The Oncologist, 2025, **30**, oyaf027
https://doi.org/10.1093/oncolo/oyaf027
Advance access publication 27 March 2025
Original Article

Analysis of tumor budding as a prognostic factor for recurrence in patients with stage II and III colon cancer. Experience in a tertiary hospital

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Abstract

Background: Stage II and III colorectal cancer (CRC) poses a significant challenge due to rising global incidence and mortality rates. Despite advancements in screening and treatment, there's a pressing need for reliable prognostic biomarkers. Tumor budding emerges as a promising marker associated with poor prognosis and higher recurrence. However, its incorporation into clinical guidelines differs when considering adjuvant treatment. This study assesses tumor budding's prognostic value for recurrence in stage II and III CRC, exploring its implications for risk stratification.

Methods: This retrospective study encompassed patients with stage II-III CRC at Hospital Universitario La Paz from October 2016 to January 2022. Tumor budding was assessed according to the 2016 ITBCC guidelines and categorized as low, intermediate, or high. The primary outcomes, time to recurrence (TTR) and overall survival (OS), were analyzed using Kaplan-Meier and Cox regression models.

Results: A total of 390 patients were included in the final analysis. They were predominantly male (55%) with an average age of 75 years (range 35-95). Fifty percent of patients were stage II. Tumor budding was categorized as low, intermediate, and high in 186 (48%), 110 (28%), and 94 (24%) patients, respectively. After a median follow-up of 46.1 months, there were 71 recurrences and 96 deaths. Time to recurrence (TTR) was significantly shorter for patients with high tumor budding. At 24 months, freedom from recurrence was 92%, 84%, and 69% for low, intermediate, and high tumor budding groups, respectively. Median TTR was not reached in any group. Multivariate analysis revealed high-grade budding as an independent predictor of recurrence with a hazard ratio (HR) of 2.39 ($P = .01$; 95% CI, [1.42-4.04]).

Conclusion: Our study highlights the prognostic value of tumor budding in predicting recurrence in both stage II and III colorectal cancer patients, reinforcing its potential as an important biomarker beyond stage II CRC.

Key words: colorectal cancer; tumor budding; prognosis; adjuvant therapy; recurrence.

Implication for practice

Our research underscores the pivotal role of high-grade tumor budding as a critical prognostic factor in guiding adjuvant treatment decisions for colorectal cancer patients. By identifying high-grade tumor budding, clinicians can more accurately stratify patients and optimize adjuvant treatment selection, ultimately improving patient outcomes.

Received: 9 April 2024; Accepted: 24 December 2024.

Introduction

The management of Stage II and III CRC continues to present a significant clinical challenge.[1,2] Despite an increase in the proportion of patients diagnosed at early stages, attributable to enhanced population screening initiatives, the mortality rate is escalating due to a rising incidence globally. This situation, juxtaposed with an aging population, where the implications of toxicity cannot be underestimated, underscores the necessity for improvements in adjuvant strategies.

Established CRC prognostic parameters for Stage II involve both major factors, such as lymph node sampling (<12) and pT4 stage, as well as minor factors including high-grade tumors, vascular, lymphatic, and perineural invasions, tumor obstruction, and elevated preoperative CEA levels per the 2020 ESMO guidelines.[3] However, better biomarkers are needed to guide risk assessment and therapy stratification. In this context, tumor budding has emerged as a promising pathological biomarker in CRC and represents the only neoplasm where there is a consensus between pathologist to guide the standardization of tumor budding assessment and reporting, the 2016 International Tumour Budding Consensus Conference (ITBCC).[4]

Tumor budding is defined as single cells or cell clusters of up to 4 or 5cells at the invasive margin of colorectal cancer.[4-6] It represents the pathological footprint of partial epithelial-mesenchymal transition (EMT)[7] and thus is related to worst prognosis.[7,8] There is evidence of a strong relationship between high levels of tumor budding in CRC and the overall rate of local or metastatic recurrence,.[9-11] It has also been reported that high tumor budding is associated with worse 5-year overall survival OS.[8,12]

However, despite all this evidence, budding has been included as a prognostic marker in some, but not all, clinical practice guidelines for localized colon cancer.[3] Thus, we designed this study with the aim of evaluating the prognostic value of tumor budding for recurrence in patients with stage II and III CRC.

