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# Tumor aggression-defense index–a novel indicator to predicts recurrence and survival in stage II-III colorectal cancer

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## Abstract

**Background** Although the TNM staging system plays a critical role in guiding adjuvant chemotherapy for colorectal cancer (CRC), its precision for risk stratification in stage II and III CRC patients with proficient DNA mismatch repair (pMMR) remains limited. Therefore, precise predictive models and research on postoperative treatments are crucial for enhancing patient survival and improving quality of life.

**Methods** This retrospective study analyzed 1051 pMMR CRC patients who underwent radical resection and were randomly assigned to training (n = 736) and validation (n = 315) groups. Immunohistochemistry and hematoxylin and eosin staining were utilized to evaluate regulatory-Immunoscore (RIS), tertiary lymphoid structures (TLS), and tumor budding (TB). The Tumor Aggression-Defense Index (TADI) was derived through a multi-factor COX regression model. Subgroup analysis demonstrated potential of TADI in guiding personalized adjuvant therapy for stage II and III CRC.

**Results** Univariate and multivariate Cox analysis indicated that TADI was an independent prognostic indicator. Among stage II CRC, chemotherapy was significantly correlated with improved recurrence times in individuals with intermediate (95% CI 0.19–0.59, P < 0.001) and high (95% CI 0.36–0.95, P = 0.031) TADI. In stage III CRC receiving adjuvant chemotherapy, a duration of 3 months or longer was notably associated with a prolonged time to recurrence in those with high TADI (95% CI 0.40–0.98, P = 0.041) compared to durations of less than 3 months.

**Conclusion** The TADI serves as an effective parameter for predicting the survival outcomes of stage I-III pMMR CRC patients and guiding precision treatment strategies.

**Keywords** Colorectal cancer, Regulatory-immunoscore, Tertiary lymphoid structures, Tumor budding, Tumor aggression-defense index

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## Introduction

Colorectal cancer (CRC) remains one of the leading causes of cancer-related morbidity and mortality worldwide. Despite advances in surgical and adjuvant therapies, recurrence remains a significant barrier to long-term survival, affecting 10%–20% of stage II and 30%–40% of stage III CRC patients [1, 2]. The majority of these cases are DNA mismatch repair-proficient (pMMR), which tend to show poor responses to adjuvant chemotherapy treatment (ACT) [3]. Traditional prognostic models, such as the Tumor-Node-Metastasis (TNM) staging system, have demonstrated limited accuracy in predicting patient outcomes, underscoring the need for more precise approaches [4, 5]. Several tumor stratification markers, including CpG island methylator phenotype (CIMP) [6, 7] and mutations in APC, KRAS, and BRAF genes [8], have been investigated; however, their predictive accuracy remains suboptimal, and their clinical applicability is limited. This highlights the urgent need for robust and accessible predictive models to guide personalized therapy for pMMR CRC patients.

Tumor invasion and the tumor microenvironment (TME) are central to CRC progression and therapeutic response. Tumor-intrinsic invasive factors drive malignancy, while the TME plays a pivotal role in promoting anti-tumor immunity and supporting tumor suppression [9]. Tumor budding (TB), a hallmark of aggressive cancer subtypes [10], is frequently associated with poor prognosis and higher recurrence rates [11]. In contrast, protective components within the TME, such as tertiary lymphoid structures (TLS) and tumor-infiltrating lymphocytes (TILs), enhance anti-tumor immunity and contribute to tumor suppression [12]. The organized zones of follicular B cells, CD3 + T cells, and LAMP + dendritic cells within TLS facilitate efficient tumor antigen presentation and the generation of effector T cells and antibody-producing plasma cells [13, 14]. Additionally, high density of TILs has been closely associated with favorable prognosis, as it typically indicates a robust immune response [15]. It has been correlated with improved clinical outcomes, underscoring the critical need to understand the balance between tumor aggression and immune defense mechanisms in CRC.

Integrating tumor invasion markers and immune microenvironmental factors provides a comprehensive framework for evaluating CRC prognosis. By assessing both aggressive tumor characteristics, such as TB, and protective immune components like TLS and TILs, it is possible to develop more precise and actionable prognostic models. This study introduces the Tumor Aggression-Defense Index (TADI), a novel approach that quantifies both invasive and defensive factors. Rigorous validation demonstrates that TADI holds significant potential for improving survival prediction, guiding risk stratification, and optimizing adjuvant chemotherapy for stage II-III pMMR CRC patients. This model not only addresses the limitations of existing prognostic markers but also captures the multifaceted, dynamic interplay between tumor-intrinsic invasive factors and the complex immune responses within the TME, marking a significant advancement in personalized cancer management.

## Material and methods

### Patients and endpoints

This study retrospectively analyzed stage I-III pMMR CRC patients who underwent curative resection at Harbin Medical University Cancer Hospital (HMUCH), from January 4, 2013 to December 30, 2015 (Fig. 1). Detailed inclusion and exclusion criteria are provided in the Supplementary Materials. Following stratification by primary tumor location, tumor differentiation, T stage, N stage and relapse, 1051 patients were randomly divided (7:3) into a training set (n = 736) and a validation set (n = 315) (Fig. 1). The primary endpoint of this study was to assess time to recurrence (TTR), which was defined as the duration from the date of surgery to the initial diagnosis of disease recurrence. Additionally, the secondary endpoint was overall survival (OS), which was defined as the duration from the date of surgery to death from any cause. This study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of the Harbin Medical University Cancer Hospital (KY2022-20).

### Clinical indicators

Patient demographics and tumor characteristics were systematically recorded. This included age, gender, Eastern Cooperative Oncology Group performance status (ECOG PS), preoperative carcinoembryonic antigen (CEA) levels, and tumor location, along with detailed pathological data such as differentiation grade (poor-undifferentiation, moderate, well), T stage (T1-4), N stage (N0-2), TNM stage (re-staged according to the 8th AJCC staging system), and VELIPI. Tumors exhibiting positive biomarkers for venous emboli, lymphatic, or perineural invasion were classified as VELIPI + . Follow-up data on subsequent treatments, including adjuvant chemotherapy treatment (ACT), were also gathered.

### Pathological processing and evaluation

Formalin-fixed, paraffin-embedded surgical specimens were processed into 4-μm-thick slices for hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) (Fig. 2A). To ensure consistency, sections representing the deepest extent of tumor invasion were selected for analysis. Following staining, slides were

