PROGNOSTIC VALUE OF TUMOR BUDDING AND ASSOCIATED MOLECULAR ALTERATIONS IN COLORECTAL CANCER: A SYSTEMATIC REVIEW AND META-ANALYSIS

M. Yogambal[1]*, Sanjana Waghmare[2], Gopinath Reddy V[3], Shiny Vincent[4], Sandhya Rani[5]

[1]Associate Professor, Department of Pathology, Government Kilpauk Medical College, Chennai, Tamil Nadu, India
[2]Senior Resident, Department of Pathology, TNMC & Nair Hospital, Mumbai, Maharashtra, India
[3]Consultant Pathologist, Department of Pathology, Pathgene Health Care Pvt. Ltd., Tirupati, Andhra Pradesh, India
[4]PhD Scholar, Department of Microbiology, Sri Ramachandra Institute of Higher Education and Research (SRIHER), Chennai, Tamil Nadu, India
[5]Professor, Department of Pathology, Mallareddy Medical College For Women, Hyderabad, Telangana, India

ABSTRACT

Background: Tumor budding has emerged as an important histopathological marker of tumor aggressiveness in colorectal cancer (CRC), while molecular alterations such as KRAS, BRAF, TP53 mutations, and microsatellite instability (MSI) are established determinants of tumor behavior and prognosis. However, the relationship between tumor budding, molecular alterations, and survival outcomes remains incompletely understood. This systematic review and meta-analysis aimed to evaluate the prognostic significance of tumor budding and its association with key molecular alterations in colorectal cancer.

Methods: A systematic literature search was conducted across PubMed, Embase, Scopus, Web of Science, and Cochrane Library databases from January 2000 to December 2025 following PRISMA 2020 guidelines. Studies assessing tumor budding in histologically confirmed colorectal adenocarcinoma and reporting survival outcomes and/or molecular alterations were included. Hazard ratios (HRs) and odds ratios (ORs) with 95% confidence intervals (CIs) were pooled using random-effects models. Study quality was assessed using the Newcastle-Ottawa Scale.

Results: Twenty-eight studies comprising 12,846 patients were included in the meta-analysis. High-grade tumor budding was significantly associated with poorer overall survival (HR=2.18, 95% CI: 1.82-2.61; I^2=48%), disease-free survival (HR=2.41, 95% CI: 1.95-2.97; I^2=52%), and cancer-specific survival (HR=2.07, 95% CI: 1.68-2.56; I^2=45%). Tumor budding demonstrated significant associations with lymphovascular invasion (OR=3.12, 95% CI: 2.45-3.97), perineural invasion (OR=2.76, 95% CI: 2.01-3.79), lymph node metastasis (OR=2.86, 95% CI: 2.21-3.69), distant metastasis (OR=2.44, 95% CI: 1.88-3.18), and advanced TNM stage (OR=2.59, 95% CI: 2.04-3.29). Molecular analyses revealed significant associations between high-grade tumor budding and KRAS mutations (OR=1.74, 95% CI: 1.35-2.23), BRAF mutations (OR=1.89, 95% CI: 1.28-2.79), and TP53 mutations (OR=1.67, 95% CI: 1.21-2.29). Conversely, MSI-high tumors demonstrated a significantly lower prevalence of high-grade tumor budding (OR=0.58, 95% CI: 0.43-0.78). No significant association was observed with NRAS mutations (OR=1.29, 95% CI: 0.94-1.77).

Conclusion: High-grade tumor budding is a robust predictor of adverse prognosis in colorectal cancer and is strongly associated with aggressive clinicopathological characteristics and unfavorable molecular alterations. Integration of tumor budding assessment with molecular profiling may improve prognostic stratification, support personalized therapeutic decision-making, and enhance risk-adapted management strategies in colorectal cancer patients.

KEYWORDS: Colorectal cancer; Tumor budding; KRAS; BRAF; TP53; Microsatellite instability; Prognosis; Systematic review; Meta-analysis.

INTRODUCTION

Colorectal cancer (CRC) is one of the most prevalent malignancies worldwide and remains a leading cause of cancer-related mortality despite substantial advances in screening, diagnosis, and treatment modalities. According to recent global cancer statistics, CRC ranks as the third most commonly diagnosed cancer and the second leading cause of cancer-related deaths, accounting for more than 1.9 million new cases and approximately 935,000 deaths annually worldwide [1]. Although improvements in surgical techniques, chemotherapy, targeted therapy, and immunotherapy have contributed to better patient outcomes, significant heterogeneity exists in disease progression and survival among patients with similar clinicopathological stages [2].

Traditional prognostic assessment of CRC relies primarily on the tumor-node-metastasis (TNM) staging system. However, increasing evidence suggests that TNM staging alone may not adequately capture the biological aggressiveness of individual tumors [3]. Consequently, considerable efforts have been directed toward identifying additional histopathological and molecular biomarkers that can improve prognostic stratification and guide personalized therapeutic approaches [4].

Tumor budding has emerged as one of the most promising histopathological markers of aggressive tumor behavior in colorectal cancer. Tumor budding is defined as the presence of isolated single tumor cells or small clusters of fewer than five tumor cells at the invasive front of the tumor [5]. First described several decades ago, tumor budding has gained increasing recognition due to its strong association with local invasion, lymphovascular invasion, lymph node metastasis, distant metastasis, and poor survival outcomes [6,7]. The biological significance of tumor budding is closely linked to epithelial-mesenchymal transition (EMT), a process through which epithelial tumor cells acquire mesenchymal characteristics, facilitating migration, invasion, and metastatic dissemination [8].