Methodology

In this retrospective observational study, we selected patients treated at Hospital Universitario La Paz between October 2016 and January 2022, who had their tumor budding grade systematically assessed according to the 2016 ITBCC in the pathology report.[4] Inclusion criteria included patients aged >18 years with a pathologically confirmed diagnosis of stage II-III colorectal adenocarcinoma who underwent resection with curative intent.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Hospital Universitario La Paz (PI-3607).

We used formalin-fixed paraffin-embedded (FFPE) tumor samples stained with H&E. To assess the budding grade, the cancer slides were scanned at ×10 objective and the hotspot field was chosen. In this field, buds, as previously defined, were counted at ×20 objective. Then, tumor budding was scored as 1-low (<5 buds), 2-intermediate (5-9 buds), and 3-high (≥10 buds) respectively as described in the ITBCC [4] (Figure 1).

Clinical-pathological variables were obtained from the patients' health records and the corresponding pathology report. The variables that we included are listed in Table 1. Adjuvant treatment was decided by assessing the functional status of the patient and grading the overall risk of recurrence based on clinical guidelines at the time.

Outcome variables were TTR and OS. TTR was calculated from the date of the surgery until the date of tumor recurrence or last follow-up. OS was defined as the time between the date of diagnosis and the date of death or last follow-up. SPSS statistical software, Version 24 (SPSS Inc. Chicago, Illinois, USA) was used. The $\chi 2$ test and t test for unpaired data were applied to compare frequencies and means, respectively. The interaction among clinicopathologic parameters was first analyzed using univariate logistic regression. Survival curves were estimated using the Kaplan-Meier method, and the log-rank test was used for the difference assessment. A multivariate Cox proportional hazard model was used to identify independent prognostic factors for survival.

Results

Population characteristics

A total of 390 patients were included in this study. Baseline characteristics are depicted in Table 1. The mean age of the patients was 72 years, with a range of 35-95 years. There were 213 (55%) male patients and tumors were distributed 51% vs 49% in the right and left colon, respectively. Fifty percent of the patients were classified as stage II and 50% as stage III. Deficient mismatch repair (dMMR) was observed in 14% of the tumors. 51% of the patients received adjuvant chemotherapy. Specifically, 74% of stage II tumors did not receive adjuvant therapy, while 75% of stage III tumors did. Regarding TB, 186 (48%) had low-grade budding, 110 (28%) had intermediate-grade budding, and 94 (24%) had high-grade budding.

Recurrence and survival analysis

During a median follow-up period of 46.1 months a total of 71 events of recurrence and 96 deaths were observed. At 24 months, 69% of patients with high-grade TB, 84% with intermediate-grade, and 92% with low-grade were free from recurrence. At 48 months, 59% of patients with high-grade tumor budding were free from recurrence. Table 2. Median time to recurrence was not reached in either of the budding groups (Figure 2).

A multivariate Cox proportional hazards analysis was performed to determine the predictors of global recurrence in the study population. The results revealed that high-grade budding was the sole independent predictor of recurrence, with a hazard ratio (HR) of 3.66 (95% confidence interval [CI]: 2.3-583, $P < 0.005$), when compared to patients with low-grade budding. Intermediate-grade budding was not associated with an increased risk of recurrence (HR = 0.91, 95% CI, 0.54-1.57, $P = 0.76$). Furthermore, T4 status, N2 status, obstruction, perforation, lymphovascular infiltration (LVI) and perineural infiltration (PNI) presence were identified as significant predictors of recurrence. However, these variables were not statistically significant in the multivariate analysis. Table 3.

A further multivariate analysis stratified by stage was performed. For stage II, T4 status (HR = 3.14, $P = .008$) and high-grade budding (HR = 2.53, $P = .04$) were significant predictors of recurrence. For stage III, also high-grade budding (HR = 2.28, $P = .01$) and T4 status (HR = 2.55, $P = .02$) were significant in the multivariate analysis.