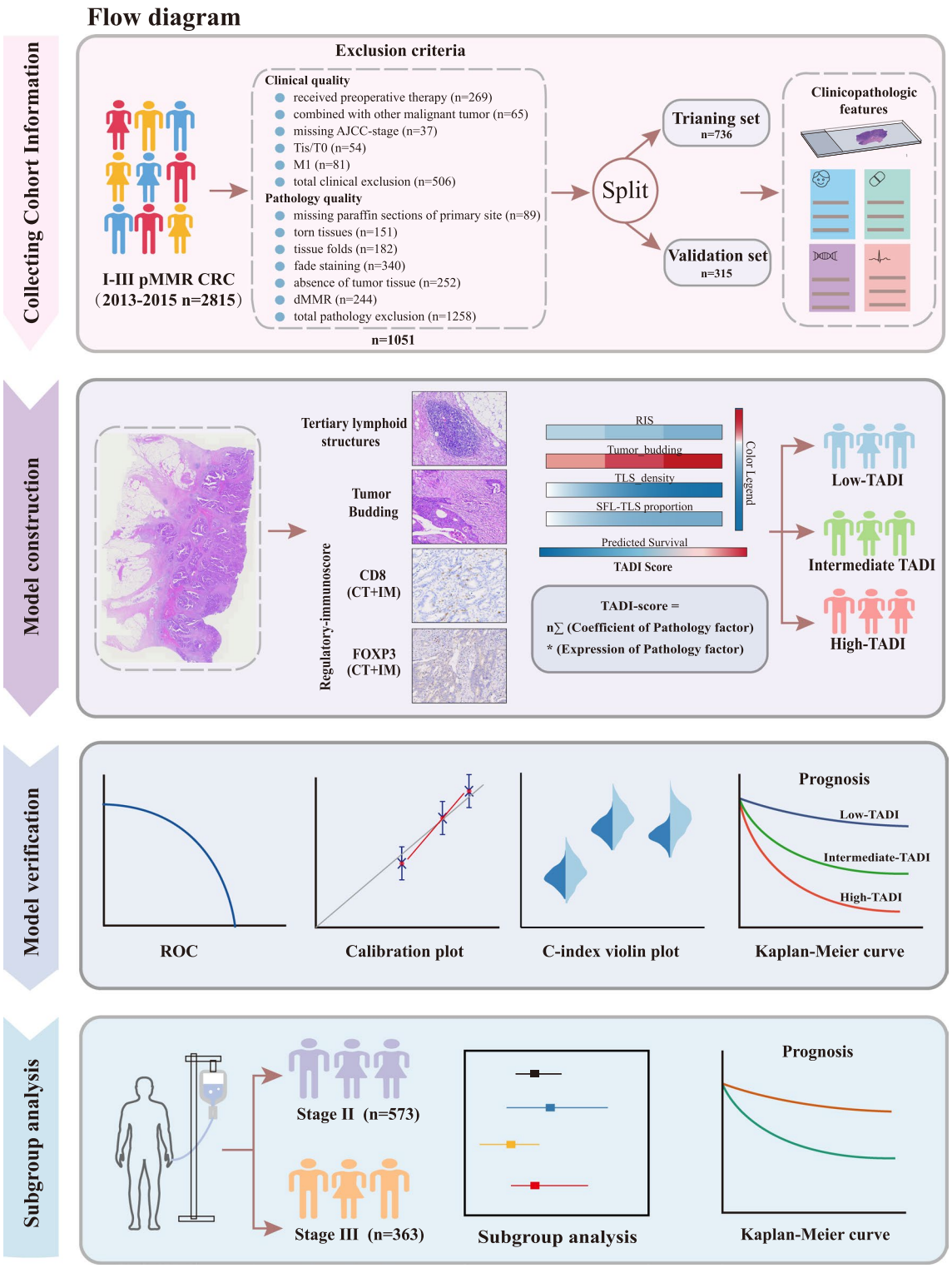



**Fig. 1** The flow chart of this study. pMMR: mismatch-repair-proficient, CRC: colorectal cancer, RIS: regulatory-Immunoscore, TLS: tertiary lymphoid structures, SFL-TLS: secondary follicle-like stage TLS, TADI: Tumor Aggression-Defense Index, ROC: receiver operating characteristic

digitally scanned by the MoticEasyScan Infinity system, and images were evaluated with Motic DSAssistant software for histological assessment. Tertiary lymphoid structures, tumor budding, and the regulatory Immunoscore (RIS) were defined independently by two experienced gastroenterology pathologists, who were blinded to the clinicopathological information of patients. A third expert pathologist was responsible for the final decision in case of a disagreement between the two pathologists. The full definitions of these indicators can be found in sTable 1.

#### Evaluation of tertiary lymphoid structures

Existence, abundance, location, and subtypes of tertiary lymphoid structures (TLS) were evaluated on H&E-stained slides. Pathologists standardized TLS definition and classification for consistency. TLS density was determined as the number of TLS per mm of tumor-invasive front in peritumoral regions (within 7 mm of the tumor front) [16]. The length of the invasive front of the tumor was measured by Fiji software. TLS were divided into three categories according to the morphology determined by H&E staining; (1) early stage (E-TLS): small, quasi-circular clusters of lymphocytes (Fig. 2B); (2) primary follicle-like stage (PFL-TLS): large clusters without germinal center formation (Fig. 2C); and (3) secondary follicle-like stage (SFL-TLS): large clusters with germinal center formation (Fig. 2D). The numbers of TLS in each maturation stage were counted and expressed as a proportion from all TLS within each patient.

#### Evaluation of tumor budding

Tumor budding (TB) was defined as the presence of a single isolated tumor cell or a small cluster of tumor cells (up to four cells) at the invasive margin of the tumor. TB was assessed on digitally scanned H&E-stained tissue slides, at 20 × magnification to examine a single hotspot field, with an area normalized to 0.785 $mm^2$ at the invasive margin of the tumor, in accordance with the ITBCC 2016 guidelines. Categories for TB scoring were: Bd1 (0–4 buds: low), Bd2 (5–9 buds: intermediate), and Bd3 (more than 10 buds: high) (Fig. 2E–G) [10, 17].

#### Evaluation of regulatory immunoscore

Regulatory Immunoscore (RIS) was used to assess the immune microenvironment by evaluating the balance between cytotoxic T cells (CD8 +) and regulatory T cells (FOXP3 +). The IHC staining was performed to identify CD8 + T cells (Abcam, ab101500, 1:500) and FOXP3 + T cells (Abcam, ab200334, 1:500). The densities of CD8 + and FOXP3 + T cells in central tumor (CT) and invasive margin (IM) tissues (cells/$mm^2$) were estimated by the Fiji/ImageJ platform at 20 × magnification (Fig. 2H–I) [18]. Each patient was categorized into high or low immune cell density groups based on the estimated densities in each tumor region. The thresholds for categorization are specified in Supplementary Table 1. Specifically, a high density of immune cells was recorded as score 1, while a low density was recorded as score 0. The RIS was defined by summing the scores of two immune parameters (CD8 + and FOXP3 + lymphocyte densities) across the two regions (CT and IM). RIS was categorized into three groups, with scores ranging from 0, 1 to 3 and 4 representing low, intermediate and high, respectively (Fig. 2J) [19].

#### Statistical analysis

Statistical analyses were performed by utilizing R software (version 4.3.2). Mann–Whitney tests were utilized for comparing continuous variables, while Chi-square or Fisher's exact tests were employed for analyzing categorical variables. Kaplan–Meier survival curves were analyzed with log-rank tests to assess TTR and OS. To evaluate the prognostic significance of clinical and pathological factors, a multivariate Cox proportional hazards regression model was employed. This method allows for the simultaneous assessment of multiple covariates on time-to-event outcomes while accommodating censored data, which is particularly advantageous in retrospective studies where follow-up durations and event occurrences

(See figure on next page.)

