this study was to evaluate RKIP expression and to determine its association with clinicopathological features, including EMT in form of tumor budding in pancreatic ductal adenocarcinoma (PDAC).
Methods. Staining for RKIP was performed on a multipunch Tissue Microarray (TMA) of 120 well-characterized PDACs with clinicopathological, follow-up and adjuvant therapy information. RKIP-expression was assessed separately in the main tumor body and in the tumor buds. Another 3 TMAs containing normal pancreatic tissue, precursor lesions (Pancreatic Intraepithelial Neoplasia, PanINs) and matched lymph node metastases were stained in parallel. Cut-off values were calculated by receiver operating characteristic (ROC) curve analysis.
Results. We found a significant progressive loss of RKIP expression between normal pancreatic ductal epithelia (average: 74%), precursor lesions (PanINs; average: 37%), PDAC (average: 20%) and lymph node metastases (average: 8%, p<0.0001). RKIP expression was significantly lower in tumor buds (average: 6%) compared to the main tumor body (average: 20%, p<0.005). RKIP loss in the tumor body was associated with higher tumor grade (p=0.0389), increased T-stage (p=0.045) as well as high-grade peritumoral (p=0.0051) and intratumoral budding (p=0.0227). RKIP loss in the buds was associated with increased T stage (p=0.0091) and showed a trend towards worse patient survival (p=0.0763).
Conclusions. The progressive loss of RKIP seems to play a major role in the neoplastic transformation of pancreas and correlates with aggressive features in PDAC, especially characterized by the presence of EMT in form of tumor budding.

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FP33
Intratumoural budding (ITB) in preoperative biopsies predicts the presence of lymph node and distant metastases in colon and rectal cancer patients

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Background. In the inter-disciplinary pre-operative management of colorectal cancer patients, the pathologist's role is limited to a confirmation of malignancy from the biopsy; other relevant histomorphological parameters apply only to the surgical resection. Tumour budding (the presence of detached tumour cells) has consistently been associated across studies with lymph node (LN) and distant metastasis. Interestingly, tumour budding can also be detected in biopsies (intra-tumoural budding; ITB) and may have similar clinical importance. The aim of this study was to investigate whether ITB in pre-operative colon and rectal cancer biopsies can be translated into daily diagnostic practice.
Methods. Pre-operative biopsies from 152 patients with primary colorectal cancer underwent immunohistochemistry for AE1/AE3 to facilitate visualization of tumour buds. All biopsies from each patient were evaluated; the region containing the densest number of buds was identified. All buds in this "hot-spot" were counted at 40× (high-power field, HPF).
Results. In non-neoadjuvantly treated patients, a greater number of tumour buds in the biopsy was associated with pT stage (p=0.0143), LN metastasis (p=0.0007), lymphatic (p=0.0065) and venous vessel invasion (p=0.0318) and distant metastasis (cM1; p=0.0013). Using the logistic regression equation, a probability "scale" was developed to estimate the probability of LN and distant metastasis using the number of tumour buds (e.g. 30 buds/HPF: 86% chance of LN metastasis). Although only 19 patients received neoadjuvant therapy, a greater number of tumour buds in the pre-treatment biopsy was related to lymphatic invasion (p=0.0337). The marginal significance for pT (p=0.0623) and tumour regression grade (p=0.0778) requires confirmation on a larger cohort. The interobserver agreement was excellent (ICC: 0.813).
Conclusions. Tumour budding can be assessed in the preoperative biopsy of colon and rectal cancer patients. It is practical to evaluate, reproducible and predictive of LN and distant metastasis. ITB qualifies for further investigation in the prospective setting.

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FP34
TrkB expression in colorectal cancers highlights anoikis-resistance as a possible survival mechanism of tumor budding (EMT-like) cells

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Background. Tumour buds in colorectal cancer represent an aggressive subgroup of non-proliferating and non-apoptotic tumour cells. We hypothesize that survival of tumour buds is dependent on resistance to anoikis (cell death due to detachment from the extracellular matrix). Here we investigate the role of neurotrophic tyrosine kinase receptor TrkB, a promoter of epithelial-mesenchymal transition (EMT) and anoikis-resistance, in facilitating a pro-tumour budding phenotype.
Methods. TrkB immunohistochemistry was performed on a multiple-punch tissue microarray of 211 colorectal cancer patients. Cytoplasmic (cTrkB) and nuclear (nTrkB) staining were evaluated in tumour and tumour buds. KRAS/BRAF mutations were investigated.
Results. cTrkB and nTrkB were strongly inversely correlated in tumour (r=−0.38; p<0.0001) and tumour buds (r=−0.41; p<0.0001). cTrkB was associated with high-grade tumour budding (p<0.0001), KRAS mutation (p=0.0008) and expressed frequently in tumour buds (100/154 cases; p<0.0001). In low-grade budding cases, cTrkB was significantly associated with lymph node positivity (p=0.0555) and venous invasion (p=0.0394) with marginal significance for lymphatic invasion (p=0.0879), after adjusting for adjuvant therapy. cTrkB positive tumours (p=0.049) and those with positive

buds (p=0.0282) had more unfavorable survival time. nTrkB was expressed in low-grade budding cases (p=0.0073), BRAF wild-type tumours (p=0.0519) and expressed infrequently in tumour buds (34/154; p<0.0001).
Conclusions. TrkB appears to be involved in the promotion of tumour budding, particularly in a KRAS wild-type context and highlights a subgroup of aggressive tumour buds at the invasion front of colorectal cancers. These findings underline the potential role of anoikis-resistance in survival of EMT-like tumour budding cells in these patients.