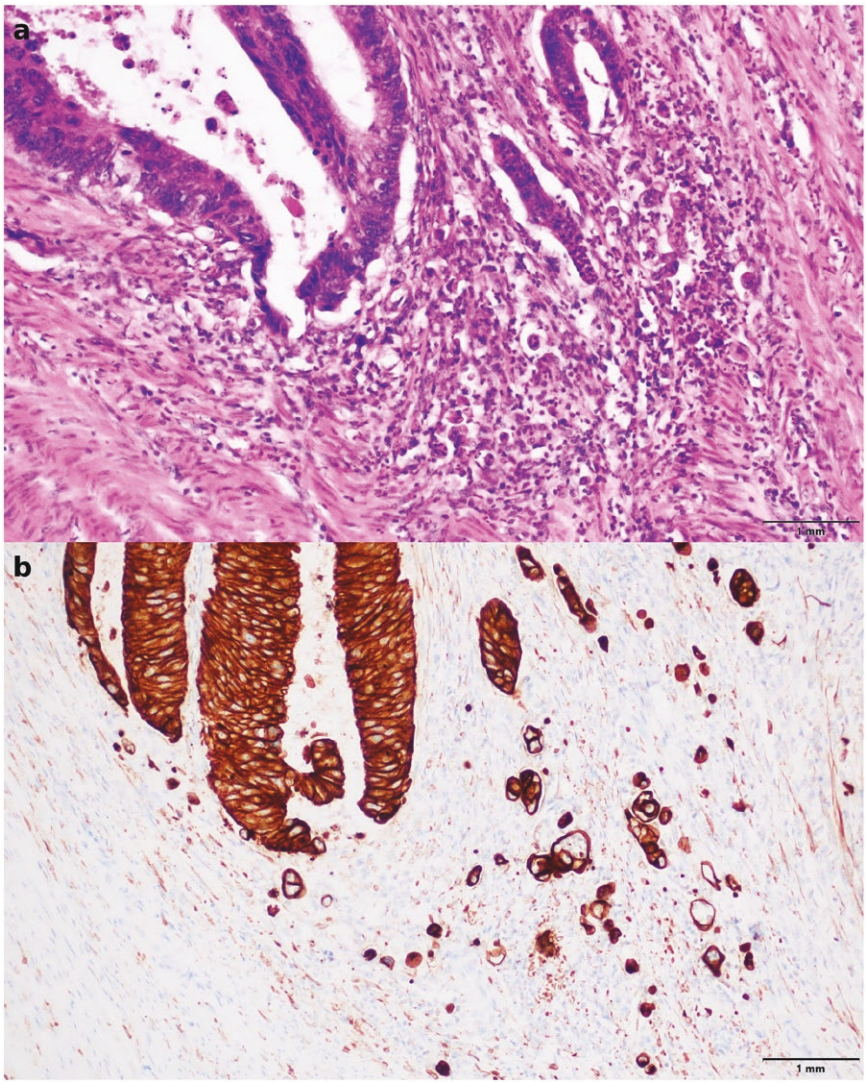

Figure 1. (A) Tumor budding (single cells or clusters of as many as 4 tumor cells at the invasive margin of the tumor). Original magnification, ×200. (B) Tumor budding, immunostaining for CKAE1/AE3. Original magnification, ×200.

Age was the only parameter that had a significant association with OS, with a hazard ratio of 1.08 (95% CI, 1.06-1.11, $P < .005$). The T4 stage, obstruction, grade 3 differentiation, and high-grade budding were also explored for their association with a higher hazard of death. The HR for T4 stage, obstruction, grade 3 differentiation, and high-grade tumor budding were 1.63 (95% CI, 0.45-5.84, $P = .46$), 1.61 (95% CI, 0.94-2.78, $P = .09$), 1.69 (95% CI, 0.93-3.09, $P = .09$), and 1.34 (95% CI, 0.84-2.15, $P = .22$), respectively. Despite these associations, none of these variables were statistically significant in the analysis. The number of lymph nodes removed was not found to be associated with OS.

Survival according to TNM stage at diagnosis

At 24 months, among stage III patients, 70% with high-grade budding, 80% with intermediate-grade, and 92% with low-grade were free from recurrence. Likewise, for stage II patients, 68% with high-risk budding, 88% with intermediate-risk, and 91% with low-risk were recurrence-free. Survival at others time intervals is shown in Table 2. Median time to recurrence was not reached in either of the budding groups (Figure 3 and 4).

