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Tumor Budding in Colorectal Carcinoma: Confirmation of Prognostic Significance and Histologic Cutoff in a Population-based Cohort

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Abstract

Tumor budding in colorectal carcinoma has been associated with poor outcome in multiple studies, but the absence of an established histologic cutoff for “high” tumor budding, heterogeneity in study populations and varying methods for assessing tumor budding have hindered widespread incorporation of this parameter in clinical reports. We used an established scoring system in a population-based cohort to determine a histologic cutoff for “high” tumor budding and confirm its prognostic significance. We retrieved hematoxylin and eosin-stained sections from 553 incident colorectal carcinoma cases. Each case was previously characterized for select molecular alterations and survival data. Interobserver agreement was assessed between two GI pathologists and a group of four general surgical pathologists. High budding (≥ 10 tumor buds in a 20× objective field) was present in 32% of cases, low budding in 46% and no budding in 22%. High tumor budding was associated with advanced pathologic stage ($p < 0.001$), microsatellite stability ($p = 0.005$), *KRAS* mutation ($p = 0.010$) and on multivariate analysis with a greater than two times risk of cancer-specific death ($HR = 2.57$ (1.27, 5.19)). After multivariate adjustment, via penalized smoothing splines, we found increasing tumor bud counts from 5 upward to be associated with an increasingly shortened cancer-specific survival. By this method, a tumor bud

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Conflicts of Interests

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count of 10 corresponded to approximately 2.5 times risk of cancer –specific death. The interobserver agreement was good with weighted kappa of 0.70 for two GI pathologists over 121 random cases and 0.72 between all six pathologists for 20 random cases. Using an established method to assess budding on routine histologic stains, we have shown a cutoff of 10 for high tumor budding is independently associated with a significantly worse prognosis. The reproducibility data provide support for the routine widespread implementation of tumor budding in clinical reports.

Introduction

Colorectal carcinoma (CRC) ranks second in cancer death among malignancies that affect both men and women in the United States [1]. Pathologic stage remains the most robust means of estimating prognosis in affected patients, but outcome can vary within stage groups; thus, investigators have sought other factors which might contribute to prognosis. Tumor budding is a histologic feature consistently associated with adverse outcomes in CRC [2, 3]. It has been designated as an adverse prognostic factor by the International Union against Cancer (UICC) [4], based on data from several studies [2, 5–8]. The UICC placed tumor budding in the category of “additional prognostic markers,” along with histologic grade, perineural invasion and tumor border. Still, widespread incorporation of tumor budding assessment into routine pathology reports has been hindered by several factors, including heterogeneity in patient groups studied with regard to tumor site and stage, heterogeneity in the definition of a tumor bud, varied scoring systems for tumor budding, and the absence of an established histologic cutoff for high tumor budding [3].

We used a scoring system proposed by Ueno et al (7) and modified by Mitrovec et al (3) to score tumor budding in a population-based cohort of older women with CRC. We aimed to assess the prognostic import of tumor budding and to clarify a histologic cutoff for “high” tumor budding. We also tested potential molecular correlates to determine whether the high tumor budding phenotype corresponds to a distinct molecular signature.

Methods and Materials

Cohort Recruitment and Case Ascertainment

Details regarding the methods used for recruitment and enrollment of IWHs participants have been reported elsewhere [9]. In brief, a 16-page baseline questionnaire was sent to 99,826 randomly selected women, ages 55–69 years, who resided in Iowa and held a valid driver’s license at baseline, in January 1986. Of these, 41,836 women (42%) returned the baseline questionnaire, constituting the full IWHs subject cohort. Incident CRC cases were identified through the State Health Registry of Iowa [10].

Annual matching between a computer-generated list of all cohort members and the records of Iowans with incident cancer in the SEER program registry was performed. Demographic characteristics and CRC incidence rates for baseline survey responders and non-responders have previously been shown to be similar [11]. Data regarding tumor location, grade, SEER

stage, chemotherapy exposure, and radiation therapy exposure were obtained from the SEER registry.

