

## CHARACTERIZATION OF TUMOR BUDDING AND THE TUMOR MICROENVIRONMENT IN COLORECTAL CANCER USING HYPERPLEX IMMUNOFLUORESCENCE

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**Background** Current colon cancer classification, prognostication, and therapy decisions are based mainly on cancer staging.<sup>1-2</sup> However, additional biomarkers are needed to improve patient stratification and complement treatment decision-making strategies. Tumor budding is recognized as an independent prognostic factor in a variety of solid cancers.<sup>3-4</sup> Tumor buds (TBs) are isolated single tumor cells or groups of up to four tumor cells, located both peri- and intra-tumorally. A higher tumor bud count correlates with poor prognosis in colorectal cancer (CRC), and it is hypothesized that a subset of TBs represents, at least in part, an Epithelial-Mesenchymal Transition (EMT) state.<sup>5</sup> To explore this hypothesis further, we developed a sequential immunofluorescence (seqIF™)-based panel for a more spatial approach to characterizing TBs and the tumor microenvironment.

**Methods** Human CRC sections from different cohorts underwent initial pre-processing on PT Module™ (Thermo Fisher) followed by automated cycles of seqIF and imaging performed on COMET™ (Lunaphore Technologies). Hyperplex panels consisting of >20 protein biomarkers were generated using off-the-shelf antibodies and served to characterize the tumor-stroma interactions in a preliminary cohort of samples. In a second stage, the spatial screening was performed on larger cohorts of neoadjuvantly treated vs. untreated CRC patient samples. The final characterization of TBs and their interaction with the surrounding milieu was performed by downstream image analysis of the stained tissues.

**Results** With COMET™ we built an optimized panel including >20 biomarkers for characterization of the tumor and the surrounding stroma. The panel robustness was tested in 15 different CRC cases and resulted in outstanding staining quality with high signal-to-background ratio and elution efficiency (>98%) for all biomarkers. The developed panel allowed the visualization of inter- and intra-tumoral heterogeneity of stained cases and the identification of cell populations including immune cells, cancer-associated fibroblasts, and tumor cells. In line with previously described EMT phenotype of TBs,<sup>6-7</sup> our observations showed loss of EpCAM and E-Cadherin in a group of TBs verifying decreased epithelial phenotype (figure 1).

**Conclusions** The >20-plex panels generated on COMET allowed (i) to discriminate TBs signatures within the depth of tumor-stroma interactions and (ii) to extract valuable TBs features in terms of marker expression. Next, crucial info about TBs phenotypes and their cellular neighborhood will be obtained through an unsupervised analysis approach. This will finally serve to better identify novel immunograms of these cellular entities, thus better defining their therapeutic potential for a personalized medicine approach.

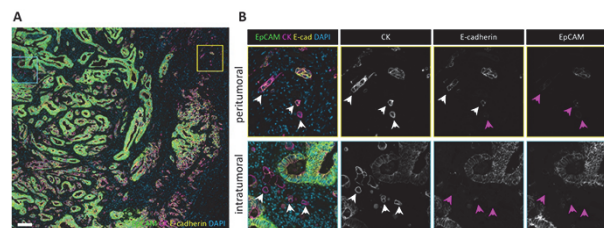
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### REFERENCES

1. Brierley JD, Gospodarowicz MK, Wittekind C. TNM Classification of Malignant Tumours. 8th edition. Wiley Blackwell, 2017.
2. Amin MB, et al. (eds). AJCC Cancer Staging Manual. 8th edition. Springer, 2017.
3. Berg KB, Schaeffer DF. Tumor budding as a standardized parameter in gastrointestinal carcinomas: more than just the colon. *Mod. Pathol* 2018;**31**:862-872.

4. Almangush A, Salo T, Hagstrom J, Leivo I. Tumour budding in head and neck squamous cell carcinoma – a systematic review. *Histopathology* 2014;**65**:587-594.
5. Rogers AC, et al. Systematic review and meta-analysis of the impact of tumour budding in colorectal cancer. *Br. J. Cancer* 2016;**115**:831-840.
6. Grigore AD, et al. Tumor budding: the name is EMT. Partial EMT. *J. Clin. Med.* 2016;**5**(5):51.
7. De Smedt L, et al. Expression profiling of budding cells in colorectal cancer reveals an EMT-like phenotype and molecular subtype switching. *Br. J. Cancer* 2017;**116**:58-65.

**Ethics Approval** The study was approved by Kantonale Ethik-kommission Bern, approval number KEK#2017-01803.



**Abstract 1459 Figure 1** Loss of epithelial markers in peri- and intra-tumor

A. Selected composite panel showcasing the loss epithelial markers with remarkable loss of EpCAM in the tumor front. Scale bar is 200 μm. B. Zoom-in images of representative areas for peri- and intra-tumoral buds. Tumor buds are shown with arrowheads and the TBs with loss of E-cadherin or EpCAM are highlighted with magenta-colored arrowheads.

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