The univariate HR results showed that high-grade budding was significantly associated with a higher risk of tumor global recurrence in both stage II 4.65 (95% CI, 1.96-9.44, $P < .005$) and stage III 3.0 (95% CI, 1.68-5.38, $P < .005$) patients. Intermediate-grade budding was not associated with an increased risk of tumor recurrence in either stage II patients, with an HR of 0.77 (95% CI, 0.29-2.06, $P < 0.005$), or stage III patients, with an HR of 0.92 (95% CI, 0.49-1.77, $P = 1.23$). Complete multivariate analysis is shown in Table 3.

Discussion

Despite being listed as a biomarker to consider for patients when calculating risk of recurrence in several international guidelines,[13,14] high-grade tumor budding is not yet included as a risk marker in ESMO guidelines.[3] In many cases, it is still not considered when stratifying populations in high-impact clinical trials, such as the DYNAMIC trial,[15] which evaluated the utility of ctDNA in guiding adjuvant treatment in stage II CRC.

In this study, we reaffirm the capability of tumoral budding to predict tumor relapse independently of tumor stage. The

Table 1. Baseline patient characteristics.

Patient count	390	100%
Age		
Mean	72	
Range	35-95	
<= 70	153	39
> 70	237	61
Sex		
Male	213	55
Female	177	45
Location		
Right	197	51
Left	193	49
T status		
T1	2	1
T2	15	4
T3	209	53
T4	163	42
Grade		
Grade 1	13	3
Grade 2	334	88
Grade 3	32	8
Harvested lymph ndes		
>= 12	342	89
<= 12	44	11
N status		
N0	198	51
N1	141	36
N2	51	13
Obstruction		
No	346	89
Yes	44	11
Perforation		
No	358	92
Yes	32	8
Linfovascular invasion		
No	206	53
Yes	184	47
Perineural invasion		
No	303	78
Yes	87	22
Adyuvant chemotherapy		
Yes	197	51
No	192	49
Mismatch repair status		
Proficient	316	81
Deficient	55	14
Unkown	19	5

Table 2. Combined recurrence-free survival (RFS) rates by budding grade and clinical stage at 24, 36, and 48 months

	24 Months (%)	36 Months (%)	48 Months (%)
Global			
High Grade	69	63	59
Intermediate	84	80	80
Low Grade	92	92	90
Stage II			
High Grade	68	68	68
Intermediate	88	88	88
Low Grade	93	93	91
Stage III			
High Grade	70	60	52
Intermediate	80	74	74
Low Grade	92	90	88

TTR in patients with high-grade budding tumors is surprising, as there are only slight differences (68% vs 66%) between patients with stage III and II, respectively.

This finding confirms its potential as a biomarker that goes beyond the anatomical nature of the TNM classification and highlighting the relevance of the tumor biology. In this case high-grade tumor budding represents a biological distinct cohort of patients as is one of the hallmarks of the EMT.[5,7] To the best of our knowledge, this is the only study in the literature that analyzes the recurrence rate differentially based on tumor budding in a mixed population of stage II and III patients.

We found a global RFS of 87% in patients with stage II, similar data were obtained in the BT subgroup analysis of the SACURA trial,[16] where the RFS was 88% in patients with stage II at 24 months. RFS in the stage III population was also in line with the IDEA phase III trial post-hoc analysis for tumor budding.[17]

Prior to the introduction of the 2016 consensus, numerous studies were conducted analyzing tumor budding as a prognostic biomarker in patients with stages I-III CRC. A study conducted at St Mark's Hospital[9] demonstrated the predictive capacity of this marker; however, tumor budding pathological analysis was not standardized at that time and its results cannot be generalized to the current time. Since 2016, standardization in reporting budding has resulted in multiple studies analyzing it. In most of these studies,[16,18-20] budding was the only or one of the only biomarkers predicting recurrence in multivariate analysis, with hazard ratios ranging from 2.6 to 5.55 for patients with stage II disease.

In the stage III population, the prognostic capabilities of tumor budding diminish, as seen in the post-hoc analysis of the IDEA trial,[17] where the hazard ratio is 1.41. This contrasts with our research, where we found that the hazard ratio remains consistent for both stages II and III, with values of 2.53 and 2.28, respectively.

Despite the evidence provided by our study and analogous research, suggesting a higher recurrence risk in patients with high-grade budding, the quantifiable benefit of adjuvant treatment for this group remains indeterminate. As we know, budding is a histopathological marker of tumor biology and more specifically of EMT. We know that EMT is associated with the formation of cancer stem cells and resistance to therapy,[21-23] so a possible benefit to adjuvant therapy is not clear in this context.

