

Tumor budding assessment in CRC: why and how

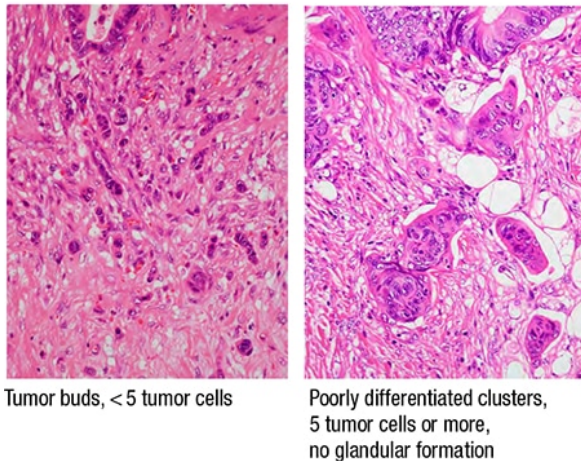
Karen Lusky

August 2020—Tumor budding is a robust prognostic marker that should be reported at least in pT1 and stage II colorectal carcinomas and taken into account with other risk factors. Further evidence is needed for tumor budding assessment in specimens taken after neoadjuvant therapy, says Heather Dawson, MD, senior staff GI pathologist at the Institute of Pathology, University of Bern in Switzerland.

Dr. Dawson, whose group has studied budding in CRC for more than 15 years, made those points and others in a recent CAP TODAY interview and in a CAP19 session on prognostic factors in CRC.

Tumor budding is defined as single tumor cells or clusters of up to four tumor cells at the invasive margin of CRC. “We know from many studies they play a role in the tumor microenvironment,” Dr. Dawson says. They’re involved in the epithelial-to-mesenchymal transition and viewed as the “morphological correlate of this process.”

Fig. 1. Tumor budding and poorly differentiated clusters
Buds’ big brother



Ueno H, Kajiwar Y, Shimazaki H, et al. New criteria for histologic grading of colorectal cancer. *Am J Surg Pathol*. 2012;36(2):193-201. <https://journals.lww.com/ajsp/pages/default.aspx>. The Creative Commons license does not apply to this content. Use of the material in any format is prohibited without written permission from the publisher, Wolters Kluwer Health, Inc. Please contact permissions@lww.com for further information.

Tumor buds are an independent prognostic factor in most studies, she says, but their predictive value isn’t yet known. Anything with five or more tumor cells qualifies as a poorly differentiated cluster, or PDC. “PDCs are basically tumor buds’ big brother, and they have been shown to have a prognostic value on their own. There is no consensus upper limit of the PDC. Many studies just use an arbitrary cutoff of up to 20 cells.” (**Fig. 1**).

If there are a lot of tumor buds, the tumor appears to act in a more malignant fashion, says Raul S. Gonzalez, MD, associate professor of pathology at Harvard Medical School, who spoke on tumor deposits in the CAP19 session (CAP TODAY, July 2020). “The tumor metastasizes more readily and has a worse prognosis,” he says.

When pathologists calculate a pT and pN classification for colorectal cancer, “the TNM classification system works very well but has one weakness,” Dr. Dawson cautions, “in that it only fits into the anatomical distribution within

the body.” This is especially apparent in stage II colorectal cancers. “You have a wide span of prognosis within the same stage. And a lot of stage II patients actually do worse than their stage III counterparts. So this is why we need additional biomarkers for better risk stratification within a certain tumor stage,” she says.

Few potential biomarkers fill all of the requirements needed to be implemented into practice, Dr. Dawson says, and the REMARK guidelines were put in place to promote a higher level of quality in biomarker reporting in studies. So what can be expected from an optimal biomarker? she asks. “This would be a marker that is driven by hypothesis and backed by a considerable level of evidence in the literature. It needs to be reproducible, and it certainly has to have some sort of meaning, a prognostic effect, and ideally also predictive power. It has to be cost-effective and easy to implement. So we are looking for tumor budding as a biomarker to check all of these boxes” (Altman DG, et al. *PLoS Med.* 2012;9[5]:e1001216).