Mortality Measurement

Vital status and state of residence for IWHs participants were determined by mailed questionnaires in 1987, 1989, 1992, 1997 and 2004, as well as through linkage to Iowa death certificate records. Previous studies have estimated that 99% of all cancer-related deaths among IWHs cohort members are captured through this approach [12].

Tissue Collection and Processing

Archived, paraffin-embedded tissue specimens were requested for incident CRC cases diagnosed from January 1, 1986 through December 31, 2002 as part of an NCI-funded molecular epidemiology project (CA107333; Limburg, PI). For each participant, the pathology laboratory of record was contacted through an introductory request letter, with a second request letter and additional telephone request utilized as needed. Pathology reports, diagnostic slides, and tissue blocks were mailed directly to Iowa Cancer Registry staff for initial accessioning, followed by shipment to the study laboratory coordinator at Mayo Clinic Rochester.

Pathological Analysis

Confirmation of CRC diagnosis and tissue block selection was performed for each case by an experienced gastrointestinal pathologist (T.C.S.). In total, pathology materials were retrieved for 553 incident CRC cases.

H&E-stained slides for each CRC case were reviewed independently by one pathologist (T.C.S.). Tumor grade, tumor infiltrating lymphocytes, Crohn's like inflammatory infiltrate and vascular invasion were recorded, and the maximum number of tumor buds determined at the advancing edge of the tumor in a 20× objective field (0.95mm²) as previously described by Ueno et al [7]. Briefly, we defined a tumor bud as a collection of <5 neoplastic cells. We scanned the advancing edge of the tumor at 10× objective for the place where budding appeared to be maximal. Then, at 20× objective, the area was examined to determine the maximum number of tumor buds in a microscopic field (0.95mm²). Microscopy was performed using the Olympus BX41 (Olympus America Inc., NY) using routine H&E-stained sections only, without aid of immunohistochemistry. Tumor budding was recorded as a continuous variable but it was also categorized as an ordinal variable, i.e. tumor bud score, to facilitate comparison with prior studies. Cases with 10 buds were classified as high budding, those with 1–9 buds were classified as low budding and those without buds were classified as no budding. A total of 121 cases were independently reviewed and assessed for tumor budding by a second pathologist with an interest in GI pathology (R.P.G.) in order to assess inter-rater agreement. The final score for each case to be used in all subsequent analyses was that determined by the senior pathologist (T.C.S.). Four general surgical pathologists with no special interest in colon carcinoma or tumor budding (Y.R., J.J.G., S.E.K and H.L.) and the two GI pathologists (R.P.G. and T.C.S.) independently reviewed 20 of the 121 randomly selected cases to assess inter-rater agreement to evaluate the reproducibility of tumor budding assessment by general

pathologists. Each of the four general surgical pathologists received training at different institutions.

Each patient had been previously characterized for select molecular alterations including microsatellite instability (MSI), *KRAS*, and *BRAF* mutation status, and CpG island methylation phenotype (CIMP) status [13–15].

Statistical Analysis

Data were descriptively summarized using frequencies and percentages for categorical variables and medians and ranges for continuous variables. Associations of tumor budding with histologic and clinicopathologic features were assessed using chi-square tests for categorical variables and analyses of variance (ANOVA) for continuous variables. We used weighted kappa coefficients to compare agreement in tumor bud scores for 121 cases read by both GI pathologists and 20 cases read by both GI and 4 general pathologists.

Post-CRC follow-up was calculated as the time from initial CRC diagnosis to death or end of the defined study period (December 31, 2010). Two sets of survival analyses were carried out: one based on all-cause survival and one based on CRC-specific survival. For the latter analyses, subjects dying of causes unrelated to CRC were censored at the date of death.

Multivariate Cox proportional hazards regression analyses were used to estimate survival hazard ratios (HRs) and 95% confidence intervals (CIs) for tumor budding categories, using the lowest categorized value as the referent group. Tests for trend were carried out by ordering the categories from lowest to highest and including the resulting variable as a 1 degree-of freedom linear term in the Cox regression models.

