rising incidence observed in younger demographics. Despite surgical resection remaining the cornerstone of treatment, metastatic CRC poses significant therapeutic challenges. Immunotherapy, a mode of treatment that leverages the patient's immune system, presents a promising frontier in CRC management, particularly for late-stage cases with limited treatment options. The study was aimed to elucidate the relationships between genetic profiles and predictive biomarkers in CRC patients to inform immunotherapy decisions and improve outcomes.

Methods: We conducted a large-scale study involving 660 patients with CRC, analyzing genetic profiles and predictive biomarkers for immune checkpoint inhibitors (ICIs) using next-generation sequencing (NGS) and immunohistochemistry (IHC). The study focused on assessing the association between gene mutations and markers such as microsatellite instability (MSI) status, tumor mutational burden (TMB), and programmed death ligand 1 (PD-L1) expression.

Results: Analysis revealed a diverse mutational landscape in CRC, with *TP53* (73.64%), *APC* (67.58%), and *KRAS* (46.82%) being the most frequently mutated genes. We observed significant associations between *KRAS* mutations and co-occurrences with *FBXW7*, *PIK3CA*, and *SMAD4* mutations, while *KRAS* mutations were mutually exclusive with *TP53* mutations. *KRAS* mutations were enriched in the PD-L1 tumor proportion score (TPS) ≥1% population (P=0.03), whereas *APC* mutations were enriched in the PD-L1 TPS <1% population (P=0.10) as compared to their wild types. Additionally, specific mutations such as *KRAS* p.A146T, *PIK3CA* p.H1047R, and *BRAF* p.V600E were significantly associated with higher TMB and MSI-high status, indicating potential benefits from ICI therapy.

Conclusions: Our findings underscore the importance of genetic profiling in guiding treatment decisions for patients with CRC, particularly in the era of immunotherapy. Understanding the complex interplay between genetic alterations and immune markers is critical for optimizing therapeutic strategies and improving clinical outcomes. Further research is warranted to validate these findings and explore personalized treatment approaches in CRC.

Keywords: Colorectal cancer (CRC); microsatellite instability (MSI); tumor mutational burden (TMB); programmed death ligand 1 (PD-L1); immunotherapy

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Introduction

Colorectal cancer (CRC) involving the colon and rectum ranks as the third most common cancer worldwide, accounting for 10.2% of all cancer cases, with a mortality rate of 9.2% (1,2). Recently, there has been an upward trend in the incidence of CRC among younger individuals (3). The progression of CRC typically unfolds slowly, often without symptoms, and follows a complex, multistep process. It is characterized by diverse mechanisms, including somatic cell mutations, genetic fusion, genetic instability, and epigenetic changes (4). These mechanisms induce histological and morphological alterations that lead to carcinogenesis, eventually spreading to lymph nodes and adjacent or distant organs (5). Although surgical resection remains the mainstay treatment for CRC, options are limited for metastatic cases (6). Despite chemotherapy being commonly recommended, targeted therapies, such as inhibitors targeting specific mutation spectra, such as vascular endothelial growth factor (VEGF)/epidermal growth factor receptor (EGFR), are feasible only for a minority of cases (4,7). Hence, there is a pressing need for alternative and effective treatment modalities for patients with CRC.

Immunotherapy represents a novel approach to treating CRC, and it harnesses the patient's own immune system to combat tumor cells (8,9). It addresses the issue of specificity, which is a major limitation of chemotherapy and radiation therapy. The primary genetic alteration in CRC depends on damage to DNA mismatch repair (MMR) activity, leading to approximately 15% of tumors exhibiting a microsatellite instability (MSI) phenotype (10). MSI serves as a molecular predictive factor for DNA MMR deficiency and a predictive biomarker for immunotherapy response. Recent studies have delved into immune cells within the CRC microenvironment, revealing diverse immune landscapes based on microsatellite status and other factors. Patients with the MSI phenotype tumors exhibit heightened tumor mutational burden (TMB) and neoantigen load, facilitating immune effector cell infiltration and yielding stronger antitumor immune responses compared to microsatellite stable (MSS) tumors with fewer immune cell infiltrates (11). Research has shown that functional immune cell infiltrates within specific subgroups of these tumors are associated with improved postoperative prognosis and reduced risk of recurrence in patients with CRC, supporting the view that immunotherapy-based treatments should offer clinical benefits, particularly for late-stage patients with extremely poor prognosis (12,13). Immunotherapy has revolutionized medical oncology, particularly with immune checkpoint inhibitors (ICIs) showing promising and enduring clinical responses in certain CRC cases (14,15). However, the efficacy of ICIs in treating CRC is influenced by various factors, including genetic and immune markers. Thus, investigating the genetic characteristics and distribution of predictive biomarkers for immunotherapy in patients with CRC is crucial for assessing the clinical utility of ICIs in these patients.

Highlight box

Key findings

- The study identified *TP53*, *APC*, and *KRAS* as the most frequently mutated genes in colorectal cancer (CRC), with TP53 being the most common.
- KRAS mutations were found to be significantly enriched in the programmed death ligand 1 tumor proportion score ≥1% population, indicating a potential response to immune checkpoint inhibitor (ICI) therapy.
- Specific mutations such as *KRAS* p.A146T, *PIK3CA* p.H1047R, and *BRAF* p.V600E were associated with higher tumor mutational burden and microsatellite instability-high status, suggesting a benefit from ICI therapy.

What is known and what is new?

- It is known that CRC is a prevalent cancer with a rising incidence in younger populations and that immunotherapy is a promising treatment for metastatic CRC.
- The novelty of this study is the detailed genetic profiling and its association with ICIs, which may provide a more personalized approach to CRC treatment.

What is the implication, and what should change now?

- The implications of this study are significant for the field of CRC treatment, particularly in tailoring immunotherapy strategies based on genetic profiles.
- The findings suggest that genetic profiling should be integrated into standard CRC treatment protocols to optimize patient outcomes.
- Further research is needed to validate these findings and to explore how these insights can be applied in personalized medicine for patients with CRC.

In this large-sample study, we analyzed the genomic profiles and their associations with the predictive biomarkers for ICIs, including MSI status, TMB, and programmed death ligand 1 (PD-L1) expression through next-generation sequencing (NGS) and immunohistochemistry (IHC) staining in 660 patients. Our findings can contribute to informing the diagnostic decision-making of clinicians and provide guidance for selecting treatment options for patients with CRC, particularly systemic treatments including targeted therapies and immunotherapy. We present this article in accordance with the MDAR reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-24-748/rc).

