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Predictive Value of Combined Detection of Peripheral Blood Treg and IL-10 for Disease Progression in Patients with Colorectal Cancer

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Abstract

Objective

Regulatory T cells (Treg) and interleukin-10 (IL-10) play critical roles in the tumor immune microenvironment. This study evaluated the independent and combined predictive value of peripheral blood Treg proportion and plasma IL-10 for disease progression (PD) in colorectal cancer (CRC) patients, and investigated the association between distinct immune phenotypes and PD.

Methods

Treg proportion and plasma IL-10 were measured in 76 newly diagnosed CRC patients. Associations with TNM/clinical stage and their correlation were analyzed. Patients were stratified into four immune phenotypes using median values as cutoffs. PD distribution among phenotypes was tested by chi-square with post-hoc Fisher's exact test and Bonferroni correction. Logistic regression, ROC curves, calibration, and DCA evaluated model performance.

Results

Treg and IL-10 showed a significant negative correlation (r_s = -0.238, P = 0.0476). Elevated Treg was associated with male sex, M1 stage, and stage IV, whereas decreased IL-10 correlated with lymph node metastasis, M1 stage, stage IV, and postoperative status. The "high Treg/low IL-10" phenotype was enriched in the PD group (adjusted P = 0.030, OR = 5.05). Multivariate regression identified Treg (OR = 1.43, P = 0.005) and IL-10 (OR = 0.78, P = 0.037) as independent predictors. The combined model achieved the highest AUC (0.767, 95% CI: 0.660–0.874), outperforming IL-10 model (P = 0.024). Calibration and DCA confirmed good agreement and net benefit; a nomogram enabled individualized prediction.

Conclusions

Treg and IL-10 are independent predictors of PD in CRC. The "high Treg/low IL-10" phenotype may serve as a biomarker for high-risk patients. The combined model showed superior predictive performance and clinical utility over single indicators, supporting its value for early risk stratification and treatment guidance.

Introduction

Colorectal cancer (CRC) is one of the most common malignancies worldwide, ranking third in incidence and second in mortality [1]. Notably, the incidence of early-onset CRC (diagnosed at age < 50 years) is steadily increasing globally, particularly in high-income countries [2, 3]. Despite advances in screening and treatment, a considerable proportion of patients still experience progressive disease (PD) during treatment, leading to reduced survival rates [4]. Mechanistically, The tumor microenvironment (TME) plays a critical role in tumor initiation, progression, and therapeutic resistance, wherein immune cells and their secreted cytokines form a complex regulatory network [5, 6].

Regulatory T cells (Tregs) are key immunosuppressive cells in the TME that suppress anti-tumor immune responses through multiple mechanisms (e.g., secretion of IL-10, TGF-β), thereby promoting tumor immune evasion [7]. Multiple studies have confirmed that elevated Treg proportions in peripheral blood and tumor tissues of CRC patients are significantly associated with tumor progression, distant metastasis, and poor prognosis [8, 9].

IL-10 is a pleiotropic cytokine with dual regulatory roles in tumor immunity [10]: high concentrations can suppress antigen-presenting cell function and effector T cell activity, exerting immunosuppressive effects; whereas under specific conditions, IL-10 can enhance anti-tumor immunity by activating $CD8^+$ T cells and natural killer (NK) cells [11, 12]. However, the specific role of IL-10 in CRC progression and its synergistic relationship with Tregs remain unclear. Clinical studies have yielded inconsistent findings regarding the prognostic value of IL-10, indicating that its predictive value alone is limited and a comprehensive analysis integrating other immune indicators is warranted [13, 14].

Treg and IL-10 exhibit a complex interplay: Tregs can secrete IL-10, and IL-10 further promotes Treg differentiation and functional maintenance, forming a positive feedback loop [15–17]. Meanwhile, IL-10 exerts negative immunoregulatory effects by suppressing

effector T cells and antigen-presenting cells [18]. In CRC, Tregs can be divided into functionally distinct subsets based on IL-10 secretion: the IL-10$^+$ Treg subset exerts anti-tumor effects by inhibiting pro-tumorigenic IL-17$^+$ effector T cells, correlating with favorable clinical outcomes; whereas the IL-10$^-$ Treg subset primarily mediates immunosuppression and promotes tumor immune evasion [19].

Notably, the role of Tregs in CRC exhibits a stage-dependent pattern—suppressing excessive inflammation in early tumorigenesis while promoting immunosuppression in established tumors [20]. This functional divergence suggests that Tregs' prognostic value depends on subset composition and disease stage [19, 20]. We hypothesize that predominance of the IL-10$^-$ Treg subset with concurrent deficiency of the IL-10$^+$ subset may represent an "immune imbalance" state associated with poor prognosis [19]. However, this combined pattern has not been systematically evaluated in CRC, as previous studies have predominantly analyzed Treg proportion or IL-10 concentration as independent indicators [21, 22], leaving the clinical significance of the high Treg/low IL-10 phenotype unclear. Elucidating the Treg–IL-10 interplay in CRC may provide insights into immune evasion mechanisms and inform the development of immunotherapeutic strategies.

Accordingly, this study aims to: (1) evaluate the independent predictive value of peripheral blood Treg proportion and plasma IL-10 level for PD in CRC; (2) explore the association between the high Treg/low IL-10 phenotype and PD risk; and (3) construct and compare predictive models based on Treg, IL-10, and their combination to provide a feasible biomarker panel for clinical risk stratification.

Methods

2.1 Study Population and Design

After obtaining written informed consent, this study retrospectively enrolled 76 patients with CRC (age range: 32–83 years) who were diagnosed and treated at the Tianjin Cancer Hospital Airport Hospital from July 2025 to March 2026. All patients were first-time admissions, and peripheral blood samples were collected simultaneously prior to treatment for both assays. Inclusion criteria were: pathologically confirmed diagnosis and complete clinicopathological data. Exclusion criteria were: (1) concurrent other malignancies or autoimmune diseases; (2) concurrent severe infection; (3) recent neoadjuvant therapy, anticoagulant therapy, or long-term NSAID use; (4) history of other malignancies within 5 years; (5)severe hepatic, renal, or hematological dysfunction; (6) inflammatory bowel disease or cardio-cerebrovascular diseases [23]. Based on imaging and clinical evaluation, patients were divided into a PD group and a Non-PD group. All patients were fully informed of the study purpose and procedures and provided written informed consent before enrollment. PD Status was assessed by [18F]FDG PET-CT scanning. The PERCIST 1.0 criteria were used to define PD, which included: (1) a 30% or greater increase in the sum of the [18F]FDG-SUVmax of target lesions; (2) appearance of new [18F]FDG-avid lesions typical of cancer; or (3) progression of non-target disease. All PET-CT images were independently evaluated by two experienced nuclear medicine physicians who were blinded to the patients' clinical outcomes.

2.2 Detection Methods

Treg cell detection: Peripheral blood was collected in EDTA-K_2 anticoagulant tubes. 50 μL of whole blood were used for flow cytometry staining. Each sample was incubated with 10 μL of antibodies, including FITC-conjugated anti-CD3, APC-conjugated anti-CD4, PE-Cy5.5-conjugated anti-CD25, and PE-conjugated anti-CD127 (all from Beckman Coulter), and washed with PBS. The CD3$^+$CD4$^+$CD25^highCD127^low phenotype was used to define Tregs, as this gating strategy has been validated to specifically enrich FoxP3$^+$ natural Tregs while avoiding non-specific inclusion of activated conventional T cells [24]. Treg proportion was calculated as the percentage of CD3$^+$CD4$^+$CD25^highCD127^low cells among total CD4$^+$ T cells.