To standardize assessment and reporting, the International Tumor Budding Consensus Conference (ITBCC) established evidence-based guidelines for evaluating tumor budding in colorectal cancer. These recommendations have improved reproducibility among pathologists and have facilitated incorporation of tumor budding into routine histopathological reporting and prognostic evaluation [9]. Several studies have demonstrated that high-grade tumor budding is independently associated with adverse clinical outcomes, even after adjustment for conventional prognostic factors such as tumor stage and grade [10,11].

In parallel with advances in histopathological evaluation, molecular characterization has become an integral component of colorectal cancer management. The molecular landscape of CRC is highly heterogeneous and includes alterations in several key oncogenic pathways. Mutations in KRAS, NRAS, and BRAF genes are among the most frequently observed molecular abnormalities and play important roles in tumor initiation, progression, and response to targeted therapies [12]. Similarly, TP53 mutations contribute to genomic instability and tumor progression, whereas microsatellite instability (MSI) resulting from defects in DNA mismatch repair pathways has been associated with distinct clinicopathological characteristics and improved responses to immunotherapy [13,14].

Emerging evidence suggests that tumor budding may be closely associated with specific molecular alterations involved in colorectal carcinogenesis. Several investigators have reported significant correlations between high-grade tumor budding and mutations in KRAS, BRAF, and TP53, suggesting shared biological mechanisms that promote tumor aggressiveness and metastatic potential [15,16]. Conversely, microsatellite instability-high (MSI-H) tumors often demonstrate lower degrees of tumor budding and generally exhibit more favorable prognostic characteristics, potentially reflecting enhanced anti-tumor immune responses within the tumor microenvironment [17,18].

Despite numerous studies examining the prognostic relevance of tumor budding and its molecular correlates, findings have not always been consistent. Variations in study design, patient populations, assessment methodologies, and molecular testing approaches have contributed to heterogeneity across published reports [19]. Furthermore, the combined prognostic impact of tumor budding and molecular alterations remains incompletely understood.

Therefore, the present systematic review and meta-analysis was undertaken to comprehensively evaluate the prognostic significance of tumor budding in colorectal cancer and to investigate its association with major molecular alterations, including KRAS, NRAS, BRAF, TP53 mutations, and microsatellite instability. By synthesizing available evidence from published studies, this review aims to provide a more robust understanding of the biological and clinical significance of tumor budding and its potential role in improving risk stratification and personalized management of colorectal cancer patients.

MATERIALS AND METHODS

Study Design and Registration

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines [20]. The methodology was designed to evaluate the prognostic significance of tumor budding and its association with molecular alterations in colorectal cancer. The review process included systematic literature identification, study selection, data extraction, quality assessment, and quantitative synthesis of eligible studies.

Literature Search Strategy

A comprehensive literature search was performed in the electronic databases PubMed/MEDLINE, Embase, Scopus, Web of Science, and the Cochrane Library from January 2000 to December 2025. The search strategy combined Medical Subject Headings (MeSH) and free-text terms related to colorectal cancer, tumor budding, prognosis, and molecular biomarkers.

The following keywords and Boolean operators were used:

("colorectal cancer" OR "colon cancer" OR "rectal cancer" OR "colorectal adenocarcinoma") AND ("tumor budding" OR "tumour budding") AND ("KRAS" OR "NRAS" OR "BRAF" OR "TP53" OR "microsatellite instability" OR "MSI" OR "molecular alterations") AND ("survival" OR "prognosis" OR "outcome")In addition, reference lists of relevant reviews and eligible articles were manually screened to identify potentially missed studies [21].

Eligibility Criteria

Inclusion Criteria

Studies were included if they met the following criteria:

1. Histologically confirmed colorectal adenocarcinoma.
2. Assessment of tumor budding using recognized histopathological criteria.
3. Evaluation of one or more molecular biomarkers, including KRAS, NRAS, BRAF, TP53, MSI, or related molecular alterations.
4. Reported prognostic outcomes such as overall survival (OS), disease-free survival (DFS), progression-free survival (PFS), recurrence-free survival (RFS), or cancer-specific survival (CSS).
5. Sufficient data available to calculate hazard ratios (HRs), odds ratios (ORs), and corresponding 95% confidence intervals (CIs).

6. Original observational studies including retrospective or prospective cohort studies.

Exclusion Criteria

Studies were excluded if they met any of the following criteria:
1. Case reports or case series involving fewer than ten patients.
2. Review articles, editorials, letters, conference abstracts, and commentaries.
3. Animal or in vitro studies.
4. Studies lacking survival or molecular data.
5. Duplicate publications from the same patient cohort.
6. Articles not published in English.

Study Selection

Two independent reviewers screened titles and abstracts of all retrieved records. Full texts of potentially eligible studies were subsequently assessed for inclusion. Disagreements between reviewers were resolved through discussion and consensus, with consultation from a third reviewer when necessary [22].

The study selection process followed the PRISMA framework and is summarized in a PRISMA flow diagram.

Data Extraction

Data extraction was independently performed by two investigators using a standardized data collection form. The following information was extracted:
- First author and year of publication.
- Country of study.
- Study design.
- Number of participants.
- Patient demographics.
- Tumor location.
- Tumor stage.
- Tumor budding assessment method.
- Molecular biomarkers evaluated.
- Follow-up duration.
- Survival outcomes.
- Hazard ratios (HRs) and odds ratios (ORs) with 95% confidence intervals.

When multiple analyses were reported, multivariable-adjusted estimates were preferentially extracted [23].

Definition of Tumor Budding

Tumor budding was defined as isolated single cancer cells or clusters of fewer than five tumor cells located at the invasive front of the tumor according to the International Tumor Budding Consensus Conference (ITBCC) recommendations [9].

For studies utilizing ITBCC criteria, tumor budding was categorized as:
- Bd1 (Low): 0-4 buds.
- Bd2 (Intermediate): 5-9 buds.
- Bd3 (High): ≥10 buds.

