Tumor Budding in Colorectal Carcinoma

Translating a Morphologic Score Into Clinically Meaningful Results

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Context.—Tumor budding has received increasing recognition as an important independent prognostic factor in colorectal carcinoma. Prominent tumor budding in adenocarcinoma arising in a polyp has been shown to be a risk factor for lymph node involvement. The variability in methods used for evaluating tumor budding in different studies and lack of standardized guidelines have impeded routine inclusion of tumor budding in pathology reports. This changed last year with consensus guidelines based on the International Tumor Budding Consensus Conference (ITBCC). These guidelines have been included in the recent College of American Pathologists (CAPs) Colorectal Cancer Protocol. The consensus methodology will allow uniform reporting of this finding, but challenges in interpretation in the setting of intense inflammation, fibrosis, or gland fragmentation need to be addressed in future guidelines.

THE CLINICAL CONTEXT

Tumor budding in colorectal carcinoma (CRC) has been studied extensively and many recent reviews have highlighted the key studies that have brought tumor budding into the clinical realm.1–5 The clinical significance of tumor budding as an independent risk factor for adverse outcomes in CRC has been well demonstrated in numerous studies as well as meta-analyses.6–16 The goal of this review is not to exhaustively reiterate the information provided in these publications, but to provide a brief overview of the clinical and historical context and emphasize the practical aspects of its implementation on a routine basis.

Objective.—To provide a brief overview of the known clinical significance of tumor budding in colorectal carcinoma and discuss the practical aspects of its implementation on a routine basis.

Data Sources.—English-language pathology literature.

Conclusions.—Tumor budding has been shown to be an independent prognostic marker in colorectal carcinomas and the routine reporting of tumor buds is now advocated by using the approach outlined by the ITBCC guidelines. Tumor budding is included in the CAP protocol as a recommended element. Presence of prominent tumor budding in an adenocarcinoma in a polyp may have implications for management, such as additional resection, while it serves as a prognostic factor in other settings.

Adenocarcinomas arising in polyps (“malignant polyps”), tumor budding has been associated with increased risk of lymph node metastasis in multiple studies.20–23 In one study by the Study Group for Budding/Sprouting in Colorectal Cancer of the Japanese Society for Cancer of the Colon and Rectum (JSCCR), “high-grade tumor budding,” defined as 5 or more foci of isolated cancer cells or a cluster comprising fewer than 5 cells at the invasive front, was associated with a 3.14 odds ratio for lymph node metastasis and was proposed as a predictive parameter that could be helpful in clinical management (ie, facilitate the decision for additional surgical resection after endoscopic treatment).23

Current clinical practice guidelines in oncology issued by the National Comprehensive Cancer Network (NCCN; version 2.2017) include incorporation of tumor budding in...
clinical treatment, advising that “tumor budding has been shown to be an adverse histological feature associated with adverse outcome and may preclude polypectomy as an adequate treatment of endoscopically removed malignant polyps.” Similar guidelines are currently used in the management of patients with colorectal carcinoma in Japan (JSCCR) and Europe (European Society for Medical Oncology) \(^{25,26}\).

Tumor budding has been studied predominantly at the invasive front and is referred to as **peritumoral tumor budding**. Tumor buds can also be observed within the tumor, which has been referred to as **intratumoral tumor budding**. Intratumoral tumor budding can be evaluated in preoperative biopsies and has also been associated with the presence of lymph node metastasis, higher tumor grade, and lymphovascular invasion in the resection specimen, as well as distant metastasis. \(^{21,27-31}\) In rectal tumors treated with neoadjuvant chemotherapy, intratumoral tumor budding in preoperative biopsy has been associated with nonresponse to therapy as well as decreased survival. \(^{28}\)

**Tumor Budding in Resection Specimens: Stage I and Stage II Colorectal Carcinoma**

Similar to malignant polyps, multiple reports \(^{20,23,32-41}\) have demonstrated the association between high tumor budding and increased risk for nodal metastasis in resected stage I (pT1/2 N0 M0) CRC.