**Fig. 2** Evaluation of RIS, Tumor budding and TLS according by hematoxylin/eosin (H&E) staining and immunohistochemistry (IHC). **A** Representative sections for evaluating RIS, Tumor budding and TLS. **B–D** Representative images of TLSs in each maturation category as evaluated by H&E. **E–G** Representative images of Tumor budding in each category as evaluated by H&E. **H–I** Representative immunohistochemistry of CD8 + cells stained with CD8 antibody and FOXP3 + cells stained with FOXP3 antibody. **J** The RIS scoring system is based on the counting of two lymphocyte subsets (CD8 and FOXP3) in two locations (CT and IM) of the primary tumor. All patients were divided into high (H in a dark circle) or low (L in a light circle) groups for the density of each marker in each region. A high density of immune cells was recorded as a score of 1, and a low density was recorded as a score of 0. Patients were stratified according to a score of 0, 1–3 or 4, representing low, intermediate, or high RIS, respectively. For example, score 0 refers to a tumor with low densities of CD8 + and FOXP3 + cells in CT and IM regions. Score 4 refers to a tumor with high densities of CD8 + and FOXP3 + cells in CT and IM regions. TLS, tertiary lymphoid structures; E-TLS, early-stage TLS; PFL-TLS, primary follicle-like stage TLS; SFL-TLS, secondary follicle-like stage TLS; CT, center of the tumor; IM, invasive margin; RIS, regulatory-Immunoscore

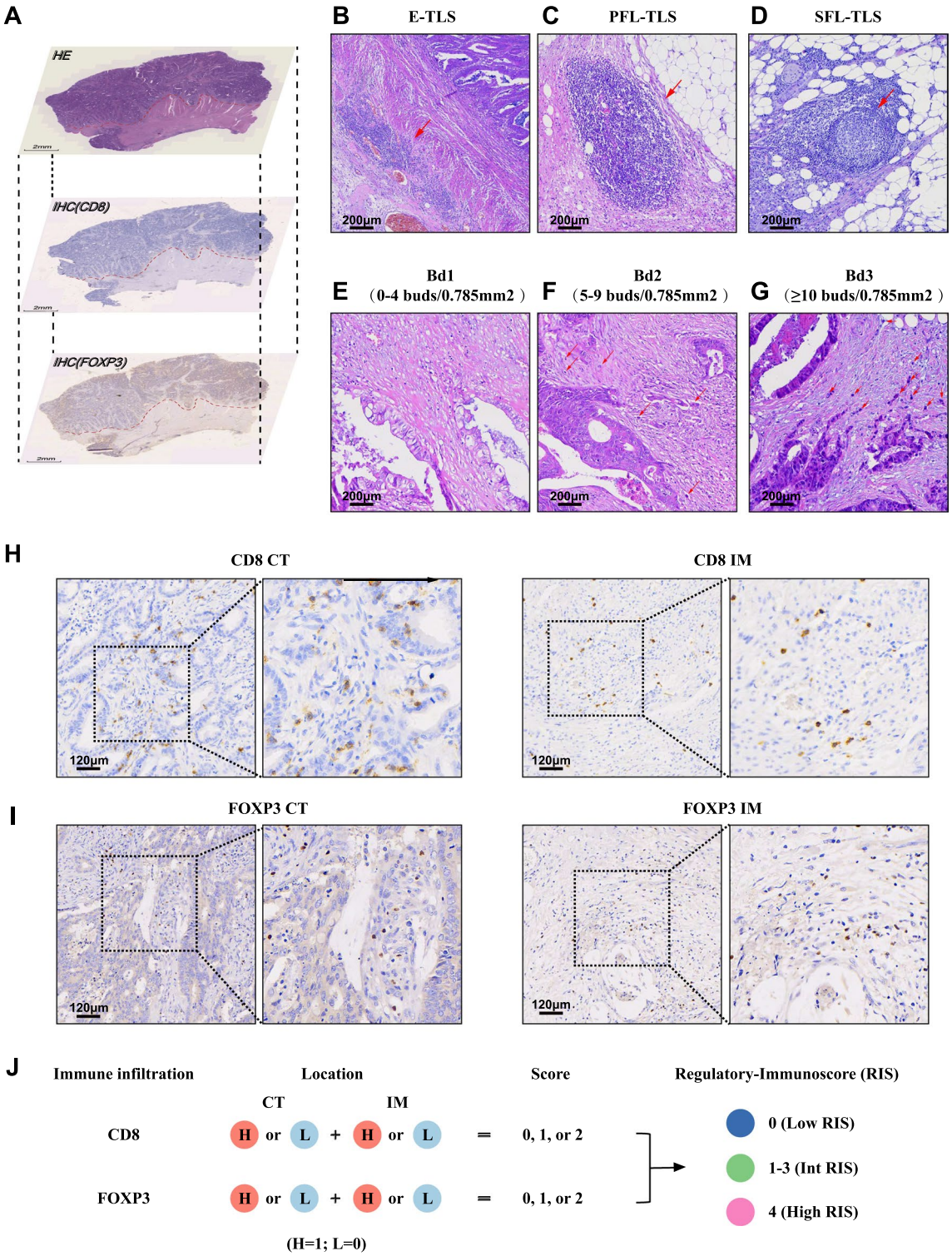



**Fig. 2** (See legend on previous page.)

vary [20]. Variables such as RIS, tumor budding, TLS density, and SFL-TLS proportion were selected based on their potential prognostic relevance. Model performance was evaluated using Harrell's C-index and ROC curve analysis, with calibration curves confirming alignment between predictions and observed outcomes. Statistical significance was set at $p < 0.05$ for all tests.

## Results

### Clinical characteristics

A total of 1051 patients with stage I-III pMMR CRC were included following rigorous clinical and biomarker quality control measures. Among them, 115 (10.95%) patients had stage I CRC, 573 (54.5%) had stage II CRC, and 363 (34.5%) had stage III CRC. In total, 268 (46.8%) of the patients with stage II CRC and 272 (74.9%) of the patients with stage III CRC received adjuvant chemotherapy. The median follow-up period was 89.0 months (95% CI 87.0–91.6), providing a sufficient duration for assessing recurrence and survival outcomes with reliable follow-up data. Clinicopathologic factors were consistent between the training and validation sets, ensuring the robustness of the comparative analyses (Table 1).

### Univariate and multivariate analyses of pathological risk factors

In the training cohort, univariate analysis demonstrated significant correlations between TTR and several factors, including RIS, tumor budding, TLS density, E-TLS proportion, PFL-TLS proportion, and SFL-TLS proportion. Multivariate analysis identified RIS, tumor budding, TLS density, and SFL-TLS proportion as independent risk factors for recurrence (Table 2). Notably, RIS scores exhibited a gradual decline from TNM stage I to III and from T1 to T4, suggesting that both TNM and T stages influence tumor-infiltrating lymphocyte infiltration (sFigure 1A, E). Similarly, high-grade tumor budding increased progressively from TNM stage I to III and from T1 to T4 (sFigure 1B, F). Intragroup analysis of TLS distribution across TNM stages revealed that TLS density varied significantly, with higher density observed in stage II patients compared to other stages. However, no significant differences were noted among the T stages, indicating that the TNM stage, rather than the T stage, likely influences TLS density (sFigure 1C, G). Further analysis demonstrated that the proportion of SFL-TLS differed across TNM and T stages, with a higher proportion in stage III patients and a decreasing trend from T1 to T4 stages, indicating that both TNM and T stages may affect TLS maturity (sFigure 1D, H).