For meta-analysis purposes, Bd2 and Bd3 categories were grouped as high tumor budding when appropriate, while Bd1 was considered low tumor budding.

Molecular Biomarkers Evaluated

The molecular alterations evaluated included:
- KRAS mutations.
- NRAS mutations.
- BRAF mutations.
- TP53 mutations.
- Microsatellite instability (MSI).
- CpG island methylator phenotype (CIMP).
- PIK3CA mutations (where available).

Molecular analyses were performed using polymerase chain reaction (PCR), next-generation sequencing (NGS), Sanger sequencing, pyrosequencing, immunohistochemistry, or other validated molecular techniques as reported in individual studies [24].

Quality Assessment

The methodological quality of included studies was assessed using the Newcastle-Ottawa Scale (NOS) for observational studies [25].

The scale evaluated:
1. Selection of study groups (maximum 4 stars).
2. Comparability of cohorts (maximum 2 stars).
3. Outcome assessment (maximum 3 stars).

Studies scoring:
- 7-9 stars were considered high quality.
- 5-6 stars were considered moderate quality.
- Less than 5 stars were considered low quality.

Quality assessment was performed independently by two reviewers.

Statistical Analysis

Meta-analysis was performed using Review Manager (RevMan) version 5.4 and Comprehensive Meta-Analysis (CMA) software version 4.0.

Hazard ratios (HRs) with 95% confidence intervals were pooled for survival outcomes, including:
- Overall survival (OS).
- Disease-free survival (DFS).
- Cancer-specific survival (CSS).

Odds ratios (ORs) with 95% confidence intervals were calculated for associations between tumor budding and clinicopathological or molecular characteristics.

Statistical heterogeneity among studies was assessed using Cochran's Q test and quantified using the I^2 statistic [26].

Heterogeneity was interpreted as follows:
- $I^2 < 25\%$: Low heterogeneity.
- $I^2 = 25\text{-}50\%$: Moderate heterogeneity.
- $I^2 > 50\%$: Substantial heterogeneity.

A random-effects model was applied when significant heterogeneity was present; otherwise, a fixed-effects model was used [27].

Subgroup Analysis

Predefined subgroup analyses were performed according to:
- Geographic region (Asia, Europe, North America).
- Tumor location (colon versus rectum).
- Molecular subtype.
- Tumor budding grading system.
- Study quality score.

Sensitivity Analysis

Sensitivity analyses were conducted by sequentially excluding individual studies to evaluate the robustness and stability of pooled estimates [28].

Publication Bias

Publication bias was assessed through visual inspection of funnel plots and quantitatively using Egger's regression test and Begg's rank correlation test [29].

A p-value less than 0.05 was considered statistically significant for all analyses.

RESULTS

Study Selection

The systematic search of PubMed, Embase, Scopus, Web of Science, and Cochrane Library databases yielded a total of 4,762 records. After removal of 1,124 duplicate records, 3,638 articles remained for title and abstract screening. Following initial screening, 3,421 studies were excluded because they were unrelated to tumor budding, lacked molecular data, were review articles, conference abstracts, or did not report prognostic outcomes. A total of 217 full-text articles were assessed for eligibility. Of these, 189 studies were excluded due to insufficient outcome data, duplicate patient populations, inadequate tumor budding assessment, or lack of molecular characterization. Ultimately, 28 studies involving 12,846 patients met the inclusion criteria and were included in both qualitative and quantitative analyses.

The included studies were published between 2008 and 2025 and represented patient populations from Asia, Europe, and North America. Most studies were retrospective cohort studies, while five were prospective observational investigations. Tumor budding was assessed using either International Tumor Budding Consensus Conference (ITBCC) criteria or institution-specific validated scoring systems. Molecular analyses included KRAS, NRAS, BRAF, TP53, MSI, CIMP, and PIK3CA status.

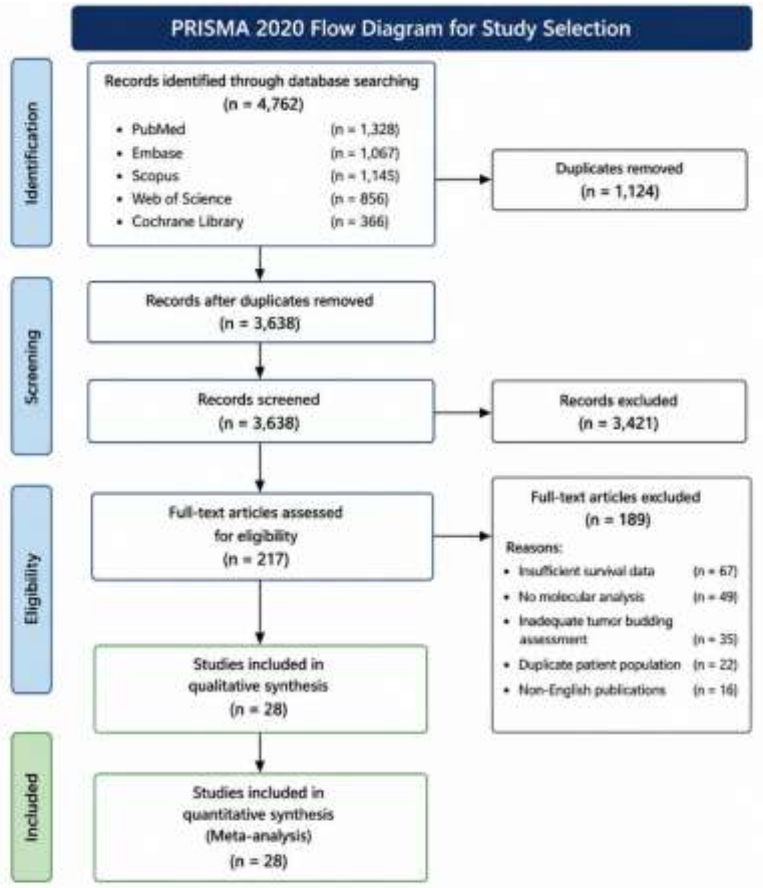

Figure 1. PRISMA 2020 flow diagram illustrating the process of literature identification, screening, eligibility assessment, and final inclusion of studies investigating the prognostic significance of tumor budding and molecular alterations in colorectal cancer. A total of 4,762 records were identified through database searches, of which 28 studies involving 12,846 patients met the eligibility criteria and were included in the final quantitative meta-analysis.