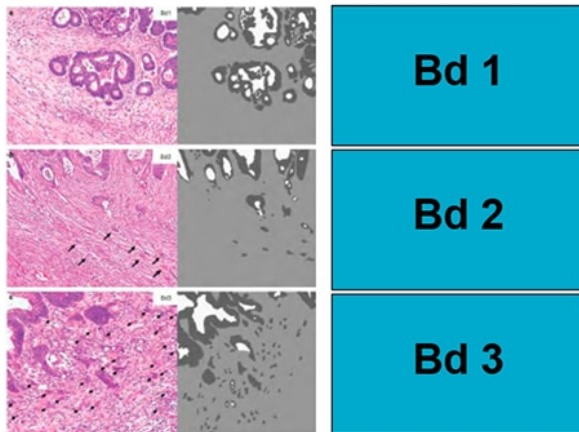
For tumor budding to be used in routine reporting, consensus was needed, Dr. Dawson says, and important questions needed to be addressed. Where should tumor budding be assessed within a tumor? “What will be the optimal field number and size of the field where we should be counting tumor budding? Should we even be counting tumor budding or just eyeballing it? And should this be done on H&E or immunohistochemistry?”

Different groups advocated for different methods over the years. “So there were a lot of potential scoring systems out there, and we knew that if tumor budding was ever going to make it into the clinic, we would need to have some sort of consensus scoring method,” Dr. Dawson says. That was the goal of the 2016 International Tumor Budding Consensus Conference (ITBCC) in Bern, Switzerland (Lugli A, et al. *Mod Pathol.* 2017;30[9]:1299-1311). “We wanted to establish a set of guidelines that was based on the highest level of evidence in the literature.”

The first step in the ITBCC consensus method for reporting tumor budding in colorectal cancer is to define the field (specimen) area for the 20× objective lens of the microscope based on the eyepiece field number diameter. When looking at the slides and inspecting the invasive front for lymphovascular invasion, tumor grade, depth of invasion, perineural invasion, and so on, make a “mental note” of where the most buds are, Dr. Dawson says, adding, “This can really be done by eyeballing.” Then return to that slide and scan all of the invasive material. “Do that until you feel comfortable that you have identified the hotspot. At that hotspot, go up to 20× and then count the tumor buds you see. Then you will end up with a number.”

The pathologist has to determine whether to normalize this number. “So what is this normalization? The ITBCC method is standardized for an area of 0.785 millimeters square,” she says. And that is the area that pathologists are seeing at 20×, if their field number diameter is 20 mm. “In Japan, a lot of pathologists use 20-millimeter field number diameter eyepieces. And unfortunately in North America and Europe, many of us use 22-millimeter field number diameter eyepieces.” The area at 20× is 0.950 mm². “So you need to divide the number of buds by 1.21.” Next check whether the number of buds corresponds to Bd1, Bd2, or Bd3. (Bd1 is zero to four buds, Bd2, five to nine buds, and Bd3, 10 buds or more.) “This is low-, intermediate-, or high-grade tumor budding.”

Fig. 2. ITBCC 2016
Tumor budding grades



Reprinted by permission from Copyright Clearance Center: Springer Nature, *Modern Pathology*. Lugli A, Kirsch R, Ajioka Y, 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. Copyright 2017.

Members of the ITBCC intentionally chose a three-tier system because it accommodates clinical situations that have different clinical endpoints. “So you can use a three-tier system for endoscopically resected pT1 colorectal cancers where having Bd1 is okay, but as soon as you get into Bd2 or Bd3, this is considered a risk factor for lymph node metastases.” A three-tier system can also be used for stage II patients, where the endpoint is tumor recurrence and patient survival, Dr. Dawson says. “So you have to set your threshold a bit higher. And in these cases, Bd1 and Bd2 are tolerated, and only Bd3 is considered a risk factor.” (**Fig. 2**).

At the consensus conference, there was also dialogue about whether pathologists should report the number of buds they see, or just the cutoffs, which would be Bd1, Bd2, and Bd3. “Cutoffs are very convenient for clinicians because it makes clinical management so much easier if something falls into a category,” she says, “but the truth is that budding lies on a biological spectrum. So cutoffs will ultimately mischaracterize the extent of risk variation within a certain group.” For example, say two patients have colorectal cancer, one with 11 tumor buds and the other with 200. “They are both high-grade budders, but which of the tumors is more aggressive?” It is the patient with 200 buds because the risk rises per bud. So pathologists should report the number of buds and the grade category, she says.