### Construction and validation of the tumor aggression-defense index

Multivariate Cox regression was employed to develop a clinical prediction score model called the Tumor Aggression-Defense Index (TADI) a prognostic model integrating four independent risk factors: RIS, tumor budding, TLS density, and SFL-TLS proportion. Each factor was assigned a weighted score based on its regression coefficient, which was then summed to calculate the predicted probability of TTR (sFigure 2A). The model demonstrated robust predictive performance, with AUC of 0.717 (95% CI 0.680–0.755) in the training set and 0.733 (95% CI 0.675–0.791) in the validation set (sFigure 2B, C). Patients were stratified into three groups—low-TADI, intermediate-TADI, and high-TADI—based on their TADI scores, with cutoff points selected at the first and second tertiles of the TADI score distribution. Representative histopathological images are shown in sFigure 3. Critical data on RIS, TLS, and TB scores across the different TADI groups are summarized in sTable 2. Comparative analyses revealed that TADI achieved a superior C-index for predicting recurrence risk (training: 0.69, 95% CI 0.66–0.72; validation: 0.71, 95% CI 0.67–0.76) compared to individual pathological risk parameters, including TNM stage, RIS, tumor budding, TLS density, and SFL-TLS proportion (Fig. 3A). Incorporating TADI into a combined model with TNM staging significantly improved TTR prediction, as evidenced by higher time-dependent AUC (tAUC) values in both training and validation sets (Fig. 3B). Calibration curves showed strong agreement between predicted and observed recurrence probabilities at one, three, and five years (Fig. 3C, D), while decision curve analysis demonstrated a greater net clinical benefit of TADI across a wide range of threshold probabilities (sFigure 2D, E).

### Prognostic efficacy of TADI in stage I-III pMMR colorectal cancer

Univariate analysis demonstrated that TADI, presence of VELIPI, advanced TNM stage, and poor tumor grade were associated with an unfavorable prognosis in the cohort. Multivariate Cox regression analysis for TTR and OS demonstrated that TADI remained an independent prognostic factor (TTR: HR = 2.31, 95% CI 1.86–2.85, $P < 0.001$; OS: HR = 2.22, 95% CI 1.77–2.80, $P < 0.001$). These results suggest that an elevated TADI score is associated with a notably increased risk of disease recurrence and mortality. Notably, other clinically relevant tumor characteristics, including TNM stage and tumor grade, lost their significance in the multivariate model, thereby underscoring the superior predictive value of TADI in this cohort (Fig. 4A, B). Kaplan–Meier survival curves showed that patients with low TADI scores exhibited significantly longer TTR and OS compared to intermediate- and high-TADI groups ($P < 0.001$), with consistent trends across training and validation cohorts (Fig. 5A–D). Moreover, TADI scores were found to be elevated in patients who subsequently experienced recurrence or death, further supporting its potential as a reliable prognostic biomarker (Fig. 5E–J). Subgroup analyses stratified by age, sex, VELIPI, tumor site, TNM stage, and tumor differentiation consistently demonstrated that elevated

**Table 1** Clinicopathologic factors in patients with pMMR colorectal cancer

| Clinicopathological features | Total (n = 1051) | Training set (n = 736) | Validation set (n = 315) | *P value* |
|---|---|---|---|---|
| Age (years), n (%) | | | | 0.692 |
| < 70 | 865 (82.3%) | 603 (81.9%) | 262 (83.2%) | |
| > = 70 | 186 (17.7%) | 133 (18.1%) | 53 (16.8%) | |
| Sex, n (%) | | | | 0.305 |
| Female | 427 (40.6%) | 307 (41.7%) | 120 (38.1%) | |
| Male | 624 (59.4%) | 429 (58.3%) | 195 (61.9%) | |
| ECOG PS | | | | 0.177 |
| ≥ 2 | 212 (20.2%) | 157 (21.3%) | 55 (17.5%) | |
| 0–1 | 839 (79.8%) | 579 (78.7%) | 260 (82.5%) | |
| CEA at diagnosis | | | | 0.164 |
| < 5 ng/mL | 645 (61.4%) | 456 (62.0%) | 189 (60.0%) | |
| ≥ 5 ng/mL | 399 (38.0%) | 273 (37.1%) | 126 (40.0%) | |
| Unknown | 7 (0.67%) | 7 (0.95%) | 0 (0.00%) | |
| Adjuvant chemotherapy | | | | 0.929 |
| No | 501 (47.7%) | 352 (47.8%) | 149 (47.3%) | |
| Yes | 550 (52.3%) | 384 (52.2%) | 166 (52.7%) | |
| Period number | | | | 0.983 |
| < 3 month | 260 (24.7%) | 181 (24.6%) | 79 (25.1%) | |
| ≥ 3 month | 290 (27.6%) | 203 (27.6%) | 87 (27.6%) | |
| untreated | 501 (47.7%) | 352 (47.8%) | 149 (47.3%) | |
| Tumor location, n (%) | | | | 0.051 |
| Left sided | 197 (18.7%) | 128 (17.4%) | 69 (21.9%) | |
| Right sided | 285 (27.1%) | 192 (26.1%) | 93 (29.5%) | |
| Rectum | 569 (54.1%) | 416 (56.5%) | 153 (48.6%) | |
| VELIPI, n (%) | | | | 0.903 |
| Absent | 830 (79.0%) | 580 (78.8%) | 250 (79.4%) | |
| Present | 221 (21.0%) | 156 (21.2%) | 65 (20.6%) | |
| TNM stage, n (%) | | | | 0.371 |
| I | 115 (10.9%) | 82 (11.1%) | 33 (10.5%) | |
| II | 573 (54.5%) | 391 (53.1%) | 182 (57.8%) | |
| III | 363 (34.5%) | 263 (35.7%) | 100 (31.7%) | |
| Tumor grade, n (%) | | | | 0.451 |
| Poor-undifferentiation | 169 (16.1%) | 125 (17.0%) | 44 (14.0%) | |
| Moderate | 803 (76.4%) | 555 (75.4%) | 248 (78.7%) | |
| Well | 79 (7.52%) | 56 (7.61%) | 23 (7.30%) | |
| RIS, n (%) | | | | 0.753 |
| Low | 122 (11.6%) | 87 (11.8%) | 35 (11.1%) | |
| Intermediate | 629 (59.8%) | 435 (59.1%) | 194 (61.6%) | |
| High | 300 (28.5%) | 214 (29.1%) | 86 (27.3%) | |
| CD8 CT | 154 (168) | 155 (170) | 154 (162) | 0.946 |
| CD8 IM | 273 (226) | 272 (230) | 275 (215) | 0.847 |
| Treg CT | 53.6 (58.9) | 53.4 (59.1) | 54.2 (58.5) | 0.823 |
| Treg IM | 97.2 (88.7) | 95.8 (85.7) | 101 (95.5) | 0.434 |
| Tumor budding, n (%) | | | | 0.855 |
| Bd1 | 477 (45.4%) | 338 (45.9%) | 139 (44.1%) | |
| Bd2 | 407 (38.7%) | 283 (38.5%) | 124 (39.4%) | |
| Bd3 | 167 (15.9%) | 115 (15.6%) | 52 (16.5%) | |
| TLS density | 0.46 (0.59) | 0.44 (0.58) | 0.52 (0.60) | 0.057 |
| E-TLS proportion | 0.43 (0.42) | 0.42 (0.42) | 0.46 (0.42) | 0.284 |
| PFL-TLS proportion | 0.13 (0.23) | 0.13 (0.23) | 0.13 (0.22) | 0.715 |
| SFL-TLS proportion | 0.07 (0.17) | 0.07 (0.16) | 0.07 (0.18) | 0.474 |

**Table 1** (continued)

ECOG PS: Eastern Cooperative Oncology Group performance status; CEA: carcinoembryonic antigen; pMMR: proficient mismatch repair, VELIPI: venous emboli, lymphatic invasion, or perineural invasion; RIS: regulatory-Immunoscore; CT: central tumor; IM: invasive margin; TLS: tertiary lymphoid structures; E-TLS: early-stage TLS; PFL-TLS: primary follicle-like stage TLS; SFL-TLS: secondary follicle-like stage TLS

