576P Prognostic values of a modified diagnostic biopsy-adapted immunoscore based on double immunohistochemical staining in patients with locally advanced rectal cancer

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Background: Tumor immune contexture plays a crucial role in the outcome of cancer patients. The aim of this study was to evaluate the prognostic value of the modified diagnostic biopsy-adapted immunoscore (mIS_b) for patients with locally advanced rectal cancer (LARC).

Methods: We included 181 LARC patients from a single sub-center of a prospective study (NCT02533271), with 151 (83.4%) receiving surgical treatment and 30 (16.6%) following a watch-and-wait (W&W) strategy. Tumor biopsy tissues were stained using dual immunohistochemistry for CD8+ and CD3+ T cell densities, and the CD8/CD3 ratio was calculated. mISb was developed using mean percentile values of CD8+ T cell density and CD8/CD3 ratio, and patients were classified into low (0%-25%), intermediate (>25%-70%), and high (>70%-100%) mIS_b groups. Spearman correlation analysis was performed. Kaplan-Meier survival analysis, multivariate Cox regression models were used to assess the prognostic value of mIS_b for disease-free survival (DFS), and the role of mIS_b in the W&W strategy was further investigated.

Results: A strong correlation was found between CD8+ and CD3+ T cell densities (R=0.86, $P<0.001$), and a weaker correlation with the CD8/CD3 ratio (R=0.45). In the low, intermediate, and high mIS_b groups, there were 35 (23.2%), 84 (55.6%), and 32 (21.2%) patients, respectively. The respective 3-year DFS rates for these groups were 59.0%, 69.5%, and 80.1% ($P=0.01$). Multivariate analysis in surgically treated patients revealed mIS_b as an independent prognostic factor for DFS (intermediate vs. high: HR=2.38, 95% CI: 0.92-6.16, $P=0.08$; low vs. high: HR=3.69, 95% CI: 1.35-10.07, $P=0.01$). Among W&W patients, 6 (37.5%) in the $mIS_b \leq 50\%$ group experienced local-region or distant metastases, while only 1 (7.1%) in the $mIS_b > 50\%$ group had local-region recurrence.

Conclusions: mIS_b, as a potentially valuable prognostic indicator, demonstrates significant importance in assessing the prognosis of LARC patients. Future research is needed to further investigate the clinical application of mIS_b in rectal cancer prognosis, particularly in the context of the W&W strategy.

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577P Systematically assessing the intratissue microbiota in 937 patients with colorectal cancer

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Background: Dysbiosis of the fecal microbiota has been closely related to colorectal cancer (CRC) and has increasingly proven to be associated with CRC prognosis. However, a significant knowledge gap still exists regarding intestinal tissue-resident microbes and their associations with host genetic alterations, gene expressions and prognosis in CRC.

Methods: We undertook a comprehensive analysis of the tissue-resident microbes in CRC patients from the U-CAN cohort, a large prospective longitudinal study of adult cancer patients in Sweden, using treatment-naïve whole-genome (average 53x coverage) and whole-transcriptome sequencing data of tumors (n=937) and their adjacent normal tissues (n=475).

Results: In total, 301 genera and 420 species were identified as common tissue-resident taxa. We showed for the first-time distinct enrichment patterns of tissue-resident microbiome in the right- and left-sided colons. Importantly, these patterns were highly consistent between tumor and normal tissues, with right-sided colons enriched with *Clostridium spp* and left-sided colons enriched with *Akkermansia muciniphila* and *Bifidobacterium spp*. We identified 94 tumor-enriched bacteria, and 38 were significantly associated with host hypermutation (HM) status. Previously known CRC-enriched bacteria, such as *Fusobacterium* groups and *Campylobacter*, were more prevalent in right-sided and HM tumors, and strongly correlated with mutations in host drivers and DNA damage repair genes. CRC driver genes that correlated with identified intratissue bacteria including *CASP8, TP53* and *SMAD2,* with *CASP8* significantly associated with multiple CRC-enriched taxa in all and in HM samples. Finally, risk scores derived from tumor or normal-resident taxa could both predict CRC prognosis and significantly improve the prognosis performance of the consensus molecular subtypes (CMS).

Conclusions: Our study provides new insights into the interactions between tumor-enriched microbiota with CRC and identifies potential bacteria markers that could improve the accuracy of CRC prognosis prediction. These findings can guide future efforts to exploit the performance of intratissue bacteria on CRC prognosis prediction.

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578P Tissue-resident microbiota characterization in colorectal cancer metastases

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Background: Early evidence suggested a role of tissue-resident microbiota (TRM) in the development of colorectal cancer. However, its presence in colorectal cancer metastases (CRCM) is poorly reported. Here, we aim to explore and characterize the TRM in CRCM.

Methods: From several biobanks in Belgium, we retrospectively collected 237 frozen samples, including 104 CRC primary tumor (PT) and 133 patient-matched CRCM (liver CRCM=98). Hepatocellular carcinoma (HCC, n=50), cholangiocarcinoma (CGC, n=23) and breast liver metastases (BLM, n = 16) were also collected. 82 CRCM were collected prospectively. The V4 region 16S rRNA gene was amplified and sequenced. We strictly applied sterile conditions from sample collection to sequencing, with the integration of negative controls (n=253). Additionally, 257 duplicated random samples were processed twice by independent experimenters. Sequencing results were processed with DADA2 to identify bacterial amplicon sequence variants (ASVs).

Results: ASVs were identified in all tissues. Microbial composition was significantly different between tumor samples and negative controls (PERMANOVA, p=0.0001). PT presented the highest bacterial biomass. ASV sharing frequency between PT and CRCM was significantly higher within than between patients (Wilcoxon test, p=0.007), suggesting potential bacterial translocation from PT to CRCM. The most prevalent PT bacteria were also the most shared in matched CRCM. No difference was observed between CRCM locations (liver, others). TRM of HCC, CGC and BLM were characterized by an ultra-low bacterial biomass, and distinct phyla compared to CRCM. In CRCM, the presence of Ruminoccocus gnavus (Rg) was associated with overall survival after CRCM resection (median 83.6 vs 41.6 months, HR: 0.35 IC95:0.13-0.90, p=0.024).

Conclusions: We observe a specific TRM in CRCM, shared with matched PT and suggesting bacterial translocation from PT to CRCM. In this cohort, the presence of Rg in CRCM seems prognostic after CRCM resection. Further experiments are needed to confirm and detail these results.

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