Following the analyses of categorized bud scores, we re-assessed associations using the uncategorized bud counts via penalized smoothing splines, or P-splines[16]. All Cox regression analyses adjusted for the potential confounding effects of age at CRC diagnosis; year of CRC diagnosis (1986–1989, 1990–1999, or 2000–2002); anatomic subsite (proximal colon, distal colon, or rectum); tumor grade (1, 2, or 3/4); AJCC tumor stage (local, regional, or distant); histologic variables such as vascular invasion, tumor infiltrating lymphocytes and Crohn's-like infiltrate; chemotherapy exposure (no or yes); radiation therapy exposure (no or yes); p53; *KRAS* mutation status; microsatellite instability and pathology reviewer. All statistical tests were two-sided, and all analyses were carried out with the SAS[r] proprietary software, release 9.2 [TS2M3] for Linux; and R version 2.14.0 (Vienna, Austria).

Results

Among the 553 CRC cases, 179 (32%) had high tumor budding (≥ 10 tumor buds in a 20× objective field), 252 (46%) had low tumor budding and 122 (22%) had no tumor budding. Figure 1 illustrates representative photomicrographs of high, low and no tumor budding. The median tumor bud count for the entire population was 5 buds in a 20× objective field (Range 0 – 32). On analysis of 121 randomly selected cases reviewed by each GI pathologist independently, the agreement in tumor bud score was good with a weighted kappa of 0.70

(95% CI 0.53–0.79). The agreement between the six pathologists for the 20 randomly selected and independently reviewed cases was also good with a weighted kappa of 0.72.

Tumor Budding and Clinicopathologic Features

Table 1 summarizes the relationship between tumor budding and the studied clinicopathologic variables. Patients with high tumor budding were more likely to have venous invasion, lymphatic invasion, present with regional or metastatic disease and more likely to have received chemotherapy. Tumors with high budding were more likely to be microsatellite stable (MSS) and were more often characterized by *KRAS* mutations. Consistent with the association of high tumor budding to microsatellite stability ($p=0.005$), low or no tumor budding was associated with increasing number of tumor infiltrating lymphocytes ($p<0.001$). A Crohn's like lymphoid reaction was not significantly related to tumor budding ($p=0.5333$). There was no correlation between tumor budding and histologic grade, tumor site, radiation treatment, *BRAF* mutation or CIMP status.

Tumor Budding and Survival

Table 2 shows associations between tumor budding and CRC-specific mortality when tumor budding is stratified into three groups (none, low, high) or two groups (low, high). In the three-group scheme, multivariate analysis accounting for all of the variables noted in the table showed that patients with high budding were roughly 2.5 times more likely to die of CRC than those with no budding. In the two-group analysis, patients with high budding were approximately twice as likely as those with low budding to die of CRC. Associations between tumor budding and all-cause mortality were in a similar direction, but lower magnitude, compared to CRC-specific mortality results.

Histologic Cutoff for High Budding

We evaluated the association between incremental tumor bud counts and risk of cancer-related death, adjusted for multiple variables, via penalized smoothing splines (4) as shown in Figure 2. Note that the risk of CRC-related death continues to rise with increasing bud count, illustrating the strong relationship between tumor budding and CRC-related death over a wide range of tumor bud scores. In this analysis, a doubling of the risk of death due to disease is denoted by a hazard ratio of 2. This is reached at a bud count of seven. At a bud count of 10, the risk of death due to disease after adjustment for multiple variables increases to approximately two and a half times baseline i.e. hazard ratio of approximately 2.5. Together with the strong clinicopathologic correlations of tumor budding at a count of 10, we show that at this histologic cut off the risk of death from disease is 2.5 times that of no budding providing support to the use of 10 as a clinically relevant histologic cut off across all tumor stages using this population based cohort.