IL-10 detection: EDTA-K_2 anticoagulated peripheral blood was centrifuged at 2,000 rpm for 10 minutes to separate plasma. 25 μL of plasma were collected and processed strictly according to the instructions of the Six Cytokine Detection Kit (flow cytometric fluorescence method, Unimed). All plasma samples were processed within 24 hours of collection and stored at 4°C before acquisition to ensure stability of cytokine levels.

Data analysis: Peripheral blood samples from each subject were simultaneously analyzed for Treg and IL-10 using a DxFLEX flow cytometer (Beckman Coulter), and data were processed with Kaluza software (Beckman Coulter). Representative flow cytometry plots illustrating the gating strategy for Treg identification (Fig. 1A) and the standard curve for IL-10 quantification (Fig. 1B) are presented in Fig. 1.

2.3 Immunophenotype Grouping

Using the median values of Treg proportion and IL-10 level as cutoff points, all patients were classified into four immunophenotype groups based on their levels above or below the respective medians: low Treg low IL-10, low Treg high IL-10, high Treg low IL-10, and high Treg high IL-10. This median-based stratification approach has been widely employed in CRC immunophenotyping studies to define immune subgroups for prognostic analysis[21]. The distribution of these four immunophenotype groups was subsequently compared between the PD and Non-PD groups to evaluate their association with PD.

2.4 Statistical Analysis

Statistical analyses were performed using GraphPad Prism 10.6.1 (GraphPad Software, San Diego, CA, USA) and R version 4.3.3 (R Foundation for Statistical Computing, Vienna, Austria). Normality was assessed by the Shapiro–Wilk test (GraphPad Prism). Normally distributed data were expressed as mean ± SD, skewed data as median (IQR). Differences in Treg or IL-10 levels between two groups were compared using independent samples t-test or Mann–Whitney U test, as appropriate (GraphPad Prism). Spearman's correlation was evaluated using GraphPad Prism. The distribution of four immunophenotype groups between PD and Non-PD groups was compared using Pearson's chi-square (χ^2) test in R. If significant, post-hoc Fisher's exact tests with Bonferroni correction (adjusted P = original P × 4) were performed. Univariate and multivariate logistic regression models (with PD as the dependent variable) were constructed in R, and odds ratios (ORs) with 95% confidence intervals (CIs) were computed. Receiver operating characteristic (ROC) curves were constructed using the pROC package(version 1.19.0.1) [25]in R; AUCs with 95% CIs were calculated and compared via DeLong test models [26]. Residual diagnostics and the immunophenotype distribution plot were generated using the ggplot2 package (version 4.0.2). All P values were two-sided, $P < 0.05$ was considered statistically significant. Data are presented as median (IQR) or mean ± SD as appropriate.

A nomogram was constructed based on the multivariate logistic regression model to visualize the prediction model for individualized risk assessment. Calibration curves were generated to assess the agreement between predicted probabilities and observed outcomes using 1,000 bootstrap resamples. Decision curve analysis (DCA) was performed to evaluate the clinical net benefit of each model across a range of threshold probabilities. All above analyses were implemented using the rms package (version 6.7-1) and the rmda package (version 1.6) in R.

Results

3.1 Baseline characteristics of the study cohort

A total of 76 patients were included in this study, with a median age of 60 years (range: 32–83 years). The cohort comprised 46 males (60.5%) and 30 females (39.5%). Among the patients, 27 (35.5%) had clinical stage III and 37 (48.7%) had stage IV; 17 (22.4%) had N0 stage and 59 (77.6%) had N1/N2 stage; 38 (50.0%) had M0 stage and 38 (50.0%) had M1 stage. Baseline demographic and clinicopathological characteristics of the study cohort are summarized in Table 1.

Table 1

Baseline clinical and pathological characteristics of colorectal cancer patients stratified by Treg proportion and IL-10 level. Notes: Tregs, Regulatory T cells. Data for Treg are expressed as mean ± SD and analyzed by independent samples t-test. For IL-10, data in Gender, Clinical stage, and T stage are normally distributed and presented as mean ± SD (t-test); data in Age, N stage, and M stage are non-normally distributed and presented as median (IQR) (Mann–Whitney U test).

P < 0.05 was considered statistically significant.

Characteristics	n	Treg(CD3⁺CD4⁺CD25⁺CD127^low)/CD4+ T cells (%)	*P*-value	IL-10 (pg/mL)	*P*-value
Age		Mean ± SD		Median (IQR)	
≤ 60	44	10.570 ± 2.552	0.3447	5.107 (3.443–6.733)	0.1337
>60	32	10.040 ± 2.193		6.065 (5.003–7.064)	
Gender		Mean ± SD		Mean ± SD	
Male	46	10.830 ± 2.522	**0.0440**	5.710 ± 2.578	0.5216
Female	30	9.6930 ± 2.088		5.348 ± 2.095	
Clinical stage		Mean ± SD		Mean ± SD	
III	27	9.637 ± 2.057	**0.0299**	6.020 ± 2.124	**0.0433**
IV	37	10.95 ± 2.511		4.876 ± 2.237	
Tumor (T stage)		Mean ± SD		Mean ± SD	
T3	46	10.430 ± 2.428	0.9251	5.524 ± 2.676	0.9911
T4	28	10.370 ± 2.398		5.518 ± 1.929	
Lymph node metastasis (N stage)		Mean ± SD		Median (IQR)	
N0	17	9.947 ± 2.474	0.4386	6.142 (5.638–7.448)	**0.0369**
N1/N2	59	10.460 ± 2.396		5.110 (3.755–6.824)	
Distant metastasis (M stage)		Mean ± SD		Median (IQR)	
M0	38	9.739 ± 2.200	**0.0263**	6.065 (4.940–7.461)	**0.0295**
M1	38	10.960 ± 2.478		5.093 (2.983–6.251)	

3.2 Associations of Treg and IL-10 with clinicopathological characteristics

Treg proportion was significantly associated with sex: male patients had a significantly higher Treg proportion than females (10.830 ± 2.522% vs. 9.693 ± 2.088%, *P* = 0.0440) (Fig. 2B). Treg proportion was also significantly higher in patients with M1 stage than in those with M0 stage (10.960 ± 2.478% vs. 9.739 ± 2.200%, *P* = 0.0263) (Fig. 2E), and in patients with clinical stage IV than in those with stage III (10.950 ± 2.511% vs. 9.637 ± 2.057%, *P* = 0.0299) (Fig. 2F). No significant associations were observed between Treg proportion and age, T

stage, or N stage (all $P > 0.05$). These results indicate that elevated Treg proportion is associated with male sex, distant metastasis, and advanced tumor stage.

IL-10 level was significantly lower in patients with lymph node metastasis (N1/N2) than in those without metastasis (N0) [median: 5.110 (IQR: 3.755–6.824) vs. 6.142 (IQR: 5.638–7.448) pg/mL, $P = 0.0369$] (Fig. 3D). IL-10 level was also significantly lower in patients with M1 stage than in those with M0 stage [median: 5.093 (IQR: 2.983–6.251) vs. 6.065 (IQR: 4.940–7.461) pg/mL, $P = 0.0295$] (Fig. 3E), and in patients with clinical stage IV than in those with stage III (4.876 ± 2.237 vs. 6.020 ± 2.124 pg/mL, $P = 0.0433$) (Fig. 3F). No significant differences in IL-10 levels were observed with respect to age, sex, or T stage (all $P > 0.05$). These results indicate that reduced IL-10 level is associated with lymph node metastasis, distant metastasis, and advanced tumor stage.

3.3 Correlation between Treg proportion and IL-10 level

Spearman correlation analysis revealed a significant negative correlation between peripheral blood Treg proportion and plasma IL-10 level ($r_s = -0.2376$, $P = 0.0476$) (Fig. 4). The scatter plot showed that as Treg proportion increased, IL-10 levels tended to decrease. The significant negative correlation between Treg and IL-10 suggested a potential synergistic interaction in modulating tumor immunity. This prompted us to investigate whether combining these two indicators could enhance predictive accuracy beyond that of each individual parameter. Accordingly, we constructed a combined model integrating both Treg and IL-10 and compared its predictive performance with that of the single-marker models.

