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Prognostic Implications of Chemokine Signaling Pathway Mutations for Immune Checkpoint Inhibitor Therapy in Colon Adenocarcinoma

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ABSTRACT

In recent years, immunotherapy has gained prominence both as a therapeutic option and as a major topic of cancer research. Despite this progress, the effectiveness of immune checkpoint inhibitors (ICI) in colorectal cancer remains limited, largely because current biomarkers can identify only a small fraction of patients who will benefit. Since chemokine signaling plays a key role in guiding immune cell recruitment and shaping antitumor immunity, this study examined whether alterations in chemokine-related genes influence outcomes in colon adenocarcinoma (COAD) patients treated with ICIs.Clinical and genomic data from an ICI-treated COAD cohort were obtained from cBioPortal and integrated with datasets from The Cancer Genome Atlas (TCGA). These resources were used to characterize mutation patterns, immunogenic features, and tumor microenvironment (TME) differences associated with distinct chemokine mutation profiles. Cox regression analyses indicated that patients with a high chemokine-mutation burden experienced significantly better survival following ICI therapy. According to CIBERSORT results, this subgroup displayed greater infiltration of M1 macrophages, neutrophils, and activated natural killer (NK) cells. Measures of immunogenicity—including tumor mutation burden (TMB), neoantigen load (NAL), DNA damage repair (DDR) pathway mutations, and microsatellite instability–high (MSI-H)—were also elevated in the high-mutation group. Overall, our findings suggest that the mutational status of the chemokine signaling pathway is strongly associated with ICI treatment outcomes in COAD. Enhanced genomic instability and a more immune-active TME may underlie the improved prognosis observed in patients with a high chemokine-mutation load, offering a potential direction for refining immunotherapy selection in COAD.

Keywords: Immune checkpoint inhibitors, Chemokine, Mutations, Colon adenocarcinoma, Tumor microenvironment

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Introduction

Colorectal cancer is a major malignancy of the gastrointestinal tract arising in the colon or rectum. Globally, it ranked as the third most frequently diagnosed cancer in 2020, representing roughly 10% of all new cancer cases, and the second leading cause of cancer-related mortality (9.4%) [1]. Colon adenocarcinoma (COAD), accounting for nearly 95% of large intestinal malignancies, represents the predominant histological subtype [2].

Recent advances have brought immunotherapy—particularly immune checkpoint inhibitors (ICIs)—to the forefront of cancer treatment. Despite this progress, ICI-based therapy has not yet produced major breakthroughs in colorectal cancer. Currently, mismatch repair deficiency (dMMR) or microsatellite instability–high (MSI-H) status remains the most reliable biomarker for identifying COAD patients likely to benefit from ICIs, and responses in this subgroup are often remarkable. However, patients who are mismatch repair proficient (pMMR), microsatellite stable (MSS), or have microsatellite instability–low (MSI-L) disease typically lack predictive biomarkers and show limited clinical benefit [3]. Since only about 15% of colorectal cancers exhibit dMMR–MSI-H status [3], the majority of patients remain without effective prognostic markers for ICI treatment.

Additional candidates such as tumor mutation burden (TMB) and POLE P286R mutations have been proposed as emerging biomarkers [4-6]. Nevertheless, each has limitations: studies of TMB have largely focused on dMMR/MSI-H cases [5, 6], and no clear immunological distinction between POLE-mutant and POLE–wild-type tumors has been demonstrated [4]. Consequently, identifying novel and dependable biomarkers to guide ICI application in COAD remains an urgent priority.

Chemokines are small signaling proteins that orchestrate the migration and accumulation of immune cells, thereby shaping antitumor immunity. For example, CXCR5 drives B-cell activation [7], whereas CXCL9/10/11 promote the infiltration of NK cells and T lymphocytes into tumor sites [7]. Chemokines and their receptors have also been implicated in predicting responses to ICI therapy across cancer types. CXCR3, for instance, has been reported as a key indicator of response to anti-PD-1 treatment in melanoma [8], while elevated expression of CXCL9 and CXCL10 has been associated with improved outcomes in patients receiving anti-PD-1 or anti-CTLA-4 agents [9]. Despite these observations, the prognostic significance of mutations within the chemokine signaling pathway for patients undergoing ICI therapy remains poorly explored.

To address this gap, we investigated whether mutational alterations in chemokine-related genes correlate with the therapeutic efficacy of ICIs in COAD. Using data from the TCGA and MSKCC cohorts, we assessed the prognostic value of high chemokine-pathway mutation status and employed bioinformatic analyses to identify potential mechanisms linking gene-level alterations to immune and microenvironmental changes.

Materials and Methods

ICI-treated cohort and TCGA-COAD cohort

Clinical information and somatic mutation profiles—including survival outcomes under immunotherapy—were obtained for a COAD cohort from cBioPortal [10], hereafter referred to as the ICI-treated cohort. Additionally, clinical data and mutation files for COAD samples were retrieved from The Cancer Genome Atlas (TCGA) using the TCGAbiolinks R package [11], designated as the TCGA-COAD cohort. These datasets formed the basis of the primary analyses.

To further validate our findings, we downloaded several additional datasets from cBioPortal, including non-immunotherapy COAD and immunotherapy cohorts for esophageal carcinoma (ESCA), non–small cell lung cancer (NSCLC), and skin cutaneous melanoma (SKCM) [10, 12, 13].

Gene set enrichment analysis (GSEA)

Raw count gene expression data for COAD were obtained through the *TCGAbiolinks* package in R. Differential expression between the high-mut and low-mut groups was assessed using the *edgeR* package [14]. Functional enrichment—including KEGG pathways, Reactome processes, and Gene Ontology (GO) categories—was performed with *clusterProfiler* [15], applying a significance threshold of $p < 0.05$. Gene sets used for GSEA were sourced from the MSigDB repository [16].

Tumor microenvironment (TME) analysis

Expression matrices from TCGA-COAD were submitted to the CIBERSORT web platform to estimate the relative abundance of 22 immune cell populations within the tumor microenvironment [17]. Data on immune checkpoint genes and immune-related scores were retrieved from Thorsson *et al.* [18]. Microsatellite instability status was inferred using the MANTIS algorithm, which stratifies tumors into MSI-L and MSI-H groups [19]. Immune infiltration scores were calculated using the ESTIMATE method [20].

Connectivity map (CMap) analysis

Differentially expressed genes between high-mut and low-mut groups in the TCGA dataset were transformed into GPL96 identifiers. The top 500 upregulated and downregulated genes were exported in GRP format and analyzed using the Connectivity Map (Build 02) to identify potential compounds associated with the observed gene expression signatures [21].

Statistical analysis

Univariate and multivariate Cox proportional hazards models were applied to examine the impact of chemokine pathway mutations on survival outcomes in COAD patients receiving ICI therapy, yielding hazard ratios (HRs) and 95% confidence intervals (CIs). Differences in TMB and NAL between mutation groups were assessed using

the Mann–Whitney U test, whereas Fisher's exact test was used to compare mutation frequencies among the top 20 most frequently altered genes. Overall survival was evaluated using Kaplan–Meier curves, with significance determined by the log-rank test. A p-value < 0.05 was considered statistically significant. All analyses and visualizations were performed in R.