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FP35
CpG promoter hypermethylation of CTNNB1 (β-catenin) and TWIST2 promote a high-grade tumor budding phenotype in colorectal cancer

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Background. Tumor budding in colorectal cancer is an epithelial-mesenchymal transition (EMT)-like process characterized by activation of Wnt signaling underlined by nuclear β-catenin and loss of E-cadherin expression. Here, we determine whether deregulation of WNT-related genes and known repressors of E-cadherin via CpG promoter hypermethylation promotes a pro-tumor budding phenotype.
Methods. Methylation analysis for APC, CDH1 (E-cadherin), CTNNB1 (β-catenin), TWIST, ZEB1 and ZEB2 by pyrosequencing was performed using bisulfite-converted DNA from 209 colorectal cancer patients with full clinical and pathological data. Mutational analysis for KRAS and BRAF was carried out. Tumor budding was assessed according to the 10-in-10 method. Immunohistochemistry for β-catenin, E-cadherin, VEGF and pAKT was performed using a multi-punch tissue microarray.
Results. High-grade budding was observed in 64% of CTNNB1-methylated cancers. 80% of CTNNB1-negative/TWIST-methylated cases were low-grade [p<0.0001; OR: 2.61 (95% CI:1.7–4.0)]. CTNNB1 methylation correlated with loss of E-cadherin expression (p=0.0026), and overexpression of pAKT (p=0.005) and VEGF (p=0.002). Although 66.7% of CTNNB1-methylated cases showed nuclear β-catenin versus 36.4% of CTNNB1-negative cancers, this difference was not significant (p=0.1461).
Conclusions. Hypermethylation of CTNNB1/TWIST may be involved in the process of tumor budding providing for the first time a link between epigenetic changes and a pro-budding (EMT-like) phenotype in colorectal cancer patients.

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FP36
CD8/CD45RO T-cell infiltration in pre-operative biopsies of colorectal cancer predicts histopathologic features of the matched resection specimen and survival outcome

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Background. Reliable prognostic markers based on biopsy specimens of colorectal cancer (CRC) are currently missing. We hypothesize that CD8+ T-effector and CD45RO+ memory T-cell infiltrates in biopsies may be able to predict survival outcome of CRC patients and the histopathological features of the CRC resection specimen in the pre-operative setting. We therefore aimed to develop a prognostic score based on biopsy specimens to allow earlier risk stratification of CRC patients in clinical practice.
Methods. 346 patients treated from 2002–2011 for primary CRC at the Bern University Hospital were included in the study, 185 of which had matched biopsies. Cases were re-reviewed for histopathological features including T, N, M, G, L, V and Pn. Clinical data on age, gender, tumor location, time to progression/metastasis and post-operative therapy was retrieved from patient records. Biopsy specimens were immunostained for pancytokeratin and CD8 or CD45RO. Stromal (s) and intraepithelial (i) CD8+ and CD45RO+ T-cells were assessed in 5 HPF of highest density of infiltration. T-cell infiltrates were assessed for prediction of pTNM-stage of the matched resection specimen and patient survival.
Results. A greater number of total (t) CD8 counts in the preoperative biopsy was significantly associated with more favorable survival outcome (p=0.0139) in patients with primary CRC independently of pT, pN or pM-stage and adjuvant therapy [HR=0.043 (0.26–0.71); p=0.001]. Using Classification and Regression Tree Analysis (CART), full pathological TNM stage including additional histopathologic prognostic parameters of the resection specimen could be predicted by assessment of tCD8 and sCD45RO in the pre-operative biopsy: High numbers of tCD8 in the biopsy predicted earlier T-stage (p<0.0001) in the resection specimen as well as absence of lymphatic invasion (p=0.0165). Infiltration by sCD45RO+ cells was highly predictive of the absence of nodal metastasis (p=0.0243), absence of distant metastasis (p=0.0321) absence of vascular invasion (p=0.0321) and was significantly associated with lower tumor grade (p=0.0156).
Conclusions. Evaluation of CD8+ and CD45RO+ T-cell infiltration in pre-operative biopsy specimens of CRC predicts full TNM-stage of the matched resection specimen and survival outcome. This may allow risk stratification of CRC patients at the time of diagnosis.

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