388P Investigating the association between crown-like structures and body composition parameters on the tumour-immune microenvironment in early high-risk primary breast cancer

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Background: Breast cancer (BC) remains the leading cause of cancer-related mortality among women globally. Antibody therapy such as anti-HER2 and immune-checkpoint therapies have improved clinical outcomes in patients with BC, however, responses are variable and treatment resistance is common, highlighting the need to identify novel biomarkers that predict response to treatment. Obesity is considered an established risk factor for BC and is associated with poor patient outcomes, particularly in aggressive subtypes such as HER2+ and triple-negative BC. This is partly mediated via systemic inflammation that leads to metabolic dysfunction which may promote tumour immune evasion. A characteristic hallmark feature of obesity is the presence of crown-like structure (CLS), characterised by macrophages encircling enlarged and necrotic adipocytes in a crown-like pattern. We have shown that CD32B+ CLSs present at the adipose-tumour border are associated with reduced time to metastatic disease in obese patients treated with trastuzumab therapy. However, the prognostic significance of CLS and its mechanistic role in high-risk BC subtypes is still not clear. We aim to investigate how CLS-driven inflammation promotes immuno-metabolic changes and influences antibody therapy responses within the tumour-microenvironment.

Methods: Patient clinico-pathological data were analysed from the BeGIN BC cohort. Definiens Architect XD was used for ROI segmentation and macrophage density quantification. Zeiss Zen was used to analyse CD163+ CLS in IHC-stained patient tumour sections. Descriptive statistical analysis was performed using GraphPad Prism.

Results: CLSs were associated with a higher density of CD163+ macrophages at the border (B-CLS) ($p<0.01$) and were strongly associated with higher body mass ($p<0.01$), fat mass ($p=0.01$), and fat-free mass ($p<0.01$). In addition, there was an association between high B-CLS density and chemotherapy toxicity ($p<0.0001$), lymphovascular invasion ($p=0.0004$), higher tumour grade and nodal metastasis.

Conclusions: CD163+ B-CLS are associated with higher patient body composition parameters and may serve as prognostic biomarkers in BC.

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390P Tumor cell-released autophagosome (TRAP) induce alveolar macrophages that suppress T cell function to promote breast cancer pulmonary metastasis by releasing L-lactate

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Background: Lung metastasis is the primary cause of breast cancer-related mortality. Lactate are involved in the progression of breast cancer. However, the mechanism of lactate rpoduction is not fully understood. This study posits that tumor cell-released autophagosomes (TRAPs) play a crucial role in this process.

Methods: TRAPs were isolated from breast cancer cell lines to analyze their impact on lactate production in mouse alveolar macrophages. The study used both in vitro and in vivo models, including Toll-like receptor 4 ($TLR4^{-/-}$) mice and engineered breast cancer cell lines. Immunofluorescence, ELISA, Western blotting, RNA sequencing, and flow cytometry were employed to dissect the signaling pathways leading to lactate production and to explore their immunosuppressive effects, particularly focusing on the impact of lactate on T-cell function. The therapeutic potential of targeting TRAP-induced lactate and their immunosuppressive functions was evaluated using oxamate and αPD-L1 antibodies. Clinical relevance was assessed by correlating circulating levels of TRAPs and lactate level with lung metastasis in patients with breast cancer.

Results: This study showed that TRAPs induced the formation of lactate in mouse alveolar macrophages by using the high mobility group box 1 and activating the TLR4-Myd88-ERK/p38 signaling axis. More importantly, TRAP-induced lactate inhibited T-cell function in vitro and in vivo, thereby contributing to the formation of lung premetastatic niche (PMN) immunosuppression. In contrast, Becn1 KD-4T1 breast tumors with decreased circulating TRAPs in vivo reduced the production of lactate, which in turn attenuated the immunosuppressive effects in PMN and resulted in a reduction of breast cancer pulmonary metastasis in murine models. Treatment with αPD-L1 in combination with oxamate that degraded lactate restored T-cell function and significantly reduced tumor metastasis.