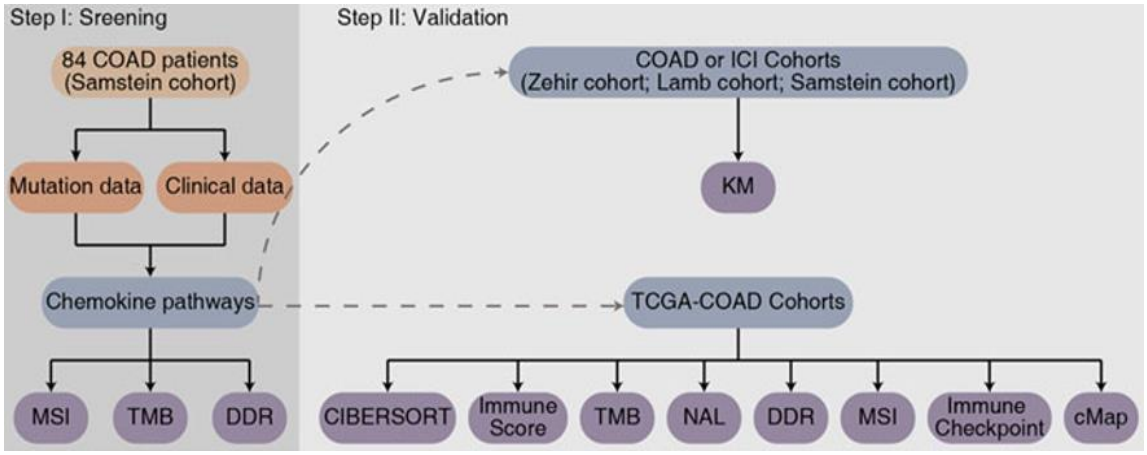
Results and Discussion

Chemokine pathway mutational load identifies COAD patients more likely to benefit from ICI therapy

Patients in the ICI-treated cohort were classified into two groups according to the number of non-synonymous alterations detected in chemokine-related genes. When these groups were examined using a univariate Cox model, neither age category nor tumor sampling site showed a meaningful association with survival under immunotherapy. In contrast, patients with a higher burden of chemokine pathway mutations demonstrated a clear survival advantage **(Figure 1b)**.

After adjusting for other clinical variables, the multivariate Cox analysis confirmed that the high-mutation category independently predicted better outcomes following ICI therapy **(Figure 1b)**. To explore this effect more directly, we compared overall survival across the 84 individuals included in the ICI-treated cohort. A pronounced separation of the survival curves was observed: individuals with high-mut status lived significantly longer than those with few or no chemokine pathway mutations (log-rank p = 0.01; HR = 0.41; 95% CI: 0.21–0.78) **(Figure 1d)**.

However, when the same mutation-based grouping strategy was applied to the TCGA-COAD dataset—where patients had not been uniformly treated with ICIs—no prognostic differences emerged **(Figures 1c and 1e)**. This contrast implies that chemokine pathway alterations do not intrinsically dictate tumor aggressiveness in COAD but appear to modulate how tumors respond immunologically when exposed to immune checkpoint blockade.



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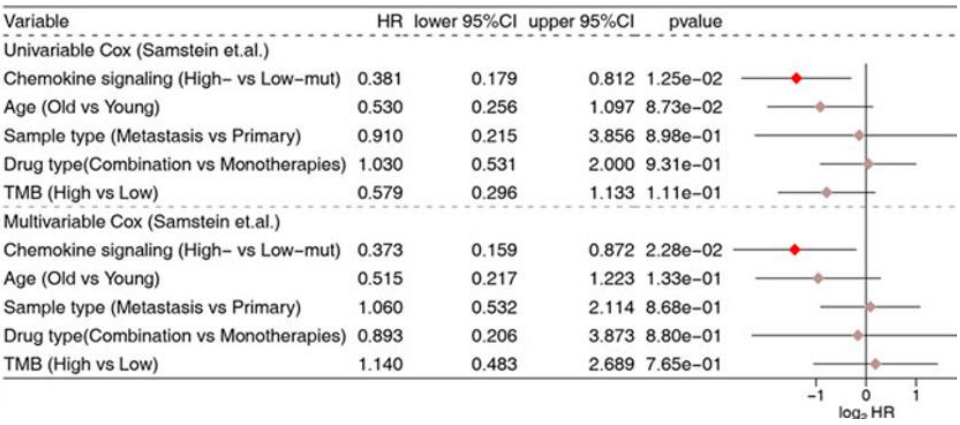

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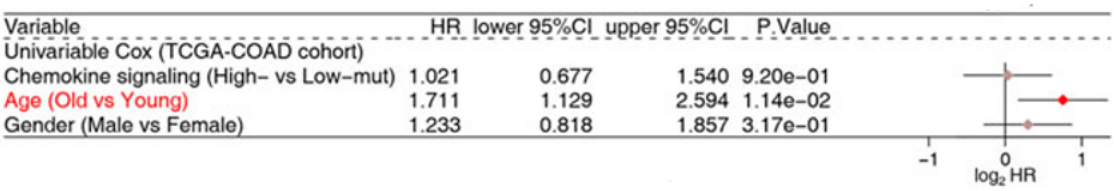

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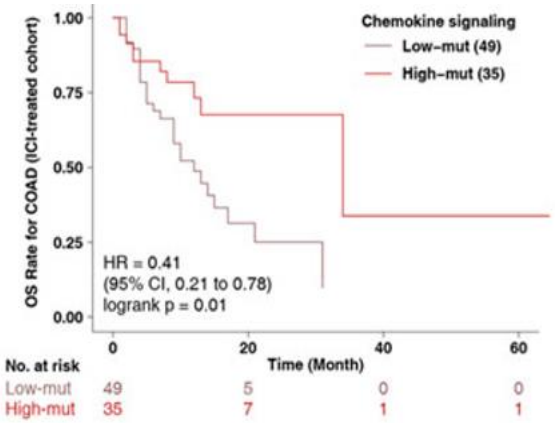
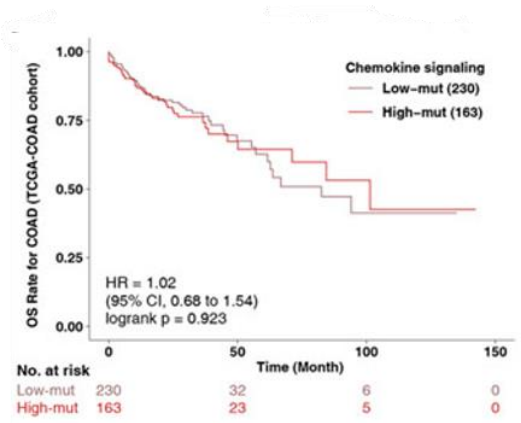




d) e)

Figure 1. Predictive significance of clinical variables and chemokine pathway mutation status for ICI response.