Methods

Patient samples

A total of 660 patients with CRC were included in our analysis. The patients were from the Hospital of Chengdu University of Traditional Chinese Medicine, Sichuan Provincial People's Hospital, and Chengdu BOE Hospital, and their tumor tissue samples were submitted to Shanghai Tongshu Biotechnology Co., Ltd. for NGS molecular profiling from September 2022 to December 2023. The characteristics of patients are shown in Table S1. This study was conducted in accordance with the guidelines of the Helsinki Declaration (as revised in 2013). The study was approved by the Medical Ethics Committee of Hospital of Chengdu University of Traditional Chinese Medicine (No. 2024KL-192). The other two participated hospitals were informed and agreed with this study. Individual consent for this retrospective analysis was waived.

NGS and alteration identification

Genomic DNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor samples was subjected to NGS using the NextSeq platform (Illumina, San Diego, CA, USA). A custom-designed panel (Tongshu BioTech, Shanghai, China) comprising 556 cancer-related genes covering all solid tumors with the optimal features in terms of functional and clinical relevance, including prognostic impact and targeting, was employed. The average sequencing depth exceeded 10,000×. Alignment of these sequences to the human reference genome GRCh37/hg19 was performed using the Burrows-Wheeler Aligner (BWA). Strelka2 was used for the detection of single-nucleotide variants (SNVs) and insertions and deletions (indels) with default parameters. Detected somatic mutations included SNVs, small indels, copy number variations, and gene fusions.

Assessment of TMB

We define TMB as the number of somatic mutations and indels per megabase of coding region detected in tumor tissue. TMB analysis exclusively involved sequencing data from a panel of 556 cancer-related genes, with the upper quartile TMB value of tumor tissue samples being used as the threshold to distinguish between high and low TMB levels.

IHC staining

PD-L1 tumor proportion score (TPS) was calculated based on the percentage of tumor cells with membranous PD-L1 staining on each slide. The PD-L1 combined positive score (CPS) was defined as the ratio of the total number of PD-L1-positive tumor cells, lymphocytes, and macrophages to the total number of tumor cells in the entire slide. The positive cutoff values for PD-L1 TPS and CPS were 1% and 1 (median), respectively. Tumors were categorized into high-density and low-density groups based on the median number of positively stained immune cells per unit area. Quantitative analysis of IHC images was performed using Image Pro Plus version 6.0 (Media Cybernetics, Rockville, MD, USA).

Statistical analysis

Statistical analysis was performed using SPSS 25.0 software (IBM Corp., Armonk, NY, USA). A P value <0.05 was considered statistically significant. Categorical variables were expressed as numbers and percentages, normally distributed continuous data were expressed as the mean and standard deviation (SD), and nonnormally distributed continuous data were expressed as the median and interquartile range (IQR). The Pearson Chi-squared test, Fisher exact test, and nonparametric tests were employed to compare the distribution of TMB and immune markers. Correlation analysis was conducted using Pearson correlation analysis and Spearman rank correlation analysis.

Results

Mutational spectrum of Chinese patients with CRC

In our study, we examined somatic variations in 556 genes across 660 samples from patients with CRC using a targeted

Figure 1 Gene mutation spectrum of the top 30 mutated genes in CRC. The top panel represents the TMB, and the middle panel represents the matrix of frequently mutated genes. Columns represent samples, and the clinicopathological characteristics of individual patients are presented below. MSI, microsatellite instability; PD-L1, programmed death ligand 1; CPS, combined positive score; TPS, tumor proportion score; SNV, single-nucleotide variant; CNV, copy number variation; MSI-H, microsatellite instability-high; MSS, microsatellite stable; CRC, colorectal cancer; TMB, tumor mutational burden.

NGS panel. The analysis revealed a total of 556 gene somatic variations, with the notable alterations highlighted in *Figure 1*. Among these, *TP53* (73.64%), *APC* (67.58%), *KRAS* (46.82%), *PIK3CA* (21.82%), *SMAD4* (17.12%), *FBXW7* (16.67%), *LRP1B* (15.91%), *TCF7L2* (12.88%), *GNAS* (10.76%), and *SOX9* (10.61%) emerged as the top 10 mutated genes. Nonsynonymous SNVs were the most prevalent, with C>T substitutions being the most common (*Figures 1*,*2A*). Correlation analysis indicated significant associations between *KRAS* mutations and co-occurrences with *FBXW7* (P=0.003), *PIK3CA* (P<0.001), and *SMAD4* (P<0.001) mutations, while they were mutually exclusive with *TP53* mutations (P<0.001) (*Figure 2B*). Pathway enrichment analysis indicated significant associations of these mutated genes with 10 oncogenic pathways, including RTK-RAS, PI3K, cell cycle, NOTCH, WNT, TGF-beta, Hippo, TP53, MYC, and NRF2 (*Figure 2C*).

Further investigation into mutation subtypes revealed distinct patterns. We conducted a detailed analysis of the predominant mutation subtypes associated with key targetable alterations in CRC (*Figure 2D-2I*). Among the 330 *KRAS* mutations identified, the most prevalent was *KRAS* G12D (32.1%), followed by G12V (17%), G13D (13.9%), A146T (8.48%), G12S (5.76%), G12C (4.55%), G12A (3.03%), and Q61H (1.82%). NRAS exhibited a different profile, with Q61X being the most frequently mutated isoform. For *PIK3CA* mutations (n=182), the top mutant subtypes were p.E542K (16.3%), E545K (12%), and H1047R (8.15%). *BRAF* mutations (n=60) were categorized into three classes: class I (V600D/E/K/R), class II (G464V/G469X/E586K/L597X/K601X), and class III (G466V/N581X/D594X/G596R). Specifically, we identified 37 class I (V600E), 3 class II (G469X/K601X), and 5 class III (D594X) *BRAF* mutations. Notably, copy number variations were most frequently observed in the *ERBB2* and *EGFR* genes. Furthermore, *ERBB2* p.R678Q and *EGFR* p.T790M