3.4 Association between Immunophenotype and disease progression

Combined analysis of Treg proportion and IL-10 level was performed to evaluate their impact on PD. The distribution of the four immunophenotypes in the PD and Non-PD groups is shown in Fig. 5A and Fig. 5B. The overall chi-square test revealed a statistically significant difference in immunophenotype distribution between the two groups ($\chi^2 = 8.40$, df = 3, $P = 0.0385$). Standardized residual analysis indicated that the “high Treg low IL-10” phenotype was the primary contributor to the overall difference: this phenotype was significantly enriched in the PD group (standardized residual = + 2.782) and significantly underrepresented in the Non-PD group (standardized residual = − 2.782) (Fig. 5C). After Fisher’s exact test with Bonferroni correction, only this phenotype showed a statistically significant difference between the two groups (corrected $P = 0.0302$), with a proportion of 38.5% (15/39) in the PD group versus 10.8% (4/37) in the Non-PD group, yielding an OR of 5.05 (95% CI: 1.38–23.55)(Fig. 5D). The distribution of the remaining three phenotypes did not differ significantly between groups (corrected $P > 0.05$).

3.5 Logistic Regression Analysis

Univariate logistic regression showed that each unit increase in Treg proportion was associated with a 41% increase in the risk of PD (OR = 1.41, 95% CI: 1.13–1.81, $P = 0.004$); each unit increase in IL-10 level was associated with a 21% decrease in the risk of PD (OR = 0.79, 95% CI: 0.62–0.96, $P = 0.030$). The multivariate joint model revealed that both Treg (OR = 1.43, 95% CI: 1.13–1.87, $P = 0.005$) and IL-10 (OR = 0.78, 95% CI: 0.61–0.97, $P = 0.037$) were independent predictors of PD. The AIC value of the joint model was 96.16, which was lower than that of the Treg-only model (99.10) and the IL-10-only model (103.94), indicating the best model fit.Likelihood ratio tests showed that the addition of IL-10 to the Treg-only model ($\chi^2 = 4.936$, df = 1, $P = 0.026$) and the addition of Treg to the IL-10-only model ($\chi^2 = 9.782$, df = 1, $P = 0.0018$) significantly improved the model fit.

3.6 ROC Curve Analysis and Model Comparison, and Clinical Utility

ROC curve analysis was performed to evaluate and compare the predictive performance of Treg, IL-10, and their combined model for PD in CRC patients. The AUC for the Treg model was 0.692 (95% CI: 0.571–0.813) (Fig. 6A); the AUC for the IL-10 model was 0.364 (95% CI: 0.239–0.489) (Fig. 6B); and the AUC for the combined Treg + IL-10 model was 0.767 (95% CI: 0.660–0.874) (Fig. 6C). The combined model demonstrated the highest predictive performance among the three models. Because IL-10 was identified as a protective factor (OR < 1), its univariate model predicted the opposite direction of the outcome. After reversing the prediction direction (modeling high IL-10 as predicting non-PD), the AUC was 0.636, indicating moderate discriminative ability for identifying patients without disease progression.

The DeLong test showed that the AUC of the combined model was significantly higher than that of the IL-10 alone model (Z = 2.254, P = 0.024), while the difference compared with the Treg alone model approached but did not reach statistical significance (Z = 1.913, P = 0.056). The difference in AUC between the two univariate models (Treg vs. IL-10) was not statistically significant (Z = 0.638, P = 0.523) (Table 2). A nomogram integrating Treg and IL-10 was constructed to enable individualized prediction of PD risk (Fig. 6E). Calibration

curves demonstrated good agreement between predicted and observed probabilities across all models, with the combined model showing the closest fit to the ideal calibration line(Fig. 6F).. DCA further confirmed that the combined model provided the greatest net benefit across a wide range of threshold probabilities, supporting its clinical utility(Fig. 6G). These results indicate that the combined model outperformed IL-10 alone, with a trend toward superiority over Treg alone, suggesting that combining Treg and IL-10 provides incremental predictive and clinical value for identifying patients at high risk of PD.

Table 2

DeLong's test results for pairwise comparisons of ROC curves. Note: AUC = area under the receiver operating characteristic curve; CI = confidence interval. Significance level set at α = 0.05 (two-sided).

Comparison	AUC1 (Combined)	AUC2 (Single)	AUC difference	95% CI of difference	Z value	p value	Conclusion
Combined vs Treg	0.767	0.692	0.075	(− 0.0018, 0.1522)	1.913	0.056	Borderline significant
Combined vs IL-10	0.767	0.636	0.131	(0.0171, 0.2449)	2.254	0.024	**Significant**
Treg vs IL-10	0.692	0.636	0.056	(− 0.1155, 0.2270)	0.638	0.523	Not significant

Discussion

This study systematically evaluated the value of combining a specific Treg subset, defined by flow cytometry as the percentage of CD3⁺CD4⁺CD25^highCD127^low cells among CD4⁺ T cells in peripheral blood, together with plasma IL-10 level in predicting PD in patients with CRC. This Treg subset is widely recognized as regulatory T cells with high suppressive activity; the combination of CD25^high and CD127^low enables more specific enrichment of FoxP3⁺ natural Tregs, thereby avoiding the non-specificity associated with the traditional CD25 single-positive definition[7]. To our knowledge, this study is the first to report, in a single-center cohort, a significant association between the "high Treg low IL-10" immunophenotype and PD. The main findings were as follows: Treg proportion and IL-10 level were significantly negatively correlated (r_s = -0.2376, P = 0.0476); elevated Treg was associated with male sex, distant metastasis, and advanced tumor stage, whereas decreased IL-10 was associated with lymph node metastasis, distant metastasis, and advanced tumor stage; the "high Treg low IL-10" phenotype was significantly enriched in the PD group (corrected P = 0.030, OR = 5.05); multivariate logistic regression identified both Treg (OR = 1.43, P = 0.005) and IL-10 (OR = 0.78, P = 0.037) as independent predictors of PD; and the combined model outperformed either single marker. These findings suggest that combined detection of Treg and IL-10 may facilitate early identification of patients at high risk for PD and reflect specific immune microenvironment characteristics during CRC progression. The negative correlation between Treg and IL-10 further supports their synergistic role in immunomodulation. Previous studies have reported that Tregs can suppress immune responses through mechanisms including IL-10 secretion[7]; the negative correlation observed in this study provides clinical evidence supporting this immunoregulatory mechanism.

The CD4⁺CD25^highCD127^low Treg subset defined in this study is widely recognized as a Treg population with potent immunosuppressive functions[24]. Previous studies have reported that Tregs suppress antitumor immune responses through multiple mechanisms, including secretion of IL-10 and TGF-β, consumption of IL-2, and expression of CTLA-4, thereby promoting tumor immune escape and progression[7]. Numerous studies have confirmed that increased peripheral blood Treg proportion is associated with poor prognosis in various solid tumors[8], and in CRC, it has been reported to be positively correlated with disease stage and lymph node metastasis [19]. Our results are consistent with these findings: each unit increase in Treg proportion was associated with approximately a 41% increase in PD risk, and elevated Treg was significantly associated with male sex, M1 stage, and stage IV. However, no significant correlation was observed between Treg proportion and lymph node metastasis, which may be attributable to the relatively small sample size. Furthermore, the AUC of Treg alone was 0.692, indicating moderate discriminatory ability and suggesting limited predictive performance of a single biomarker, consistent with previous meta-analyses[8].