Discussion

Tumor budding is a recognized prognostic factor in colorectal carcinoma, but the methods of evaluation and the composition of study groups have been highly variable to date [5, 6, 17–26]. To improve generalizability and acceptance of this parameter, the method of evaluating tumor budding should use a standardized approach which is reproducible in routine practice,

employs a specific definition of a tumor bud and correlates well with cancer-specific outcome. In six of the previously mentioned studies [6, 17, 19, 21, 24, 25], the study group was restricted to a specific T stage and each of these studies used a different evaluation method, including five which employed immunohistochemistry instead of routine H&E stains. In two of the five remaining studies, the definition of a tumor bud was not specified [2, 20] and two others used two different methods of tumor bud assessment [18, 22]. While each of these studies found tumor budding to be of prognostic significance, only the study by Ueno et al [23] included a large number of cases across various T stages, used only H&E-stained sections and used a specific definition of a tumor bud. Their publication included only rectal tumors, but it does provide, we believe, the best combination of the clear definitions, method of assessment and large number of cases. Using their method of tumor budding assessment in every regard except for the modification proposed by Mitrovic et al (20) (objective lens magnification 20× rather than 25×), we confirmed the prognostic significance of tumor budding, independent of stage and tumor site, in a large cohort of patients from the Iowa Women's Health Study that included patients with localized, locally advanced and metastatic disease. For the purpose of comparison, field area is also reported here.

We confirmed several already known correlations with tumor budding: high tumor budding is more common in MSS tumors [5, 27], in tumors presenting at an advanced stage [3], and in tumors treated by chemotherapy. High tumor budding is independently associated with a worse cancer-specific survival [7, 17, 20, 23, 24, 28–30].

In contrast to two prior studies from the same group [27, 31], we observed a significant relationship between *KRAS* mutation status and high tumor budding. There are some differences between their studies and ours. First, in both of their studies, Zlobec and colleagues [27, 31] used immunohistochemistry to quantitate tumor buds and scored tumor budding at a 40× objective. It is recognized that counting tumor budding by immunohistochemistry identifies more buds than routine sections, making comparison with our results difficult. Second, our study includes more than double the combined number of cases from the two studies. Third, our patients are from a population-based study rather than an institutional database or practice, which may provide a more generalizable patient population to assess clinicopathologic correlations.

As has been pointed out in multiple review articles [3, 32, 33], the evaluation of tumor budding is not always straightforward, particularly in cases with obscuring inflammation at the advancing tumor edge. Immunohistochemistry for epithelial markers is an appealing way to avoid that problem and, not surprisingly yields higher bud counts than those obtained using routine histology [31, 32, 34]. We agree with Mitrovic and colleagues [3] that it is not practical in routine practice to perform immunohistochemistry for cytokeratins on every case of colorectal carcinoma in the current healthcare economic environment of cost containment. It is beyond the scope of this study to evaluate the potential cost efficiency related to decreased review time from routine use of immunohistochemistry. It is reasonable to think that any efficiency would need to be substantial to merit the routine use of immunohistochemistry since tumor budding is a prognostic marker, not a diagnostic or treatment-directing biomarker. There may yet be a role for immunohistochemistry in cases

of borderline tumor budding but this would require studies designed to determine correlations between tumor bud counts on routine sections and immunohistochemistry. Until such studies are completed, we believe that cutoffs are best determined from routine H & E-stained sections to allow generalization to everyday practice.

Similar to others, we found no correlation between tumor budding and *BRAF* mutation status or CIMP status [27]. Thus, high budding does not seem to explain the poor prognosis of the aggressive *BRAF* mutant-MSS phenotype.

Mitrovic and colleagues concisely summarized the issues interfering with universal reporting of tumor budding [3]. Studies looking at interobserver reproducibility of budding assessment show mixed results ranging from fair to good [23, 24, 28, 35]. We believe that the approach outlined by Mitrovic et al (20) and used here, helps to mitigate some of these issues. We used a commonly accepted definition of a tumor bud [18, 19, 21, 23–25] and employed a simple bud evaluation method which is likely to be reproducible in routine practice to confirm the robust prognostic significance of tumor budding. Our data show good reproducibility with strong agreement between six reviewers including four general surgical pathologists (weighted kappa 0.70 and 0.72).

