Tumor Budding in Colorectal Adenocarcinomas

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Budding means different things to different people!
Tumor budding – from a historical point of view

• Described for the first time in the 1950’ as ‘tumor sprouting’ by Imai

• Was introduced in English literature by Hase et al in 1993

• Loosely defined as:
  • Single tumor cells or small groups of tumor cells (often< 5) at the invasive border of adenocarcinomas (peri-tumoral budding - PTB) or within the main tumor (intra-tumoral budding - ITB).

• Is most often used in colorectal carcinomas
The biology of tumor budding is only partly known, but a main theory is that this process is a part of the epithelial-mesenchymal transition (EMT)
Scoring of tumor budding – from a historical perspective

• In 2002 Ueno et al published a paper on tumor-budding - using systematic quantification of the number of ‘buds’ per field of vision

• A budding focus represents a single tumor cell or a group of maximum 4 tumor cells at the invasive margin

  • The distinction between low and high degree of budding varies between publications, but the cut off is often around 9-10 foci per field of vision using 20-25X magnification

• However, if the number is below the chosen cut off, some authors refer to it as absence of budding
The problem with scoring of tumor budding – low degree of reproducibility

• Scoring was/is often performed by eyeballing on H&E stained randomly chosen tumor slides

• It has been shown in several studies that the reproducibility was/is low to moderate using H&E stained slides

• Several authors have recommended the use of cytokeratin staining to enhance the number of budding foci and the reproducibility of scoring
H&E vs. cytokeratin staining – pitfalls can be inflammation and fragmentation of tumor due to necrosis. In single cells, a nucleus must be visible for the cell to count as a ‘budding cell’.
Many pathologists felt like this.......
Since then data kept piling up for the importance of tumor budding in colorectal cancer – despite the method used for scoring

**TUMOR BUDDING**

*Potential clinical scenarios*

- **Malignant polyps**
  - Tumor budding as a predictor of lymph node metastases
  - Clinical implication: surgical resection

- **Stage II CRC**
  - Tumor budding as an adverse prognostic factor
  - Clinical implication: risk adapted follow up and adjuvant therapy

- **Pre-operative biopsies of colon and rectal cancer**
  - Tumor budding as an adverse prognostic factor and predictor of lymph node and distant metastasis
  - Clinical implication: neo-adjuvant therapy and risk adapted surgery
The International Tumor Budding Consensus Conference (ITBCC) 2016

• On the initiative of Professor Alessandro Lugli, The Department of Pathology, Bern, Switzerland this meeting took place in April 2016.

• 25 participants (22 pathologists, 2 surgeons and 1 translational researcher) from Europe, Canada, the United States and Japan.

• The ITBCC entailed 9 sessions with presentations, a pre-meeting survey, and an e-book including the main literature on tumor budding in CRC.
The following 10 statements achieved consensus

- Tumor budding is defined as a single tumor cell or a cell cluster consisting of 4 tumor cells or less.

- Tumor budding is an independent predictor of lymph node metastasis in pT1 colorectal cancer.

- Tumor budding is an independent predictor of survival in stage II colorectal cancer.

- Tumor budding should be taken into account along with other clinico-pathological features in a multidisciplinary setting.
The following 10 statements achieved consensus

• Tumor budding is counted on H&E (can be supplemented with panCK)

• Tumor budding is assessed in one hotspot (in a field measuring 0.785 mm²) at the invasive front

• A three-tier system should be used along with the budding count in order to facilitate risk stratification in colorectal cancer
  
  • Bd1 (low): 0-4 buds
  
  • Bd2 (intermediate): 5-9 buds
  
  • Bd3 (high): ≥10 buds
The following 10 statements achieved consensus

• Tumor budding and tumor grade is not the same
• Intra-tumoral budding exists in colorectal cancer and is related to lymph node metastasis
• Tumor budding should be included in guidelines/protocols for colorectal cancer reporting
Recommendations for reporting tumor budding in colorectal cancer

- Define the field (specimen) area of the 20X objective lens of your microscope based on the eyepiece field number (FN) diameter (* See table)
- Select the H&E slide with the greatest degree of budding at the invasive front
- Scan 10 individual fields (20X objective) to identify ‘the hot spot’ at the invasive front
- Count the buds in the selected hot spot (20X objective)
Recommendations for reporting of tumor budding in colorectal cancer

• Divide the bud count by the normalization factor to determine the tumor bud count per 0.785 mm$^2$
  • Bud count per 0.785 mm$^2$ = Bud count 20X objective/Normalization factor (*See Table)

• Select the budding (Bd) category based on the bud count and indicate the absolute count per 0.785 mm$^2$
  • Example: Bd3 (high), count 14 per 0.785 mm$^2$
To adjust the tumor bud threshold to your field of view:
Multiply by the normalization number

To standardize your tumor bud count data for submission:
Divide by the normalization number

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ITBCC International working groups - subjects

- Stage II projects
- pT1 projects
- Preoperative biopsies
- MSI cancers and metastasis
- Digital Pathology
- Poorly differentiated clusters
Comprehensive assessment of tumour budding by cytokeratin staining in colorectal cancer.


• Introducing the concept of overall tumor budding (OTB) that seems just as valuable as PTB and ITB

• Easier to define on a panCK stained, digitalized slide (no definition of invasive margen)
Danish Guidelines from the Danish Colorectal Cancer Group (DCCG)

• Follow the guidelines from ITBCC with a possibility to indicate bud count on both H&E and PanCK

• Will be evaluated in working groups this fall and implemented from January 1st 2018

• Digital training sets will be prepared and sent to Danish GI pathologists in the DCCG working group

• A workshop for the Danish GI pathologists in the DCCG working group is planned in the fall 2017
Digital analysis of tumor budding – APP developed in collaboration with Visiopharm

- 10117 - PCK, Tumor Budding, Colorectal Cancer


- Work in progress
  - Refinement and adjustment of the APP according to the international guidelines
  - Testing on new patient samples
Define the invasive border manually (panCK staining)  
Zoom in on the invasive border
Elimination of large structures

Zoom in on elimination of large structures
Define tumor buds (green) – avoiding diffuse areas (pink) some with correct size, but with a weak panCK staining (necrosis?)
10 hotspots coloured from blue to red – red being the ‘hottest’

Zoom in on hotspots
Hopefully we can become