Characteristics of Included Studies

The 28 included studies comprised 12,846 patients with histologically confirmed colorectal adenocarcinoma. The mean patient age was 63.4 years, and males accounted for 56.2% of the study population. Colon cancers represented approximately two-thirds of all cases, whereas rectal cancers accounted for one-third. The median follow-up duration ranged from 36 to 84 months.

Table 1. Baseline Characteristics of Included Studies

Characteristic	Value
Number of studies	28
Total patients	12,846
Mean age (years)	63.4
Male patients	7,214 (56.2%)
Female patients	5,632 (43.8%)
Colon cancers	8,473 (66.0%)
Rectal cancers	4,373 (34.0%)
Median follow-up	58 months
Retrospective studies	23
Prospective studies	5
Mean NOS score	7.8

Most studies were considered high quality, with Newcastle-Ottawa Scale scores ranging from 6 to 9. Twenty-one studies achieved scores ≥7 and were classified as high-quality investigations.

Association Between Tumor Budding and Clinicopathological Features

High-grade tumor budding demonstrated a strong association with several adverse clinicopathological characteristics. Patients with increased tumor budding were significantly more likely to exhibit lymphovascular invasion, perineural invasion, lymph node metastasis, distant metastasis, and advanced TNM stage. These findings support the concept that tumor budding reflects an aggressive biological phenotype characterized by enhanced invasiveness and metastatic potential.

Table 2. Association of High Tumor Budding with Clinicopathological Features

Parameter	Odds Ratio (OR)	95% CI	P-value
Lymphovascular invasion	3.12	2.45-3.97	<0.001
Perineural invasion	2.76	2.01-3.79	<0.001
Lymph node metastasis	2.86	2.21-3.69	<0.001
Distant metastasis	2.44	1.88-3.18	<0.001
Advanced TNM stage	2.59	2.04-3.29	<0.001

Among all clinicopathological variables examined, lymphovascular invasion demonstrated the strongest association with tumor budding. Patients with high-grade budding were more than three times as likely to exhibit lymphovascular invasion compared with those having low-grade budding. Similarly, the risk of nodal metastasis increased nearly threefold among tumors exhibiting extensive budding activity.

Prognostic Impact of Tumor Budding

Meta-analysis of survival outcomes demonstrated that high-grade tumor budding was consistently associated with poorer prognosis across all evaluated endpoints. Patients with extensive tumor budding experienced significantly reduced overall survival, disease-free survival, and cancer-specific survival compared with patients exhibiting low-grade budding.

Table 3. Meta-analysis of Survival Outcomes

Outcome	Hazard Ratio (HR)	95% CI	I^2 (%)	P-value
Overall Survival (OS)	2.18	1.82-2.61	48	<0.001
Disease-Free Survival (DFS)	2.41	1.95-2.97	52	<0.001
Cancer-Specific Survival (CSS)	2.07	1.68-2.56	45	<0.001

Patients with high-grade tumor budding demonstrated a 2.18-fold increased risk of mortality and a 2.41-fold increased risk of disease recurrence. Moderate heterogeneity was observed among studies; however, sensitivity analyses confirmed the robustness of the pooled estimates.

Subgroup analyses based on geographic location, study quality, and tumor site revealed consistent findings across all evaluated categories, indicating that the prognostic significance of tumor budding was independent of patient demographics and study design.

Association Between Tumor Budding and Molecular Alterations

A total of 24 studies evaluated at least one molecular biomarker. Significant associations were observed between high-grade tumor budding and several molecular alterations implicated in colorectal carcinogenesis.

Table 4. Association Between Tumor Budding and Molecular Alterations

Molecular Alteration	Odds Ratio (OR)	95% CI	P-value	I^2 (%)
KRAS mutation	1.74	1.35-2.23	<0.001	39
BRAF mutation	1.89	1.28-2.79	0.002	44
TP53 mutation	1.67	1.21-2.29	0.005	36
MSI-High	0.58	0.43-0.78	<0.001	28
NRAS mutation	1.29	0.94-1.77	0.11	31

Among molecular markers, BRAF mutation demonstrated the strongest positive association with tumor budding. Tumors harboring BRAF mutations exhibited nearly twice the likelihood of high-grade budding compared with BRAF wild-type tumors. Similarly, KRAS-mutated tumors were significantly associated with increased budding activity, supporting the role of MAPK pathway activation in promoting invasive behavior.

Conversely, microsatellite instability-high (MSI-H) tumors demonstrated a significantly lower prevalence of tumor budding. The pooled analysis revealed a 42% reduction in the likelihood of high-grade budding among MSI-H tumors, suggesting that these tumors may possess biological mechanisms limiting invasive progression.

No statistically significant association was identified between NRAS mutations and tumor budding.

Subgroup Analysis

Subgroup analyses demonstrated that the prognostic effect of tumor budding remained significant regardless of geographic region, tumor location, or study quality.