The ITBCC criteria were implemented in the 2017 version of the CAP protocol for the examination of specimens from patients with primary carcinoma of the colon and rectum. “That was a very important step toward getting tumor budding entered and into routine reporting,” Dr. Dawson says. “We report tumor budding because it has potential impact on prognosis and benefit to patients. And besides the CAP protocol, it was also important for us to get tumor budding into other major guidelines. And we were happy to see tumor budding listed as an additional prognostic factor by the Union for International Cancer Control.”

pT1 colorectal cancers are the clinical scenarios that have been best studied for tumor budding. “Here we are interested in budding as a predictor of lymph node metastases,” she says, and the clinical decision that has to be made is whether the patient needs a resection. “How high is the risk of the patient having lymph node metastases? Or can we have a good conscience and sleep well at night just leaving the patient alone?”

A systematic review published in 2013 found the strongest independent predictors of lymph node metastasis to be lymphatic invasion, submucosal invasion ≥ 1 mm, tumor budding, and poor histological differentiation (Bosch SL, et al. *Endoscopy*. 2013;45[10]:827–834).

A meta-analysis published in 2017 found a strong association between the presence of tumor budding and risk of nodal metastasis in pT1 CRC (Cappellesso R, et al. *Hum Pathol.* 2017;65:62–70).

Another clinical scenario is stage II CRC where the pathologist isn't forecasting lymph node metastasis, she says, but instead looking at tumor budding as a factor for tumor progression and patient survival. "The clinical decision that needs to be made here is can or should a patient receive adjuvant chemotherapy?"

In stage II CRC, Dr. Dawson says, "it is a real matter of debate if patients should get chemotherapy or not." Stage II has a "huge spectrum" of patients, she says, some of whom live a long time with their disease and others who have aggressive tumors. Pathologists "are under pressure to select patients who have aggressive disease. That is where tumor budding comes in because high-grade tumor budding predicts aggressive disease."

Numerous studies have found that "tumor budding is prognostically relevant independent of stage. That's why you can argue that tumor budding is important information across all stages of colorectal cancer. But for treatment management decisions, tumor budding will not play a role in stage III and stage IV." The level of evidence in the literature is sufficient to support reporting of tumor budding in pT1 and stage II colorectal cancer.

Preoperative biopsies of colon and rectal cancer are the third clinical scenario and one she describes as promising. "If we could take information from a preoperative biopsy and predict clinical response to neoadjuvant therapy, this would be very useful for patients," she says. Intratumoral budding is associated with higher T stage, higher N stage, and other aggressive features such as lymphovascular invasion. It is also associated with peritumoral budding and with survival. Here too the increased number of tumor buds presents a greater risk. "Again this is all on a biological spectrum," she says.

"A study by colleagues in Ireland was able to demonstrate that a higher number of buds seen in a preoperative biopsy was associated with a poorer response to neoadjuvant therapy. This is the information we want to have" (Rogers AC, et al. *Mod Pathol.* 2014;27[1]:156–162).

"We know that budding in biopsies correlates with budding in the resection specimen, and that budding in biopsies predicts lymph node and distant metastases, as well as patient survival," Dr. Dawson says. "But it's going to be tough to get this implemented because there are going to be a lot of questions in terms of quality measurement that need to be addressed.

"For instance, how many biopsies are going to be needed? How much invasive tumor do we need? How deep do the biopsies need to be taken, et cetera?" And most pathologists know that diagnosing invasive colorectal cancer on biopsies can sometimes be challenging, she notes. "So these are things that will definitely need to be addressed by subsequent studies before we use budding in biopsies."

In speaking about the biology of tumor budding, Dr. Dawson says tumor buds have different protein expression profiles than the main tumor body. "And it appears that tumor buds show overexpression of markers related to epithelial-to-mesenchymal transition, cell migration and cell survival, and cell differentiation and cell proliferation. Ki-67, for example, is downregulated in these cells in comparison to the main tumor body.

"Most of this has to do with Wnt pathway deregulation," Dr. Dawson says. It begins with a mutation of the *APC* gene and leads to the internalization in nuclear translocation of beta-catenin. This creates a complex that acts as a transcription factor causing the upregulation of genes, for instance, involved in migration. Tumor budding was recently shown to be associated with *BRAF* and *KRAS* mutations (Trinh A, et al. *Br J Cancer.* 2018;119[10]:1244–1251).

A study of 238 mismatch repair deficient colorectal carcinomas found that budding can be seen less in MMR-d CRC. "This probably also has to do with the lymphocyte infiltration," she says, noting that budding-to-lymphocyte ratio could be assessed in the future. "Nevertheless, when seen in these tumors, tumor budding retains its prognostic value" (Ryan É, et al. *Am J Surg Pathol.* 2018;42[1]:60–68).