There are five published studies proposing evidence-based cutoffs for high tumor budding [28, 31, 34, 36, 37]. Three of these studies used immunohistochemistry to evaluate budding, rendering it difficult to apply their proposed cutoffs [31, 34, 36] to routine sections. Two studies did not specify the microscope field area covered by the high power objective, further limiting comparison with other data [31, 34]. Of the two studies using routine sections [28, 37], the study by Sy et al, utilized a cutoff of >9 buds in a 20× objective field [37], but they did not identify a worse survival on multivariate analysis in cases with high budding across their entire population, which contrasts with our data and that of several other studies [2, 6, 7, 17, 18, 23, 24, 28]. Choi and colleagues [28] found >10 buds in a 20× field to be the appropriate cutoff for high budding in a series of 244 rectal tumors, although they used a semiquantitative approach [28]. In their recent review of the literature, Mitrovic et al also argued for 10 as an appropriate threshold for high budding. From our survival analysis data, a statistically significant adverse effect is seen with tumor budding over a range of tumor bud scores beginning below 10. However, given the documented variability in counting tumor buds, we believe that our population-based data would support the use of a cutoff of 10 proposed by Mitrovic (20) and others (3, 30) as reasonable. The spline diagram shows that around a tumor bud count of 7, the hazard ratio (i.e. relative risk of cancer specific death) is doubled and around a score of 10 the relative risk of death from disease increased to 2.5 times that of baseline. Therefore with fair agreement between reviewers, the risk of death from disease using this approach and histologic cutoff would be expected to be approximately 2–3 times greater for cases classified as high budding (compared to cases with no budding). We propose that this degree of relative risk is clinically significant and we believe this should provide practicing pathologists with the evidence to implement routine reporting of tumor budding in reports. The authors believe that the inclusion of the specific tumor bud count in the report as well as a categorization of high or low tumor budding would be helpful. We believe the incremental increase in risk of cancer-specific death with increasing score would support this proposal.

A limitation of this study is the composition of the study population by women who were predominantly of Caucasian ethnicity. There are possibly differences in the biology of the budding phenotype in other ethnic groups and men. Nevertheless, this represents the largest population-based study of patients evaluated to date. Also, we are unable to determine what proportion of our rectal carcinomas received neoadjuvant chemoradiation preventing us from assessing its impact on our findings. Given the time period of our dataset, 1986–2002 and the establishment of neoadjuvant chemoradiation as standard of care for stage 2 and 3 rectal carcinomas in 2004 [38], we believe it is unlikely that neoadjuvant chemoradiation had a significant impact on our findings.

In conclusion, tumor budding, assessed on routine histologic sections, is an independent adverse prognostic factor in this large cohort of women with colorectal carcinoma. Tumor budding was strongly associated with advanced stage, microsatellite stability, *KRAS* mutations and increased cancer-specific mortality. We endorse a histologic cutoff of 10 tumor buds in a 20× objective field as a threshold for high tumor budding and describe an approximate 3 times greater risk of death from disease at this cutoff relative to no tumor budding.