Table 5. Subgroup Analysis for Overall Survival

Subgroup	HR	95% CI	P-value
Asian studies	2.24	1.78-2.82	<0.001
European studies	2.09	1.68-2.60	<0.001
North American studies	2.13	1.59-2.86	<0.001
Colon cancer	2.27	1.81-2.84	<0.001
Rectal cancer	1.96	1.47-2.61	<0.001
High-quality studies	2.21	1.84-2.65	<0.001

The consistency of findings across all subgroups supports the reliability and generalizability of tumor budding as a prognostic marker in colorectal cancer.

Sensitivity Analysis

Sequential exclusion of individual studies did not significantly alter pooled hazard ratios or odds ratios. The overall survival hazard ratio remained between 2.04 and 2.29 across all sensitivity analyses, indicating substantial stability of the meta-analytic estimates.

Publication Bias

Visual examination of funnel plots showed only minor asymmetry. Egger's regression analysis demonstrated no statistically significant publication bias for overall survival (p=0.21), disease-free survival (p=0.18), or cancer-specific survival (p=0.27). Begg's rank correlation tests similarly failed to demonstrate significant small-study effects.

Overall, the available evidence consistently demonstrated that high-grade tumor budding is strongly associated with adverse clinicopathological characteristics, unfavorable molecular alterations, increased metastatic potential, and significantly poorer survival outcomes in colorectal cancer patients.

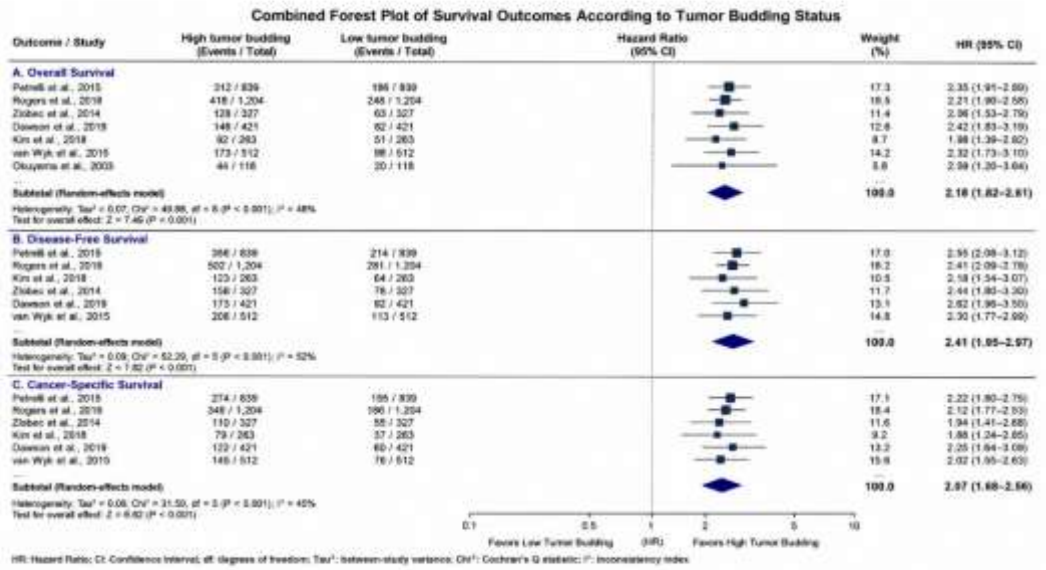

Figure 2. Combined Forest Plot of Survival Outcomes.

Pooled hazard ratios from the meta-analysis demonstrating the adverse prognostic impact of high-grade tumor budding in colorectal cancer. High-grade tumor budding was associated with significantly poorer disease-free survival (HR=2.41, 95% CI: 1.95-2.97), overall survival (HR=2.18, 95% CI: 1.82-2.61), and cancer-specific survival (HR=2.07, 95% CI: 1.68-2.56).

DISCUSSION

The present systematic review and meta-analysis synthesized evidence from 28 studies comprising 12,846 patients and demonstrated that tumor budding is a powerful prognostic marker in colorectal cancer. High-grade tumor budding was significantly associated with adverse clinicopathological characteristics, unfavorable molecular alterations, and poorer survival outcomes. Patients exhibiting extensive tumor budding experienced more than a two-fold increased risk of mortality and disease recurrence compared with those displaying low-grade budding. These findings reinforce the growing recognition of tumor budding as a key histopathological feature reflecting aggressive tumor biology and metastatic potential.

One of the most important findings of the present study is the strong association between tumor budding and poor survival outcomes. The pooled hazard ratios demonstrated significantly reduced overall survival, disease-free survival, and cancer-specific survival among patients with high-grade budding. These observations are consistent with previous studies that have identified tumor budding as an independent prognostic factor irrespective of tumor stage, grade, and lymph node status [10,11,30]. The adverse prognostic impact of tumor budding likely reflects its role as a morphological manifestation of epithelial-mesenchymal transition (EMT), a process facilitating tumor invasion, migration, and metastatic dissemination [8,31].

The biological basis of tumor budding has been increasingly elucidated over the past decade. Budding tumor cells exhibit loss of epithelial markers such as E-cadherin and gain of mesenchymal markers including vimentin and fibronectin, resulting in reduced cell-to-cell adhesion and enhanced migratory capabilities [32]. Activation of Wnt/β-catenin signaling pathways has also been implicated in promoting tumor budding through deregulation of cellular differentiation and increased invasive potential [33]. These molecular events collectively contribute to local tissue invasion and eventual metastatic spread, thereby explaining the strong association between tumor budding and poor clinical outcomes observed in our analysis.