Conclusions: Our results demonstrate a novel role of TRAPs in the production of lactate, which may provide a new strategy for early detection and treatment of pulmonary metastasis in patients with breast cancer.

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391P T cells and neoplastic cells FOXP3+ in the tumor microenvironment of breast cancer: Changing the prognostic

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Background: The TNM staging and immunohistochemistry (IH) are determinants of survival in breast cancer patients. The inflammatory infiltrates in the tumor microenvironment (TME) can influence prognosis, either positively or negatively, depending on the cellular subtype.

Methods: The prospective study included 81 women with breast cancer, all treated according to TNM and IH. The quantitative analysis of CD8+, CD4+ T lymphocytes, FOXP3+ regulatory T cells (Tregs), and FOXP3+ tumor cells in the TME used the tissue microarray (TMA), method of multiple paraffin-embedded tumor specimens. Patient survival was measured and their correlation with these cells in the TME.

Results: Patient survival correlated with TNM and IH findings, following the expected pattern. Low or high expression of CD4+ T lymphocytes was detected in 82.3% of cases, CD8+ T lymphocytes in 89.5%, and FOXP3+ T lymphocytes in 62.4% in the TME, with significant correlations. The expression of FOXP3+ T lymphocytes ranged from ≥1% to 40%. Additionally, FOXP3+ was expressed in 42% of the TME in tumor cells, varying between 10% and 90%. Correlations were found between FOXP3+ and the estrogen receptor (inverse correlation: rho = -0.2922, p = 0.0085) and Ki67 (direct correlation: rho = +0.2398, p = 0.0322). Patients with ≤10% FOXP3+ expression in lymphocytes (Log Rank $\chi^2 = 5.871$, p = 0.015) and in tumor cells (Log Rank $\chi^2 = 5.101$, p = 0.024) had longer progression-free survival (PFS) compared to those with higher expression (>10%). Patients with Luminal B tumors with up to 30% CD4 T cells in the TME had longer PFS (Log Rank $\chi 2$: 4.874, p = 0.027; Breslow $\chi 2$: 4.002, p = 0.045). In the joint analysis of FOXP3+ expressed in lymphocytes and tumor cells, a longer PFS was found in patients with ≤10% FOXP3+ (low/low) versus the other subgroups (low/high; high/low; high/high) (Log Rank $\chi 2$: 9.691, p = 0.021). Low FOXP3 expression, versus high, implied a longer PFS in both univariate (p = 0.006) and multivariate (p = 0.018) Cox regression analyses.

Conclusions: TNM and IH define the prognosis of patients with breast cancer. However, the higher expression of FOXP3+ in lymphocytes and tumor cells in the TME can worsen this prognosis, and the lower expression of CD4+ T lymphocytes, in the Luminal B subtype, can improve the prognosis.

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392P Single-cell sequencing reveals immune features of treatment response to neoadjuvant chemoradiotherapy plus immunotherapy in locally advanced rectal cancer

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Background: Neoadjuvant chemoradiotherapy plus immunotherapy (NCRIT) shows promise in locally advanced rectal cancer (LARC), but many patients fail to respond. The immune—stromal mechanisms driving differential outcomes remain unclear.

Methods: This retrospective study included 30 patients with LARC treated with NCRIT. scRNA-seq with paired TCR-seq was performed on 5 pre- and 11 post-treatment tumors. Post-treatment tumors from all 30 patients underwent bulk RNA-seq. Pre- and post-treatment FFPE samples were assessed by HE, IHC, and mIF.

Results: We profiled 112,562 high-quality cells and identified diverse immune, stromal, and epithelial subsets. In T cells, non-responders exhibited enrichment and clonal expansion of exhausted $CD8^+$ T cells and activated $FOXP3^+$ regulatory T cells (Treg), while responders were enriched for $CD8^+$ tissue-resident memory T cells (TRM), effector $CD8^+$ T cells, and NK cells. Bulk RNA-seq and mIF confirmed these findings and