WHAT SHOULD WE DO WITH TUMOUR BUDDING IN EARLY COLORECTAL CANCER?

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DISCLOSURE

None

CANCER STAGING
TNM and prognosis in CRC

COLORECTAL CANCER
Prognostic factors grid

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>Tumour related</th>
<th>Host related</th>
<th>Environment related</th>
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<tbody>
<tr>
<td>Essential</td>
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<td>Age</td>
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<tr>
<td></td>
<td>N category</td>
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<td>Screening programme</td>
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<tr>
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<td>M category</td>
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<td>CRM</td>
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<tr>
<td>Additional</td>
<td>Vascular/lymphatic invasion</td>
<td>Race</td>
<td>Socioeconomic status</td>
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<td></td>
<td>Perineural invasion</td>
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<td>Centre volume and experience</td>
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<tr>
<td></td>
<td>Tumour grade</td>
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<td>Tumour budding</td>
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<td>Perforation</td>
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<td>KRAS</td>
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<td>MSI</td>
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<td>New and promising</td>
<td>Molecular profile</td>
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</table>
TUMOUR BUDDING IN CRC

At a glance

Definition
Single tumour cells or clusters of up to 4 tumour cells at the invasive margin

Biological aspect
Presence in the TME
Involved in EMT
Interaction tumour buds/immune cells

Clinical aspect
Biomarker of tumour progression
Independent prognostic factor
Predictive value: not known yet

META-ANALYSIS OF TUMOUR BUDDING IN CRC STAGE I-IV

Endpoint: lymph node metastases

1993-2016
34 papers
n=7821

OR 4.94, 95% CI 3.96–6.17, p<0.00001

Rogers et al, BJC 2016

META-ANALYSIS OF TUMOUR BUDDING IN CRC STAGE I-IV

Endpoint: recurrence and cancer-related death

OR 5.50
95% CI 3.64–8.29
p<0.00001

OR 4.51
95% CI 2.55–7.99
p<0.00001

Rogers et al, BJC 2016
POTENTIAL CLINICAL SCENARIOS FOR TUMOUR BUDDING IN CRC

pT1 CRC
- Tumour budding as a predictor of lymph node metastases
  Clinical implication: resection

Stage II CRC
- Tumour budding as a factor of tumour progression and survival
  Clinical implication: adjuvant therapy

Pre-operative biopsies of colon and rectal cancer
- Tumour budding as a factor of tumour progression and predictor of regression grade and (non) response to neo-adjuvant therapy
  Clinical implication: neo-adjuvant therapy

RISK FACTORS FOR AN ADVERSE OUTCOME IN EARLY INVASIVE CRC

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of Patients</th>
<th>Beta (95% CI)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Lymphatic invasion</td>
<td>121</td>
<td>7.3 (5.3-9.5)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Lymph node invasion</td>
<td>78</td>
<td>13.1 (10.2-16.4)</td>
<td>0.022</td>
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<tr>
<td>Submucosal invasion</td>
<td>75</td>
<td>7.3 (5.4-9.4)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Poor histological differentiation</td>
<td>75</td>
<td>7.3 (5.4-9.4)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Cappellesso et al, Hum Path 2017

TUMOR BUDDING AS A RISK FACTOR FOR NODAL METASTASIS IN pT1 CRC
A META-ANALYSIS

Table 4

<table>
<thead>
<tr>
<th>Factor</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>OR</th>
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<tr>
<td>Tumour budding</td>
<td>80.1</td>
<td>71.8</td>
<td>26.7</td>
<td>54.9</td>
<td></td>
</tr>
<tr>
<td>Submucosal invasion ≥ 1mm</td>
<td>80.1</td>
<td>71.8</td>
<td>26.7</td>
<td>54.9</td>
<td></td>
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</tr>
</tbody>
</table>

Bosch et al, Endoscopy 2013

PREDICTING LN METASTASIS IN pT1 CRC:
A systematic review for risk factors providing rationale for therapy decisions

17 studies, 3621 patients; strongest independent predictors of LNM:
- Lymphatic invasion (RR 5.2, 95% CI 4.0 – 6.8)
- Submucosal invasion ≥ 1mm (RR 5.2, 95% CI 1.6 – 15.4)
- Tumour budding (RR 5.1, 95% CI 3.6 – 7.3)
- Poor histological differentiation (RR 4.6, 95% CI 3.3 – 6.9).

Ueno et al, Gastroenterology 2004

41 studies
N=10137
Tumour budding and LNM:
OR 6.44
95% CI 5.26-7.87
p<0.0001
How should tumour budding be reported?

Lack of consensus for...

- best topographic area of assessment
- field number and size (1, 5 or 10 HPF; 20x or 40x magnification)
- evaluation methods (semiquantitative, quantitative with cutoffs or continuous scoring)
- H&E vs Immunohistochemistry

International Tumour Budding Consensus Conference (ITBCC)

Project started at the USCAP in Boston 2015

Pre-meeting survey (8 questions)
9 sessions
7 presentations & texts
Literature / e-book
Applied method: GRADE

ITBCC 2016

How to report tumour budding

1. Define the field (or fields) that will be used for counting tumor buds
2. Select an area with the greatest degree of budding and the tumor interface (or areas)
3. Count the number of buds in the selected area (or areas)

Lugli et al, Modern Pathology 2017
**ADDITIONAL PRACTICAL ASPECTS**

Histological subtypes
- Signet-ring cell and mucinous carcinoma
- Micropapillary carcinoma

Peritumoral inflammation
- Medullary carcinoma
- MSI-H CRC

Glandular fragmentation

Neo-adjuvant therapy in rectal cancer

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**RESULT OF THE ITBCC 2016**

Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016

TUMOUR BUDDING IN CRC

Validation and clinical implementation

TME score (buds vs immune cells)