**Table 2** Univariate and multivariate analysis of pathological risk factors in the training set

| Characteristics | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | Hazard ratio (95% CI) | Hazard ratio (95% CI) | *P value* | *P value* |
| RIS | | | | |
| Intermediate vs Low | 0.57 (0.41–0.78) | 0.63 (0.46–0.88) | 0.006 | 0.001 |
| High vs Low | 0.31 (0.21–0.46) | 0.41 (0.27–0.62) | < 0.001 | < 0.001 |
| Tumor budding | | | | |
| Bd2 vs Bd1 | 1.93 (1.46–2.55) | 1.78 (1.35–2.35) | < 0.001 | < 0.001 |
| Bd3 vs Bd1 | 2.68 (1.93–3.73) | 2.19 (1.57–3.06) | < 0.001 | < 0.001 |
| TLS density | 0.37 (0.27–0.51) | 0.43 (0.28–0.67) | < 0.001 | < 0.001 |
| E-TLS proportion | 0.70 (0.52–0.94) | 1.25 (0.89–1.76) | 0.204 | 0.019 |
| PFL-TLS proportion | 0.53 (0.30–0.95) | 1.43 (0.82–2.51) | 0.209 | 0.033 |
| SFL-TLS proportion | 0.05 (0.01–0.18) | 0.25 (0.07–0.87) | 0.029 | < 0.001 |

RIS: regulatory-Immunoscore; TLS: tertiary lymphoid structures; E-TLS: early-stage TLS; PFL-TLS: primary follicle-like stage TLS; SFL-TLS: secondary follicle-like stage TLS

TADI levels were associated with poorer prognosis across all subgroups, reinforcing the generalizability and clinical relevance of TADI as a prognostic tool (sFigure 4).

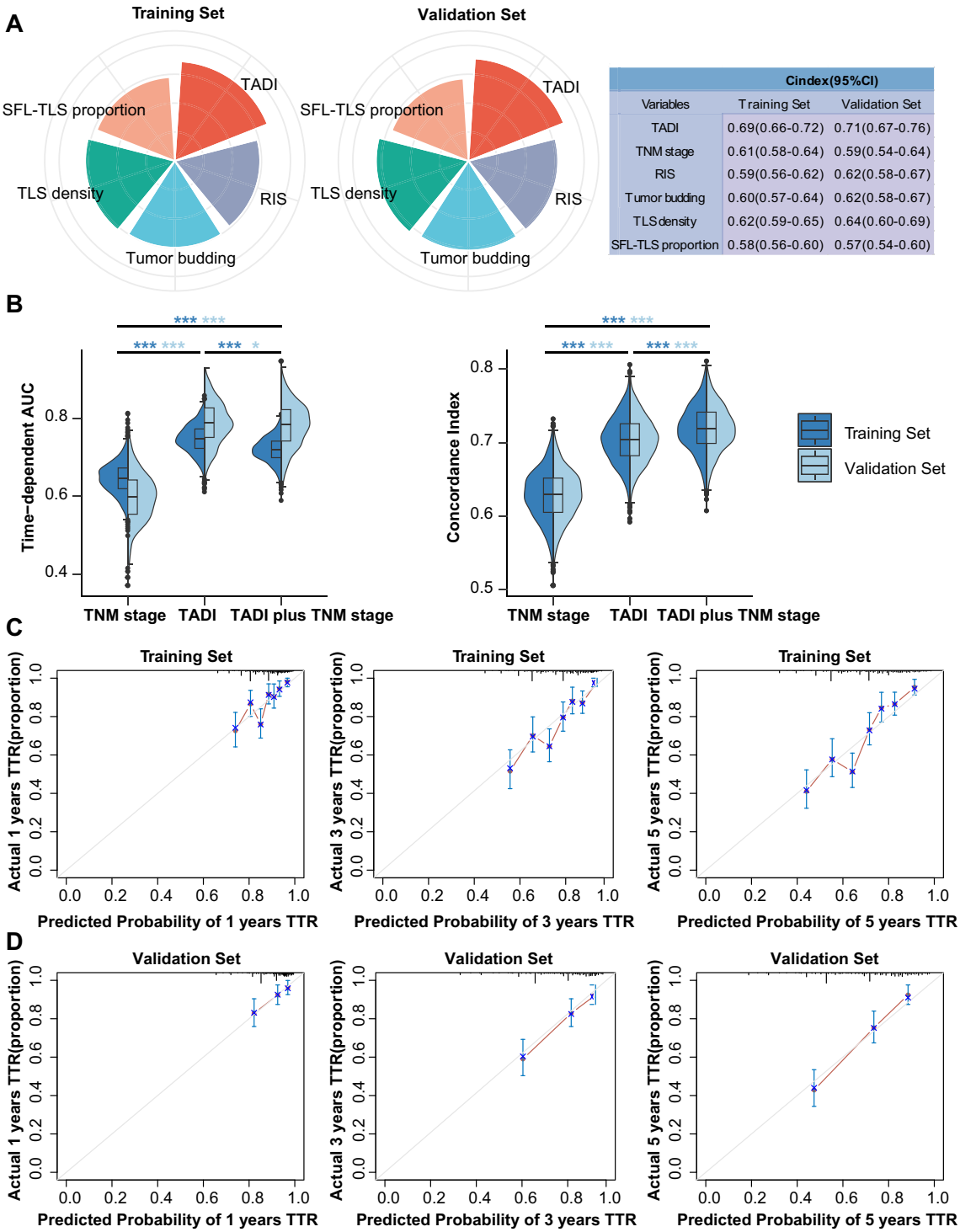
### Predictive value of TADI in stage II-III pMMR colorectal cancer for ACT

In stage II pMMR colorectal cancer (CRC) patients (n = 573), Kaplan–Meier survival analysis revealed significant differences in both TTR and OS across the low, intermediate, and high TADI groups (All Patients: TTR, P < 0.001; OS, P < 0.001). The low TADI group had the best survival outcomes, followed by the intermediate and high TADI groups with progressively worse outcomes. This trend was consistent in both the training and validation sets (sFigure 5A). Among these patients, 268 (46.8%) received adjuvant chemotherapy (ACT) (sTable 3). ACT was associated with significant improvements in both TTR and OS for patients with intermediate and high TADI scores compared to those who did not receive ACT (Intermediate: TTR: HR = 0.34, 95% CI 0.19–0.59, P < 0.001; OS: HR = 0.25, 95% CI 0.14–0.47, P < 0.001; High: TTR: HR = 0.58, 95% CI 0.36–0.95, P = 0.031; OS: HR = 0.44, 95% CI 0.26–0.74, P = 0.002) (Fig. 6). Specifically, Patients with intermediate and high TADI scores who underwent ACT exhibited significantly improved survival outcomes, with markedly reduced recurrence and mortality risks. However, ACT did not show a significant benefit in the low TADI group (TTR: P = 0.144) (Fig. 6C).