As previously mentioned, a retrospective analysis of the SACURA trial conducted by Ueno et al.[16] is to date the most

powerful study in analyzing the risk of recurrence regarding tumor budding in CRC. A total of 991 patients were included. Its strength lies in being a retrospective analysis of a randomized clinical trial in the adjuvant setting with oral tegafur-uracil vs. surgery alone. This work allows us to analyze the ability of adjuvant therapy to prevent recurrence in those patients with high-grade budding by administering adjuvant chemotherapy. Both the intermediate and high-grade groups showed an improvement in the 5-year recurrence rate in the adjuvant chemotherapy group compared to the surgery-alone group by approximately 5%, but the difference was statistically nonsignificant.

Mitrovic et al. conducted a retrospective analysis of patients from the QUASAR trial,[24] a comparable study conducted before the 2016 consensus. They examined a total of 1575 patients with high tumor budding and observed a trend towards increasing chemotherapy efficacy with increasing bud counts. However, the association did not reach statistical significance.

Recent advances in our understanding of cancer molecular biology, particularly the EMT in CRC, may pave the way for the development of more efficacious treatments, especially in the adjuvant setting. Among the promising therapies to inhibit EMT are galunisertib[25] and resveratrol,[26] which have demonstrated in vitro capabilities to inhibit the TGF-β type I receptor kinase. This inhibition can consequently downregulate the phosphorylation of Smad2, effectively blocking the canonical TGF-β pathway.[26-29]

With an increasing number of emerging molecules in development, there is a greater need than ever for a rapid, simple, and standardized marker to serve as a surrogate for the complex EMT mechanism[30,31]. This is where our study contributes to the existing evidence by expanding the prognostic power of tumor budding across the stage II/III population.

However, it is essential to acknowledge the limitations of our research. Our investigation is limited by its retrospective nature, which opens the door to various biases that may have influenced the results. We did not incorporate the evaluation of recurrence based on adjuvant therapy due to the presence of a mixed population of stages II/III. This led to a significant correlation between age and the administration of adjuvant therapy, which might indicate a potential bias. It is likely that adjuvant therapy was preferentially provided to patients with improved performance status and functional capacity. The fact that BT was not a universal and established factor for the administration of adjuvant chemotherapy during the study period could also overestimate its prognostic role compared to the classic poor prognostic factors.

Although we consider it one of the strengths of our study, having a mixed cohort of stage II/III patients can, depending on the context, be seen as a limitation as it does not represent the current use of tumor budding in international guidelines. At the moment, there are no specific recommendations for using tumor budding as a prognostic marker in patients with stage III disease.

We acknowledge the relatively short follow-up compared to the median follow-up of 69.7 months reported by Ueno et al. (2019) in the SACURA trial.[16] Despite this discrepancy, it is crucial to underline that the robust number of events captured ensures ample statistical power to discern meaningful differences and to establish reliable correlations.