This study reveals a significant association between the peripheral blood "high Treg/low IL-10" phenotype and PD in CRC, suggesting that Treg-mediated immunosuppression may predominate, while IL-10 exhibits complex duality. On one hand, the combination of low IL-10 and high Treg increases PD risk, possibly reflecting Treg compensation through contact-dependent suppression and TGF-β secretion. On the other hand, univariate analysis showed that higher IL-10 levels were associated with a lower risk of PD (OR = 0.78), and decreased IL-10 levels were significantly associated with N1/2, M1, and stage IV disease, suggesting that the protective immunomodulatory function of IL-10 may be attenuated or lost during CRC progression, thereby exhibiting characteristics of a missing "protective factor"[27].

The high Treg/low IL-10 phenotype reflects an immune imbalance, where Treg-mediated immunosuppression synergizes with weakened antitumor function due to reduced IL-10, likely suppressing cytotoxic CD8⁺ T cell activity and promoting tumor progression[19, 27, 28].

The dual role of IL-10 in tumor immunity has been widely reported: within the tumor microenvironment, IL-10 can promote immune escape by inhibiting effector T cells and antigen-presenting cells[27]; conversely, $CD8^+$ T cell-derived IL-10 can coordinate early NK cell recruitment and play a key antitumor role in combination with anti-angiogenic therapy and PD-1 blockade[28]. This functional divergence may stem from differences in tumor type, disease stage, and specimen type[29]. In the present study, higher IL-10 levels were associated with lower PD risk, a seemingly paradoxical finding that may reflect a "non-canonical" immune-enhancing effect of IL-10, possibly representing compensatory activation of host antitumor immunity or an effect independent of Treg suppression. However, the IL-10 univariate model showed limited predictive ability (AUC = 0.364, 95% CI: 0.239–0.489), consistent with its OR < 1 in logistic regression. This confirms the limitation of a single biomarker, but IL-10 served as an independent complementary factor in the combined model.

Phenotype stratification based on combined Treg and IL-10 levels identified the “high Treg/low IL-10” phenotype as significantly enriched in the PD group (corrected P = 0.030, OR = 5.05, 95% CI: 1.38–23.55), whereas the other three phenotypes did not differ significantly between groups. This phenotype may reflect a state of immune imbalance—immunosuppression mediated by high Tregs synergizing with weakened antitumor function due to low IL-10—promoting tumor progression. The interplay between Tregs and IL-10 in the tumor microenvironment has been extensively studied in other solid tumors, including CRC[19], suggesting cross-cancer generalizability.

The identification of this high-risk phenotype further suggests that immune marker combinations may outperform single markers. Simultaneous assessment of Treg and IL-10 may more comprehensively reflect the patient’s immunosuppressive status, consistent with the concepts of the “cancer-immune set point” and Immunoscore proposed in tumor immunology[30, 31]. For example, in melanoma and lung cancer, models incorporating immune-related indicators such as $CD8^+$ T cells and Tregs have been reported to better predict immunotherapy response and patient prognosis[32]. In CRC, the internationally validated Immunoscore (based on $CD3^+$ and $CD8^+$ T cell densities) has been shown to independently predict recurrence risk and survival, with predictive performance superior to that of traditional TNM staging[33]. The finding in this study that the combined model outperformed the univariate models further supports the superiority of combined assessment.

Logistic regression and ROC analysis confirmed that the combined model (AUC = 0.767) demonstrated superior predictive performance compared with IL-10 alone (AUC = 0.364, P = 0.024), with a trend toward superiority over Treg alone (AUC = 0.692, P = 0.056). Likelihood ratio tests further confirmed that the combined model was significantly improved compared with the Treg-only model (P = 0.026) and the IL-10-only model (P = 0.0018). These findings indicate that while Treg is the primary driver of PD, the addition of IL-10 provides supplementary predictive information and improves overall discriminatory performance. The IL-10 model alone showed a protective direction (OR < 1) but exhibited limited predictive ability (AUC = 0.364). After conversion to reflect positive discrimination, the IL-10 model yielded an AUC of 0.636, suggesting moderate predictive value, though still inferior to the combined model. The nomogram incorporating Treg and IL-10 provided a practical tool for translating the regression model into individualized risk estimates, facilitating bedside application. Calibration curves demonstrated satisfactory agreement between predicted and observed outcomes, supporting the reliability of the model. Decision curve analysis further confirmed that the combined model offered superior net benefit across a range of clinically relevant threshold probabilities, underscoring its potential utility in guiding clinical decision-making. In clinical practice, simultaneous measurement of Treg proportion and IL-10 level in peripheral blood, combined with calculation of the predicted probability using the regression formula or the nomogram, may facilitate risk stratification and inform therapeutic decision-making.

Nevertheless, this study is a single-center, retrospective analysis with a limited sample size. Additionally, because the samples were derived from peripheral blood rather than tumor tissue, the functional status of peripheral blood Tregs may not fully reflect that of tumor-infiltrating Tregs[34]. Despite these limitations, preclinical and mechanistic studies targeting IL-10-related pathways have demonstrated the potential to enhance the efficacy of immunotherapy[35], suggesting that modulating the Treg–IL-10 axis, particularly in patients with the high Treg/low IL-10 phenotype, may represent a promising approach to overcome immune resistance and improve clinical outcomes in CRC. Future prospective studies are warranted to validate these findings and translate them into clinical practice.

Limitations

This study has several limitations. First, the small sample size (n = 76) limited statistical power; the DeLong test for comparing models reached only marginal significance (P = 0.056), and a larger cohort may reveal clearer differences. Second, the single-center design and median-based cut-off values limit generalizability and require external validation. Third, the cross-sectional design precludes causal inference, and potential confounders (e.g., age, stage, treatment) were not fully controlled. Fourth, peripheral blood samples were used instead of tumor tissue; Treg function and IL-10 levels in blood may not fully reflect tumor-specific immune status, as plasma IL-10 derives from multiple cellular sources. Fifth, only Treg and IL-10 were assessed; other key immune components (e.g., $CD8^+$ T cells, NK cells, PD-L1, MDSCs) were not included, and a multi-parameter approach is needed in future studies.

Conclusions

In conclusion, the "high Treg/low IL-10" phenotype is a significant risk factor for PD in CRC patients (OR = 5.05). The combined assessment of peripheral blood Treg and IL-10 achieved superior predictive performance (AUC = 0.767) compared with either marker alone, with satisfactory calibration and favorable net benefit in decision curve analysis. The nomogram integrating these two biomarkers provides a practical tool for individualized risk stratification and immunomonitoring in clinical practice. Future larger, multicenter prospective studies are warranted to validate these findings and explore the underlying mechanisms.

Declarations

Conflicts of Interest

The authors confirm that no competing interests exist.

Ethics Statement

This study was approved by the Ethics Committee of Tianjin Cancer Hospital Airport Hospital (Approval No. KY-2024-0049). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all individual participants included in the study. Clinical trial number: not applicable.

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Author Contribution

Hao Jin, Liming Xu, and Dan Wang designed the study. Dan Wang, Dongmei Han, and Caihong Wu screened and extracted the data. Dan Wang, Xin Li, and Zhenhua Ge conducted and visualized the data analysis. Dan Wang wrote the manuscript. Hao Jin and Liming Xu provided financial support and edited the manuscript. Dan Wang is the first author; Dongmei Han, Caihong Wu, and Xin Li are co-first authors. Zhenhua Ge is the second author. Hao Jin and Liming Xu contributed equally as corresponding authors. All authors reviewed and approved the final manuscript.

Data Availability

The data that support the findings of this study are available from Tianjin Cancer Hospital Airport Hospital but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the corresponding author upon reasonable request and with permission of Tianjin Cancer Hospital Airport Hospital.