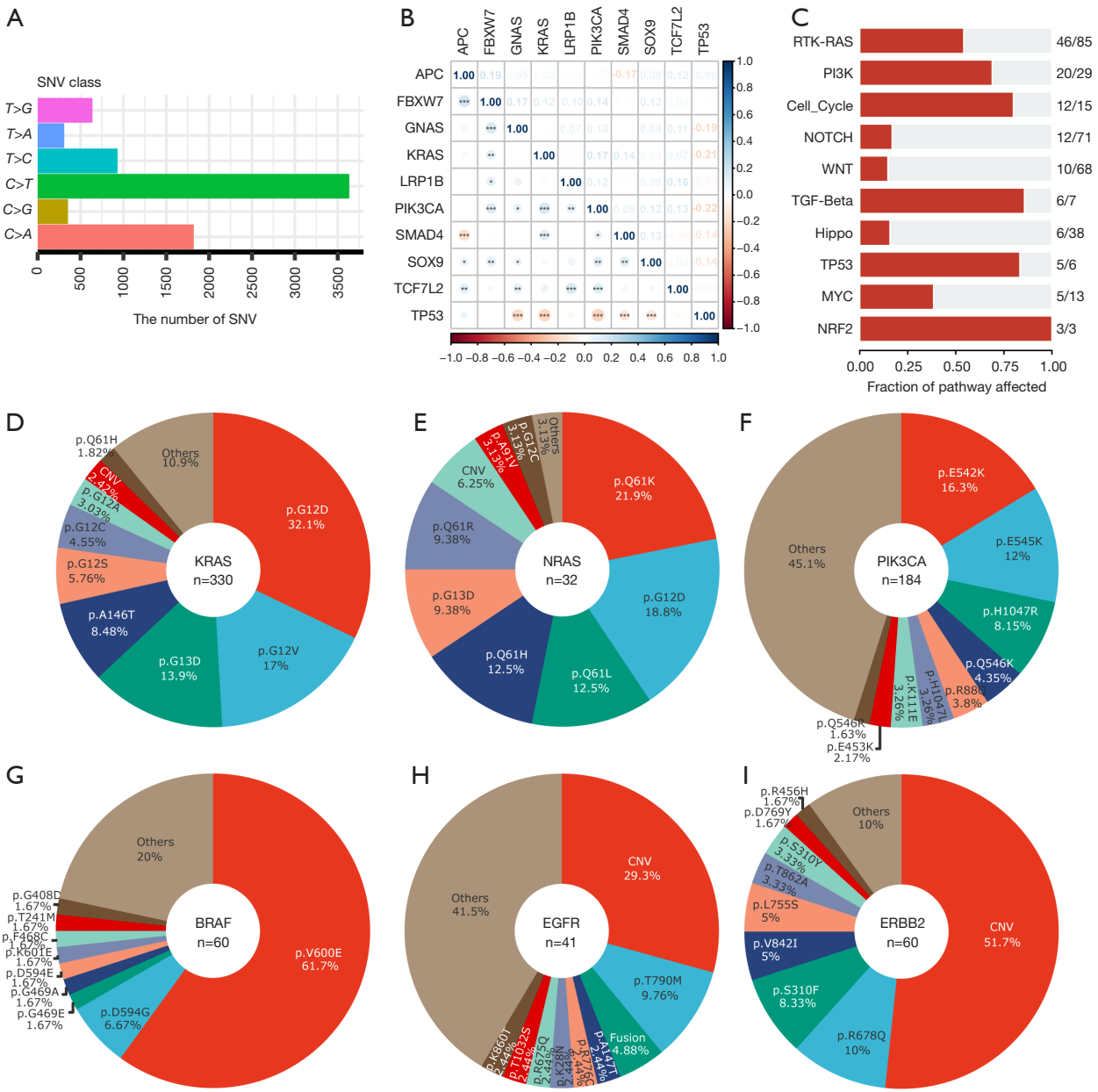

Figure 2 Gene mutation spectrum in CRC. (A) Single-nucleotide variations. (B) Correlation analysis of top 10 mutated genes. (C) The top 10 most significantly enriched pathways. Frequency distributions of (D) *KRAS*, (E) *NRAS*, (F) *PIK3CA*, (G) *BRAF*, (H) *EGFR*, and (I) *ERBB2* mutation subtypes. *, P<0.05 and ≥0.01; **, P<0.01 and ≥0.001; ***, P<0.001. CRC, colorectal cancer; SNV, single-nucleotide variant.

emerged as the most common mutation subtypes within the *ERBB2* and *EGFR* genes, respectively.

Association of the genomic landscape with TMB

The median TMB was 5.7 mutations per megabase (IQR, 3.6–7.9) in 660 patients with CRC. Tumors were stratified into TMB-high and TMB-low groups based on the upper quartile TMB value. We first investigated the association of the top 10 high-frequency mutated genes with TMB (*Figure 3*). Our analysis revealed that compared to wild-type counterparts, patients with mutations in *APC* (median: 5.7 *vs.* 5), *KRAS* (median: 6.05 *vs.* 5), *PIK3CA* (median: 7.9 *vs.* 5), *FBXW7* (median: 7.9 *vs.* 5), *SMAD4* (median: 6.4