In stage III pMMR CRC patients (n = 363), survival differences based on TADI scores were similarly observed (TTR, P < 0.001; OS, P < 0.001), with the low TADI group having the best prognosis and the high TADI group demonstrating the poorest survival. These differences were confirmed in both the training and validation sets. Clinicopathologic factors for stage III patients who received ACT are presented in sTable 4. Kaplan–Meier survival analysis revealed that patients with high TADI scores who received ACT for more than 3 months had significantly improved TTR and OS (TTR: P = 0.041; OS: P = 0.021) (sFigure 6C, F). The hazard ratios (HRs) further highlight the clinical benefit of prolonged ACT in these groups: patients with high TADI scores experienced a significantly reduced risk of recurrence (TTR: HR = 0.63, 95% CI 0.40–0.98, P = 0.041) and mortality (OS: HR = 0.56, 95% CI 0.34–0.92, P = 0.021) when receiving ACT for more than 3 months, compared to those who underwent shorter treatment durations (sFigure 6G). Conversely, patients with low and intermediate TADI scores did not exhibit improved clinical outcomes based on ACT duration, suggesting no additional benefit from prolonged chemotherapy in these groups.

## Discussion

Colorectal cancer (CRC) exhibits considerable variability in prognosis and treatment response, driven by the complex interplay between intrinsic tumor invasiveness and TME. For instance, invasive features such as tumor budding within the tumor tissue are linked to poor




| | Cindex(95%CI) | |
|---|---|---|
| Variables | Training Set | Validation Set |
| TADI | 0.69(0.66-0.72) | 0.71(0.67-0.76) |
| TNM stage | 0.61(0.58-0.64) | 0.59(0.54-0.64) |
| RIS | 0.59(0.56-0.62) | 0.62(0.58-0.67) |
| Tumor budding | 0.60(0.57-0.64) | 0.62(0.58-0.67) |
| TLS density | 0.62(0.59-0.65) | 0.64(0.60-0.69) |
| SFL-TLS proportion | 0.58(0.56-0.60) | 0.57(0.54-0.60) |

**Fig. 3** Performance of the TADI model. **A** C-index values of TADI and pathologically based risk parameters in the training and validation sets. **B** t-AUC (left) and C-index (right) for the TADI model, the anatomy-based parameters model and the anatomy-based parameters plus the TADI model. The left side of each violin plot represents the data in the training set and the right represents the data in the validation set. **C–D** Calibration plot of observed versus predicted 1-year, 3-year and 5-year time to recurrence for the TADI model in the training set and validation set. TADI, Tumor Aggression-Defense Index; RIS, regulatory-Immunoscore, TLS, tertiary lymphoid structures; SFL-TLS, secondary follicle-like stage TLS; t-AUC, the time-dependent area under the curve

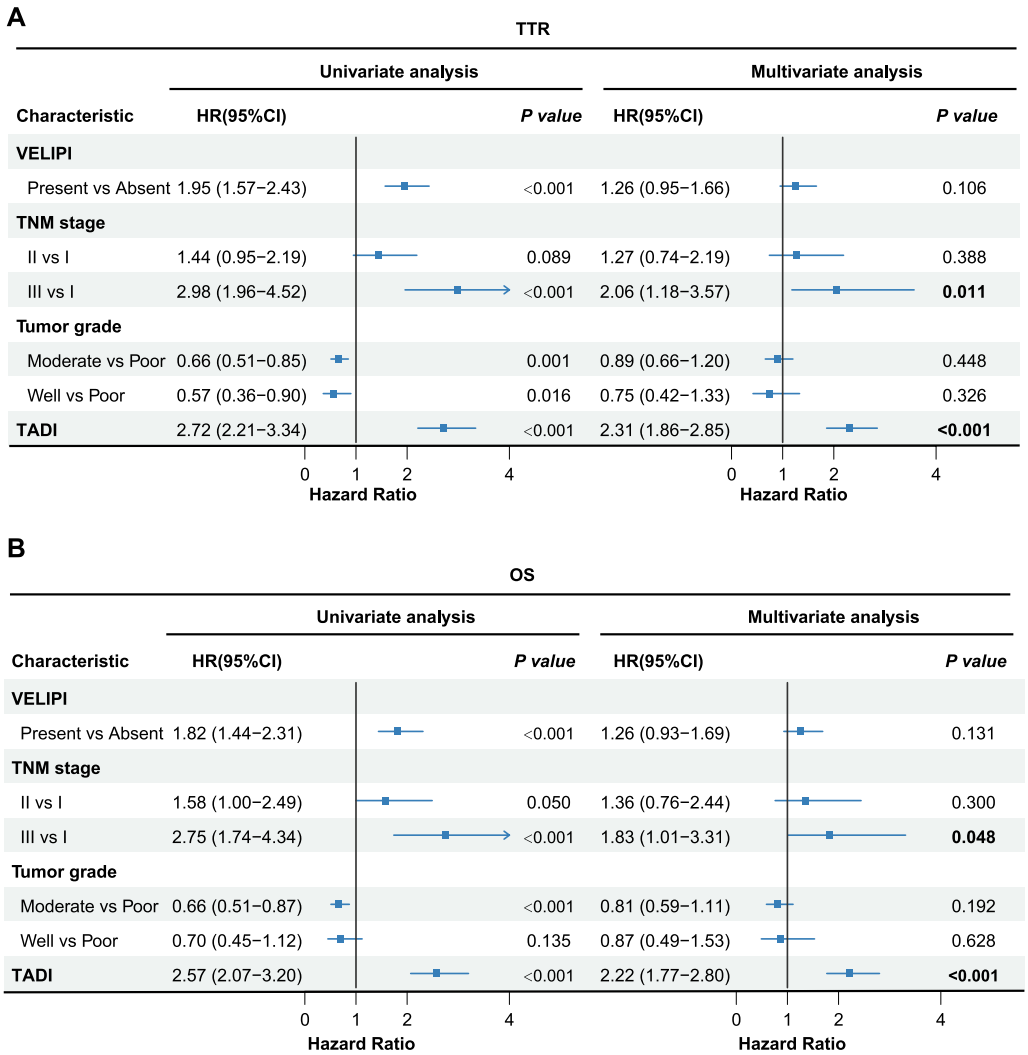



**Fig. 4** Univariate and multivariate analyses of TADI. **A** Univariate and multivariate analysis for TTR using cox regression. **B** Univariate and multivariate analysis for OS using cox regression. VELIPI: positive for the biomarkers for venous emboli, lymphatic invasion, or perineural invasion; TADI, Tumor Aggression-Defense Index; TTR, time to recurrence; OS, overall survival

prognosis, while higher levels of tumor-infiltrating lymphocytes (TILs) in the TME are generally associated with more favorable outcomes and enhanced immune activity [21]. Moreover, mismatch repair status further influences the TME, with pMMR and dMMR CRC displaying distinct immune landscapes. dMMR CRC, characterized by a high mutation burden, generate more tumor-specific neoantigens, which in turn promote increased TILs and stronger immune responses [22, 23]. In contrast, pMMR CRC, the predominant subtype, exhibit pronounced heterogeneity, contributing to variable responses to chemoradiotherapy (CRT), a nuance that traditional TNM staging fails to capture effectively [24]. While TNM staging and existing immune-based models, such as those developed by Tsikitis et al., Zhang et al., and Ueno et al., provide valuable prognostic insights, they predominantly focus on tumor characteristics and immune markers in isolation, without fully integrating both tumor invasiveness and immune response [25–27]. This study, focusing on pMMR CRC, introduces the Tumor-Associated Defense Index (TADI), a novel prognostic model integrating both invasive and immune-related biomarkers from tumor tissue and the TME. TADI outperforms conventional TNM staging in predicting prognosis, offering valuable insights for personalized adjuvant therapy decisions in stage II-III pMMR CRC patients, with the potential to optimize treatment strategies and improve clinical outcomes.

This study investigates two crucial independent defensive factors within the TME: Immunoscore and TLS.