(a) Workflow summarizing dataset collection and analytical procedures. (b) Forest plots displaying univariate and multivariate Cox regression outcomes for the ICI-treated cohort [10], and (c) for the TCGA-COAD cohort. Hazard ratios (HRs) with their corresponding 95% confidence intervals (CIs) are shown; red markers denote predictors with $p < 0.05$. Values below 1 indicate factors associated with improved survival, whereas values above 1 signify detrimental effects. (d) Kaplan–Meier curves illustrating overall survival (OS) for the 84 COAD patients who received ICI therapy. (e) Kaplan–Meier OS curves for 393 COAD cases from the TCGA dataset.

Differences in gene mutation patterns and clinical features between high- and low-mutation groups

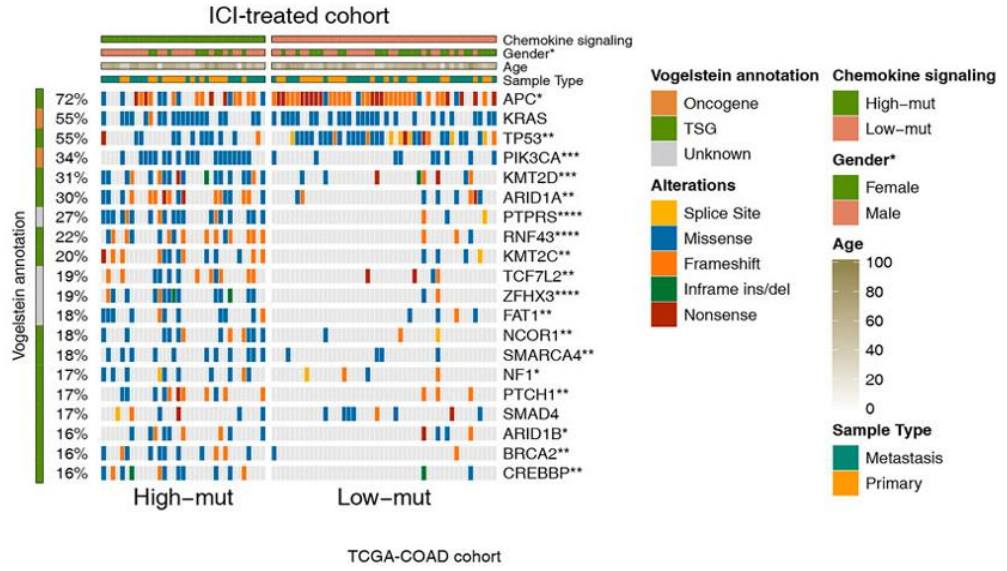
To characterize genomic differences linked to chemokine pathway mutation burden, we compared somatic mutation profiles between high-mut and low-mut groups across both the ICI-treated and TCGA-COAD cohorts. The twenty most frequently altered genes in each dataset were visualized to reveal overall mutational landscapes **(Figure 2)**.

In the ICI-treated cohort, nearly all of the highly mutated genes—except for APC, TP53, and KRAS—showed markedly elevated mutation frequencies in the high-mut subgroup **(Figure 2a)**. This included PIK3CA (0.54 vs. 0.18, $p < 0.05$), KMT2D (0.51 vs. 0.16, $p < 0.05$), ARID1A (0.49 vs. 0.16, $p < 0.05$), PTPRS (0.54 vs. 0.06, $p < 0.05$), and RNF43 (0.43 vs. 0.06, $p < 0.05$).

A similar trend was observed in the TCGA-COAD dataset, where the high-mut group showed significantly increased mutation rates for most top-ranked genes aside from APC and TP53 ($p < 0.05$); **(Figure 2b)**. Notable examples include TTN (0.72 vs. 0.39), KRAS (0.51 vs. 0.34), MUC16 (0.48 vs. 0.18), SYNE1 (0.41 vs. 0.22), and PIK3CA (0.47 vs. 0.16). Among all frequently altered genes, only KRAS and PIK3CA were proto-oncogenes.

We next assessed whether chemokine pathway mutation burden was associated with specific clinical characteristics. In the ICI-treated cohort, the proportion of male patients was higher in the high-mut subgroup ($p < 0.05$); **(Figure 3a)**, while sample type (primary vs. metastatic) did not differ significantly between groups ($p > 0.05$); **(Figure 3b)**.

In contrast, gender did not differ between mutation groups within the TCGA-COAD cohort ($p > 0.05$), despite earlier analyses suggesting that sex may influence prognosis **(Figure 1c)**. Instead, clinical stage and MSI category showed significant differences between high-mut and low-mut groups ($p < 0.05$), indicating that chemokine pathway mutation burden is not directly linked to patient sex but may associate with underlying tumor biology.



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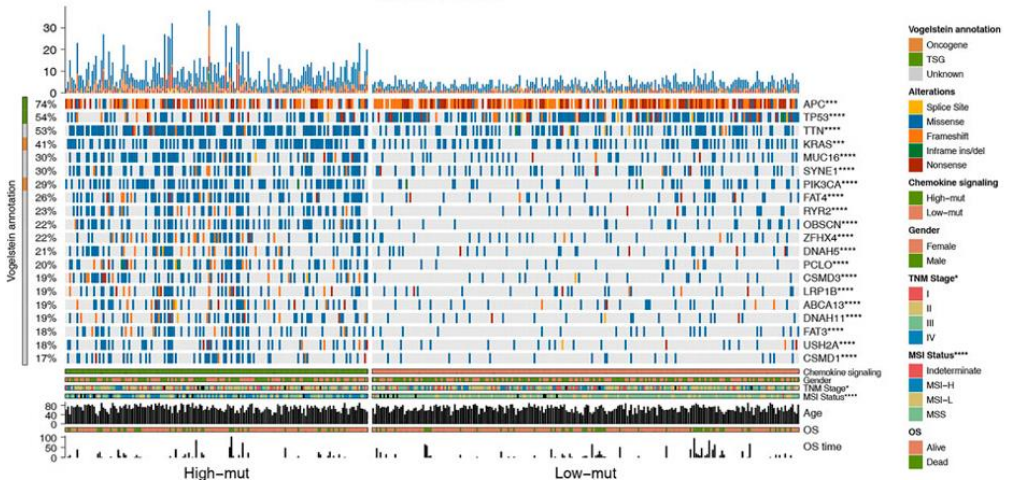

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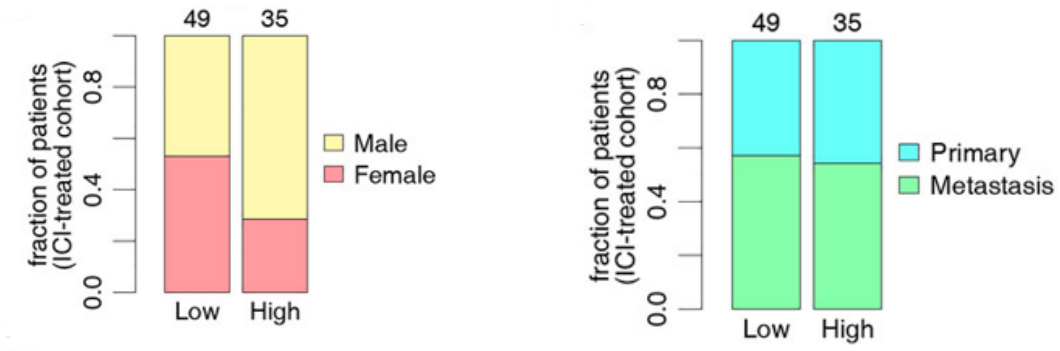
Figure 2. Genomic profiles of COAD patients in the ICI-treated cohort [10] (a) and the TCGA-COAD cohort (b). The 20 genes with the highest mutation frequencies and corresponding clinical information are shown in the figure.