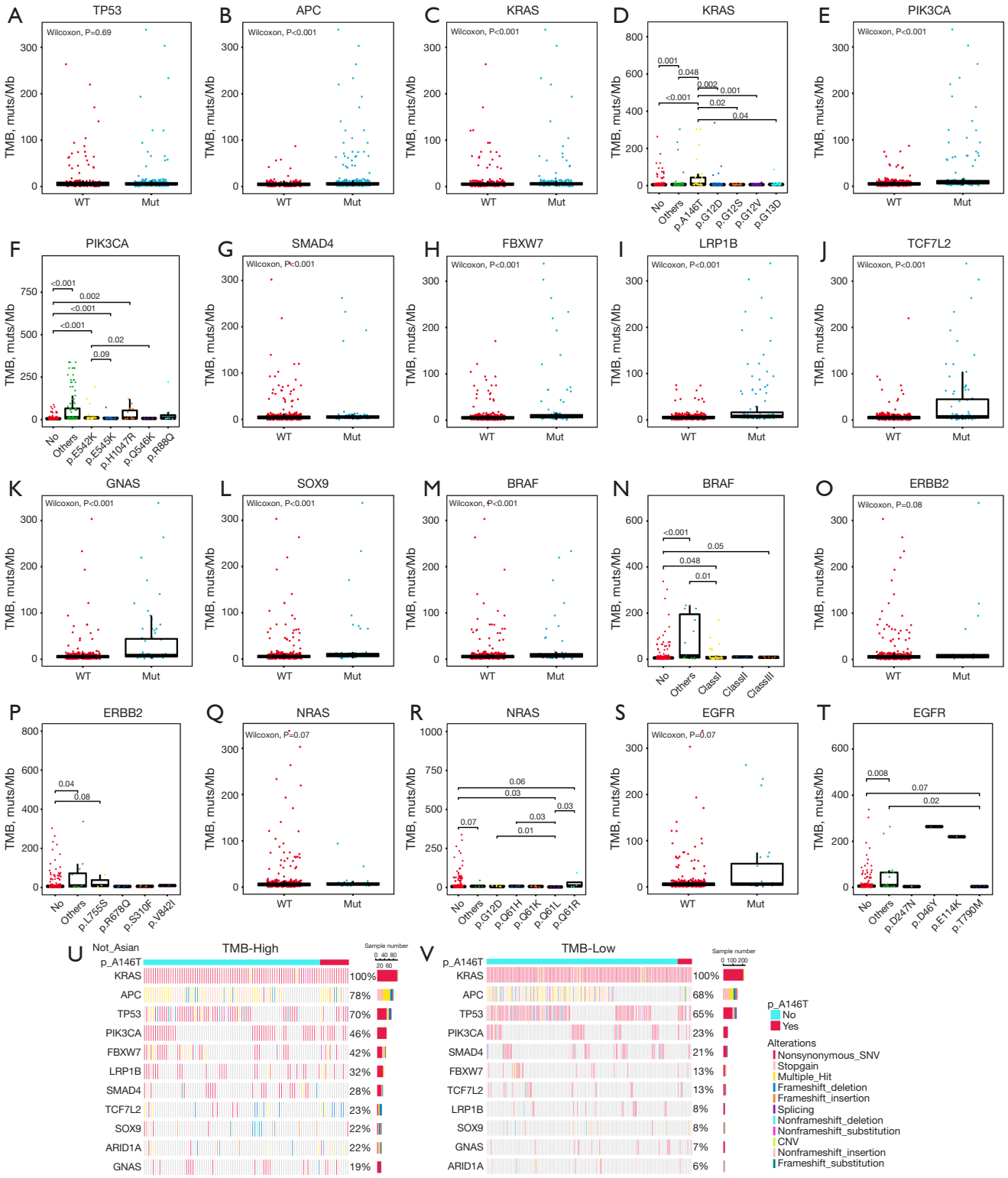

Figure 3 Genomic profile and its associations with TMB. Association of TMB with the (A) *TP53* mutation, (B) *APC* mutation, (C) *KRAS* mutation, (D) *KRAS* mutation subtypes, (E) *PIK3CA* mutation, (F) *PIK3CA* mutation subtypes, (G) *SMAD4* mutation, (H) *FBXW7* mutation, (I) *LRP1B* mutation, (J) *TCF7L2* mutation, (K) *GNAS* mutation, (L) *SOX9* mutation, (M) *BRAF* mutation, (N) *BRAF* mutation

subtypes, (O) *ERBB2* mutation, (P) *ERBB2* mutation subtypes, (Q) *NRAS* mutation, (R) *NRAS* mutation subtypes, (S) *EGFR* mutation, and (T) *EGFR* mutation subtypes. (U,V) Top 10 concomitant mutations of high-TMB and low-TMB tumors in *KRAS*-mutant patients with CRC. TMB, tumor mutational burden; WT, wild-type; Mut, mutation; CNV, copy number variation; CRC, colorectal cancer.

vs. 5.56), *LRP1B* (median: 7.9 *vs.* 5), *TCF7L2* (median: 7.1 *vs.* 5.7), *GNAS* (median: 7.9 *vs.* 5.7), and *SOX9* (median: 7.9 *vs.* 5.51) exhibited higher TMB levels (all P<0.001), whereas *TP53* showed no significant difference (*Figure 3A-3C,3E,3G-3L*). Furthermore, *KRAS*^{A146T}-mutant tumors exhibiting significantly elevated TMB levels (P<0.001), while tumors with *KRAS* G12 mutations showed TMB levels comparable to the wild type (*Figure 3D*). Similarly, specific mutation subtypes within *PIK3CA*, particularly p.E542K, p.E545K, and p.H1047R, had a higher TMB than did tumors with wild-type *PIK3CA* (P<0.001, P<0.001, and P=0.002, respectively) (*Figure 3F*).

Regarding other targetable alterations, compared to their wild-type counterparts, tumors with mutations in *BRAF* (median: 7.1 *vs.* 5.7; P<0.001), *ERBB2* (median: 6.05 *vs.* 5.7; P=0.08), *NRAS* (median: 6.4 *vs.* 5.7; P=0.07), and *EGFR* (median: 6.05 *vs.* 5.7; P=0.07) exhibited higher TMB levels. Additionally, *BRAF*^{V600E}-mutant tumors (P=0.048) and *BRAF*^{D594X}-mutant tumors (P=0.05) had a higher TMB than did the *BRAF* wild-type tumors. We found no significant difference in TMB among tumors with different *ERBB2* or *EGFR* subtypes. Notably, tumors with *NRAS* p.Q61R mutation showed a significantly higher TMB compared to their wild-type counterparts (median: 9.65 *vs.* 5.7; P=0.06), whereas p.Q61L exhibited a significantly lower TMB (median: 3.25 *vs.* 5.7; P=0.03). There was a significant difference between p.Q61L and p.G12D and between p.Q61H and p.Q61R (all P values <0.05) (*Figure 3M-3T*).

We aimed to further delineate the genetic mutational profile of *KRAS*-mutant tumors and investigate the reasons for elevated TMB in *KRAS*-mutant tumors. We observed that in high-TMB tumors, those with *KRAS* mutations exhibited a significantly higher proportion of concurrent *FBXW7*/*PIK3CA*/*LRP1B* mutations, which also predicted elevated TMB (*Figure 3U*). Furthermore, in tumors with the *KRAS* p.A146T mutation, which had a higher TMB, there were also a significantly higher proportion of concurrent *FBXW7*/*PIK3CA*/*LRP1B* mutations.

Correlation between mutational landscape and PD-L1 and MSI

We further explored the relationship between mutational landscape and the expression of PD-L1 and MSI status. Figure S1 illustrates the distribution of targeted alterations and MSI status within subgroups of PD-L1 TPS or CPS detection. Among the PD-L1 TPS ≥1% subgroup (n=49), 4.3% exhibited MSI-high (MSI-H) status with targeted alterations, whereas within the PD-L1 CPS ≥1 subgroup (n=122), 7.6% showed MSI-H with targeted alterations.

We observed significant associations between specific mutations and PD-L1 expression levels. *KRAS*-mutant tumors displayed a notably higher percentage of positive PD-L1 TPS compared to those with wild-type *KRAS* (31.9% *vs.* 17.8%; P=0.03), which may be attributed to the *KRAS* p.G12V, p.G12S, and p.G13D mutations (*Figure 4A* and Figure S2A). Conversely, tumors with *APC* mutations and *TP53* mutations showed the opposite results. Moreover, PD-L1 CPS positivity was more frequently observed in *TCF7L2*-mutant tumors (73.9% *vs.* 59.0%; P=0.25) and *PIK3CA* p.H1047R-mutant tumors (100% *vs.* 59.9%; P=0.27) (*Figure 4B* and Figure S2B).

Furthermore, we found a significant correlation between MSI-H status and specific gene mutations. The percentage of MSI-H tumors with mutations in *FBXW7* (P=0.009), *GNAS* (P<0.001), *LRP1B* (P<0.001), *PIK3CA* (P<0.001), and *TCF7L2* (P<0.001) was markedly higher compared to their wild-type counterparts. Conversely, the proportion of MSI-H tumors with wild-type *TP53* was significantly higher than that of *TP53*-mutant tumors (P<0.001) (*Figure 4C*). Additionally, the proportion of MSI-H tumors with mutations in *KRAS* p.A146T (P=0.01), *PIK3CA* p.H1047R (P<0.001), and *BRAF* p.V600E (P=0.003) were also significantly higher compared to wild-type tumors (Figure S2).