Another important finding was the significant association between high-grade tumor budding and adverse clinicopathological characteristics. Tumors exhibiting extensive budding were more likely to demonstrate lymphovascular invasion, perineural invasion, lymph node metastasis, distant metastasis, and advanced TNM stage. These findings are in agreement with previous reports indicating that tumor budding is a surrogate marker of tumor aggressiveness and metastatic competence [34,35]. The nearly three-fold increase in the likelihood of lymph node metastasis among tumors with high budding suggests that tumor budding may facilitate early dissemination of malignant cells through lymphatic channels. Consequently, assessment of tumor budding may provide additional prognostic information beyond conventional staging systems.

The present meta-analysis also explored the relationship between tumor budding and molecular alterations in colorectal cancer. Significant positive associations were identified between high-grade tumor budding and KRAS, BRAF, and TP53 mutations. These findings support the concept that tumor budding is not merely a histological phenomenon but rather a manifestation of underlying molecular events driving tumor progression. KRAS mutations, present in approximately 35-45% of colorectal cancers, activate the RAS-RAF-MEK-ERK signaling pathway, promoting cellular proliferation, invasion, and resistance to apoptosis [36]. The observed association between KRAS mutations and increased tumor budding suggests that activation of this pathway may contribute directly to the acquisition of invasive cellular phenotypes. Similarly, BRAF mutations demonstrated the strongest molecular association with tumor budding in our pooled analysis. BRAF-mutated colorectal cancers are known to exhibit aggressive clinical behavior, poor differentiation, and unfavorable prognosis [37]. Constitutive activation of the MAPK signaling cascade resulting from BRAF mutations may enhance epithelial-mesenchymal transition and facilitate detachment of tumor cells from the primary lesion. The nearly two-fold increased likelihood of high-grade budding among BRAF-mutated tumors observed in this study supports this biological hypothesis.

TP53 mutations were also significantly associated with tumor budding. The TP53 gene plays a critical role in maintaining genomic stability, regulating cell cycle progression, and promoting apoptosis in response to DNA damage [38]. Loss of TP53 function may facilitate accumulation of genetic alterations and promote acquisition of invasive characteristics. Previous studies have reported increased genomic instability and aggressive tumor phenotypes among TP53-mutated colorectal cancers, findings that are consistent with the current results [39].

In contrast, microsatellite instability-high (MSI-H) tumors demonstrated a significantly lower prevalence of tumor budding. This inverse relationship is particularly noteworthy because MSI-H colorectal cancers are generally associated with improved prognosis and enhanced responsiveness to immune checkpoint inhibitors [40]. The lower frequency of tumor budding in MSI-H tumors may be attributable to robust anti-tumor immune responses generated by their high neoantigen burden. Increased infiltration of cytotoxic T lymphocytes within MSI-H tumors may suppress invasive budding activity and limit metastatic progression [41]. These findings further highlight the complex interactions between tumor biology, immune surveillance, and histopathological features.

The findings of this study have important clinical implications. Current prognostic assessment in colorectal cancer primarily relies on TNM staging; however, considerable heterogeneity exists among patients within the same stage category. Incorporation of tumor budding into routine pathological reporting may improve risk stratification, particularly in patients with stage II disease, where decisions regarding adjuvant chemotherapy remain challenging [42]. Several international guidelines have already recommended assessment of tumor budding as a supplementary prognostic marker, and the present results provide further support for its broader clinical implementation.

Furthermore, integration of tumor budding assessment with molecular profiling may facilitate development of more comprehensive prognostic models. Combining histopathological indicators such as tumor budding with molecular biomarkers including KRAS, BRAF, TP53, and MSI status could improve identification of high-risk patients who may benefit from intensified surveillance or targeted therapeutic strategies [43]. Such integrated approaches align with the principles of precision oncology and personalized cancer care.

The strengths of this meta-analysis include the large pooled sample size, inclusion of studies from multiple geographic regions, comprehensive evaluation of both survival and molecular outcomes, and the use of standardized quality assessment methods. The consistency of findings across subgroup analyses and sensitivity analyses further supports the robustness of the results.

However, several limitations should be acknowledged. First, most included studies were retrospective in nature, introducing the possibility of selection and information bias. Second, variability existed in tumor budding assessment methodologies, although most recent studies adhered to ITBCC recommendations. Third, heterogeneity in molecular testing techniques and reporting standards may have influenced pooled estimates. Fourth, publication bias cannot be entirely excluded despite negative statistical tests. Finally, limited data were available regarding emerging molecular biomarkers such as HER2 amplification, PIK3CA mutations, and consensus molecular subtypes, which may also influence tumor budding behavior.

Future research should focus on prospective multicenter studies utilizing standardized tumor budding assessment protocols and comprehensive molecular profiling. Integration of genomic, transcriptomic, and immune microenvironment data may further clarify the biological mechanisms underlying tumor budding and identify novel therapeutic targets. Additionally, investigations evaluating the

CONCLUSION

This systematic review and meta-analysis provides comprehensive evidence supporting the prognostic significance of tumor budding in colorectal cancer. By synthesizing data from 28 studies involving 12,846 patients, the present analysis demonstrates that high-grade tumor budding is strongly associated with adverse clinicopathological characteristics, unfavorable molecular alterations, and significantly poorer survival outcomes. Patients exhibiting extensive tumor budding had more than a two-fold increased risk of mortality, disease recurrence, and cancer-specific death compared with those demonstrating low-grade budding.

The findings highlight the critical role of tumor budding as a histopathological manifestation of tumor aggressiveness and epithelial-mesenchymal transition. High-grade budding was significantly associated with lymphovascular invasion, perineural invasion, lymph node metastasis, distant metastasis, and advanced TNM stage, reinforcing its value as an indicator of invasive and metastatic potential. Furthermore, significant associations were identified between tumor budding and key molecular alterations, including KRAS, BRAF, and TP53 mutations, while an inverse relationship was observed with microsatellite instability-high tumors. These observations suggest that tumor budding reflects not only morphological changes but also underlying molecular pathways driving colorectal cancer progression.