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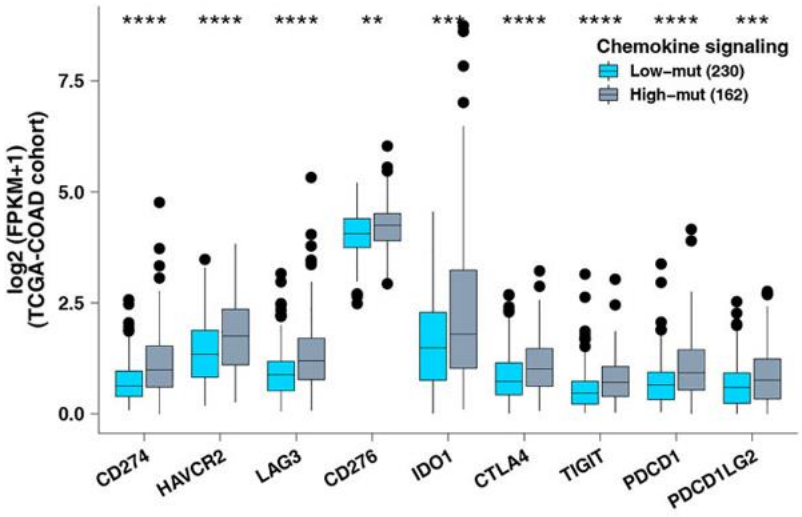

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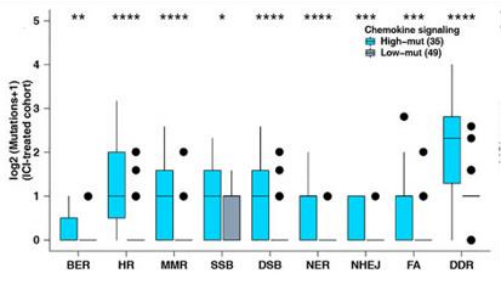
Figure 3. (a) Sex distribution in the ICI-treated cohort. (b) Distribution of primary versus metastatic samples in the ICI-treated cohort. (c) Levels of immune checkpoint gene expression in TCGA-COAD tumors.

Since immune checkpoint molecules are central targets of ICI therapy, we investigated whether their expression differed according to chemokine pathway mutation status. In the TCGA-COAD cohort, tumors harboring a high number of chemokine pathway mutations consistently showed elevated expression of multiple checkpoint genes compared with low-mut tumors ($p < 0.05$); **(Figure 3c)**, suggesting a more immunologically active tumor environment in high-mut cases.

High chemokine-pathway mutations may enhance tumor immunogenicity

Genomic instability is a key factor influencing tumor responsiveness to ICIs, with DNA damage repair (DDR) pathways serving as crucial regulators of genomic integrity. To explore potential mechanisms underlying the improved ICI outcomes in high-mut patients, we examined DDR-related gene sets from MSigDB. Across both the ICI-treated and TCGA-COAD cohorts, tumors with a high burden of chemokine pathway mutations carried significantly more nonsynonymous DDR gene alterations than low-mut tumors ($p < 0.05$); **(Figures 4a and 4b).** We also evaluated other established markers of immunogenicity, including microsatellite instability (MSI), tumor mutation burden (TMB), and neoantigen load (NAL). In the ICI-treated cohort, high-mut tumors exhibited higher MSI scores relative to low-mut cases ($p < 0.05$); **(Figure 4c).** Similarly, in TCGA-COAD samples, MANTIS-derived MSI estimates were elevated in high-mut tumors ($p < 0.05$); **(Figure 4e).** Tumor mutation burden was consistently greater in high-mut groups across both datasets ($p < 0.05$); **(Figures 4d and 4g),** and neoantigen load was also significantly increased in high-mut tumors within TCGA ($p < 0.05$); **(Figure 4f)**.

These findings indicate that tumors with elevated chemokine pathway mutational loads display heightened genomic instability and immunogenicity, which may underlie their superior responsiveness to immune checkpoint blockade.



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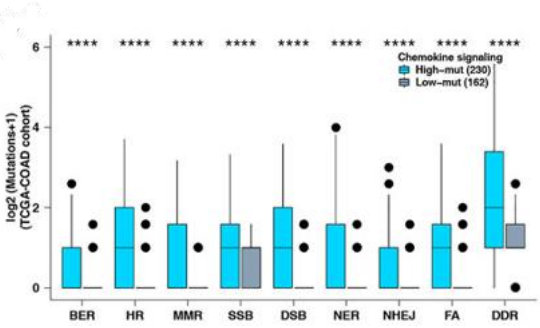

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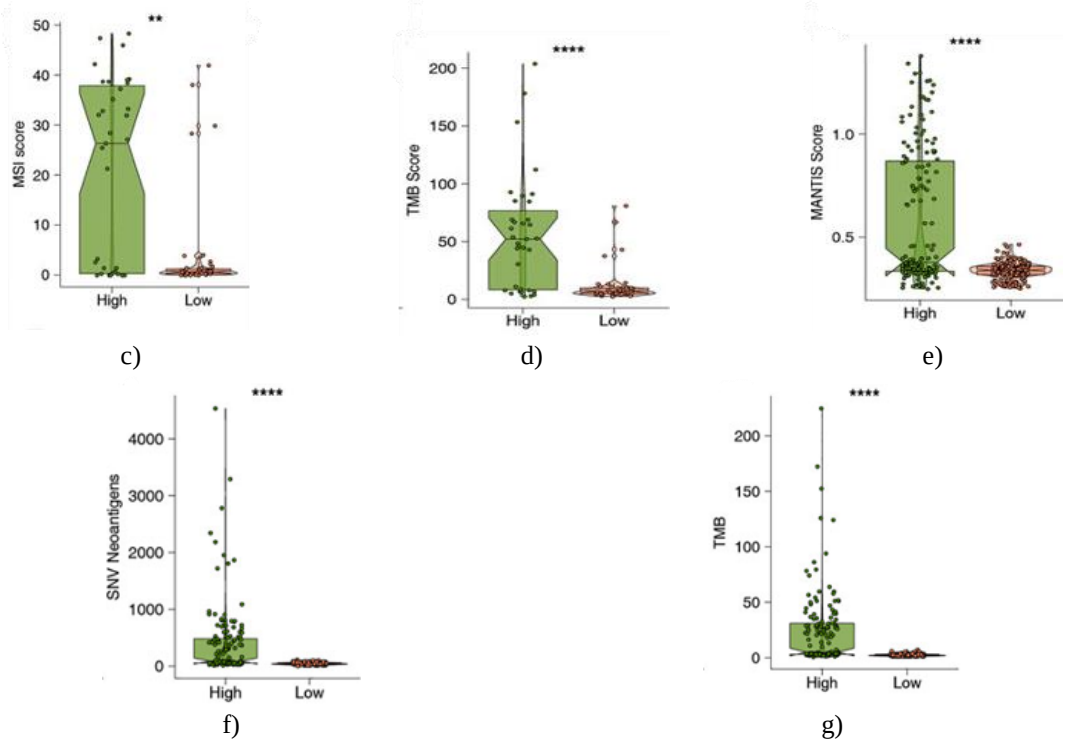