We further investigated the relationship between frequent gene mutations and these two immune markers (the expression of PD-L1 and MSI status) as a whole. Based on systematic clustering analysis of PD-L1 TPS, CPS, and MSI site proportions, three immune types were generated: PD-L1+ and MSI– (cluster 3), PD-L1– and MSI+ (cluster 1), and mixed type (cluster 2) (Figure S3). However, these gene variations showed no significant correlation with these three immune types. Nonetheless, it was observed that *ERBB2* and *GNAS* mutations occurred only in clusters 1 and 2,

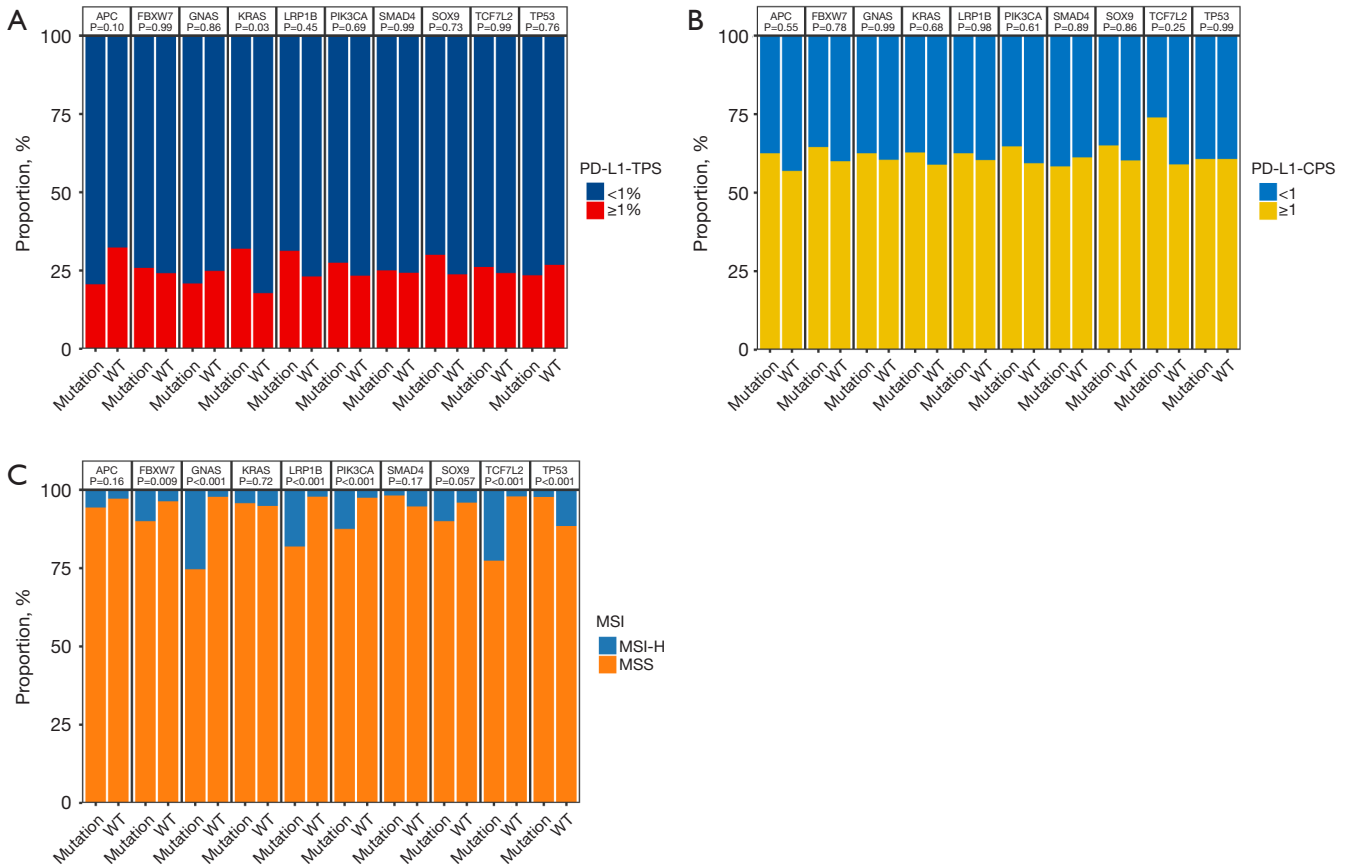

Figure 4 Correlation between the top 10 mutated genes and immune indicators. Correlation between the top 10 mutated genes and (A) PD-L1 TPS, (B) PD-L1 CPS, and (C) MSI status. WT, wild-type; PD-L1, programmed death ligand 1; TPS, tumor proportion score; CPS, combined positive score; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSS, microsatellite stable.

indicating that these tumors had greater MSI (Figure S3).

Distinct somatic mutation profile between Chinese and Western CRC

Additionally, we conducted an analysis comparing the mutational characteristics of our CRC cohort with those of non-Asian patients with CRC. The results revealed some differences in the top 10 mutated genes between the two ethnicity groups, including *APC* (P=0.001) and *GNAS* (P<0.001) mutations (*Figure 5A*). Furthermore, there was a significant disparity in TMB between the two cohorts, with the median TMB in our cohort being higher than that in non-Asian patients with CRC (median: 5.7 *vs.* 2.02; P<0.001) (*Figure 5B*). These findings suggest potential variations in mutated genes among different CRC populations, highlighting the need for further investigation with larger sample sizes.

Discussion

ICI therapy has emerged as a breakthrough in the treatment of many solid tumors, as it can lead to long-lasting remission in some patients with heavily treated late-stage metastatic disease. However, ICIs currently demonstrate activity in only a subset of patients with CRC, identified individually by MSI, TMB, and PD-L1 expression in the respective cancers (16,17). Our study focused on analyzing the genetic profiles of a cohort of 660 patients with CRC, the distribution of predictive biomarkers for ICIs, and their correlations. These findings contribute to informing diagnostic decision-making and treatment selection for patients with CRC, particularly in systemic therapies such as targeted treatments and immunotherapies.

We observed a diverse mutational landscape in patients with CRC, with notable alterations in genes including *TP53*, *APC*, and *KRAS*, which is in line with previous findings (18).

