

expression was highly variable and distinct PD-L1 driven immune phenotypes were identified based on the PD-L1 intensity on both tumor and immune cells, the distance between non-exhausted T-cell subsets (i.e. PD-1 and CTLA-4 expression on CD3<sup>+</sup>CD8<sup>+</sup> cytotoxic T-cells, CD3<sup>+</sup>CD4<sup>+</sup> T-helper cells, CD3<sup>+</sup>CD4<sup>+</sup>FOXP3<sup>+</sup> regulatory T-cells) and tumor cells as well as macrophage/(DC) subtypes. In breast cancer, the PD-L1 fluorescence intensity on tumor cells showed a significantly higher predictive performance for overall survival with an area under receiver operating curves (AUC) of 0.72 ( $p < 0.0001$ ) than the percentage of PD-L1<sup>+</sup> tumor cells (AUC: 0.54). In PD-L1 positive as well as negative breast cancers a close spatial relationship between T-cell subsets (CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup>FOXP3<sup>+</sup>PD-1<sup>+</sup>CTLA-4<sup>+</sup>) and Macrophage/DC subsets (CD68<sup>+</sup>CD163<sup>+</sup>CD11c<sup>+</sup>iNOS) was found prognostic relevant ( $p < 0.0001$ ).

**Conclusions** In conclusion, multiplex immunofluorescence PD-L1 assessment provides cutoff-free/continuous PD-L1 data which are superior to the conventional percentage of PD-L1<sup>+</sup> tumor cells and of high prognostic relevance. The combined analysis of spatial PD-L1/PD-1 data and more than 20 different immune cell subtypes of the immune tumor microenvironment revealed distinct PD-L1 immune phenotypes.

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#### P02.04 TISSUE-INFILTRATING TH9 CELLS IN HUMAN ENDOMETRIAL CANCER

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**Background** Endometrial cancer (EC) is a hormone-related carcinoma with increased morbidity among female patients of all backgrounds. The immune microenvironment of EC is uncertain.

**Materials and Methods** 102 patients were recruited in the present study. 90 postoperative specimens from the patients were analyzed by immunohistochemistry. The leukocyte landscape of endometrial cancer was mapped using high-dimensional single-cell profiling (CyTOF) for 12 patients.



Abstract P02.04 Figure 1

**Results** NK cells, MDMs, and neutrophils were enriched in adjacent normal tissue. CCR5+CD38+ PD1+Th9 cells were enriched in the invasive margin. Additionally, PD1+ESR<sub>neg</sub> T cells and Siglec1+CCR5+CD40+ESR<sup>hi</sup> macrophage were infiltrated in the tumors.

**Conclusions** Immunological landscape of EC might shed light on new immunotherapeutic approach.

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#### P02.05 COMPREHENSIVE PROFILING OF TUMOR HETEROGENEITY AND ITS MICROENVIRONMENT IN ADVANCED NON-SMALL CELL LUNG CANCER AT SINGLE CELL RESOLUTION

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**Background** Lung cancer is a highly heterogeneous disease. Cancer cells and cells within the tumor microenvironment together determine disease progression, as well as response to or escape from treatment.

**Materials and Methods** To map the cell type-specific transcriptome landscape of cancer cells and their tumor microenvironment in advanced non-small cell lung cancer (NSCLC), we analyzed 42 tissue biopsy samples from stage III/IV NSCLC patients by single cell RNA sequencing and presented the large scale, single cell resolution profiles of advanced NSCLCs.

**Results** In addition to cell types described in previous single cell studies of early stage lung cancer, we were able to identify new cell types such as follicular dendritic cells and T helper 17 cells. Tumors from different patients display large heterogeneity in cellular composition, chromosomal structure, developmental trajectory, intercellular signaling network and phenotype dominance. Our study also revealed a correlation of tumor heterogeneity with tumor associated neutrophils, which might help to shed light on their function in NSCLC.

**Conclusions** This study presented first-time the tumor heterogeneity and tumor microenvironment profile from late-stage, largely untreated NSCLC patients, and shed light on possible treatment regimes.

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#### P02.06 SEMI-AUTOMATED VALIDATION AND QUANTIFICATION OF CTLA-4 IN 90 DIFFERENT TUMOR ENTITIES USING MULTIPLE ANTIBODIES AND ARTIFICIAL INTELLIGENCE

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**Background** CTLA-4 is an inhibitory immune checkpoint receptor and a negative regulator of anti-tumor T-cell