Stage II (pT3/4 N0 M0) CRC is a heterogeneous group, with reported 5-year survival ranging from 32.3% (stage IIC adenocarcinoma of the rectum) to 66.5% (stage IIA adenocarcinoma of the colon). \(^{42}\) Adjuvant therapy in these cases is indicated only in the presence of 1 or more high-risk features, which include poorly differentiated histology, lymphatic/vascular invasion, perineural invasion, bowel obstruction, localized perforation, and close/indeterminate/positive margins. In stage II CRC, high tumor budding is associated with poor overall and disease-free survival in both retrospective and prospective studies. \(^{33-51}\) In addition, high tumor budding is associated with other known aggressive clinicopathologic features including lymphovascular space invasion, higher tumor grade, and infiltrative tumor margin. \(^{44}\) In view of these findings, the use of tumor budding as a parameter in making treatment decisions has been advocated in stage II disease but is not yet included in the NCCN guidelines. \(^{31,46}\)

**Tumor Budding in Metastatic Colorectal Carcinoma**

Very few data are available regarding the significance of tumor budding in the setting of metastasis, but one study \(^{62}\) demonstrated that tumor budding is an independent prognostic marker for lung metastasis of CRC. Another study \(^{63}\) showed that the presence of tumor budding was associated with poor response to anti-EGFR (anti–epidermal growth factor receptor) therapy in patients with metastatic CRC.

**SO HOW SHOULD WE ASSESS TUMOR BUDDING? REPORTING TUMOR BUDDING IN PATHOLOGY REPORTS**

In recognition of the growing body of literature on the prognostic importance of tumor budding, by 2008, tumor budding was included in the Recommendations for the Reporting of Surgically Resected Specimens of Colorectal Carcinoma by the Association of Directors of Anatomic and Surgical Pathology, with the recommendation that “this feature be distinguished from tumor grade and scored separately as present or absent.” \(^{74}\) However, a specific method by which to report tumor budding was not provided and routine reporting of tumor budding, though advocated by many, was hampered by the lack of consensus guidelines and standardized methodology. Numerous different methodologies have been used in the literature, ranging from qualitative assessments \(^{39}\) to semiquantitative assessments \(^{48,52}\) to more quantitative assessments based on variable field size and number of fields. \(^{31,55-57}\) Despite the multitude of methodologies, however, in meta-analyses tumor budding has continued to emerge as an independent prognostic factor in colorectal carcinomas. \(^{5-10}\)

**The International Tumor Budding Consensus Conference**

To specifically address the issue of standardized reporting of tumor budding in colorectal carcinoma, an international panel of experts was convened in Bern, Switzerland, in November 2016 for the ITBCC. The stated primary goal of the ITBCC “was to reach agreement on an international, evidence-based standardized scoring system for tumor budding in colorectal cancer.” \(^{78}\) The consensus recommendations were reported in this publication and these recommendations were incorporated into the CAP cancer protocol for the examination of specimens from patients with primary carcinoma of the colon and rectum, \(^{59}\) to be used along with the 8th edition of the American Joint Committee on Cancer (AJCC) staging manual. \(^{42}\) While not a required element, its inclusion in the new CAP cancer protocol further acknowledges the importance of tumor budding as an independent prognostic marker in colorectal carcinoma that should be assessed on a routine basis.

Of the 11 total statements, 10 statements achieved consensus, with 10 of 11 statements agreed upon by 100% of the panel members and 1 statement (No. 5 below) achieving 96% (22 of 23) agreement. These statements are as follows \(^{58}\):

1. Tumor budding is defined as a single tumor cell or a cell cluster of up to 4 tumor cells.
2. Tumor budding is an independent predictor of lymph node metastasis in pT1 colorectal cancer.
3. Tumor budding is an independent predictor of survival in stage II colorectal cancer.
4. Tumor budding should be taken into account along with other clinicopathologic factors in a multidisciplinary setting.
5. Tumor budding is counted on hematoxylin-eosin (H&E).
6. Intratumoral tumor budding in colorectal cancer has been shown to be related to lymph node metastasis.
7. Tumor budding is assessed in 1 hotspot (in a field measuring 0.785 mm²) at the invasive front.
8. For tumor budding assessment in colorectal cancer, the hotspot method is recommended.
9. A 3-tier system should be used along with the budding count to facilitate risk stratification in colorectal cancer.
10. Tumor budding should be included in guidelines/protocols for colorectal cancer reporting.
11. Tumor budding and tumor grade are not the same.