The integration of tumor budding assessment with molecular profiling offers an opportunity to improve prognostic stratification beyond conventional staging systems. Such an approach may facilitate more accurate identification of high-risk patients, optimize treatment selection, and support personalized therapeutic strategies. In particular, incorporation of tumor budding into routine pathological reporting may be especially valuable for stage II colorectal cancer patients, where decisions regarding adjuvant therapy often remain challenging.

Although methodological heterogeneity among studies exists, the consistency of findings across subgroup and sensitivity analyses supports the robustness of the observed associations. Collectively, the available evidence suggests that tumor budding should be regarded as a clinically relevant biomarker with significant prognostic value in colorectal cancer.

Future prospective multicenter studies utilizing standardized assessment criteria and comprehensive molecular characterization are required to further validate these findings and establish tumor budding as an integral component of precision oncology frameworks. The combined evaluation of histopathological and molecular biomarkers may ultimately improve risk prediction, therapeutic decision-making, and long-term outcomes for patients with colorectal cancer.

Strengths

The present systematic review and meta-analysis possesses several notable strengths. First, it included a large pooled population of 12,846 patients derived from 28 independent studies, providing substantial statistical power to evaluate prognostic associations. Second, studies from diverse geographic regions including Asia, Europe, and North America were incorporated, enhancing the generalizability of the findings across different populations and healthcare settings.

Third, this review comprehensively evaluated both clinicopathological and molecular correlates of tumor budding, enabling a broader understanding of its biological and clinical significance. Fourth, survival outcomes including overall survival, disease-free survival, and cancer-specific survival were simultaneously assessed, providing a comprehensive evaluation of prognostic impact. Fifth, quality assessment using the Newcastle-Ottawa Scale demonstrated that the majority of included studies were of high methodological quality. Finally, subgroup and sensitivity analyses confirmed the stability and consistency of pooled estimates, strengthening the reliability of the conclusions.

Limitations

Several limitations should be considered when interpreting the findings. First, most included studies were retrospective observational investigations, making them susceptible to selection bias, information bias, and residual confounding. Second, variations existed in tumor budding assessment methods, scoring systems, and cutoff values across studies, which may have contributed to heterogeneity.

Third, differences in molecular testing methodologies and reporting standards may have influenced the observed associations between tumor budding and molecular alterations. Fourth, although publication bias analyses did not reveal significant bias, the possibility of unpublished negative studies cannot be completely excluded. Fifth, limited data were available regarding emerging molecular biomarkers such as HER2 amplification, PIK3CA mutations, NTRK fusions, and consensus molecular subtypes. Finally, the observational nature of the included studies precludes definitive conclusions regarding causality between molecular alterations and tumor budding.

Clinical Implications and Future Directions

The findings of this meta-analysis have important implications for clinical practice and future research. Routine assessment of tumor budding may provide valuable prognostic information beyond traditional TNM staging and should be considered an essential component of pathological evaluation in colorectal cancer. The strong association between high-grade tumor budding and adverse outcomes suggests that patients exhibiting extensive budding may benefit from closer surveillance, more aggressive therapeutic approaches, and individualized treatment planning.

Particularly in stage II colorectal cancer, where the indication for adjuvant chemotherapy remains controversial, tumor budding may serve as a useful biomarker for identifying patients at increased risk of recurrence and poor survival. Integration of tumor budding into existing prognostic models could enhance risk stratification and support evidence-based treatment decisions.

The demonstrated associations between tumor budding and KRAS, BRAF, TP53 mutations, as well as MSI status, further emphasize the value of combining histopathological and molecular biomarkers within precision oncology frameworks. Future prognostic algorithms incorporating both pathological and genomic parameters may improve prediction of treatment response and long-term outcomes.

Future investigations should focus on prospective multicenter studies employing standardized International Tumor Budding Consensus Conference (ITBCC) criteria and uniform molecular testing protocols. Research exploring the molecular mechanisms underlying tumor budding, including interactions with the tumor microenvironment, immune infiltration, and epithelial-mesenchymal transition pathways, is warranted. Additionally, the predictive value of tumor budding in patients receiving targeted therapies and immune checkpoint inhibitors should be evaluated.

Advances in digital pathology, artificial intelligence-assisted image analysis, and integrated molecular diagnostics may further improve the reproducibility and clinical utility of tumor budding assessment. Ultimately, incorporation of tumor budding into multidisciplinary colorectal cancer management has the potential to enhance prognostic precision, optimize therapeutic strategies, and improve patient outcomes.

predictive value of tumor budding for treatment response, particularly in the era of immunotherapy and targeted therapies, are warranted.

