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implications in disease. Upon treatment of cancer cells with low-dose chemotherapy, released EVs are able to transfer phenotypic traits to other cancer cells. New treatment strategies for TNBC, like inhibitors of the ER stress pathway might impact on EV biogenesis, cargo delivery and response of cells in the cancer microenvironment. T cells, which are part of the tumour-associated immune response, also produce and respond to EVs – including functional uptake as well as direct binding on surface molecules. Our aim is to identify differences in breast cancer-derived EVs upon treatment with inhibitors of the ER stress as well as low-dose chemotherapy with particular emphasis on altered T cell immunity.

Methods Different breast cancer cell lines, known to be responsive to ER stress inhibitors and low-dose chemotherapy were used as source of EVs, which were prepared by sequential ultracentrifugation. Integrity of different EV sub-types were studied by electron microscopy (TEM) and Nanoparticle Tracking Analysis (NTA). The ImageStream (ISX) technology was used to visualize CFSE labelled EVs. T cells from healthy blood donors will be activated in serum free medium with suboptimal and optimal doses of different stimuli in the presence of labelled breast cancer-derived EVs. T cell proliferation, cytokine production and vesicle uptake will be studied.

Results Using 4 different TNBC cell lines, we were able to decrease the amount of EV depleted growth serum needed from 10% to 2%. Breast cancer cell released EVs were positive for CD63 and TSG-101 as determined by TEM and showed the expected size distribution and total particle count by NTA. Cell derived EVs were visualized by direct labelling with CFSE without immobilization on beads using the ISX technology. Preliminary data suggest altered EV release from ER stress inhibitor treated breast cancer cells.

Conclusion Serum reduced culture conditions of breast cancer cell lines were successfully implemented**.** Cell free strategies to visualize extracellular vesicles could be established by the use of imaging flow cytometry. The impact of EVs on T cell immunity is still under investigation.

P165

Phagocytic Macrophages of the Primary Tumor are Associated with Lymphogenous Metastasis in Prostate Cancer

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Prostate cancer (PC) is the second most frequently diagnosed malignant tumor and the sixth leading cause of death for cancer in the male population. Every year, over 899,000 new cases are diagnosed in the world, which is 14% of all cases of cancer. The recruitment of migratory hematopoietic cells, including tumor-associated macrophages, to the supporting stroma is essential in the progression and metastasis of tumors. One of the main macrophages function is phagocytosis of apoptotic cells, that can induce secretion of key factors implicated in tumor progression. Besides the lack of specificity and sensitivity of modern diagnostic methods leads to the search for new factors that make it possible to predict the development of metastasis in a PC patients.

To explore the role of phagocytic macrophages in prostate cancer lymph node metastasis immunohistochemical (IHC) analysis was performed.

IHC staining of the 37 human prostate cancer paraffin embedded and cutted tissue samples (13 with and 24 without lymph node metastasis) was done using mouse antibody to CD68 (1:50; Zytomed Systems). Large phagocytic "foamy" and small non-phagocytic cells were counted in 10 fields of each tissue samples using Leica DMRE light microscope with x100 magnification. GraphPad Prism 7.03 program was used for Mann Whitney U-test.

The number of large phagocytic CD68+ cells in relation to small CD68+ cells was significantly higher in samples of tissues of the primary prostate tumor of patients with lymph node metastases compered to non-metastatic patient samples (fig. A).

This marker may be useful for improving the diagnosis of metastatic prostate cancer, as well as predicting the development of lymph node metastases during biopsy in patients with primary prostate cancer after extensive research.