Figure 4. (a) DDR pathway mutations in high- versus low-mut groups, ICI-treated cohort. (b) DDR alterations in TCGA-COAD. (c) MSI comparison, ICI-treated cohort. (d) TMB comparison, ICI-treated cohort. (e) MSI comparison, TCGA-COAD. (F) NAL comparison, TCGA-COAD. (g) TMB comparison, TCGA-COAD.

High chemokine-pathway mutation burden is associated with an immune-active tumor microenvironment

To uncover why patients with a high load of chemokine pathway mutations show better responses to immune checkpoint therapy, we investigated the tumor microenvironment (TME) as a potential contributing factor. Using the TCGA-COAD dataset, immune cell composition was estimated with CIBERSORT, and patterns were compared between high-mut and low-mut groups.

Tumors with elevated chemokine pathway mutations were enriched for immune populations associated with antitumor activity, including M1 macrophages, activated NK cells, and neutrophils. Conversely, tumors with lower mutation burdens displayed higher levels of memory B cells, plasma cells, and naive CD4+ T cells. Correlation analysis revealed a positive association between mutation burden and the presence of M1 macrophages ($R = 0.19$, $p < 0.05$) and activated NK cells ($R = 0.18$, $p < 0.05$). Memory B cells ($R = -0.13$, $p < 0.05$), plasma cells ($R = -0.14$, $p < 0.05$), and naive CD4+ T cells ($R = -0.11$, $p < 0.05$) showed inverse relationships with mutation count. Neutrophils displayed a trend toward positive correlation without reaching statistical significance ($R = 0.10$, $p = 0.052$).

Interactions among these key immune populations highlighted a coordinated network of pro- and anti-tumor immune cells within the TME. To further explore therapeutic implications, Connectivity Map (cMap) analyses were conducted to identify compounds potentially modulating chemokine pathway-related mutations in COAD. Moreover, several immune activity metrics—including IFN-γ signaling, overall immune infiltration, macrophage regulation, TH1/TH2 cell activity, and leukocyte fraction—were significantly elevated in high-mut tumors ($p < 0.05$).

These findings indicate that high chemokine-pathway mutation burden correlates with an inflamed, immune-activated TME, which may underlie the observed sensitivity to immune checkpoint blockade in this patient subset.

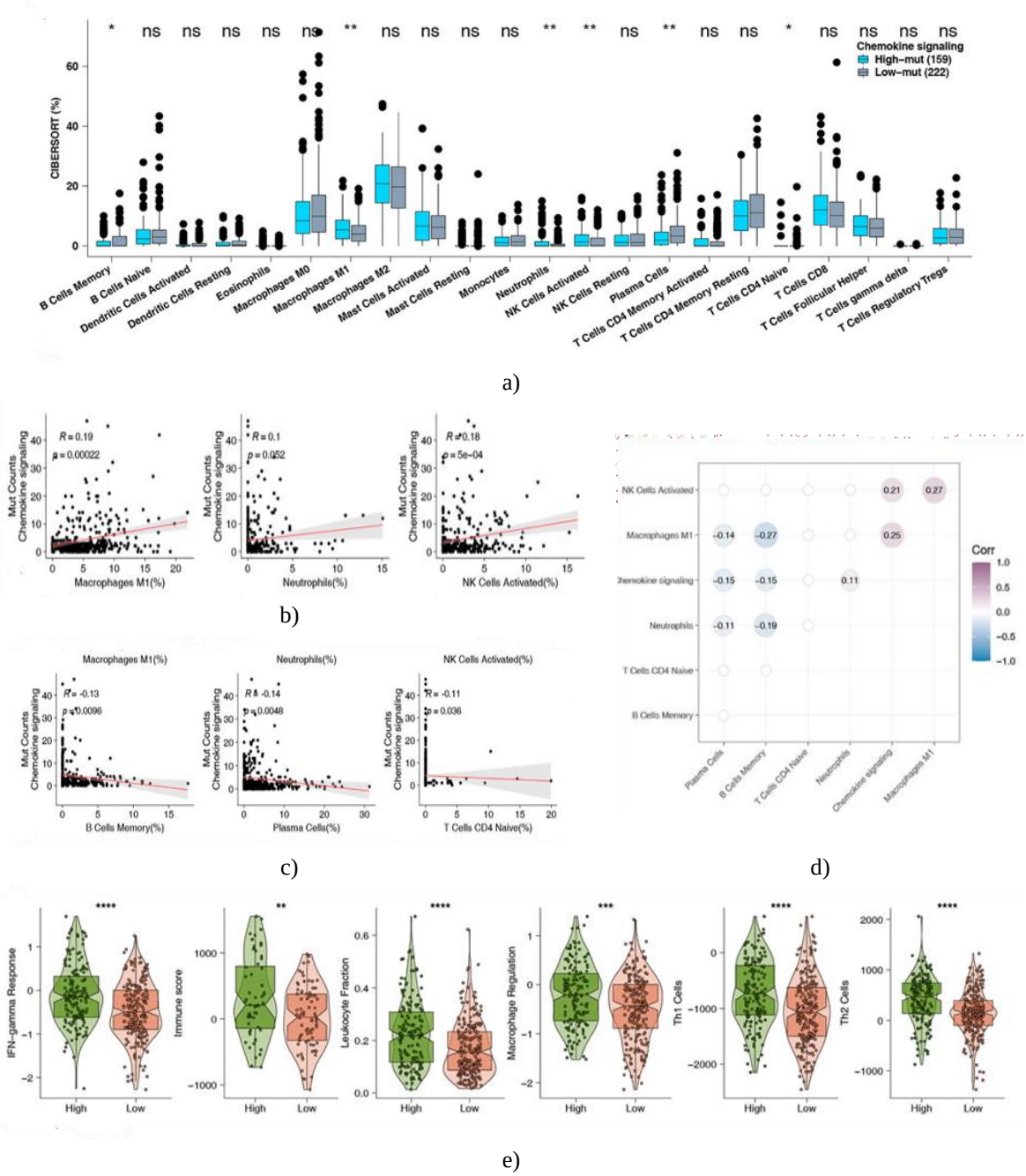

Figure 5. (a) Proportions of 22 immune cell types in high-mut versus low-mut tumors as estimated by CIBERSORT in TCGA-COAD. (b) Correlation between the abundance of highly infiltrated immune cells and DDR mutation counts in high-mut tumors. (c) Correlation between the abundance of low-infiltrated immune cells and DDR mutation counts in high-mut tumors. (d) Comparative distribution of all 22 immune cell types. (e) Immune-related scores for high-mut versus low-mut tumors, including IFN-γ response, overall immune score, leukocyte fraction, macrophage regulation, TH1, and TH2 scores.