Statements 1, 5, 7 through 9, and 11 were directly incorporated into the CAP cancer protocol as the advocated method for assessing and reporting tumor budding in
The ITBCC guidelines provide a uniform methodology for tumor budding assessment that should be limited to cases without neoadjuvant therapy, as there are insufficient data to assess the prognostic significance of tumor budding in the setting of neoadjuvant therapy.

### CHALLENGES IN TUMOR BUDDING: THE FUTURE

The ITBCC guidelines provide a uniform methodology for the assessment and reporting of tumor budding, but further work is needed to address the challenges in assessment of tumor budding.

#### Interobserver Variability

The reported interobserver variability for assessing tumor budding has ranged from moderate to very good, depending on the study. However, it is recognized that the difference in tumor budding are included, which may currently be most pathologists, at least until more experience is gained through implementation, following the guidelines included in the most recent CAP cancer protocol for colorectal carcinoma, which recommends reporting tumor budding for all carcinomas arising in polyps and in stage I and stage II carcinomas.

### Use of Cytokeratin for Tumor Budding Counts

Since most of the data in the literature were based on routine H&E staining for counting tumor buds, the ITBCC recommended H&E staining for budding counts. One of the most prominent proponents of reporting tumor budding, Alessandro Lugli, MD (who participated in the ITBCC and is the lead author of the published consensus statements), and his colleagues, have also been advocates for the use of cytokeratin immunohistochemical staining in assessing tumor budding. Their studies have demonstrated that the use of cytokeratin stains increases the tumor bud count by approximately 3-fold, although the proposed cutoff for high tumor budding in their initial studies was 10 or more averaged over 10 high-power fields. Their group, however, is one of the few to study tumor budding prospectively in patients with stage II disease. As further studies are performed and additional data become available, the methodology will likely be further refined or modified. At present, the recommendations are to perform the tumor budding counts on routine H&E staining (Figure, A and B), but to use cytokeratin immunostains as an adjunct, particularly in cases where the tumor/stroma interface may be obscured (ie, by inflammatory infiltrates; Figure, C and D).

As the use of slide scanning and other digital technology becomes more routine in pathology, manual scanning and counting of tumor buds may be superseded by automated methods.

### Absolute Tumor Bud Counts Versus Tiered Reporting

Although the current ITBCC and CAP guidelines are to report the absolute tumor bud count as well as a score based on a 3-tier system (low, intermediate, or high), it is unclear whether tumor budding is best evaluated as a continuous variable or with a tiered system (eg, 3-tier [low, intermediate, high] as in ITBCC or whether a 2-tier system [low and high]) is sufficient. The question remains of whether 10

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**Conversion Table for Standardization of Tumor Budding Count Using Different Microscope Eyepiece Diameters (×20 Objective Magnification)**

<table>
<thead>
<tr>
<th>Eyepiece FN Diameter, mm</th>
<th>Eye FN Radius, mm</th>
<th>Specimen FN Radius, mm</th>
<th>Specimen Area, mm²</th>
<th>Normalization Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>9</td>
<td>0.450</td>
<td>0.636</td>
<td>0.810</td>
</tr>
<tr>
<td>19</td>
<td>9.5</td>
<td>0.475</td>
<td>0.709</td>
<td>0.903</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>0.500</td>
<td>0.785</td>
<td>1.000</td>
</tr>
<tr>
<td>21</td>
<td>10.5</td>
<td>0.525</td>
<td>0.866</td>
<td>1.103</td>
</tr>
<tr>
<td>22</td>
<td>11</td>
<td>0.550</td>
<td>0.950</td>
<td>1.210</td>
</tr>
<tr>
<td>23</td>
<td>11.5</td>
<td>0.575</td>
<td>1.039</td>
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</tr>
<tr>
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<tr>
<td>26</td>
<td>13</td>
<td>0.650</td>
<td>1.327</td>
<td>1.690</td>
</tr>
</tbody>
</table>