Tumour buds characterization on RNA and DNA level

Digital analysis

PROGNOSTIC IMPACT OF TUMOUR BUDDING
GRADE IN STAGES 1-3 COLON CANCER

A retrospective cohort study

n = 4196
Colon Cancer
Stage I – III
ITBCC Scoring System

EDUCATIONAL AND REPORTING ASPECTS

COLLEGE of AMERICAN PATHOLOGISTS

Protocol for the Examination of Specimens From Patients With
Primary Carcinoma of the Colon and Rectum

n = 328
Colon Cancer
Stage I – IV
ITBCC Scoring System

SITE-SPECIFIC DIFFERENCES IN COLONIC ADENOCARCINOMA

KRAS mutations and high tumour budding are more frequent in cecal adenocarcinoma
HISTOLOGIC FACTORS ASSOCIATED WITH NEED FOR SURGERY IN PATIENTS WITH PEDUNCULATED T1 CRC

Table 2: Analysis of Factors in Cases with Metastases vs Matched Controls (Multivariable Metastases in Pedunculated T1 CRC)

<table>
<thead>
<tr>
<th>Factor</th>
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<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Studio</td>
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<tr>
<td>Sex</td>
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<td>Tumour size</td>
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<td>Lymphovascular invasion</td>
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<tr>
<td>Metastasis</td>
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</table>

Survival curves of Stage II colon cancer could be further stratified by TB (log-rank tests: p < 0.001).

N=135 stage II colon cancer
High-grade TB associated with pT4 (p=0.003), presence of lymphovascular invasion (p=0.001) (and DSS 89% (Bd1); 73% (Bd2); 52% (Bd3), p=0.001)

TUMOUR BUDDING AND POORLY DIFFERENTIATED CLUSTER IN PROGNOSTICATION IN STAGE II COLON CANCER
INTEROBSERVER VARIABILITY IN THE H&E-BASED ASSESSMENT OF TUMOUR BUDDING IN pT3/4 COLON CANCER

Does it affect the prognostic relevance?

6 investigators with different levels of experience

Tumor budding on H&E slides in 244 cases with primary diagnosed (2002–2011) colon carcinoma (pT3/4, N+/-, M0).

BD 3 was significantly associated with an adverse outcome (overall survival p = 0.03, cancer-specific survival p = 0.06) and the occurrence of distant metastasis (p = 0.009).

The kappa values among the investigators have a range between 0.077 and 0.357 (median 0.166). Total agreement of all investigators existed in 109 cases (44.7%).

Evaluation of tumor budding on H&E slides in pT3/4 colon cancer goes along with a considerable interobserver variability among investigators of different levels of experience.

Martin et al, Virchows Archive 2018

BUDDING AND TILS – COMBINATION OF BOTH PARAMETERS PREDICTS SURVIVAL IN CRC AND LEADS TO NEW PROGNOSTIC SUBGROUPS

The combination of both markers revealed highly significant differences in overall survival (OS) between the four groups (p=0.001).

The low budding/>5% TILs-group showed longest OS, followed by high budding/>5% TILs cases, followed by tumors with low budding/<5% TILs. OS was worst for the high budding/<5% TILs-group.

The combined score also correlated with T-, N-, M-, L-, V-staging, development of disease relapse and distant metastasis.

Lang-Schwarz et al, Hum Pathol 2018

TUMOUR MICROENVIRONMENT SCORE (TMS)

The attacker-defender approach

The combination of both markers revealed highly significant differences in overall survival (OS) between the four groups (p=0.001).

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Lang-Schwarz et al, Hum Pathol 2018

INTERNATIONAL VALIDATION OF THE CONSENSUS IMMUNOSCORE FOR THE CLASSIFICATION OF COLON CANCER

A prognostic and accuracy study

Pages et al, Lancet 2018
MOLECULAR ASPECTS OF TUMOUR BUDDING

Tumour budding in colorectal cancer: molecular rationale for clinical translation

Inti Zlobovic and Alessandro Lugli

Precise medicine and personalized healthcare call for reproducible and standardized predictive and prognostic biomarkers that can influence the clinical management of patients with cancer. In colorectal cancer, tumour budding — a histological manifestation of tumour cell invasion that is linked to epithelial-mesenchymal transition — is now emerging as one such factor.

CMS SWITCH IN TUMOUR BUDS?

5 CRC cases
Budding areas
EMT+, main tumour EMT-
CMS switch
(CMS2 ➔ CMS4)

TUMOUR BUDDING AND THE CMS CLASSIFICATION 2015

CMS SWITCH IN TUMOUR BUDS?

5 CRC cases
Budding areas
EMT+, main tumour EMT-
CMS switch
(CMS2 ➔ CMS4)

MULTICENTRIC TUMOUR BUDDING PROJECTS

DCS Grant
Lead: Iris Nagtegaal, Nijmegen (NL)
NL, CH, D, IRL, JPN, UK, F, CAN

Aim: Development and multicentre validation of digital image analysis algorithms for quantification of tumour budding from whole slide images

Swiss Cancer League Grant
Lead: Heather Dawson, Alessandro Lugli
SAGIP and IBC members

Aim: Full characterization of approx. 1000 pT1 tumours for clinical endpoint LN+ under consideration of tumour grade, depth of infiltration, ITBCC versus Pan-CK, Lymphatic invasion (double stain D2-40/Pan-CK), Blood vessel invasion (double stain CD31/Pan-CK)
The ITBCC scoring system is a promising basis for large multicentric retrospective and prospective validation studies.

Tumour budding is an additional prognostic factor in pT1 CRC and should be considered along with other clinico-pathological factors.

Tumour budding perspectives may be:
- Gene expression profiling of tumour buds
- TME score
- Digital analysis of tumour budding

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