References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229–63. 10.3322/caac.21834.
2. Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. CA Cancer J Clin. 2023;73(3):233–54. 10.3322/caac.21772.
3. Vitiello A, Zovi A. Early-onset colorectal cancer: epidemiology, risk factors, and implications for clinical practice. Clin Transl Oncol. 2026. 10.1007/s12094-026-04382-w.
4. Biller LH, Schrag D. Diagnosis and Treatment of Metastatic Colorectal Cancer: A Review. JAMA. 2021;325(7):669–85. 10.1001/jama.2021.0106.
5. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74. 10.1016/j.cell.2011.02.013.

6. Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat Med. 2018;24(5):541–50. 10.1038/s41591-018-0014-x.
7. Tanaka A, Sakaguchi S. Regulatory T cells in cancer immunotherapy. Cell Res. 2017;27(1):109–18. 10.1038/cr.2016.151.
8. Shang B, Liu Y, Jiang SJ, Liu Y. Prognostic value of tumor-infiltrating FoxP3 + regulatory T cells in cancers: a systematic review and meta-analysis. Sci Rep. 2015;5:15179. 10.1038/srep15179.
9. Saito T, Nishikawa H, Wada H, Nagano Y, Sugiyama D, Atarashi K, et al. Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. Nat Med. 2016;22(6):679–84. 10.1038/nm.4086.
10. Zotta A, Toller-Kawahisa J, Palsson-McDermott EM, O'Carroll SM, Henry ÓC, Day EA, et al. Mitochondrial respiratory complex III sustains IL-10 production in activated macrophages and promotes tumor-mediated immune evasion. Sci Adv. 2025;11(4):eadq7307. 10.1126/sciadv.adq7307.
11. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. Nat Rev Immunol. 2010;10(3):170–81. 10.1038/nri2711.
12. Mumm JB, Emmerich J, Zhang X, Chan I, Wu L, Mauze S, et al. IL-10 elicits IFNγ-dependent tumor immune surveillance. Cancer Cell. 2011;20(6):781–96. 10.1016/j.ccr.2011.11.003.
13. Li B, Wang F, Ma C, Hao T, Geng L, Jiang H. Predictive value of IL-18 and IL-10 in the prognosis of patients with colorectal cancer. Oncol Lett. 2019;18(1):713–9. 10.3892/ol.2019.10338.
14. Mazzaschi G, Perrone F, Maglietta G, Favari E, Verzè M, Pluchino M, et al. Intersecting Blood Cytokines With Cholesterol Parameters to Profile Patients With Advanced Solid Tumors Receiving Immune Checkpoint Inhibitors. J Immunother. 2024;47(9):388–94. 10.1097/cji.0000000000000534.
15. Dikiy S, Ghelani AP, Levine AG, Martis S, Giovanelli P, Wang ZM, et al. Terminal differentiation and persistence of effector regulatory T cells essential for preventing intestinal inflammation. Nat Immunol. 2025;26(3):444–58. 10.1038/s41590-024-02075-6.
16. Krishnamoorthy M, Gerhardt L, Maleki Vareki S. Immunosuppressive Effects of Myeloid-Derived Suppressor Cells in Cancer and Immunotherapy. Cells. 2021;10(5). 10.3390/cells10051170.
17. Hsu P, Santner-Nanan B, Hu M, Skarratt K, Lee CH, Stormon M, et al. IL-10 Potentiates Differentiation of Human Induced Regulatory T Cells via STAT3 and Foxo1. J Immunol. 2015;195(8):3665–74. 10.4049/jimmunol.1402898.
18. Mittal SK, Roche PA. Suppression of antigen presentation by IL-10. Curr Opin Immunol. 2015;34:22–7. 10.1016/j.coi.2014.12.009.
19. Huang X, Feng D, Mitra S, Andretta ES, Hooshdaran NB, Ghelani AP, et al. Opposing functions of distinct regulatory T cell subsets in colorectal cancer. Immunity. 2026;59(1):145–e609. 10.1016/j.immuni.2025.11.014.
20. Yu H, Yang R, Li M, Li D, Xu Y. The role of Treg cells in colorectal cancer and the immunotherapy targeting Treg cells. Front Immunol. 2025;16:1574327. 10.3389/fimmu.2025.1574327.
21. Sun J, Shi S, Sun C, Wang J, Yang X, Yang Z, et al. Predictive nomogram of the clinical outcomes of colorectal cancer based on methylated SEPT9 and intratumoral IL-10(+) Tregs infiltration. J Transl Med. 2024;22(1):861. 10.1186/s12967-024-05635-4.
22. Mohamed A, Ismail H, Zahran F, Zein N, Lindmark G, Hammarström ML, et al. High IL-10 mRNA levels in regional lymph nodes of colon cancer patients indicate poor prognosis. Front Immunol. 2025;16:1589533. 10.3389/fimmu.2025.1589533.
23. Song Z, Liu K, Zhang T, Wang B, Shi Y, Jiang Y, et al. Oncologic outcomes of single-incision laparoscopic surgery versus conventional laparoscopic surgery for colorectal cancer (CSILS): study protocol for a multicentre, prospective, open-label, noninferiority, randomized controlled trial. BMC Cancer. 2022;22(1):743. 10.1186/s12885-022-09821-9.
24. Yu N, Li X, Song W, Li D, Yu D, Zeng X, et al. CD4(+)CD25 (+)CD127 (low/-) T cells: a more specific Treg population in human peripheral blood. Inflammation. 2012;35(6):1773–80. 10.1007/s10753-012-9496-8.
25. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: an open-source package for R and S + to analyze and compare ROC curves. BMC Bioinformatics. 2011;12:77. 10.1186/1471-2105-12-77.
26. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988;44(3):837–45.
27. Ouyang W, O'Garra A. IL-10 Family Cytokines IL-10 and IL-22: from Basic Science to Clinical Translation. Immunity. 2019;50(4):871–91. 10.1016/j.immuni.2019.03.020.
28. Raskov H, Orhan A, Christensen JP, Gögenur I. Cytotoxic CD8(+) T cells in cancer and cancer immunotherapy. Br J Cancer. 2021;124(2):359–67. 10.1038/s41416-020-01048-4.
29. Mannino MH, Zhu Z, Xiao H, Bai Q, Wakefield MR, Fang Y. The paradoxical role of IL-10 in immunity and cancer. Cancer Lett. 2015;367(2):103–7. 10.1016/j.canlet.2015.07.009.

30. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature. 2017;541(7637):321–30. 10.1038/nature21349.
31. Bruni D, Angell HK, Galon J. The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. Nat Rev Cancer. 2020;20(11):662–80. 10.1038/s41568-020-0285-7.
32. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515(7528):568–71. 10.1038/nature13954.
33. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet. 2018;391(10135):2128–39. 10.1016/s0140-6736(18)30789-x.
34. Ohue Y, Nishikawa H. Regulatory T (Treg) cells in cancer: Can Treg cells be a new therapeutic target? Cancer Sci. 2019;110(7):2080–9. 10.1111/cas.14069.
35. Paluskievicz CM, Cao X, Abdi R, Zheng P, Liu Y, Bromberg JS. T Regulatory Cells and Priming the Suppressive Tumor Microenvironment. Front Immunol. 2019;10:2453. 10.3389/fimmu.2019.02453.