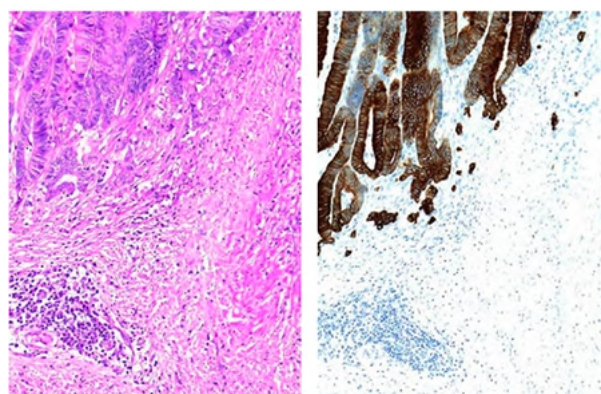
It would seem intuitive, Dr. Dawson says, that the high-grade tumor budding cases would fall into the consensus

molecular subtype 4 mesenchymal category. A 2018 study of four cohorts had “a classifier RNA transcription profiling for all of them. And it was demonstrated that in all four cohorts, high-grade tumor budders were more likely to be of the CMS4 mesenchymal subtype. So this is the best evidence we have that high-grade tumor buds are associated with and linked to the EMT process” (Trinh A, et al. *Br J Cancer*. 2018;119[10]:1244-1251).

A morphological difference can also be seen in tumor buds, she says, “especially if you can visualize tumor buds with an immunostain, say a cytokeratin stain. Then you can be surprised that those start to look a bit more spindly. So that would be a morphological correlate of transitioning more toward a mesenchymal type.”

It’s well known that certain scenarios of tumor budding assessment in CRC can be challenging and might need special explanations, she says. Examples are tumors with inflammatory infiltrate, many stromal cells, angry fibroblasts, areas of glandular fragmentation, mucinous/signet cell differentiation, and after neoadjuvant therapy (Cho SJ, et al. *Arch Pathol Lab Med*. 2018;142[8]:952-957).

Fig. 3. Tumor budding assessment
Prominent inflammatory infiltrate



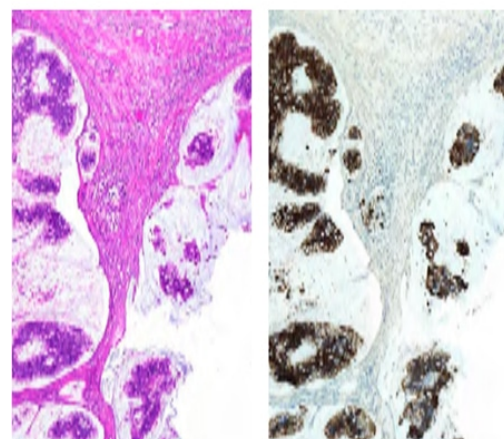
Sometimes, especially in MSI-high tumors, pathologists will see a prominent inflammatory infiltrate that can make it challenging to find the buds, Dr. Dawson warns. “A keratin stain will highlight the tumor buds, and it will make things a bit easier for you.” (**Fig. 3**).

Pathologists can be surprised at how many buds they see on the keratin stain that they didn’t identify on the H&E, Dr. Dawson says. “That’s okay. We know that you score far more tumor buds on a keratin stain. Then you have to go back to the H&E and count the tumor buds on the H&E.” She says they talked about this at length during the consensus conference and concluded that most of the evidence in the literature was based on H&E stains. “So that’s why we agreed to assess tumor budding on H&E stains, but with the option to do a keratin stain for orientation purposes only in difficult cases.”

Dr. Dawson also cautions that mucinous cancers sometimes produce a lot of mucin and the tumor cells become trapped in mucin pools. “The real tumor buds, the real deal, are able to migrate through tissue. The migration through tissue is what is going to get them into the vessels, the lymph nodes, the liver, the lungs and everywhere,” she says. “Tumor buds need to be in tissue and not in a mucin pool.” (**Fig. 4**).

In the rare instance that a colorectal cancer is only mucinous, the pathologist can say tumor budding doesn’t apply in this case and provide a comment, Dr. Dawson says. In practice, pathologists should not count tumor buds in areas of mucinous and signet ring cell differentiation and glandular fragmentation, she says.