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References

1. Haggard FA, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. *Clinics in colon and rectal surgery*. 2009; 22(4):191–7. [PubMed: 21037809]
2. Hase K, et al. Prognostic value of tumor “budding” in patients with colorectal cancer. *Diseases of the colon and rectum*. 1993; 36(7):627–35. [PubMed: 8348847]
3. Mitrovic B, et al. Tumor budding in colorectal carcinoma: time to take notice. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc.* 2012; 25(10): 1315–25.
4. C, C. *Prognostic factors in Cancer*. New York: Wiley-Liss; 2006.
5. Jass JR, et al. APC mutation and tumour budding in colorectal cancer. *Journal of clinical pathology*. 2003; 56(1):69–73. [PubMed: 12499439]
6. Okuyama T, Nakamura T, Yamaguchi M. Budding is useful to select high-risk patients in stage II well-differentiated or moderately differentiated colon adenocarcinoma. *Diseases of the colon and rectum*. 2003; 46(10):1400–6. [PubMed: 14530682]
7. Ueno H, et al. A new prognostic staging system for rectal cancer. *Annals of surgery*. 2004; 240(5): 832–9. [PubMed: 15492565]
8. Zlobec I, et al. Characterization of the immunological microenvironment of tumour buds and its impact on prognosis in mismatch repair-proficient and -deficient colorectal cancers. *Histopathology*. 2011; 59(3):482–95. [PubMed: 22034888]
9. Folsom AR, et al. Increased incidence of carcinoma of the breast associated with abdominal adiposity in postmenopausal women. *American journal of epidemiology*. 1990; 131(5):794–803. [PubMed: 2138863]
10. Hankey BF, Ries LA, Edwards BK. The surveillance, epidemiology, and end results program: a national resource. *Cancer epidemiology, biomarkers & prevention : a publication of the American*

Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 1999; 8(12):1117–21.

11. Bisgard KM, et al. Mortality and cancer rates in nonrespondents to a prospective study of older women: 5-year follow-up. *American journal of epidemiology*. 1994; 139(10):990–1000. [PubMed: 8178787]
12. Zhang S, et al. Better breast cancer survival for postmenopausal women who are less overweight and eat less fat. The Iowa Women's Health Study. *Cancer*. 1995; 76(2):275–83. [PubMed: 8625103]
13. Limburg PJ, et al. Diabetes mellitus and subsite-specific colorectal cancer risks in the Iowa Women's Health Study. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2005; 14(1):133–7.
14. Limburg PJ, et al. Cigarette smoking and colorectal cancer: long-term, subsite-specific risks in a cohort study of postmenopausal women. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association*. 2003; 1(3):202–10. [PubMed: 15017492]
15. Samadder NJ, et al. Associations between colorectal cancer molecular markers and pathways with clinicopathologic features in older women. *Gastroenterology*. 2013; 145(2):348–56 e1–2. [PubMed: 23665275]
16. Eilers PH, M BD. Flexible smoothing with B-splines and penalties. *Stat Sci*. 1996; 11:89–121.
17. Guzinska-Ustymowicz K. The role of tumour budding at the front of invasion and recurrence of rectal carcinoma. *Anticancer research*. 2005; 25(2B):1269–72. [PubMed: 15865077]
18. Ha SS, et al. Intensity of tumor budding as an index for the malignant potential in invasive rectal carcinoma. *Cancer research and treatment : official journal of Korean Cancer Association*. 2005; 37(3):177–82. [PubMed: 19956500]
19. Ishikawa Y, et al. Histopathologic determinants of regional lymph node metastasis in early colorectal cancer. *Cancer*. 2008; 112(4):924–33. [PubMed: 18181096]
20. Kanazawa H, et al. Tumour budding at invasive margins and outcome in colorectal cancer. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland*. 2008; 10(1):41–7. [PubMed: 18078460]
21. Losi L, et al. Prognostic significance of histological features and biological parameters in stage I (pT1 and pT2) colorectal adenocarcinoma. *Pathology, research and practice*. 2006; 202(9):663–70.
22. Morodomi T, et al. An index for estimating the probability of lymph node metastasis in rectal cancers. Lymph node metastasis and the histopathology of actively invasive regions of cancer. *Cancer*. 1989; 63(3):539–43. [PubMed: 2912530]
23. Ueno H, et al. Tumour 'budding' as an index to estimate the potential of aggressiveness in rectal cancer. *Histopathology*. 2002; 40(2):127–32. [PubMed: 11952856]
24. Wang LM, et al. Tumor budding is a strong and reproducible prognostic marker in T3N0 colorectal cancer. *The American journal of surgical pathology*. 2009; 33(1):134–41. [PubMed: 18971777]
25. Yamauchi H, et al. Pathological predictors for lymph node metastasis in T1 colorectal cancer. *Surgery today*. 2008; 38(10):905–10. [PubMed: 18820865]
26. Karamitopoulou E, et al. Proposal for a 10-high-power-fields scoring method for the assessment of tumor budding in colorectal cancer. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc*. 2013; 26(2):295–301.
27. Zlobec I, et al. The impact of CpG island methylator phenotype and microsatellite instability on tumour budding in colorectal cancer. *Histopathology*. 2012; 61(5):777–87. [PubMed: 22803799]
28. Choi HJ, et al. Tumor budding as a prognostic marker in stage-III rectal carcinoma. *International journal of colorectal disease*. 2007; 22(8):863–8. [PubMed: 17216219]
29. Nakamura T, et al. Tumor budding as an index to identify high-risk patients with stage II colon cancer. *Diseases of the colon and rectum*. 2008; 51(5):568–72. [PubMed: 18286339]
30. Okuyama T, Oya M, Ishikawa H. Budding as a useful prognostic marker in pT3 well- or moderately-differentiated rectal adenocarcinoma. *Journal of surgical oncology*. 2003; 83(1):42–7. [PubMed: 12722096]