Figures

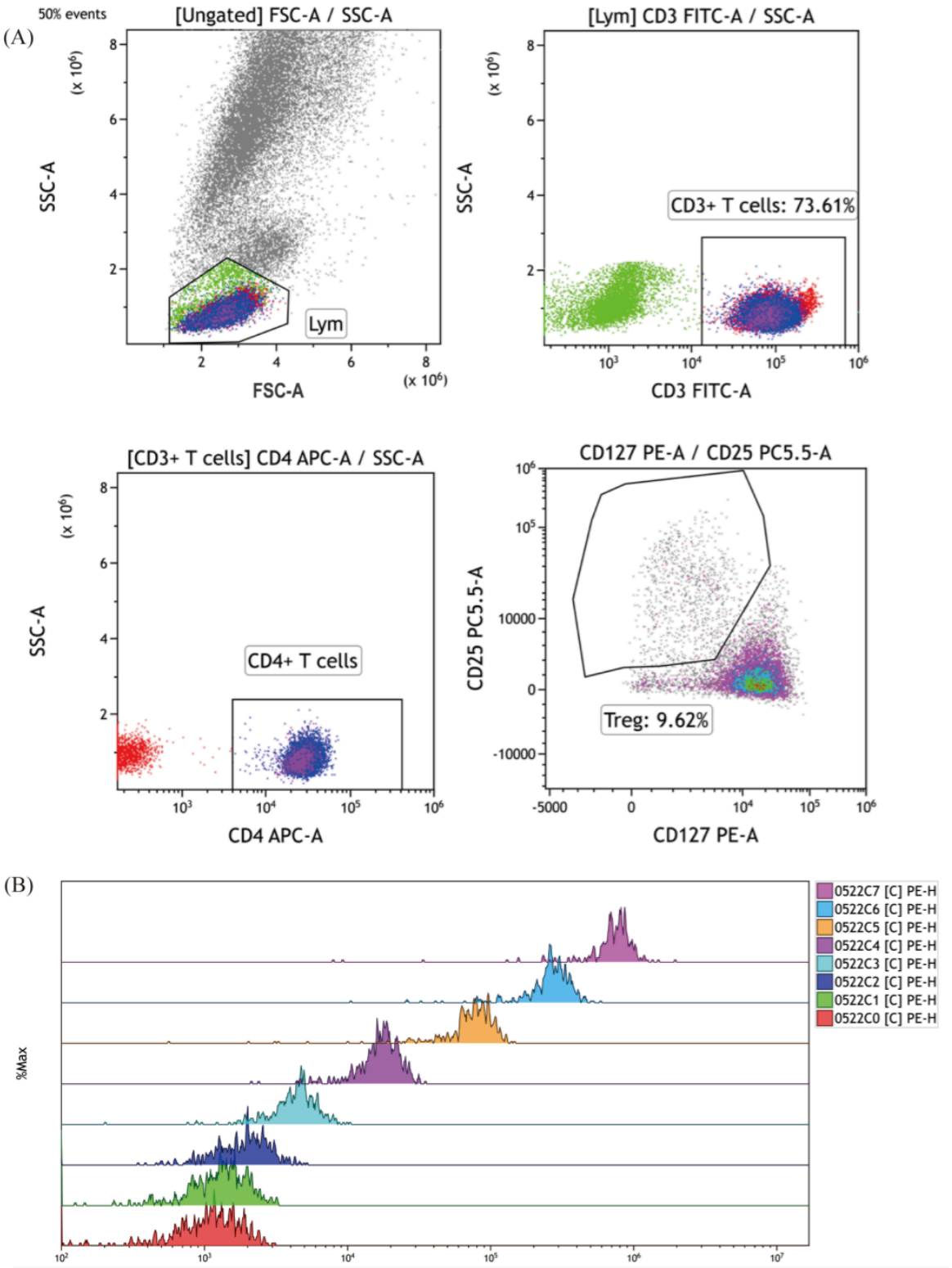


Figure 1

The gating strategy for flow cytometric analysis. (A) Representative flow cytometry gating strategy for CD3⁺CD4⁺CD25^highCD127^low Tregs. (B) Standard curve for IL-10 quantification by flow cytometry, showing the relationship between IL-10 concentration and mean fluorescence intensity (MFI), used for calculating plasma IL-10 levels in patient samples.

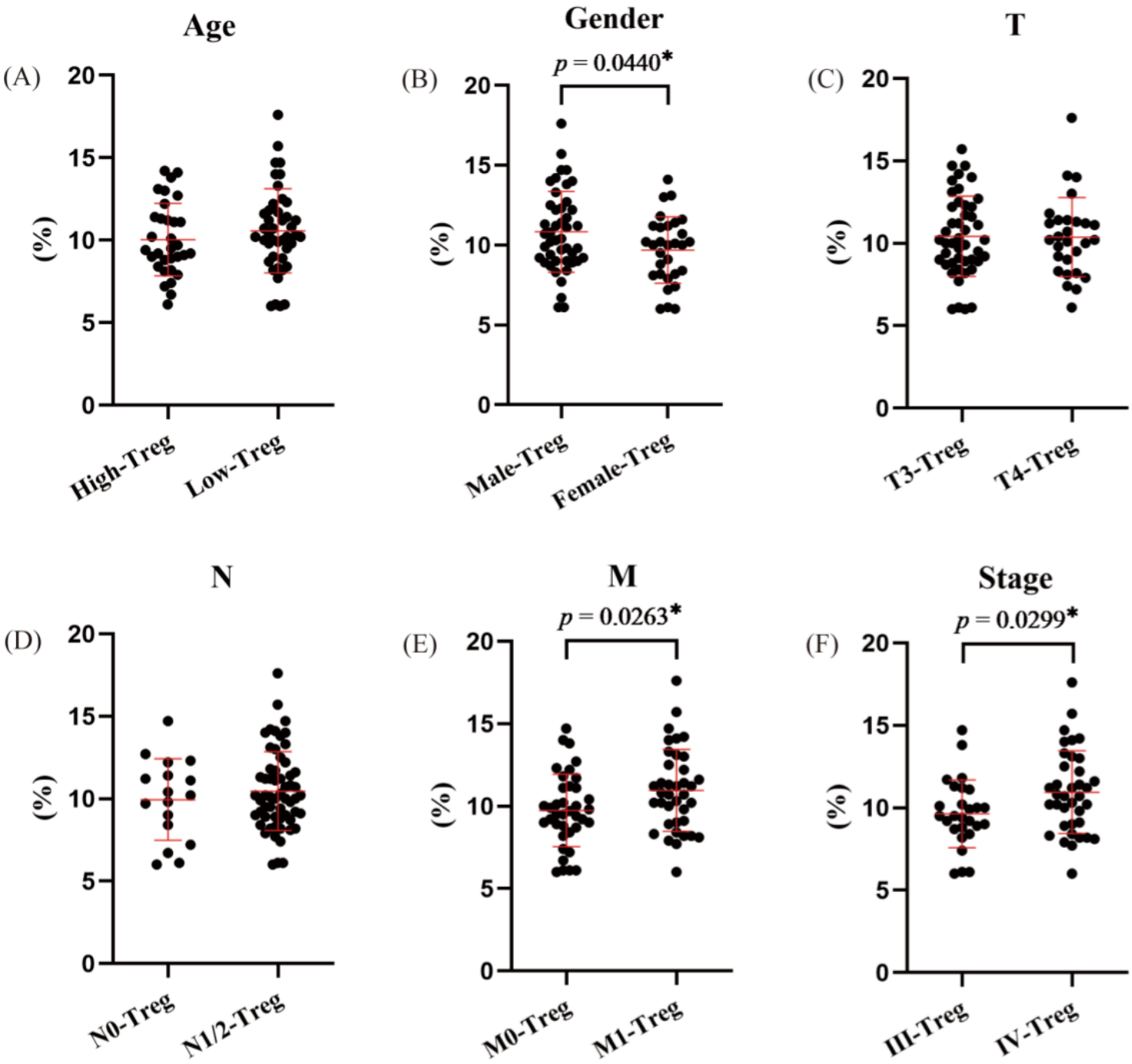


Figure 2

Association of peripheral blood Treg proportion with clinicopathological characteristics in CRC patients. Box plots showing Treg distribution stratified by age (A), gender (B), T stage (C), N stage (D), M stage (E), and clinical stage (F). Significant differences were observed for gender (*P*= 0.0440), M stage (*P* = 0.0263), and clinical stage (*P* = 0.0299). P-values were calculated using independent t-test or Mann-Whitney U test. *P < 0.05.