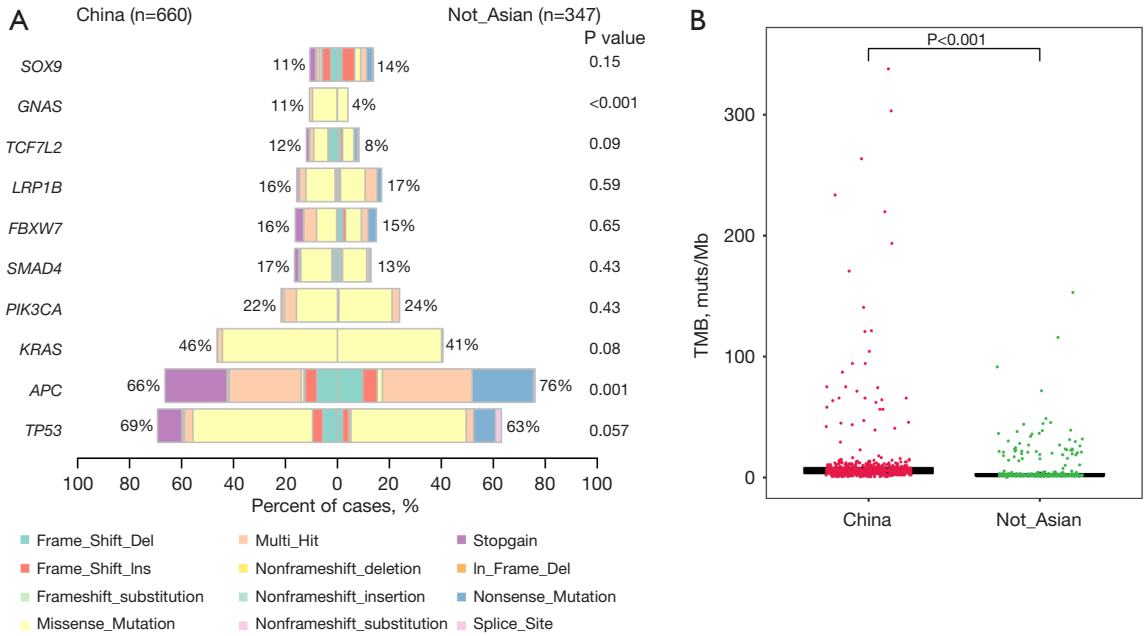

Figure 5 Comparison of somatic mutations between the Chinese and non-Asian CRC cohorts. (A) Comparison of the top 10 mutated cancer-associated genes between the CRC cohorts. (B) Comparison of the TMB between the CRC cohorts. CRC, colorectal cancer; TMB, tumor mutational burden.

In our study, we found a direct negative correlation between *KRAS* mutations and *TP53* mutations and a positive correlation with *PIK3CA* mutations, which aligns with a previous report (19). Further pathway enrichment analysis revealed the enrichment of RTK/RAS, PI3K, Wnt, and p53 pathways. Additionally, we found enrichment of the RTK/RAS pathway (*KRAS* mutations) in the PD-L1 TPS ≥1% (positive) population, while the Wnt (*APC* mutations) pathway were enriched in the PD-L1-negative population in our study. These findings may be associated with oncogenic structural changes (20,21), typically resulting in resistance to PD-1/PD-L1 therapy (22). Previous studies in CRC have found that *KRAS* mutations in the RTK/RAS pathway are associated with immune pathway suppression and reduced tumor-infiltrating lymphocyte (TIL) numbers (23), which are considered markers of poorer survival across all disease stages (24). Therefore, the association between PD-L1 positivity and the RTK/RAS pathway may contribute to the poor prognosis of PD-L1-positive CRC cohorts (25). Additionally, a recent proof-of-concept single-arm phase II clinical trial combined ICIs with the genomic stratification of the RTK/RAS pathway ($BRAF^{V600E}$) to enhance efficacy in clinically challenging MSS subtypes (26), suggesting that combining PD-L1 status and genomic features of cohorts might also improve the clinical outcomes of some combination therapies.

The PD-1 pathway plays a key role in tumor immune evasion by inhibiting T cell activation. Tumor cells upregulate PD-L1, interacting with PD-1 on T cells to dampen the immune response (27,28). Beyond the PD-1/PD-L1 axis, other immune regulatory networks contribute to T cell dysfunction, limiting anti-PD-1 therapy's efficacy (28). Only 20% of advanced cancer patients respond to anti-PD-1 treatments (28). Strategies to improve immunotherapy include combining PD-1/PD-L1 blockade with other therapies (29,30), understanding resistance mechanisms (31), and identifying predictive biomarkers like circulating exosomal PD-L1 (27). A deeper understanding of the PD-1 pathway is crucial for overcoming resistance and improving patient outcomes in cancer immunotherapy (28).

The latest clinical developments in PD-1 inhibitors have significantly impacted cancer immunotherapy. Targeting the PD-1 receptor, these inhibitors show promise in treating melanoma, lung, and bladder cancers. A study has led to their approval for first-line treatment in specific patient populations (32). Ongoing trials explore combinations with chemotherapy and other ICIs (33,34). Commercialization

of PD-1 inhibitors has rapidly increased, with multiple agents now available. Pharmaceutical companies are heavily investing in R&D to expand indications and explore earlier-stage use (35). However, high costs raise concerns about accessibility and economic burden (32). PD-1 inhibitors' use faces challenges, including immune-related adverse events (irAEs) ranging from mild (fatigue, rash, colitis) to severe (pneumonitis, endocrinopathies) (36). Understanding their safety profile is crucial for patient management. Research focuses on identifying predictive biomarkers to stratify patients based on response likelihood and adverse effect risk (34,36). PD-1 inhibitors are evolving rapidly with clinical advancements and commercialization, necessitating careful monitoring and management of toxicities.

The combination of PD-L1 and CTLA-4 inhibition has emerged as a promising strategy in metastatic refractory colorectal cancer (mCRC). A study indicates that dual blockade of these immune checkpoints enhances the immune response against tumor cells, potentially improving patient survival (37). As for efficacy, monotherapy with PD-1/PD-L1 inhibitors shows modest efficacy in mCRC, particularly in MSI-H tumors, but response rates in MSS tumors are limited. CTLA-4 inhibitors enhance T-cell activation but have a more pronounced toxicity profile. Combined inhibition has increased response rates and improved overall survival (OS) in certain patient populations, such as those with metastatic melanoma (37,38). As for safety, PD-1/PD-L1 inhibitors generally have a favorable safety profile with manageable irAEs. In contrast, CTLA-4 inhibitors are associated with a higher incidence of severe irAEs affecting multiple organ systems. Combined therapy carries an increased risk of irAEs, necessitating careful patient selection, close monitoring, and potential dose adjustments or immunosuppressant use (39,40). Identifying biomarkers for response prediction can optimize patient selection, enhancing efficacy and reducing side effects. The sequence of PD-1/PD-L1 and CTLA-4 inhibitor administration may influence outcomes, necessitating further research to determine the optimal protocol. Long-term follow-up studies are crucial to evaluate durability of response and potential late-onset side effects. While combined PD-L1 and CTLA-4 inhibition is promising for improving mCRC survival, careful consideration of patient selection, treatment sequencing, and monitoring for side effects is essential.