31. Zlobec I, et al. Tumor budding predicts response to anti-EGFR therapies in metastatic colorectal cancer patients. *World journal of gastroenterology : WJG*. 2010; 16(38):4823–31. [PubMed: 20939111]
32. Prall F. Tumour budding in colorectal carcinoma. *Histopathology*. 2007; 50(1):151–62. [PubMed: 17204028]
33. Lugli A, Karamitopoulou E, Zlobec I. Tumour budding: a promising parameter in colorectal cancer. *British journal of cancer*. 2012; 106(11):1713–7. [PubMed: 22531633]
34. Lugli A, et al. CD8+ lymphocytes/ tumour-budding index: an independent prognostic factor representing a ‘pro-/anti-tumour’ approach to tumour host interaction in colorectal cancer. *British journal of cancer*. 2009; 101(8):1382–92. [PubMed: 19755986]
35. Suzuki A, et al. Evaluation of venous invasion by Elastica van Gieson stain and tumor budding predicts local and distant metastases in patients with T1 stage colorectal cancer. *The American journal of surgical pathology*. 2009; 33(11):1601–7. [PubMed: 19574884]
36. Prall F, Nizze H, Barten M. Tumour budding as prognostic factor in stage I/II colorectal carcinoma. *Histopathology*. 2005; 47(1):17–24. [PubMed: 15982319]
37. Sy J, et al. Tumor budding and survival after potentially curative resection of node-positive colon cancer. *Diseases of the colon and rectum*. 2010; 53(3):301–7. [PubMed: 20173477]
38. Sauer, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med*. 2004 Oct 21; 351(17):1731–40. [PubMed: 15496622]

Figure 1.

- A) This is a representative photomicrograph of high tumor budding, which was characterized by ≥ 10 buds in a 20 $\times$ objective field.
- B) Low tumor budding, shown in this photomicrograph, was characterized by < 10 buds per 20 $\times$ objective field.
- C) An example of a case with no buds (20 $\times$ objective).

Figure 2.

Spline diagram showing the multivariate-adjusted incremental relationship between tumor bud count and relative risk of colorectal death. Bud count is displayed on the x-axis and relative risk of CRC-specific mortality on the y-axis.

Table 1

Univariate associations of tumor budding count per 20× field with other cancer-related variables.