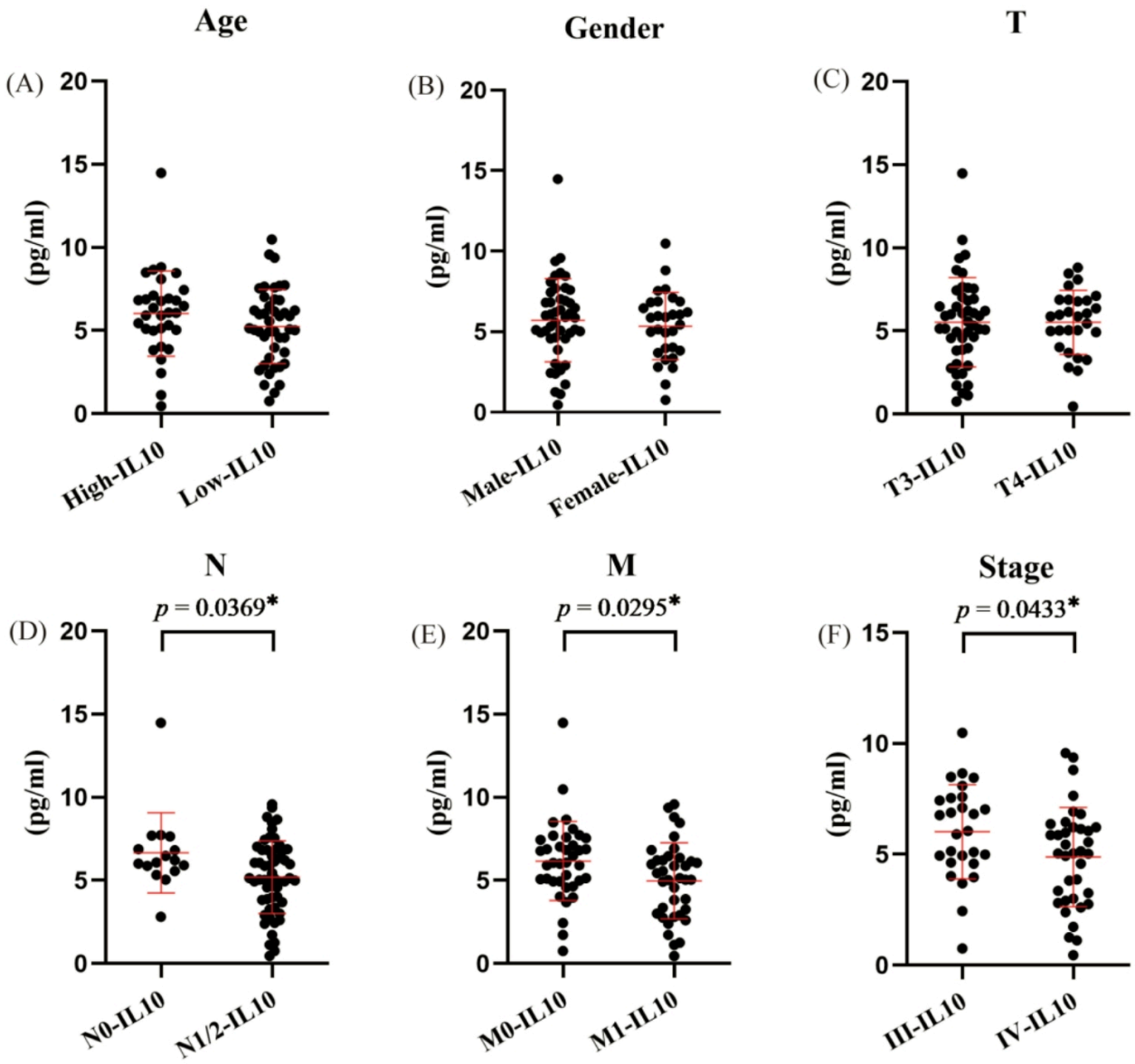


Figure 3

Association of plasma IL-10 level with clinicopathological characteristics in CRC patients. (Box plots showing IL-10 distribution stratified by age (A), gender (B), T stage (C), N stage (D), M stage (E), and clinical stage (F). Significant differences were observed for N stage (*P*= 0.0369), M stage (*P* = 0.0295), and clinical stage (*P* = 0.0433). P-values were calculated using independent t-test or Mann-Whitney U test. **P*< 0.05.

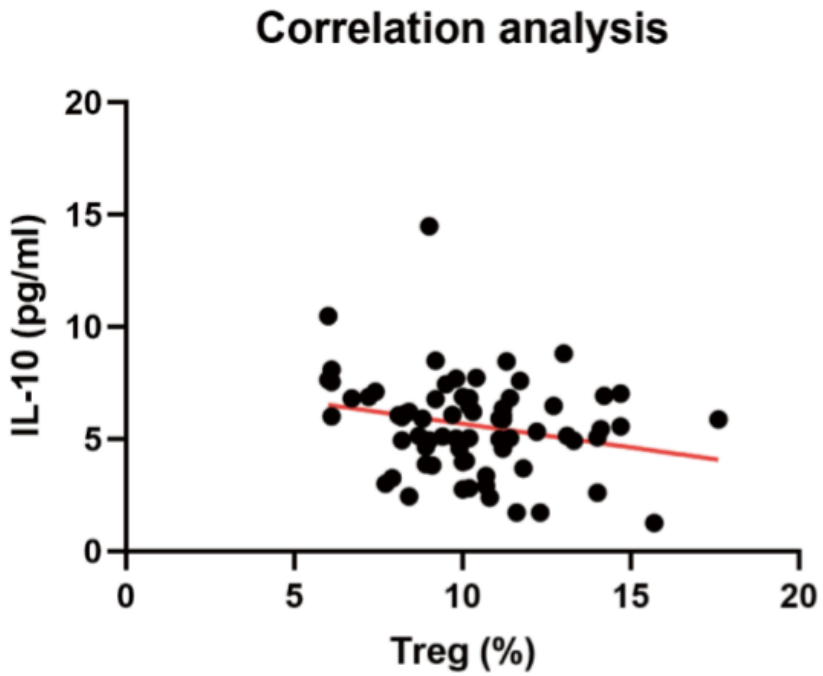


Figure 4

Correlation between peripheral blood Treg proportion and plasma IL-10 level in CRC patients. Scatter plot showing a significant negative correlation (Spearman r = -0.2376, *P* = 0.0476). Each dot represents an individual patient (n = 76). The solid line represents the linear regression fit.