Fig. 4. Tumor budding assessment
Mucinous CRC



After neoadjuvant therapy, tumor budding is not reported because it's too hard to discriminate which cells are just residual tumor cells after therapy and which ones are the tumor buds, although some studies point toward tumor budding as an adverse marker for survival in these patients, she says. The pathologist can say that tumor budding is often not applicable in these cases (**Fig. 5**).

Interobserver variability is an important issue in tumor budding, Dr. Dawson says. "Typically, interobserver variability for budding has a very wide range, from fair to very good, and this all depends on multiple criteria."

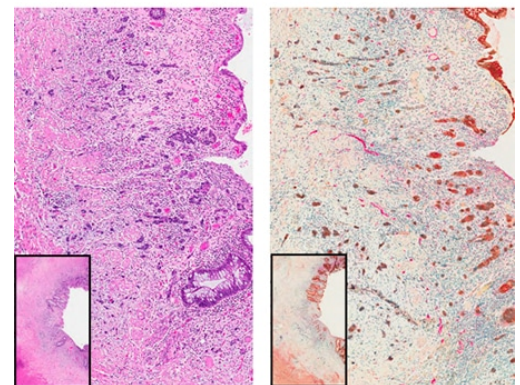
Two studies examined interobserver variability when using the ITBCC guidelines to assess and report tumor buds. The first demonstrated very poor agreement (Martin B, et al. *Virchows Arch.* 2018;473[2]:189-197), and there are two likely reasons, she says. "One was that they did the variability, but they did agreement for categories and not for numbers," she says. "So the cases where somebody would say low-grade budding and the other person would say high-grade budding [were] actually very rare."

Case mix is also a factor. "They had only very little Bd3 tumor, so most of the cases hovered around Bd1 and Bd2, and that's when they got the high interobserver variability." Interobserver variability for pT1 colorectal cancers tends to be higher, she says, probably because the invasive margin is so much smaller. "So people zoom into the same hotspot."

The second study exhibited much better interobserver variability, verified on immunohistochemistry (Barel F, et al. *Pathology.* 2019;51[1]:46-54). Based on Kappa and intra-class correlation coefficients, good to very good interobserver agreement was obtained by analyzing vertical and lateral margins, submucosal invasion, tumor differentiation, and lymphovascular invasion. It was fair based on H&E but good using IHC.

Should pathologists do budding on IHC or on H&E? "Now this is quite conflicting in the literature. There are studies that say that IHC has better results in terms of interobserver variability, and others that say it should be done on H&E. The pro of IHC is that it highlights the buds. There are also some cons. So you have to be aware of the fact that it will be more difficult to see an individual bud or to detect a tumor cell on IHC because the nucleus is often obscured, and you don't have the same morphology."

Fig. 5. Tumor budding assessment
After neoadjuvant therapy



Tumor budding is such a robust biomarker that it fundamentally works for nearly every type of solid cancer or carcinoma, Dr. Dawson says, pointing to an article that reported that tumor budding is associated with lymph node metastasis and poor survival in patients with esophageal and gastric intestinal-type adenocarcinoma (Berg KB, et al. *Mod Pathol.* 2018;31[6]:862-872). "This also has similar potential implications as in pT1 colorectal cancer." Pathologists can use tumor budding to decide whether the patient needs a resection, or if they are okay with an endoscopic mucosal resection or an endoscopic submucosal dissection specimen, she says.

"Tumor budding is also significant for oral squamous cell carcinoma and breast carcinoma. Obviously, this is limited to ductal breast adenocarcinoma because in lobular, by definition, all cells are single tumor cells and on their own, and they don't all qualify as being tumor buds," Dr. Dawson says. Tumor budding has also been shown to be prognostically relevant in lung cancer, both adenocarcinoma and squamous cell carcinoma, and in urothelial cancer.

Dr. Dawson says she isn't aware of tumor budding having been incorporated into any major reporting protocol except colorectal cancer. "So what goes beyond that is more of an experimental setting, or somebody may add it in a comment, but it's more of a free-for-all," she says. "We have generated a lot of studies on tumor budding in

pancreatic cancer, and it stratifies wonderfully in ductal pancreatic cancers. So we report that as an additional factor that our clinicians are interested in, because they help us with our studies as well. But it wouldn't be a factor that would tip your scale to treat the patient any differently at the moment." □

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