Functional pathways underlying enhanced ICI responses in high-mut tumors

To understand the molecular basis for the superior responses and prognosis observed in high-mut COAD patients treated with ICIs, we performed Gene Set Enrichment Analysis (GSEA) comparing high-mut and low-mut groups. High-mut tumors displayed strong enrichment for pathways associated with cytotoxic immune activity and tumor cell elimination. Key processes included leukocyte migration during inflammatory responses, activation of natural killer cells, and antigen processing and cross-presentation **(Figure 6a)**. In addition, multiple cytokine-related pathways were prominently upregulated in high-mut tumors, reflecting a highly active immune milieu. These included signaling related to tumor necrosis factor, interferons, interleukins, colony-stimulating factors, and

chemokines, exemplified by positive regulation of interferon-alpha production, interleukin-2 biosynthesis, IL-2 family signaling, IL-6 mediated pathways, and chemokine receptor–ligand interactions **(Figure 6b)**.

Signaling cascades central to adaptive and innate immunity were also elevated in high-mut tumors, including JAK-STAT, Toll-like receptor, B cell receptor, T cell receptor, and Fc-gamma receptor pathways **(Figure 6c)**. By contrast, low-mut tumors were enriched for metabolic and immune-suppressive processes, such as fatty acid metabolism and regulation of fatty acid transport **(Figure 6d)**, consistent with a microenvironment less conducive to immune-mediated tumor clearance.

Collectively, these findings suggest that the high-mut status not only correlates with enhanced antitumor immune infiltration but also with activation of key immune signaling pathways, providing a mechanistic rationale for the improved clinical outcomes observed in this subset of ICI-treated patients.

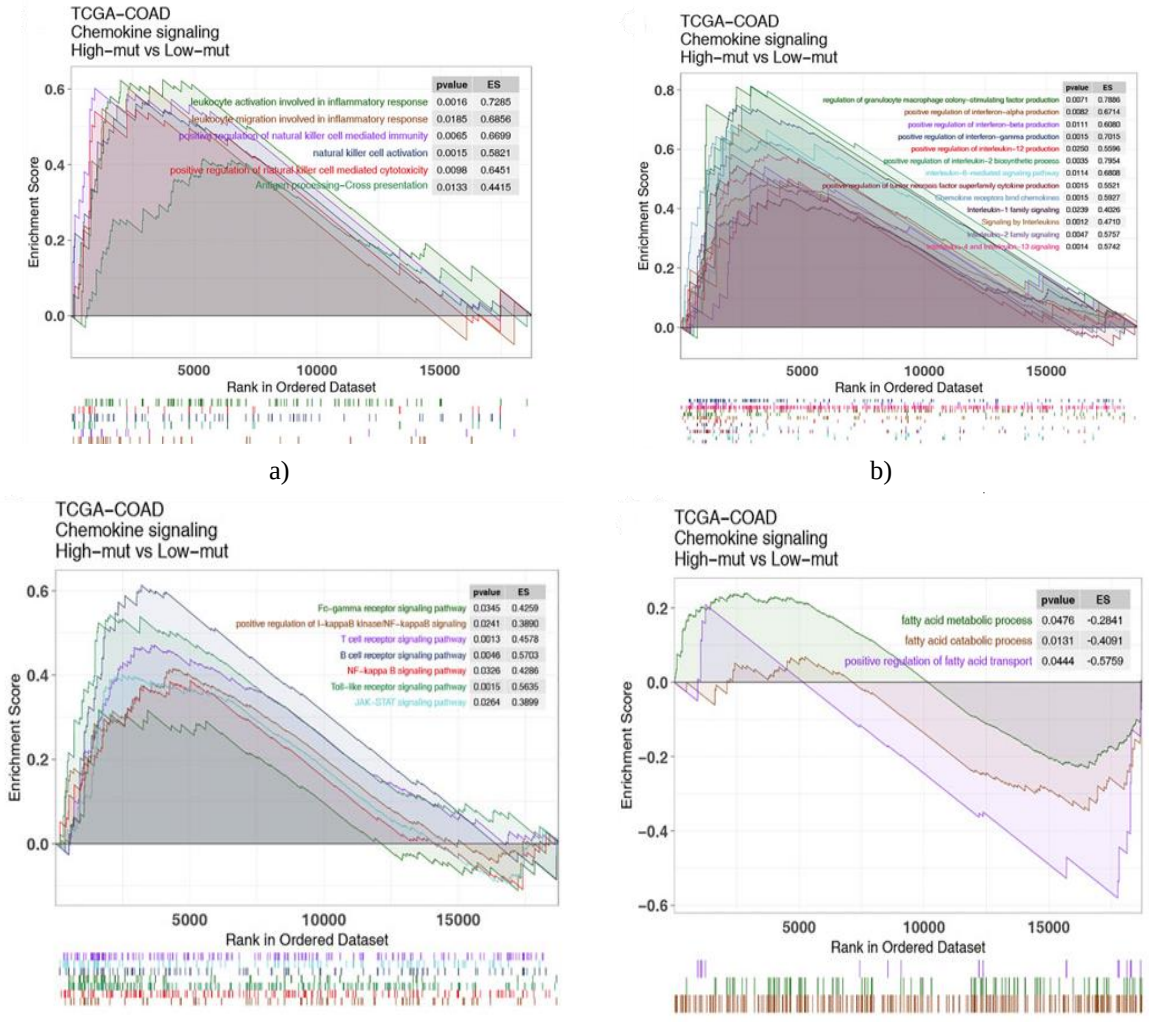

Figure 6. Biological processes differentially enriched between high-mut and low-mut tumors in the TCGA-COAD cohort as identified by GSEA. Panels show differences in immune cell activity (a), cytokine-related signaling (b), canonical immune pathways (c), and exhaustion-associated mechanisms (d).