Our study also explored the relationship between mutation landscape, TMB, and MSI status. We observed specific mutations associated with TMB levels and MSI-H status, with significant correlations being found with certain gene mutations or subtypes. Notably, tumors with *KRAS* p.A146T, *PIK3CA* p.H1047R, and *BRAF* p.V600E mutations exhibited significantly higher proportions of MSI-H and non-conflictingly corresponding high-level TMB, suggesting that these patient groups may receive greater benefit from ICI therapy. Additionally, a recent phase II study in patients with KRAS/NRAS/BRAF wild-type, MSS, metastatic CRC demonstrated promising breakthroughs with combination ICI therapy (41,42). Meanwhile, a US Food and Drug Administration (FDA)-approved KRAS inhibitor, sotorasib, showed anticancer activity in patients with *KRAS* G12C mutations in advanced solid tumors (43-45). In our study, tumors with *KRAS* G12 mutations showed TMB levels similar to the wild type and were mostly MSS, suggesting the potential benefiting from similar regimens combining ICI therapy targeting the MSS subtype.

MSI-H, a predictive biomarker for CRC immunotherapy, faces challenges in patient stratification and treatment outcome improvement (46,47). Integrating genomics, transcriptomics, proteomics, and metabolomics data offers a comprehensive view of the tumor microenvironment, revealing novel biomarkers. Single-cell sequencing technologies can uncover cell-specific biomarkers predictive of immunotherapy response by analyzing heterogeneity within the tumor microenvironment (48). Assessing immune cell composition and function, including TILs, identifies biomarkers reflecting tumor immune status. Analyzing circulating tumor DNA (ctDNA), circulating immune cells, and soluble immune mediators in blood provides real-time tumor and immune activity information, potentially serving as predictive biomarkers (46). Functional assays, like T-cell proliferation and cytokine release, reflect immune system capacity. Correlating biomarker data with clinical outcomes validates predictive value. Developing scoring systems or risk models combining multiple biomarkers aids patient stratification and guides treatment decisions, enhancing CRC immunotherapy outcomes and advancing precision medicine.

The prospects for technological advancements in CRC immunotherapy are promising. Recent progress in molecular biology and bioinformatics has deepened our understanding of the CRC tumor microenvironment and immune landscape. A key focus is chemokines like CCL5, which plays a crucial role in CRC progression and immune cell recruitment (49). Understanding CCL5's regulatory mechanisms could lead to novel biomarkers

predicting immunotherapy response. Single-cell sequencing technologies are revolutionizing CRC research by analyzing tumor heterogeneity and immune cell interactions (50). This approach identifies immune cell populations associated with better therapeutic outcomes, facilitating personalized immunotherapy strategies. By characterizing the immune landscape at a single-cell level, potential biomarkers for treatment efficacy and resistance can be uncovered. Gene fusion neoantigens, arising from chromosomal rearrangements, are gaining attention as immunogenic biomarkers in CRC immunotherapy (51). Their broad applicability across cancer types makes them attractive for biomarker development, enhancing immunotherapeutic precision and patient stratification. Imaging technologies, such as PD-L1 tracer-based imaging, offer non-invasive assessment of key biomarkers in CRC patients (50), aiding patient selection for immunotherapy and real-time treatment monitoring. Novel technologies are enhancing our understanding of CRC biology and improving therapeutic outcomes. Integration of these advancements will be crucial for developing effective, personalized CRC immunotherapy strategies.

Certain limitations to this study should be acknowledged. As we did not employ a prospective design, many patients did not have available PD-L1 expression data and were thus excluded from the analysis. Additionally, the lack of treatment and follow-up information for these patients prevented us from further confirming the impact of these molecular characteristics on the response to immunotherapy. Therefore, future prospective cohort studies are needed to validate these findings.

Conclusions

In summary, our study sheds light on the complex interplay between genetic alterations and immune markers in patients with CRC, providing valuable insights for personalized treatment strategies and highlighting the need for further research in this field. Additionally, our stratification of patients with CRC based on relevant molecular subtypes could potentially influence the efficacy of combined strategies involving ICI and targeted therapies.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-24-748/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the guidelines of the Helsinki Declaration (as revised in 2013). The study was approved by the Medical Ethics Committee of Hospital of Chengdu University of Traditional Chinese Medicine (No. 2024KL-192). The other two participated hospitals were informed and agreed with this study. Individual consent for this retrospective analysis was waived.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Biller LH, Schrag D. Diagnosis and Treatment of Metastatic Colorectal Cancer: A Review. JAMA 2021;325:669-85.

3. Nguyen M, Tipping Smith S, Lam M, et al. An update on the use of immunotherapy in patients with colorectal cancer. Expert Rev Gastroenterol Hepatol 2021;15:291-304.
4. Xu H, Liu L, Li W, et al. Transcription factors in colorectal cancer: molecular mechanism and therapeutic implications. Oncogene 2021;40:1555-69.
5. Simon K. Colorectal cancer development and advances in screening. Clin Interv Aging 2016;11:967-76.
6. Yoshino T, Arnold D, Taniguchi H, et al. Pan-Asian adapted ESMO consensus guidelines for the management of patients with metastatic colorectal cancer: a JSMO-ESMO initiative endorsed by CSCO, KACO, MOS, SSO and TOS. Ann Oncol 2018;29:44-70.
7. Miller KD, Nogueira L, Mariotto AB, et al. Cancer treatment and survivorship statistics, 2019. CA Cancer J Clin 2019;69:363-85.
8. Zhao J, Zhong S, Niu X, et al. The MHC class I-LILRB1 signalling axis as a promising target in cancer therapy. Scand J Immunol 2019;90:e12804.
9. Kong X, Fu M, Niu X, et al. Comprehensive Analysis of the Expression, Relationship to Immune Infiltration and Prognosis of TIM-1 in Cancer. Front Oncol 2020;10:1086.
10. Picard E, Verschoor CP, Ma GW, et al. Relationships Between Immune Landscapes, Genetic Subtypes and Responses to Immunotherapy in Colorectal Cancer. Front Immunol 2020;11:369.
11. Ciardiello D, Vitiello PP, Cardone C, et al. Immunotherapy of colorectal cancer: Challenges for therapeutic efficacy. Cancer Treat Rev 2019;76:22-32.
12. Nixon NA, Blais N, Ernst S, et al. Current landscape of immunotherapy in the treatment of solid tumours, with future opportunities and challenges. Curr Oncol 2018;25:e373-84.
13. Wu X, Lan X, Hu W, et al. CMTM6 expression in M2 macrophages is a potential predictor of PD-1/PD-L1 inhibitor response in colorectal cancer. Cancer Immunol Immunother 2021;70:3235-48.
14. Farkona S, Diamandis EP, Blasutig IM. Cancer immunotherapy: the beginning of the end of cancer? BMC Med 2016;14:73.
15. Kishore C, Bhadra P. Current advancements and future perspectives of immunotherapy in colorectal cancer research. Eur J Pharmacol 2021;893:173819.
16. Fan A, Wang B, Wang X, et al. Immunotherapy in colorectal cancer: current achievements and future perspective. Int J Biol Sci 2021;17:3837-49.
17. Yarchoan M, Albacker LA, Hopkins AC, et al. PD-L1 expression and tumor mutational burden are independent biomarkers in most cancers. JCI Insight 2019;4:e126908.
18. Tokunaga R, Xiu J, Goldberg RM, et al. The impact of ARID1A mutation on molecular characteristics in colorectal cancer. Eur J Cancer 2020;140:119-29.
19. Domingo E, Ramamoorthy R, Oukrif D, et al. Use of multivariate analysis to suggest a new molecular classification of colorectal cancer. J Pathol 2013;229:441-8.
20. Cheng X, Xu X, Chen D, et al. Therapeutic potential of targeting the Wnt/β-catenin signaling pathway in colorectal cancer. Biomed Pharmacother 2019;110:473-81.
21. Liao H, Li X, Zhao L, et al. A PROTAC peptide induces durable β-catenin degradation and suppresses Wnt-dependent intestinal cancer. Cell Discov 2020;6:35.
22. Yi M, Niu M, Xu L, et al. Regulation of PD-L1 expression in the tumor microenvironment. J Hematol Oncol 2021;14:10.
23. Lal N, White BS, Goussous G, et al. KRAS Mutation and Consensus Molecular Subtypes 2 and 3 Are Independently Associated with Reduced Immune Infiltration and Reactivity in Colorectal Cancer. Clin Cancer Res 2018;24:224-33.
24. Sillo TO, Beggs AD, Morton DG, et al. Mechanisms of immunogenicity in colorectal cancer. Br J Surg 2019;106:1283-97.
25. Qiu Q, Tan D, Chen Q, et al. Clinical implications of PD-L1 expression and pathway-related molecular subtypes in advanced Asian colorectal cancer patients. Am J Cancer Res 2024;14:796-808.
26. Tian J, Chen JH, Chao SX, et al. Combined PD-1, BRAF and MEK inhibition in BRAF(V600E) colorectal cancer: a phase 2 trial. Nat Med 2023;29:458-66.
27. Chen G, Huang AC, Zhang W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature 2018;560:382-6.
28. Xu-Monette ZY, Zhang M, Li J, et al. PD-1/PD-L1 Blockade: Have We Found the Key to Unleash the Antitumor Immune Response? Front Immunol 2017;8:1597.
29. Ghahremanloo A, Soltani A, Modaresi SMS, et al. Recent advances in the clinical development of immune checkpoint blockade therapy. Cell Oncol (Dordr) 2019;42:609-26.
30. Brahmer JR, Hammers H, Lipson EJ. Nivolumab: targeting PD-1 to bolster antitumor immunity. Future Oncol 2015;11:1307-26.
31. Boichard A, Pham TV, Yeerna H, et al. APOBEC-related mutagenesis and neo-peptide hydrophobicity: implications for response to immunotherapy. Oncoimmunology

