solid tumors. Previous experiments showed that tumor lactic acidosis selectively targets the signaling pathway including JNK/c-Jun and p38, resulting in inhibition of IFN-γ production. In contrast, granule exocytosis, which is regulated via the MEK1/ERK pathway, was moderately affected. Based on the contrasting effects on these two essential T cell effector activities, we investigated in more detail the effects of lactic acidosis on the killing process conducted by T cells.

**Material and Methods**

Tumor cells and cytotoxic T cells were co-cultured in lactic acid or regular culture medium and analyzed for effector function by flow cytometry and cell-mediated cytotoxicity assays. Additionally, ‘in-channel micropatterning’ in combination with artificial intelligence (AI) aided image analysis was used to visualize and analyze T cell cytotoxicity and mobility on a single cell level. Usage of collagen-matrices allowed the observation of T cell activity in a physiological three-dimensional environment. Cell metabolism was analyzed by Seahorse technology.

**Results**

In the presence of lactic acid, IFN-γ production was strongly inhibited, while degranulation was only moderately reduced. Detailed analysis of the different processes involved in T cell cytotoxicity revealed that T cell recognition of tumor cells resulted in less secretion of cytotoxins (perforin, granzyme B and granzyme A). Lytic activity against tumor cells was strongly reduced at low T cell to tumor cell ratio (1:2). This deficiency could be compensated by increasing the T cell to tumor cell ratio (10:1). Using live cell imaging we investigated underlying mechanisms that might explain how higher T cell to target cell ratios might overcome lactic acid inhibition. T cells in lactic acid covered less distance, they moved for longer time periods and made less contacts with tumor cells in comparison to T cells cultured in regular culture medium.

**Conclusions**

Micropatterning and AI based image analysis allows for detailed assessment of the processes involved in T cell-mediated killing such as mobility, speed, directionality and attachment on target cells. Lactic acidosis is hampering T cell killing activity by reducing the T cell's capacity to find its target cell and attach to it. Repeated addition of T cells or neutralization of lactic acidosis in the TME are means to overcome these deficits and hold promise to improve the outcome of T cell-based immunotherapies.

**Disclosure Information**

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**O3 HIGH-DIMENSIONAL ANALYSIS OF TUMOR ARCHITECTURE PREDICTS CANCER IMMUNOTHERAPY RESPONSE**

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**Background**

Immunotherapies have induced long-lasting remissions in countless advanced-stage cancer patients, but many more patients have not benefitted. Therefore, novel predictive markers are needed to stratify patients before treatment and select those who will most likely benefit from immunotherapy, while avoiding potentially devastating adverse effects and high treatment costs for those who will not. We reasoned that thoroughly characterizing the architecture of the tumor microenvironment (TME) at the single-cell level by highly multiplexed tissue imaging should reveal novel spatial biomarkers of immunotherapy response.

**Materials and Methods**

We used CODEX (CO-Detection by indEXing) highly multiplexed fluorescence microscopy to investigate the TME of cutaneous T cell lymphoma (CTCL) in samples from patients treated with pembrolizumab. 55 protein markers were visualized simultaneously using a tissue microarray of matched pre- and post-treatment skin biopsies from 7 pembrolizumab responders and 7 non-responders. After computational image processing and extraction of single-cell information, cell types were identified by unsupervised clustering followed by supervised curation, and cell-cell distances and ‘cellular neighborhoods’ were computed. We also performed RNA sequencing on laser-capture microdissected tissue microarray cores to extract cell-type specific gene expression profiles by CIBERSORTx analysis.

**Results**

CODEX enabled the identification and characterization of malignant CD8+ tumor cells and reactive immune cells in the CTCL TME at the single-cell level, resulting in 21 different cell type clusters with spatial information. Cluster frequencies were not significantly different between responders and non-responders pre- and post-treatment. However, advanced computational analysis of the tumor architecture revealed cellular neighborhoods (CNs) that dynamically changed during T cell therapies for cancer treatment are challenging to develop because of the complex mechanisms and cell interactions that underly T cell-mediated tumor killing. Current technologies rely on correlating phenotype, function, and gene expression based on experiments performed on different populations of T cells because no one platform is able to assess cell surface marker expression, cytokine secretion, and tumor cell killing activity of the same T cell and recover this cell for downstream genomic analysis. Here we share two use cases - CART cell functional screening and TCR sequence recovery following functional assay - that demonstrate how the T Cell Analysis Suite on the LightningTM optofluidic platform can be used to directly link T cell phenotype and function (IFNγ secretion and tumor cell killing) to genotype (TCR sequence recovery) at a single-cell level and on the same T cell, enabling deeper and more thorough characterization of how T cells mediate tumor cell death and potentially the development of more efficacious therapies.

**Disclosure Information**

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**On Demand Talks: Precision Medicine Meets Immunotherapy (Immuno-Monitoring)**

**O2 DIRECTLY LINKING SINGLE T CELL PHENOTYPE AND FUNCTION TO GENOTYPE**

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