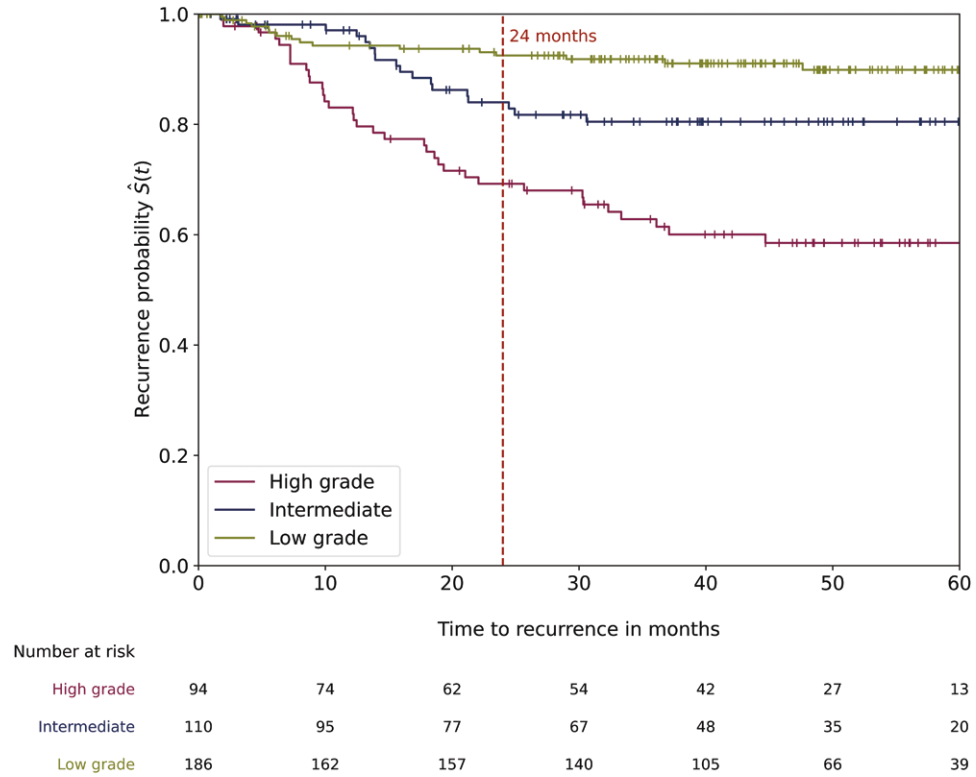

Figure 2. Kaplan-Meier plot illustrating the relationship between tumor budding grade and recurrence probability across all stages.

Table 3. Univariate and multivariate analysis of multiple risk factors for TTR both in stage II, stage III colorectal cancer and the combination of both.

Risk factor	HR univariant	IC univariant	HR multivariant	IC multivariant
Stage II and III combined				
Age	1.03 (*P* = .01)	[1.01, 1.05]	1.02 (*P* = .04)	[1.00, 1.05]
Lymph nodes	1.19 (*P* < .005)	[1.12, 1.27]	1.10 (*P* = .14)	[.97, 1.25]
LVI	2.89 (*P* < .005)	[1.74, 4.78]	1.30 (*P* = .40)	[.71, 2.37]
PNI	2.11 (*P* < .005)	[1.28, 3.46]	.94 (*P* = .83)	[.54, 1.65]
T3	.25 (*P* < .005)	[.15, .43]	1.46 (*P* = .71)	[.19, 11.05]
T4	4.67 (*P* < .005)	[2.78, 7.85]	3.63 (*P* = .21)	[.48, 27.40]
N2	3.53 (*P* < .005)	[2.12, 5.88]	1.39 (*P* = .46)	[.58, 3.31]
High grade tumor budding	3.66 (*P* < .005)	[2.30, 5.83]	2.39 (*P* < .005)	[1.42, 4.04]
Obstruction	2.94 (*P* < .005)	[1.69, 5.14]	1.29 (*P* = .42)	[.70, 2.40]
Perforation	2.10 (*P* = .04)	[1.04, 4.23]	1.20 (*P* = .63)	[.56, 2.57]
Stage II				
LVI	2.38 (*P* = .03)	[1.10, 5.16]	1.72 (*P* = .19)	[.77, 3.84]
T4	4.42 (*P* < .005)	[2.12, 9.20]	3.14 (*P* = .008)	[1.35, 7.29]
High gradetumor budding	4.30 (*P* < .005)	[2.07, 8.94]	2.53 (*P* = .04)	[1.05, 6.07]
Obstruction	3.45 (*P* = .01)	[1.28, 9.31]	1.46 (*P* = .48)	[.52, 4.10]
Stage III				
Age	1.03 (*P* = .05)	[1.00, 1.06]	1.02 (*P* = .09)	[.99, 1.06]
Lymph nodes	1.18 (*P* < .005)	[1.09, 1.28]	1.12 (*P* = .09)	[.98, 1.28]
LVI	2.79 (*P* = .004)	[1.38, 5.64]	1.07 (*P* = .88)	[.43, 2.65]
T4	4.27 (*P* < .005)	[2.12, 8.61]	2.55 (*P* = .02)	[1.15, 5.64]
N2	3.03 (*P* < .005)	[1.68, 5.46]	1.43 (*P* = .43)	[.58, 3.54]
Grade 3	2.27 (*P* = .04)	[1.04, 4.95]	1.58 (*P* = .27)	[.71, 3.52]
High grade tumor budding	3.01 (*P* < .005)	[1.69, 5.37]	2.28 (*P* = .01)	[1.21, 4.29]
Obstruction	2.33 (*P* = .01)	[1.18, 4.61]	1.31 (*P* = .48)	[.61, 2.83]

Univariate analysis has been performed. Only statistically significant values were used for multivariate analysis. Abbreviations: LVI, linfovascular invasion; N2, nodal stage N2; PNI, perineural infiltration; T3, tumor stage T3; T4, tumor stage T4; TTR, time to recurrence . Shaded rows indicate statistical significance.