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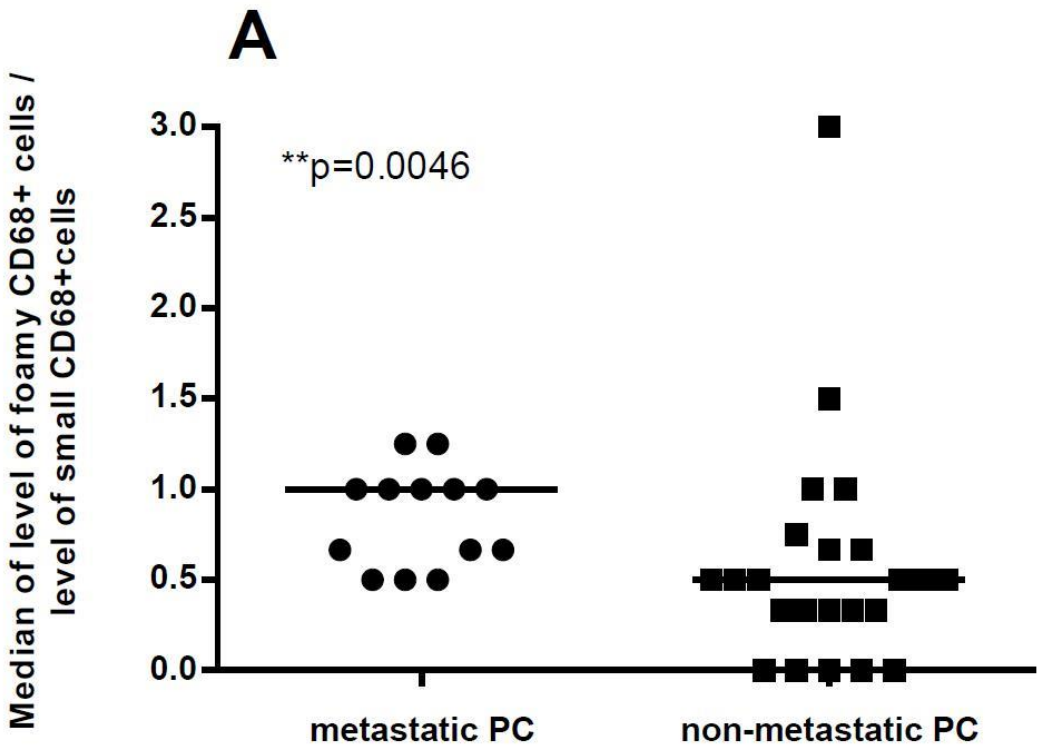
Fig.A. The relative level of "foamy" phagocytic CD68+ cells to small non-phagocytic CD68+ cells in prostate cancer tissue of patients with and without lymph node metastases.

Figure 1



P166

Effector functions of γδ T cells and their potential in cancer immunotherapy

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γδ T-cell based immunotherapy is a promising concept for the treatment of malignancies either alone or in association with new immunotherapeutical approaches as immune checkpoint blockade therapy. Aiming to improve the effectiveness of future γδ T-cell based immunotherapies against malignancies, we analyzed the expression and biological significance of inhibitory checkpoints molecules PD-1, CTLA-4, LAG-3, TIGIT and TIM-3 on colon cancer infiltrating and circulating γδ T cells. The analysis showed higher levels in tissue-resident (either normal or tumor) γδ T cells, as compared to circulating γδ T cells, while MFI analysis showed an increase of their expression from circulating cells toward infiltrating cells, reaching maximum intensity in tumor infiltrating cells. Moreover, γδ T cells infiltrating invasive tumors had higher percentages and expression of immune checkpoint molecules compared to non invasive tumors.

Blocking PD-1/PD-L1 interaction *in vitro* by Nivolumab increased the cytotoxic activity of γδ T cells against tumor cells, sustained the viability of the effector γδ T cells by reduction of their apoptosis rate but without promoting their proliferation, and induced the differentiation of γδ T cells toward a central memory phenotype. In addition, the blockade of PD-1 and CTLA-A4 signalling by Nivolumab and Ipilimumab induced IFN-γ and TNF-a production by γδ T cells upon stimulation with colon cancer cells, and restored their antitumor activity.

