L. DE SMEDT (1), S. PALMANS (1), O. GOVAERE (1), B. BOECKX (2), D. SMEETS (2), E. GALLE (2), J. WOUTERS (1), J. DE KERVEL (3), D. ANDEL (1), T. TOUSSEYN (1), G. DE HERTOGH (1), H. PRENEN (4), S. TEJPAR (4), D. LAMBRECHTS (2), X. SAGAERT (1) / [1] KU, Leuven, Belgium, Translational Cell and Tissue Research , [2] KU, Leuven, Belgium, Laboratory for Translational Genetics, [3] UZ Leuven, Leuven, Belgium, Laboratory of Hepatology, [4] KU, Leuven, Belgium, Digestive oncology

Introduction: Tumour budding is referred to as the presence of single cells or small clusters of up to five tumour cells at the invasive margin of colorectal carcinoma and is considered as an additional prognostic marker besides TNM-staging. It is generally hypothesized, but not yet proven, that the feature of budding is the histologic representation of epithelial-mesenchymal transition (EMT).

Aim: In the present study, we aim to investigate the molecular signature of tumour budding and the corresponding tumour bulk regions.

Methods: Tumour bulk and budding areas were microdissected from eight fresh-frozen (FF) colorectal cancer samples and processed for RNA-sequencing. Since little RNA was obtained from budding cells, a special low-input (~1ng) mRNA library preparation protocol was used. Gene expression profiles of budding as compared to tumour bulk were investigated for established EMT signatures, consensus molecular subtype (CMS), gene set enrichment and pathway analysis.

Results: A total of 296 genes were differentially expressed with a FDR<0.05 and at least a twofold change between tumour bulk and budding regions. Genes that were upregulated in the budding signature were mainly involved in cell migration and survival while downregulated genes were important for cell proliferation. Supervised clustering according to an established EMT gene signature categorized budding cells as EMT-positive, whereas tumour bulk samples were considered EMT-negative. TGFβ1, TGFβ3 and CTNNB1 were potential upstream regulators of the differential expression profile hinting for active signalling through WNT and TGFβ pathway. Furthermore, a shift from the consensus molecular subtype CMS2 (epithelial) to CMS4 (mesenchymal) was observed as tumour cells transit from the tumour bulk to the tumour budding regions.

Conclusions: Tumour budding regions are characterized by a phenotype switch compared to the tumour bulk, involving the acquisition of migratory characteristics and a decrease in cell proliferation. In particular, most tumour budding signatures were EMT-positive with activation of both WNT and TGFβ signalling and a switch from an epithelial subtype (CMS2) in the tumour bulk to a mesenchymal subtype (CMS4) in budding cells.

O03

Metastatic colorectal cancer has heterogeneous immune microenvironment and mutational expression

M. VAN DEN EYNDE (1), B. MLECNIK (2), G. BINDEA (2), T. FREDRIKSEN (2), L. LAFONTAINE (2), N. HAICHEUR (3), F. MARLIOT (3), A. JOURET-MOURIN (4), C. SEMPOUX (4), J. GIGOT (5), C. HUBERT (5), A. KARTHEUSER (5), C. REMUE (5), D. LEONARD (6), J. CARRASCO (7), Y. HUMBLET (8), F. PAGÈS (3), J. MACHIELS (8), J. GALON (2) / [1] Cliniques Universitaires Saint Luc, Woluwe-Saint-Lambert, Belgium, Gastroenterology, oncology, [2] Centre de Recherche des Cordeliers, Paris, France, Team 15, [3] Hôpital Européen Georges Pompidou, Paris, France, Immunology, [4] Cliniques Universitaires Saint Luc, Woluwe-Saint-Lambert, Belgium, Pathology, [5] Cliniques Universitaires Saint Luc,