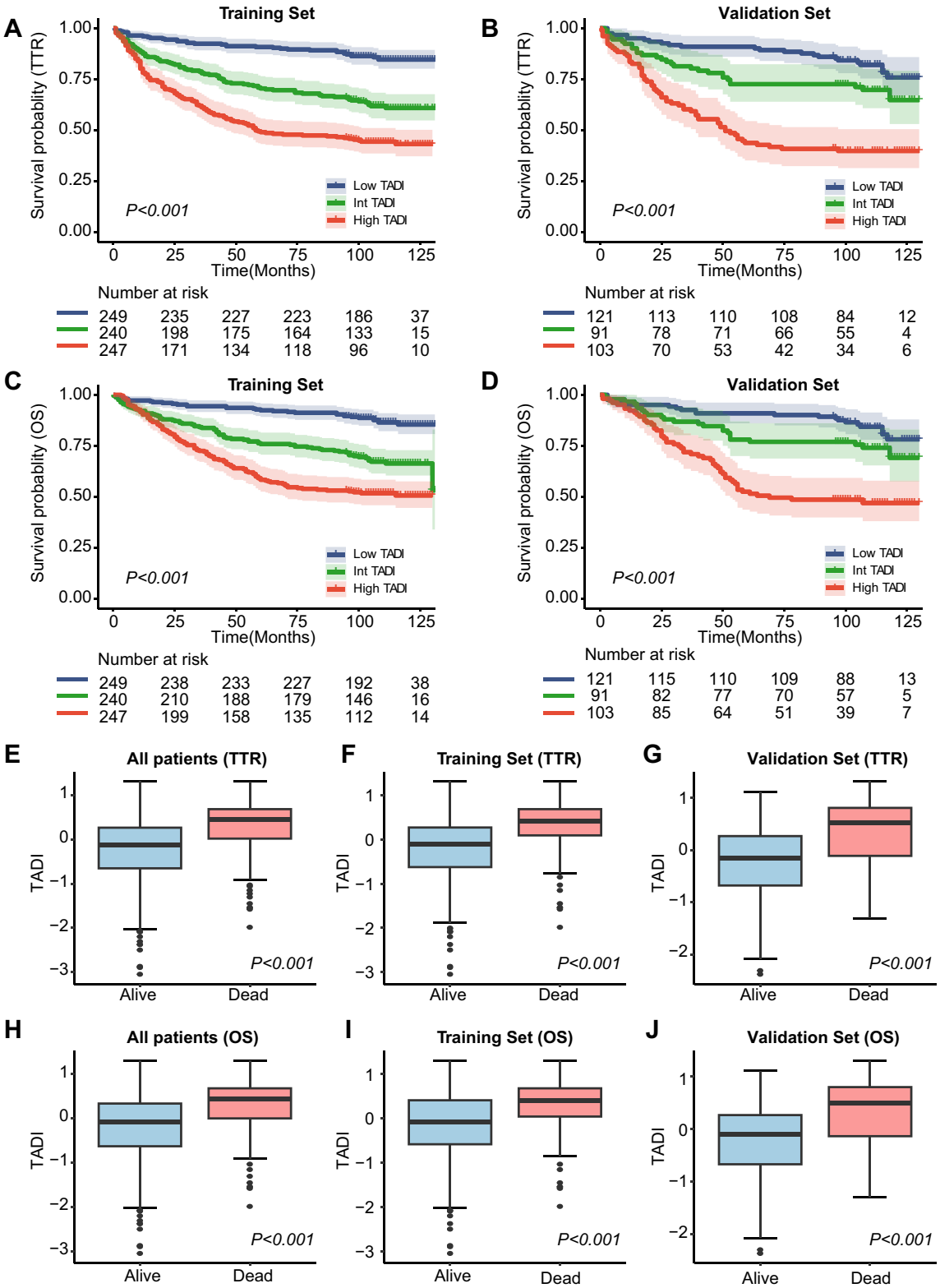



**Fig. 5** Kaplan–Meier curves for clinical events according to TADI and box plots of the distribution range of TADI for different recurrence and survival states **A** Kaplan–Meier curve for TTR according to TADI values in the training set. **B** Kaplan–Meier curve for TTR according to TADI values in the validation set. **C** Kaplan–Meier curve for OS according to TADI values in the training set. **D** Kaplan–Meier curve for OS according to TADI values in the validation set. **E–J** Box plots of the distribution range of TADI for different recurrence and survival states in all patients, training set and validation set. TADI: Tumor Aggression-Defense Index, TTR, time to recurrence; OS, overall survival