2018;8:1550341.
32. Jamy O, Sonpavde G. Emerging first line treatment options for bladder cancer: a review of phase II and III therapies in the pipeline. Expert Opin Emerg Drugs 2017;22:347-55.
33. Iwai Y, Hamanishi J, Chamoto K, et al. Cancer immunotherapies targeting the PD-1 signaling pathway. J Biomed Sci 2017;24:26.
34. Dolan DE, Gupta S. PD-1 pathway inhibitors: changing the landscape of cancer immunotherapy. Cancer Control 2014;21:231-7.
35. Wu Q, Jiang L, Li SC, et al. Small molecule inhibitors targeting the PD-1/PD-L1 signaling pathway. Acta Pharmacol Sin 2021;42:1-9.
36. Shergold AL, Millar R, Nibbs RJB. Understanding and overcoming the resistance of cancer to PD-1/PD-L1 blockade. Pharmacol Res 2019;145:104258.
37. Rotte A. Combination of CTLA-4 and PD-1 blockers for treatment of cancer. J Exp Clin Cancer Res 2019;38:255.
38. Klein-Brill A, Amar-Farkash S, Rosenberg-Katz K, et al. Comparative efficacy of combined CTLA-4 and PD-1 blockade vs. PD-1 monotherapy in metastatic melanoma: a real-world study. BJC Rep 2024;2:14.
39. Monjazeb AM, Giobbie-Hurder A, Lako A, et al. A Randomized Trial of Combined PD-L1 and CTLA-4 Inhibition with Targeted Low-Dose or Hypofractionated Radiation for Patients with Metastatic Colorectal Cancer. Clin Cancer Res 2021;27:2470-80.
40. Wojtukiewicz MZ, Rek MM, Karpowicz K, et al. Inhibitors of immune checkpoints-PD-1, PD-L1, CTLA-4-new opportunities for cancer patients and a new challenge for internists and general practitioners. Cancer Metastasis Rev 2021;40:949-82.
41. Lee MS, Loehrer PJ, Imanirad I, et al. Phase II study of ipilimumab, nivolumab, and panitumumab in patients with KRAS/NRAS/BRAF wild-type (WT) microsatellite stable (MSS) metastatic colorectal cancer (mCRC). J Clin Oncol 2021;39:abstr 7.
42. NIH-ClinicalTrials.gov. PhII Trial Panitumumab, Nivolumab, Ipilimumab in Kras/Nras/BRAF Wild-type MSS Refractory mCRC. 2020. Available online: https://classic.clinicaltrials.gov/ct2/show/NCT03442569
43. Skoulidis F, Li BT, Dy GK, et al. Sotorasib for Lung Cancers with KRAS p.G12C Mutation. N Engl J Med 2021;384:2371-81.
44. Blair HA. Sotorasib: First Approval. Drugs 2021;81:1573-9.
45. Fakih MG, Kopetz S, Kuboki Y, et al. Sotorasib for previously treated colorectal cancers with KRAS(G12C) mutation (CodeBreaK100): a prespecified analysis of a single-arm, phase 2 trial. Lancet Oncol 2022;23:115-24.
46. Hou W, Yi C, Zhu H. Predictive biomarkers of colon cancer immunotherapy: Present and future. Front Immunol 2022;13:1032314.
47. Sahin IH, Akce M, Alese O, et al. Immune checkpoint inhibitors for the treatment of MSI-H/MMR-D colorectal cancer and a perspective on resistance mechanisms. Br J Cancer 2019;121:809-18.
48. Chu X, Li X, Zhang Y, et al. Integrative single-cell analysis of human colorectal cancer reveals patient stratification with distinct immune evasion mechanisms. Nat Cancer 2024;5:1409-26.
49. Li Y, Lei Y, Sun J, et al. A promising research direction for colorectal cancer immunotherapy: The regulatory mechanism of CCL5 in colorectal cancer. Front Oncol 2022;12:1020400.
50. Bakouny Z, Flippot R, Braun DA, et al. State of the Future: Translational Approaches in Renal Cell Carcinoma in the Immunotherapy Era. Eur Urol Focus 2020;6:37-40.
51. Wang Y, Shi T, Song X, et al. Gene fusion neoantigens: Emerging targets for cancer immunotherapy. Cancer Lett 2021;506:45-54.

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