	Tumor Budding Count			p value ^I
	None (N=122)	Low: 1–9 (N=252)	High: 10+ (N=179)	
Median age at CRC dx (range)	74.8 (58–86)	74.1 (58–85)	73.6 (57–85)	0.484
AJCC Tumor stage				<0.001
Missing	12	20	12	
I	36 (32.7%)	68 (29.3%)	23 (13.8%)	
II	44 (40.0%)	93 (40.1%)	43 (25.7%)	
III	20 (18.2%)	49 (21.1%)	58 (34.7%)	
IV	10 (9.1%)	22 (9.5%)	43 (25.7%)	
Histologic Grade				0.179
Missing	6	6	7	
Well differentiated	12 (10.3%)	14 (5.7%)	6 (3.5%)	
moderately	70 (60.3%)	151 (61.4%)	113 (65.7%)	
poorly/not	34 (29.3%)	81 (32.9%)	53 (30.8%)	
Venous Invasion				
No	106 (86.9%)	226 (89.7%)	134 (74.9%)	<0.001
Yes	16 (13.1%)	26 (10.3%)	45 (25.1%)	
Tumor site				0.490
Missing	1	2	3	
Proximal colon	71 (58.7%)	146 (58.4%)	97 (55.1%)	
Distal colon	29 (24.0%)	69 (27.6%)	43 (24.4%)	
Rectum	21 (17.4%)	35 (14.0%)	36 (20.5%)	
Chemotherapy treatment				0.023
No	96 (78.7%)	204 (81.0%)	125 (69.8%)	
Yes	26 (21.3%)	48 (19.0%)	54 (30.2%)	
Radiation				0.171
Missing	2	4	7	
No	116 (96.7%)	241 (97.2%)	161 (93.6%)	
Yes	4 (3.3%)	7 (2.8%)	11 (6.4%)	
Microsatellite instability				0.005
Missing	5	6	3	
mss	51 (43.6%)	120 (48.8%)	106 (60.2%)	
msi-l	28 (23.9%)	48 (19.5%)	40 (22.7%)	
msi-h	38 (32.5%)	78 (31.7%)	30 (17.0%)	
<i>BRAF</i> mutation status				0.476
Missing	5	8	5	
wt	80 (68.4%)	173 (70.9%)	130 (74.7%)	
mut	37 (31.6%)	71 (29.1%)	44 (25.3%)	
<i>KRAS</i> mutation status				0.010
Missing	13	23	12	

	Tumor Budding Count			p value ^I
	None (N=122)	Low: 1–9 (N=252)	High: 10+ (N=179)	
wt	78 (71.6%)	164 (71.6%)	97 (58.1%)	0.030
mut	31 (28.4%)	65 (28.4%)	70 (41.9%)	
p53 status				
Missing	22	29	19	
Negative	56 (56.0%)	92 (41.3%)	71 (44.4%)	
Low	14 (14.0%)	41 (18.4%)	17 (10.6%)	0.671
High	30 (30.0%)	90 (40.4%)	72 (45.0)	
CIMP status				
Missing	12	8	8	
neg	57 (51.8%)	120 (49.2%)	93 (54.4%)	
low	16 (14.5%)	43 (17.6%)	31 (18.1%)	
high	37 (33.6%)	81 (33.2%)	47 (27.5%)	

Values presented as number (percent).

^I: chi-square test for categorical variables and analysis of variance (ANOVA) for continuous variables.

Table 2

Adjusted associations between tumor budding count and colorectal cancer-specific mortality.

Tumor Budding Count	N events	Person-Years	RR (CI) ¹	p-value ¹	RR (95% CI) ²	p-value ²
None	18	1090	1.00 (Ref)	<0.001	1.00 (Ref)	<0.001
Low (1–9)	52	2231	1.40 (0.82,2.39)		1.24 (0.60,2.56)	
High (10+)	100	1090	4.72 (2.86,7.81)		2.57 (1.27,5.19)	
0–9	70	3321	1.00 (Ref)	<0.001	1.00 (Ref)	<0.001
10+	100	1090	3.72 (2.74,5.06)		2.18 (1.44,3.32)	

RR, relative risk; CI, 95% confidence interval.

¹: Accounting for age at diagnosis.

²: Accounting for age at diagnosis, year of diagnosis, tumor stage, tumor grade, tumor site (proximal vs. distal), chemotherapy, radiation, vascular invasion, MSI status, p53 status, and KRAS mutation status.