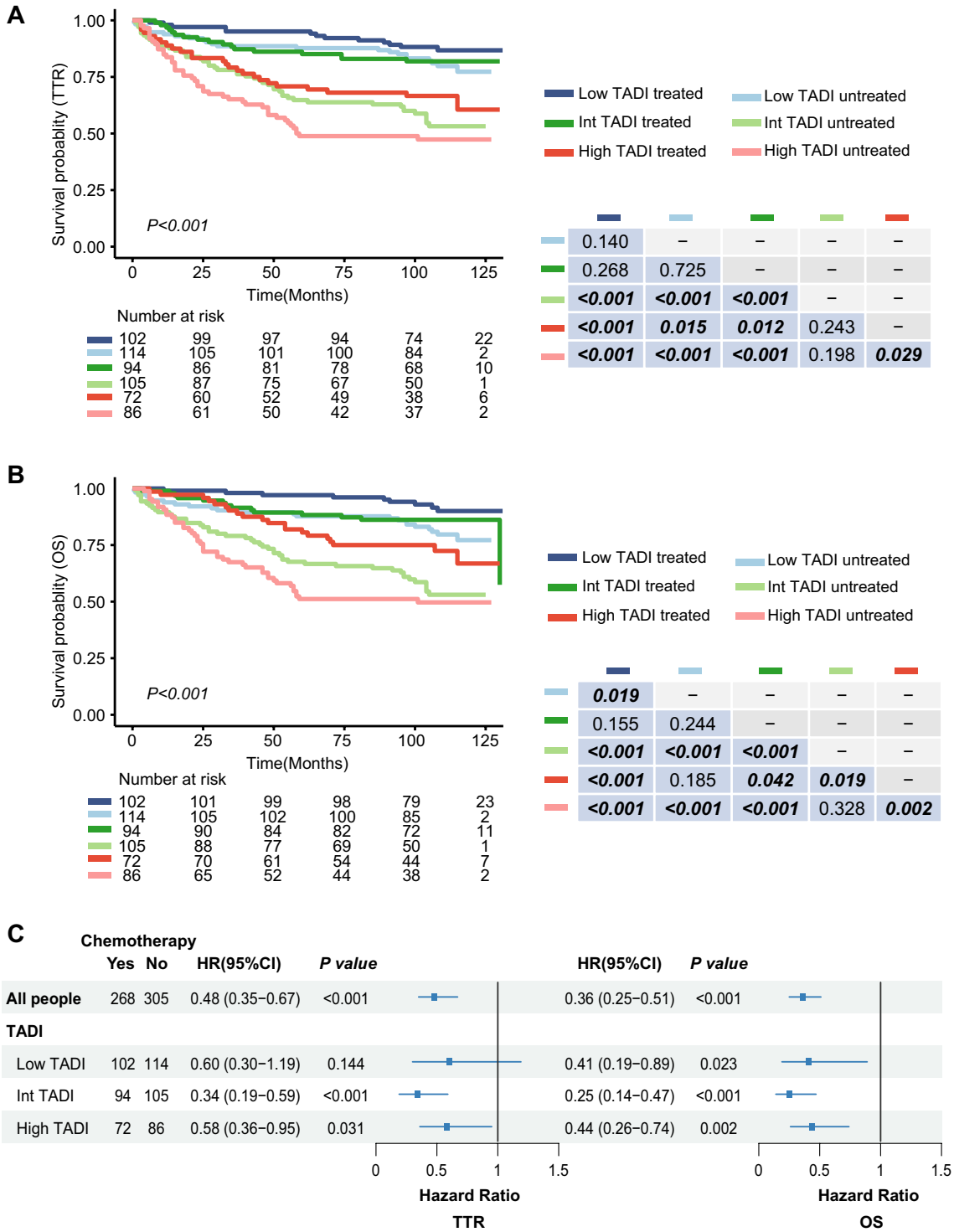



**Fig. 6** Relationship between TADI and clinical outcome among stage II colorectal cancer patients treated with ACT. **A** The Kaplan–Meier curve was used to analyze the TTR of stage II pMMR CRC based on their TADI status and receipt of ACT. **B** The Kaplan–Meier curve was used to analyze the OS of stage II pMMR CRC based on their TADI status and receipt of ACT. **C** Forest plot for time to recurrence and overall survival according to ACT (yes or no) among patients with stage II pMMR CRC. TADI, Tumor Aggression-Defense Index; ACT, adjuvant chemotherapy treatments; TTR, time to recurrence; OS, overall survival

Immunoscore quantifies lymphocyte density in the core tumor and invasive margin, serving as a robust indicator of the immune landscape. Our analysis, incorporating both CD8 + and FOXP3 + T cells, our RIS aligns with previous findings, demonstrating that higher RIS correlates with a more favorable prognosis and significantly correlates with TNM and T stages. Additionally, TLS—ectopic lymphoid structures within tumors—play a critical role in promoting anti-tumor immune responses [19]. Meta-analyses show that high TLS expression correlates with improved overall survival, recurrence-free survival, and reduced risk of tumor recurrence [28–30]. Our study further reinforces these findings, highlighting the significant association between increased TLS density and maturity and better prognosis [31]. This study highlights the critical role of TLS expression and TLS density in the anti-tumor immune response in CRC. We found that TLS density correlated with TNM stage but not T stage. Advanced TNM and higher T stages were associated with reduced TLS density and fewer mature TLS. These results emphasize how tumor progression affects TLS formation and TILs infiltration, influencing the immune landscape within the TME. This study also examines invasive tumor factors, particularly TB, a well-established marker of poor prognosis in CRC and a key feature of epithelial-mesenchymal transition (EMT) [32–34]. Our analysis further underscores the significance of TB as an indicator of tumor aggressiveness, reinforcing its prognostic value in CRC.

Moreover, TADI demonstrates potential in predicting the efficacy of ACT, particularly for stage II-III patients. Current clinical guidelines consider high-risk features like poorly differentiated tumors, perforation/obstruction, inadequate lymph node retrieval, lymph vascular invasion (LVI), and perineural invasion (PNI) for guiding adjuvant therapy in stage II CRC patients [35]. While some studies suggest that ACT may improve relapse-free survival (RFS) and OS in stage II CRC patients [36, 37], others caution that the risks and adverse effects may outweigh these benefits [38, 39]. Our stratified analysis of TADI in stage II patients suggests that those with low TADI scores derive minimal benefit from ACT, supporting the potential for more selective treatment approaches in this cohort. In stage III CRC, the duration of ACT appears to significantly impact patient prognosis. The IDEA study, for example, highlights the value of adjusting treatment duration based on T/N stages (low-risk vs. high-risk) [40]. For T1-3/N1 patients, a 3-month CAPOX regimen demonstrates efficacy with reduced toxicity. Post hoc analyses from IDEA France and CALGB/SWOG 80702 studies also suggest combining tumor deposit count and lymph node metastasis improves the accuracy of TNM staging predictions [41, 42]. However, despite advances in stratification, current research still struggles to optimize treatment duration for all stage III CRC patients. Our study indicates that patients with high TADI scores derive significant benefit from ACT durations exceeding 3 months, while patients with low to moderate TADI scores may not benefit from prolonged treatment regimens. This suggests that high TADI levels, which correlate with reduced TIL and TLS infiltration and increased tumor budding (TB), may indicate an immune-suppressive environment. We hypothesize that moderate TADI patients may not benefit from prolonged treatments due to weak immunogenicity or an immune-suppressive environment, where oxaliplatin-induced immunogenic cell death (ICD) is less effective in 'cold tumors' [43].

Currently, microsatellite instability-high (MSI-H) is the only biomarker used to guide immune checkpoint inhibitor (ICI) therapy in CRC, but its utility is limited in pMMR CRC, where ICI monotherapy is largely ineffective. While combination ICI therapy shows modest efficacy (10%−20%) in pMMR CRC [44, 45], identifying additional biomarkers is crucial to predict which patients may experience durable responses. Various factors within the tumor microenvironment (TME) influence immune therapy efficacy, including the density of tumor-infiltrating lymphocytes (TILs) [46–48]. For instance, a Phase 2 multicenter study on regorafenib plus nivolumab demonstrated that higher baseline densities of cytotoxic T cells, regulatory T cells, and macrophages were associated with improved outcomes [49]. Furthermore, TLS and B cells have been shown to enhance immune responses in melanoma and breast cancer [50, 51]. Given these insights, the TADI model, which reflects both tumor invasiveness and immune defense characteristics, may offer a valuable composite biomarker to predict responses to ICIs, such as PD-1/PD-L1 or CTLA4 inhibitors, in pMMR CRC.

This study has several limitations. First, the single-center design and the absence of an independent external validation cohort limit the generalizability and external applicability of the findings. Multicenter, prospective studies with independent validation cohorts are necessary to confirm the robustness and predictive value of TADI across diverse clinical and immunologic profiles. Secondly, the complexity of TADI scoring necessitates intricate formulas, posing challenges for clinical implementation. Simplifying the TADI scoring process would enhance its clinical feasibility. Thirdly, while TB, TLS, and RIS benefit from established assessment criteria, their scoring and quantification may still be subject to pathologists' subjective interpretations. Integrating digital pathology techniques like deep learning could mitigate observer bias in future evaluations. Despite these

limitations, our innovative stratified approach based on the attack-defense system model within TME holds promise for guiding precise and personalized treatment strategies for stage II-III pMMR CRC patients.

## Supplementary Information

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Additional file 1.

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**Author contributions**
Yanqiao Zhang, Chao Liu, and Chunhui Zhang conceived this study. Tong Wu, Lin Fang, Yuli Ruan, and Shuling Han collected all clinical data of patients. Mengde Shi, Dan Su, Yue Ma, Ming Ma, Xiaolin Lu, Bojun Wang, and Yuanyu Liao performed the data analysis. Tong Wu, Lin Fang, and Yuli Ruan wrote the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Data availability**
All data supporting the findings in this study are presented in the manuscript and the supplementary information, and additional raw data can be made available by the corresponding author upon reasonable request.

## Declarations

**Ethics approval and consent to participate**
This study complied with the principles of the Declaration of Helsinki and was approved by the ethics committee of the Harbin Medical University Cancer Hospital (retrospective study approval KY2022-20). All participants provided written informed consent.

**Consent for publication**
All authors consent for publication.

**Competing interests**
The authors report there are no competing interests to declare.

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