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*References 38, 47, 51, 53, 55, 56, 62–64.*
tumor buds versus 50 tumor buds—while both high tumor budding, according to the current proposed scoring system—would truly carry the same clinical significance. Furthermore, if a cutoff value is used for a tiered scoring system, those numbers straddling the cutoff (eg, 9 versus 11 buds if 10 buds are used as a cutoff for high tumor budding) are problematic and appear to suggest different clinical relevance where there may not be any. Rieger and colleagues have argued that tumor budding may be best assessed as a continuous variable, but advocated the reporting of both the number of tumor buds as well as a tiered score (eg, low, intermediate, high).

**Tumor Budding in Histologic Subtypes of Adenocarcinoma**

A cautionary section regarding special tumor types was included in the ITBCC consensus guidelines and these special tumor types are worth mentioning here as well. Some have suggested that signet ring cell carcinomas, by definition, demonstrate high tumor budding. Most authors have also noted that tumor budding should be assessed carefully in mucinous carcinomas so as to avoid counting

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Figure. Tumor budding at the invasive edge. A and B, Tumor buds, arrows. Only those tumor cells with identifiable nuclei should be counted. C, Assessment of tumor buds can be challenging on hematoxylin-eosin when there is an obscuring inflammatory infiltrate. D, Though not recommended for routine use, a cytokeratin immunohistochemical stain can be helpful in some cases to highlight tumor buds (hematoxylin-eosin, original magnification ×1560 [A through C]; original magnification ×1560 [D]).
tumor cells and clusters floating in pools of mucin as tumor buds. In medullary carcinomas, or even in adenocarcinomas that are not otherwise specified (NOS), a prominent inflammatory infiltrate may obscure tumor buds. In such cases where tumor bud counts cannot be determined with certainty, tumor budding can be noted as “cannot be assessed” with an explanatory note.

Micropapillary carcinomas, by definition, are composed of tumor cell clusters of varying size. Care must be taken to include only those tumor cells and clusters that meet the definition of tumor buds and exclude poorly differentiated clusters (PDCs). In this context, it is of note that while a distinction between PDCs (most often defined as clusters of 5 or more tumor cells) and tumor buds has been made in the ITBCC, investigations to assess the potential utility of PDCs as an additional prognostic factor (separate from tumor buds) are underway. Some authors have reported that PDCs are an independent prognostic marker in colorectal carcinomas, including association with increased nodal metastasis,40,67 while others have suggested that PDCs may correlate with tumor grade.48–70 Similarly, the micropapillary carcinoma subtype may also be of prognostic significance.71,72 Additional studies will be necessary to understand the significance of PDCs and micropapillary architecture as possible prognostic markers independent of tumor budding.

CONCLUDING REMARKS

The significance of tumor budding in colorectal carcinoma, as an independent prognostic factor for adverse clinical outcomes, has now been well established. The routine reporting of tumor bud counts and scores is now advocated. The ITBCC guidelines represent the first international effort at a standardized methodology for assessing and reporting tumor buds in colorectal carcinoma. However, it is widely acknowledged that additional studies will be required to further refine the methodology and address the challenges in uniform reporting of tumor budding. Definite treatment options like chemotherapy in stage II disease, based on high tumor budding, need to be assessed in prospective studies. Until further data become available, the significance of tumor budding in individual cases will need to be further assessed in a multidisciplinary setting with cross talk and discussion amongst pathologists, surgeons, and oncologists.

References

44. Horigie M, Koelzer VH, Karamitopoulos E, et al. Tumor budding score based on 10 high-power fields is a promising basis for a standardized prognostic scoring system in stage II colorectal cancer. Hum Pathol. 2013;44(5):697–705.