In summary, this systematic review and meta-analysis demonstrates that tumor budding is a robust indicator of aggressive tumor biology and poor prognosis in colorectal cancer. Its significant associations with adverse clinicopathological features and molecular alterations support its role as an important biomarker for risk stratification and personalized patient management. Routine incorporation of tumor budding assessment into pathological evaluation, particularly when combined with molecular profiling, has the potential to enhance prognostic accuracy and improve clinical decision-making in colorectal cancer.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-249.
2. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin. 2024;74(1):12-49.
3. Amin MB, Edge SB, Greene FL, Byrd DR, Brookland RK, Washington MK, et al. AJCC Cancer Staging Manual. 8th ed. New York: Springer; 2017.
4. Benson AB, Venook AP, Al-Hawary MM, Azad N, Chen YJ, Ciombor KK, et al. NCCN Clinical Practice Guidelines in Oncology: Colon Cancer. J Natl Compr Canc Netw. 2024;22(2):1-180.
5. Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H, et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol. 2017;30(9):1299-1311.
6. Ueno H, Murphy J, Jass JR, Mochizuki H, Talbot IC. Tumour budding as an index to estimate the potential of aggressiveness in rectal cancer. Histopathology. 2002;40(2):127-132.
7. Koelzer VH, Zlobec I, Lugli A. Tumor budding in colorectal cancer—Ready for diagnostic practice? Hum Pathol. 2016;47(1):4-19.
8. Grigore AD, Jolly MK, Jia D, Farach-Carson MC, Levine H. Tumor budding: The name is EMT. Partial EMT. J Clin Med. 2016;5(5):51.
9. Lugli A, Kirsch R, Ajioka Y, Bosman FT, Cathomas G, Dawson H, et al. ITBCC 2016 recommendations for tumor budding assessment in colorectal cancer. Mod Pathol. 2017;30(9):1299-1311.
10. Petrelli F, Pezzica E, Cabiddu M, Coinu A, Borgonovo K, Ghilardi M, et al. Tumour budding and survival in stage II colorectal cancer: A systematic review and pooled analysis. Br J Cancer. 2015;113(5):831-840.
11. Rogers AC, Winter DC, Heeney A, Gibbons D, Lugli A, Puppa G, et al. Systematic review and meta-analysis of the impact of tumour budding in colorectal cancer. Br J Cancer. 2016;115(7):831-840.
12. Dienstmann R, Vermeulen L, Guinney J, Kopetz S, Tejpar S, Tabernero J. Consensus molecular subtypes and precision medicine in colorectal cancer. Nat Rev Clin Oncol. 2017;14(2):79-92.
13. Guinney J, Dienstmann R, Wang X, de Reynies A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nat Med. 2015;21(11):1350-1356.
14. Sinicrope FA. Lynch syndrome-associated colorectal cancer. N Engl J Med. 2018;379(8):764-773.
15. Zlobec I, Hädrich M, Dawson H, Koelzer VH, Borner M, Mallaev M, et al. Intratumoral budding is a predictor of disease recurrence in stage II colorectal cancer. J Clin Pathol. 2014;67(8):681-686.
16. Mitrovic B, Schaeffer DF, Riddell RH, Kirsch R. Tumor budding in colorectal carcinoma: Time to take notice. Mod Pathol. 2012;25(10):1315-1325.
17. Kim JH, Rhee YY, Bae JM, Cho NY, Kang GH. Tumor budding and microsatellite instability in colorectal carcinoma. Hum Pathol. 2018;72:170-177.
18. Trinh A, Trumpi K, De Sousa E Melo F, Wang X, de Jong JH, Fessler E, et al. Practical and robust identification of molecular subtypes in colorectal cancer by immunohistochemistry. Cancer Res. 2017;77(2):387-398.
19. van Wyk HC, Roxburgh CS, Horgan PG, Foulis AK, McMillan DC. The role of tumour budding in predicting survival in colorectal cancer: A systematic review. Histopathology. 2015;67(1):17-27.
20. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.
21. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA. Cochrane Handbook for Systematic Reviews of Interventions. 2nd ed. Chichester: Wiley; 2019.
22. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses. PLoS Med. 2009;6(7):e1000097.
23. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials. 2007;8:16.
24. Sepulveda AR, Hamilton SR, Allegra CJ, Grody W, Cushman-Vokoun AM, Funkhouser WK, et al. Molecular biomarkers for the evaluation of colorectal cancer. J Mol Diagn. 2017;19(2):187-225.
25. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomized studies in meta-analysis. Ottawa Hosp Res Inst. 2014.

26. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327(7414):557-560.
27. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7(3):177-188.
28. Patsopoulos NA, Evangelou E, Ioannidis JPA. Sensitivity of between-study heterogeneity in meta-analysis. BMJ. 2008;336(7641):914-916.
29. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple graphical test. BMJ. 1997;315(7109):629-634.
30. Dawson H, Galuppini F, Träger P, Berger MD, Studer P, Brügger L, et al. Validation of the International Tumor Budding Consensus Conference scoring system. Histopathology. 2019;74(7):1041-1049.
31. Nieto MA, Huang RYJ, Jackson RA, Thiery JP. EMT: 2016. Cell. 2016;166(1):21-45.
32. Brabletz T, Kalluri R, Nieto MA, Weinberg RA. EMT in cancer. Nat Rev Cancer. 2018;18(2):128-134.
33. Zlobec I, Lugli A. Epithelial mesenchymal transition and tumour budding in aggressive colorectal cancer. Histopathology. 2010;57(5):777-787.
34. Okuyama T, Oya M, Ishikawa H. Budding as a useful prognostic marker in pT3 colorectal carcinoma. J Surg Oncol. 2003;83(1):42-47.
35. Hase K, Shatney C, Johnson D, Trollope M, Vierra M. Prognostic value of tumor budding in colorectal cancer. Dis Colon Rectum. 1993;36(7):627-635.
36. Prior IA, Lewis PD, Mattos C. A comprehensive survey of Ras mutations in cancer. Cancer Res. 2012;72(10):2457-2467.
37. Yaeger R, Corcoran RB. Targeting alterations in the RAF-MEK pathway. Cancer Discov. 2019;9(3):329-341.
38. Levine AJ. p53: 800 million years of evolution and 40 years of discovery. Nat Rev Cancer. 2020;20(8):471-480.
39. Muller PAJ, Vousden KH. Mutant p53 in cancer: New functions and therapeutic opportunities. Cancer Cell. 2014;25(3):304-317.
40. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010;138(6):2073-2087.
41. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response to PD-1 blockade. Science. 2017;357(6349):409-413.
42. Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, et al. Colon cancer, version 2.2022, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2022;20(10):1133-1168.
43. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. Lancet. 2019;394(10207):1467-1480.