Chemokine pathway mutations as context-dependent prognostic markers

To evaluate whether chemokine pathway mutations have predictive value beyond ICI-treated COAD, we analyzed survival across additional COAD cohorts lacking immunotherapy, as well as other tumor types undergoing immune checkpoint therapy. Kaplan–Meier analyses revealed that in COAD patients not receiving immunotherapy, chemokine mutation status did not correlate with overall survival **(Figure 7a)**. In contrast, high-mut status effectively predicted improved outcomes in other malignancies treated with ICIs, including esophageal carcinoma, non-small cell lung cancer, and skin cutaneous melanoma **(Figures 7b–7d)**.

These findings indicate that chemokine pathway mutations exert prognostic significance primarily in the context of immunotherapy. While their predictive value appears limited in untreated COAD, the consistent association with favorable outcomes across diverse ICI-treated tumors underscores a broader immunomodulatory role and highlights the potential for further mechanistic and translational investigations.

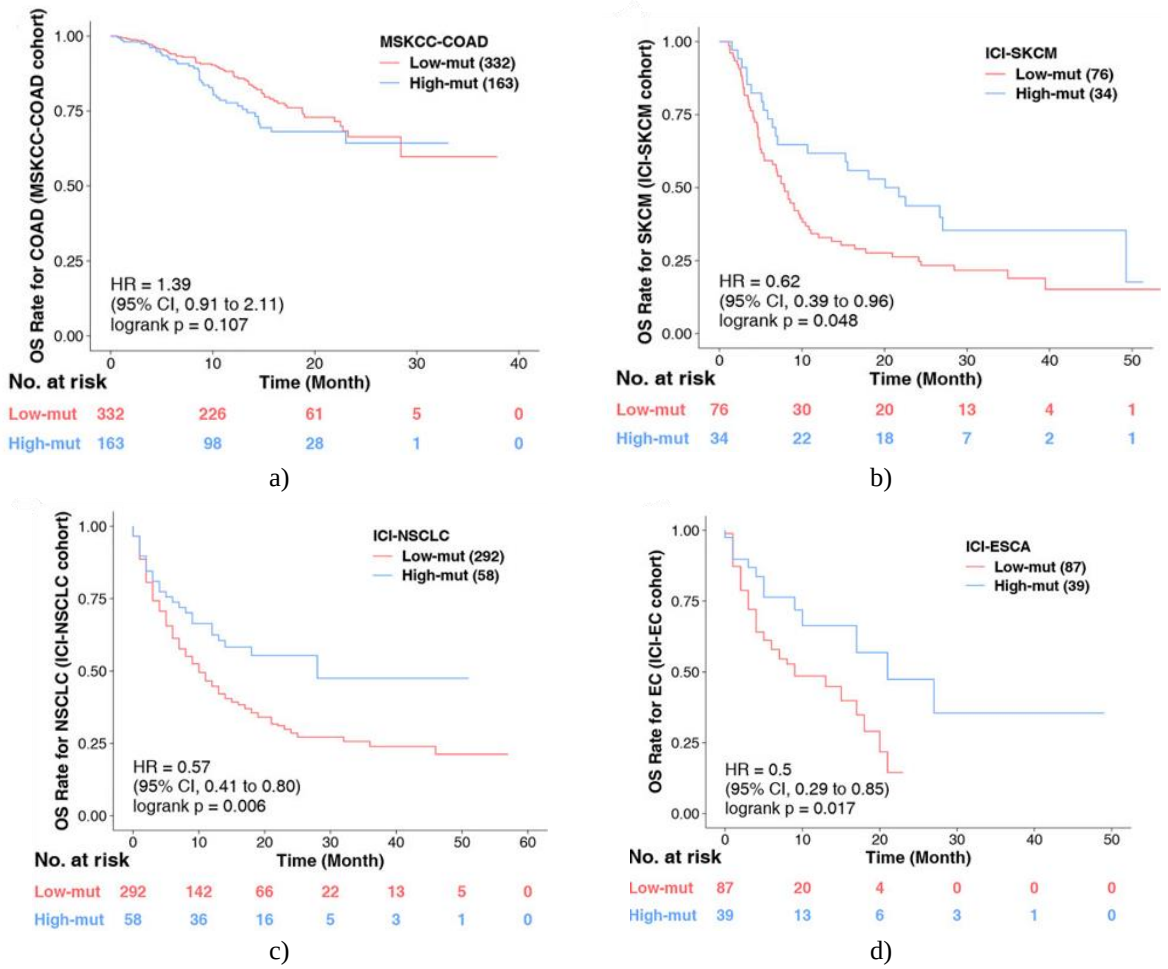

Figure 7. **(**a) KM survival curves for OS in 495 COAD patients from the MSKCC-COAD cohort [13]. (b) KM survival curves for OS in 110 COAD patients from the ICI-SKCM cohort [13]. (c) KM survival curves for OS in 350 COAD patients from the ICI-NSCLC cohort [21]. (d) KM survival curves for OS in 126 COAD patients from the ICI-ESCA cohort [10]

This study highlights the potential of chemokine signaling pathway mutations as predictors of immunotherapy outcomes in colon adenocarcinoma. By integrating clinical outcomes and somatic mutation profiles from a COAD cohort receiving immune checkpoint inhibitors, we observed that patients with a higher load of chemokine pathway mutations experienced markedly improved overall survival compared with patients harboring fewer mutations. These findings suggest that mutation burden within this pathway reflects a tumor state more responsive to immune-mediated therapy.

High-mut tumors also exhibited elevated levels of immune checkpoint gene expression. While these molecules are traditionally implicated in tumor immune evasion, their overexpression may paradoxically indicate an immune-activated microenvironment poised to respond to checkpoint blockade. This association implies that chemokine pathway mutations contribute to a more immunogenic tumor milieu, enhancing antitumor immunity and supporting patient responsiveness to ICIs.

Taken together, these results propose that counting nonsynonymous chemokine pathway mutations could serve as a practical biomarker for identifying COAD patients likely to benefit from immunotherapy. Beyond prognostication, this insight provides a foundation for mechanistic studies exploring how chemokine signaling

influences tumor–immune interactions and may guide the design of personalized immunotherapy strategies in colorectal cancer.