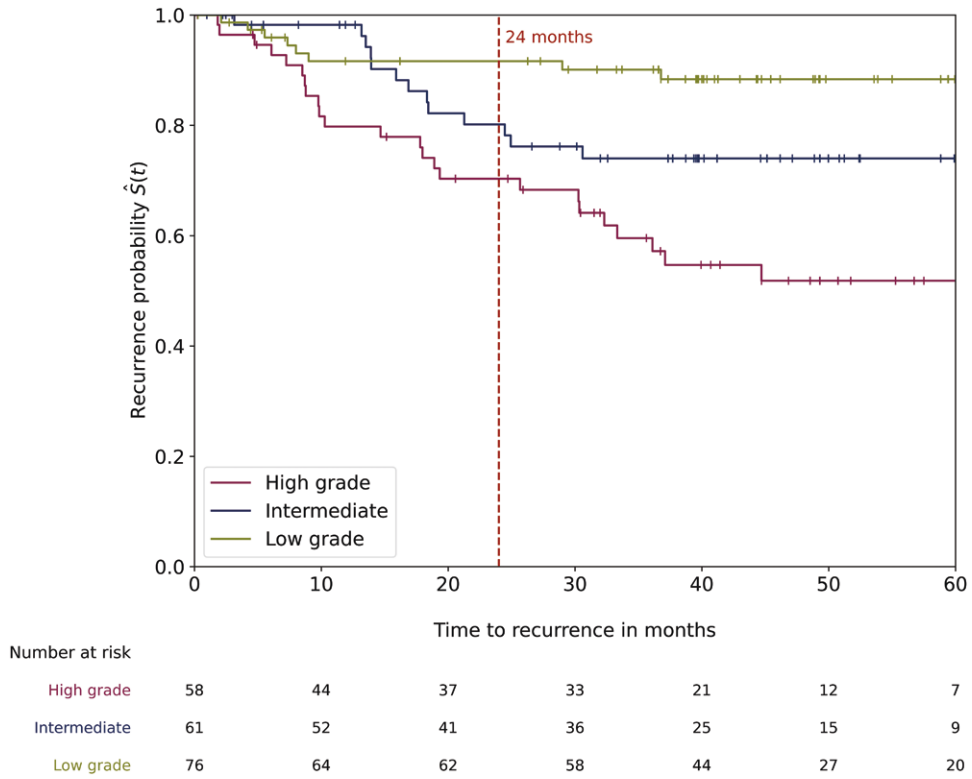

Figure 3. Kaplan-Meier plot illustrating the relationship between tumor budding grade and recurrence probability across in stage II patient.