Woluwe-Saint-Lambert, Belgium, Surgery, [6] Cliniques Universitaires Saint Luc, Woluwe-Saint-Lambert, Belgium, Sugery, [7] Grand Hopital de Charleroi, Charleroi, Belgium, Oncology, [8] Cliniques Universitaires Saint Luc, Woluwe-Saint-Lambert, Belgium, Oncology
Introduction: Understanding the colorectal cancer (CRC) metastasis process is a major clinical challenge for treatment efficacy. CRC cells must successfully negotiate a series of complex steps for progression and establishment in a foreign tissue environment.
Aim: We plan to analyze the immune microenvironment within all resected metastases (Ms) and corresponding primary tumor (PT) to investigate the possible tumor immune heterogeneity and the consequence for personalized-medicine approaches.
Methods: We performed a full whole-slide quantification of immune cell densities (CD3/CD8/CD45RO/CD20/FoxP3) in the core (CT) and invasive margin (IM) on all resected synchronous (Sync) and metachronous (Metac) Ms (n=338) and available corresponding PT (n=69) from a cohort of 114 operated metastatic CRC patients (pts). The mean density value was calculated for each marker in each tumor region (CT/IM) with a dedicated image analysis software on whole-slide images. Additionally, a somatic mutation profiling (Ion Torrent PGM technology) of 50 onco- and tumor suppressor genes from Ms (Sync and Metac) and PTs was performed on a subgroup of 12 pts with the highest tumor immune heterogeneity. Comparisons were made using the t-test and Wilcoxon-Mann-Whitney test
Results: The global immune infiltration was mostly heterogeneous in each tumor region (CT/IM) within and across Ms (Sync/Metac) and PTs from all pts. CD3, CD8 and CD45RO densities were significantly higher in the Ms (CT/IM, Sync/Metac) compared to the PTs ($p<0.05$). Conversely, higher CD20 and FOXP3 densities were observed in PTs (CT; $p<0.05$). For pts with multiple Ms, the global T-cell infiltration was increased in small-sized Ms (CT/IM; $p<0.05$). The analysis of the highest infiltrated metastasis per patient, showed that pts with a large number of Ms had a significantly higher number of infiltrating immune cells compared to pts with few Ms ($p<0.05$). Focusing on the group of pts with the highest tumor immune heterogeneity, a diverse mutational expression (TP53, PIK3CA, KDR, KIT, APC and KRAS) was observed between Sync and Metac Ms and PTs.
Conclusions: A global heterogeneity of the tumor immune environment and mutational expression was observed in metastatic CRC, suggesting a genetic evolution of tumor clones during progression. It could pose major challenge to personalized-medicine and makes tumor-biopsy challenging for biomarker discovery.

O08
Proteins in early CRC stages - from proteomic discovery to immunohistochemistry tissue distribution characterisation
F. QUESADA CALVO (1), M. MEUWIS (2), V. BERTRAND (3), R. LONGUESPÉE (3), N. BLÉTARD (4), J. SOMJA (4), G. MAZZUCCHELLI (5), N. SMARGIASSO (5), D. BAIWIR (6), M. DE PAUW-GILLET (7), P. DELVENNE (4), M. MALAISE (8), C. COIMBRA MARQUES (9), M. POLUS (2), E. DE PAUW (3), E. LOUIS (2) / [1] Centre Hospitalier Universitaire de Liège, Liège, Belgium, Gastro-entérologie, hépatologie et oncologie digestive , [2] Centre Hospitalier Universitaire de Liège, Liège, Belgium, Gastro-entérologie, hépatologie et oncologie digestive, [3] University of Liege, Liège, Belgium, GIGA-Systems Biology & Chemical Biology, Laboratory of Mass Spectrometry, [4] Centre Hospitalier Universitaire de Liège, Liège, Belgium, Anatomie et cytologie pathologiques , [5] University of Liege, Liège, Belgium, Chemistry, Laboratory of Mass Spectrometry, [6] University of Liege, Liège, Belgium,