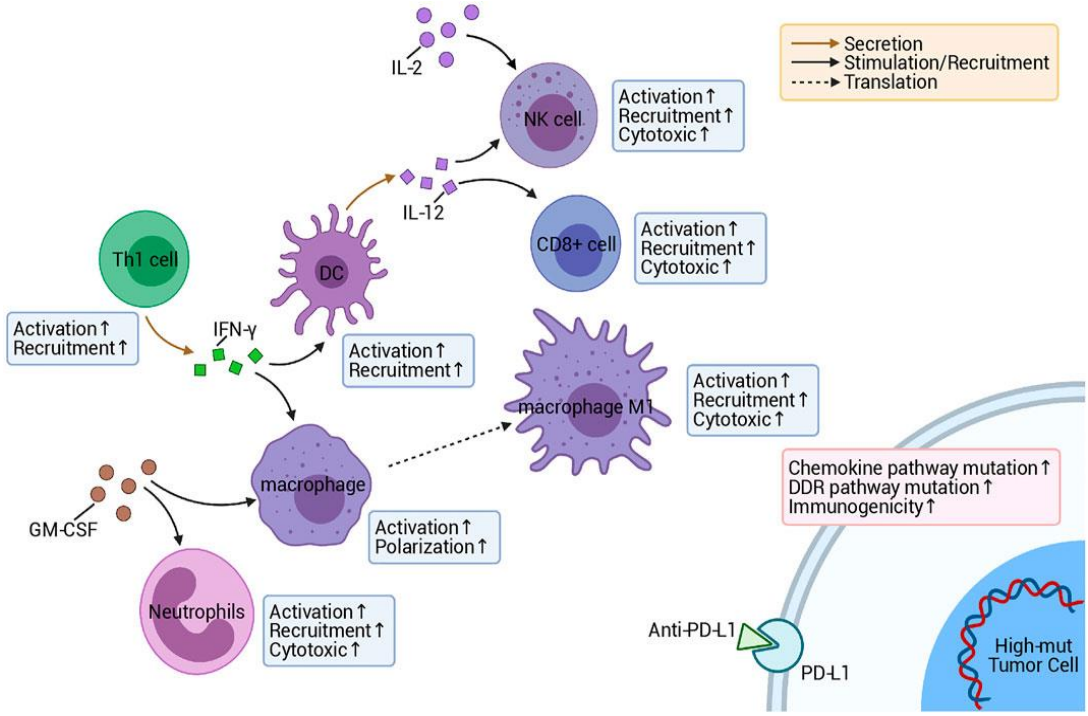

Figure 8. Proposed mechanism explaining the enhanced immunotherapy response and favorable prognosis in high-mut COAD tumors.

Mechanistic insights into improved ICI response in high-mut tumors

High immunogenicity appears to play a central role in enhancing the recognition of tumor cells by the immune system, thereby improving clinical outcomes for patients treated with immune checkpoint [22]. To investigate this, we compared indicators of immunogenic potential—including tumor mutation burden (TMB), neoantigen load (NAL), DNA damage repair (DDR) pathway mutations, and microsatellite instability (MSI)—between high-mut and low-mut tumors. Across all measures, high-mut tumors displayed significantly elevated TMB, NAL, DDR mutation counts, and MSI scores.

TMB has emerged as a robust biomarker for predicting ICI efficacy [23], with higher TMB generally correlating with an increased repertoire of tumor-specific neoantigens that facilitate immune recognition and improve therapeutic response [24-26]. DDR pathway defects can further increase TMB by compromising genomic stability, thereby enhancing tumor immunogenicity and responsiveness to immunotherapy [27]. Similarly, MSI-high status has been consistently associated with better outcomes under ICI treatment, confirming its predictive relevance [28].

Beyond intrinsic immunogenicity, the composition of the tumor microenvironment (TME) is another critical determinant of immunotherapy outcomes. Using CIBERSORT to profile 22 immune cell populations, we observed that high-mut tumors were preferentially infiltrated by pro-inflammatory and cytotoxic immune cells, including M1 macrophages, activated NK cells, and neutrophils. Notably, the abundance of these effector cells correlated positively with the number of chemokine pathway mutations. M1 macrophages produce inflammatory cytokines such as IL-6, IL-12, and TNF-α, which are pivotal for antitumor immunity [29]. NK cells exert direct cytotoxic effects, and their activity has been linked to the efficacy of anti-PD-1/PD-L1 therapy [30]. While neutrophils can have dual roles in cancer, in colorectal cancer they predominantly contribute to antitumor activity [31].

Supporting these observations, GSEA revealed that immune-mediated cytotoxicity pathways were markedly upregulated in high-mut tumors. Key immune signaling cascades—including JAK-STAT, Toll-like receptor, and Fc-γ receptor pathways—were also enriched, providing mechanistic insight into immune cell activation. JAK-STAT signaling is known to promote M1 macrophage differentiation and enhance NK cell function [32, 33], Toll-like receptors contribute to early immune responses and macrophage polarization [34], and Fc-γ receptor

activation drives cytotoxic activity across NK cells, macrophages, and other immune effectors [35]. Collectively, these findings suggest that chemokine pathway mutations facilitate both a highly immunogenic tumor state and a pro-inflammatory, cytotoxic TME, providing a biological explanation for the superior responses observed in high-mut COAD patients receiving ICIs.

Cytokine signaling and immunotherapy response

Cytokines play a central role in orchestrating the antitumor immune response, and the pronounced activation of cytokine-related pathways in high-mut tumors likely contributes to their enhanced sensitivity to immune checkpoint inhibitors. Previous research has demonstrated that the IL-12–IFN-γ axis potentiates anti-PD-1 responses, while activation of noncanonical NF-κB signaling can further stimulate IL-12 production [36]. Consistent with these findings, our GSEA results highlighted enrichment in pathways associated with leukocyte activation during inflammatory responses, NK cell-mediated immunity, IFN-γ and IL-12 production, and NF-κB signaling. Additionally, pathways controlling granulocyte-macrophage colony-stimulating factor (GM-CSF) production and chemokine receptor-ligand interactions were upregulated, suggesting a role for these factors in promoting immune cell proliferation and activity within high-mut tumors [37-40]. Conversely, pathways related to fatty acid metabolism and transport, which have been linked to immune exhaustion and metastatic progression, were downregulated in the high-mut group [41, 42], further supporting a favorable immune landscape.

Study limitations

This study has several limitations. First, only a single COAD cohort treated with ICIs was available, reflecting the scarcity of publicly available immunotherapy datasets for colorectal cancer. Nevertheless, similar trends were observed in other tumor types treated with ICIs **(Figures 7b–7d),** suggesting that the observed associations are not incidental. Future studies should include additional COAD cohorts to validate and extend these findings.

Second, we did not perform integrated survival analyses combining chemokine mutation status with TMB or MSI due to insufficient subgroup sizes, although multivariate Cox regression demonstrated that chemokine mutations are an independent prognostic factor **(Figure 1b).** Larger datasets will allow these combined analyses in the future.

Finally, the diversity of mutation sites within chemokine signaling pathways complicates mechanistic interpretation. While we compared mutated versus wild-type tumors and integrated GSEA results with prior experimental evidence, the precise molecular mechanisms by which chemokine pathway mutations influence the TME remain to be elucidated and warrant further investigation.

Conclusion

Our study identifies chemokine pathway mutation status as a robust predictor of immunotherapy response in COAD. Patients with high-mut tumors exhibited significantly longer overall survival compared with low-mut counterparts. Elevated TMB, NAL, DDR pathway mutations, and a pro-inflammatory, cytotoxic tumor microenvironment likely underlie this improved prognosis. These findings position chemokine pathway mutations as a promising biomarker for patient stratification in ICI therapy and provide a foundation for future research aimed at optimizing personalized immunotherapy strategies for colorectal cancer.

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