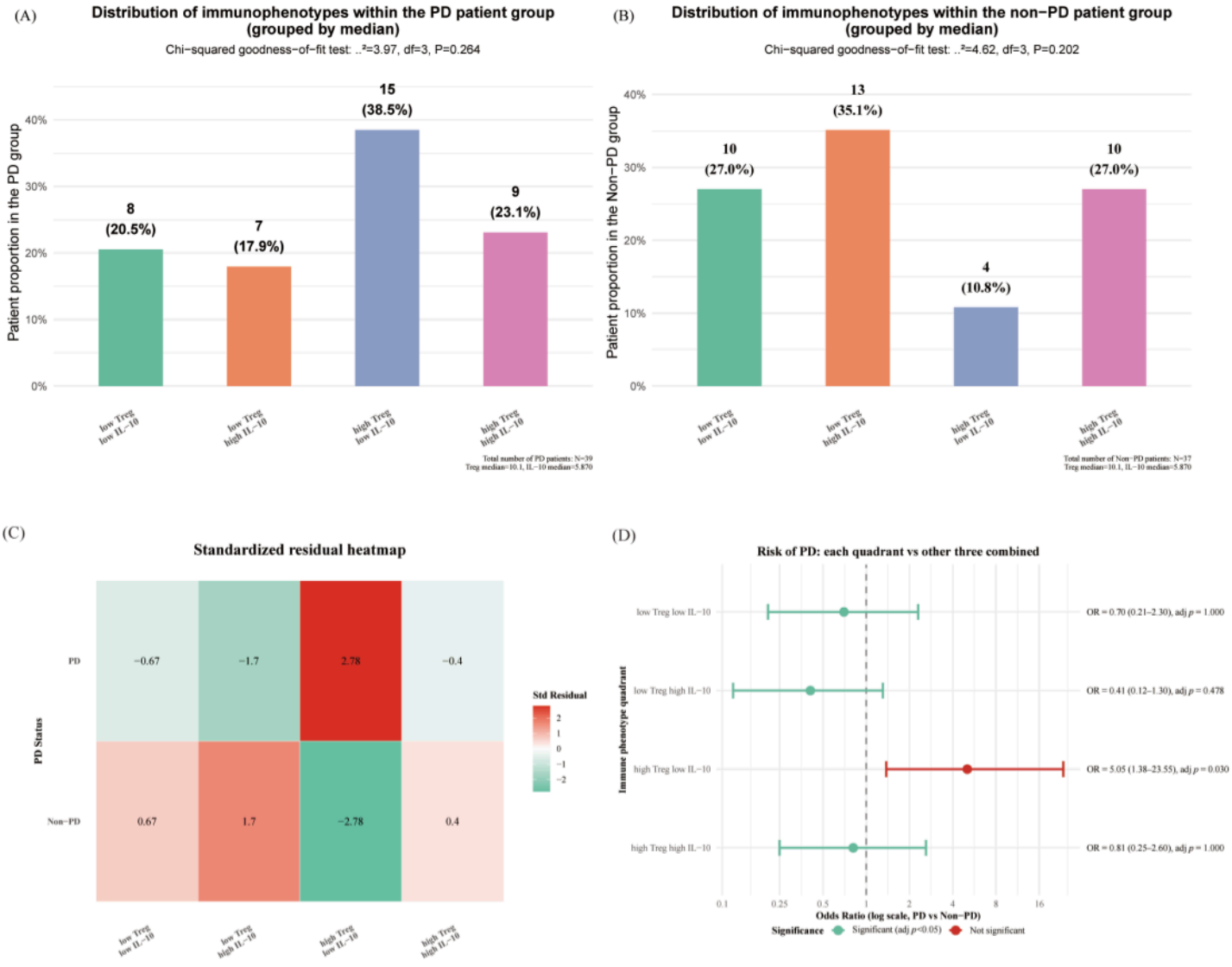


Figure 5

Immunophenotype distribution and PD risk analysis. (A, B) Proportions of patients with different Treg/IL-10 phenotypes in the PD and non-PD groups, along with chi-squared test results. (C) Standardized residual heatmap showing deviations of observed distributions from expected values (red indicates positive deviation). (D) Forest plot depicting the risk of PD for the "high Treg / low IL-10" phenotype versus the other phenotypes. The red dot indicates statistical significance (adjusted *P* =0.030, OR = 5.05).

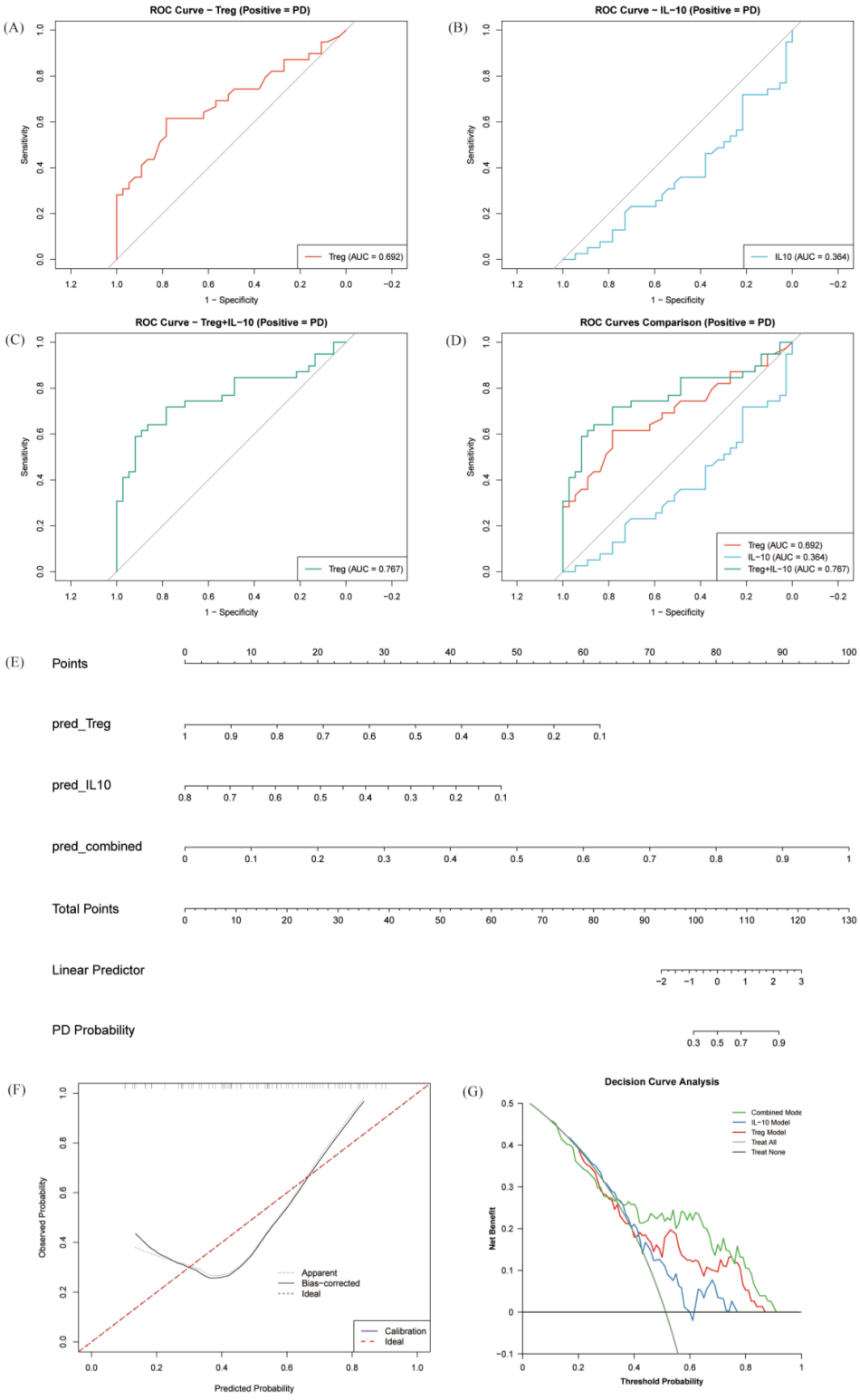


Figure 6

Comprehensive comparison of three prediction models for PD status. This figure includes four evaluation panels:(A-D) ROC curves for the Treg, IL-10, and combined models, respectively, with AUC values measuring discriminative ability. (E) Nomogram, illustrating the scoring conversion of each model variable and the quantitative relationship between total points and PD probability; (F) Calibration curves, assessing agreement between predicted probabilities and observed outcomes, with the diagonal line representing perfect calibration; (G) DCA, evaluating the clinical net benefit of each model across threshold probabilities, with "Treat All" and "Treat None" as reference strategies; The combined model (green) demonstrated superior performance across all evaluation metrics, suggesting that combining Treg with IL-10 improves PD risk prediction.