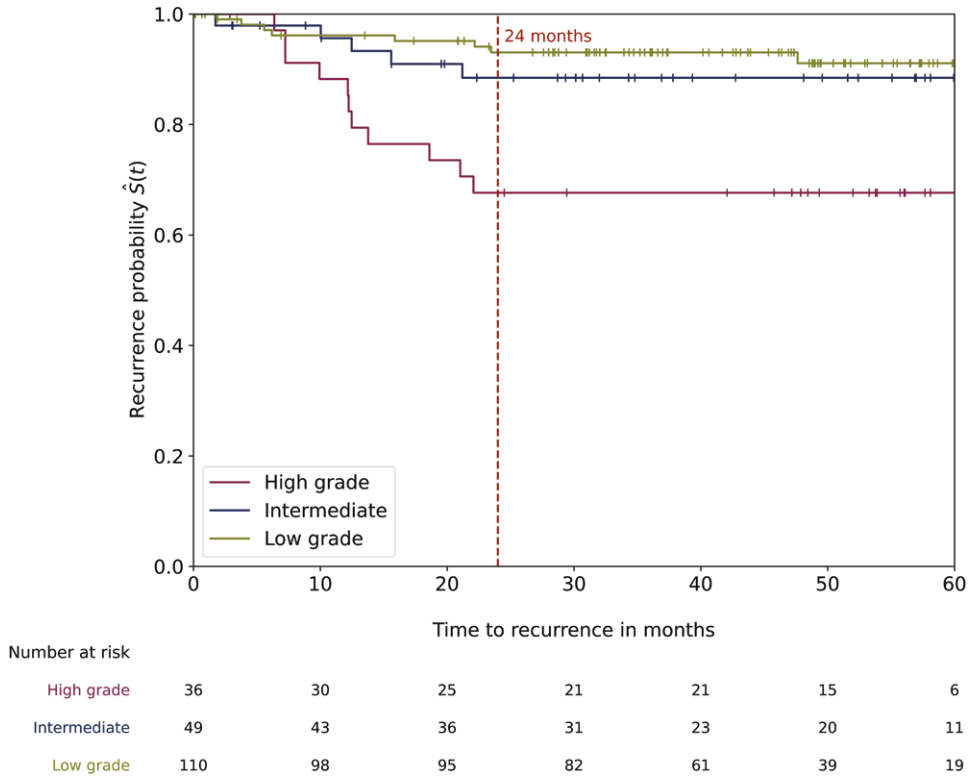

Figure 4. Kaplan-Meier plot illustrating the relationship between tumor budding grade and recurrence probability in stage III patients.

Conclusion

Our study highlights the prognostic value of tumor budding in predicting recurrence in both stage II and III colorectal cancer patients, reinforcing its potential as an important biomarker beyond stage II CRC. Despite the limitations of our retrospective investigation, the findings contribute to the growing body of evidence on the role of tumor budding and the underlying biological mechanisms, such as EMT, in colorectal cancer. The need for a rapid, simple, and standardized marker as a surrogate for the complex EMT mechanism is more pressing than ever, given the emergence of novel therapeutic molecules targeting this process.

The optimal use of adjuvant therapy in patients with high-grade budding remains unclear, as EMT is also associated with therapy resistance and cancer stem cell formation.

Acknowledgments

We thank all individuals and institutions who supported this research and contributed to its development.

Author contributions

ARL: Conception/Design, Collection and/or assembly of data, Data analysis and interpretation, Manuscript writing; **DV:** Conception/Design, Collection and/or assembly of data, Data analysis and interpretation, Manuscript writing. **DMP:** Data analysis and interpretation, Collection and/or assembly of data. **MAG:** Collection and/or assembly of data. **GMM:** Collection and/or assembly of data. **SMR:** Collection and/or assembly of data. **JPL:** Collection and/or assembly of data. **DJB:** Collection and/or assembly of data. **IRG:** Collection and/or assembly of data. **AGL:** Collection and/or assembly of data. **PZC:** Collection and/or assembly of data. **IG:** Manuscript writing, Collection and/or assembly of data. **ABC:** Collection and/or assembly of data. **PPW:** Collection and/or assembly of data. **LGS:** Collection and/or assembly of data. **MEP:** Provision of study material or patients. **JF:** Manuscript writing, Final approval of manuscript. **NRS:** Manuscript writing, Final approval of manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure

During the preparation of this work the author used GPT-4 to aid with the grammar and readability of the manuscript. After using this service, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Conflict of interests A.R.L.: has no financial conflicts of interest to disclose. D.V.: has no financial conflicts of interest to disclose. D.M.P.: has no financial conflicts of interest to disclose. M.A.G.: has no financial conflicts of interest to disclose. G.M.M.: has no financial conflicts of interest to disclose. S.M.R.: has no financial conflicts of interest to disclose. J.P.L.: has no financial conflicts of interest to disclose. D.J.B.: has no financial conflicts of interest to disclose. I.R.G.: has no financial conflicts of interest to disclose. A.G.L.: has no financial conflicts of interest to disclose. P.Z.C.: has no financial conflicts of interest to disclose. IG: Consulting/Advisory relationship: Servier and Astrazeneca—Honoraria: Servier, Merck, Astrazeneca, MSD and Amgen. A.B.C.: has no financial

conflicts of interest to disclose. P.P.W.: has no financial conflicts of interest to disclose. LGS: has no financial conflicts of interest to disclose. M.E.P.: has no financial conflicts of interest to disclose. J.F.: has no financial conflicts of interest to disclose. N.R.S.: has no financial conflicts of interest to disclose.

Data availability

The data that support the findings of this study will be made available upon reasonable request to the corresponding author.

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