156 ESTABLISHMENT OF AN IMMUNE CELL PHENOTYPING MULTIPLEXED IMMUNOFLUORESCENCE ASSAY AND DIGITAL IMAGE ANALYSIS WORKFLOW TO INVESTIGATE THE TUMOR MICROENVIRONMENT IN SOLID TUMORS

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Background The advent of immune-oncology had a significant impact on the stratification of cancer patients, in the past few years. Immune phenotyping of the tumor microenvironment is becoming a tool for the identification of novel predictive biomarkers for cancer immunotherapy. One promising approach is the usage of multiplexed immunofluorescence (mIF) assays for the semi-quantitative assessment of spatial distribution patterns.

Methods Akoya's Opal fluorophores were optimized for the Ventana Discovery ULTRA for use with human control tissues as well as several cancer tumors. The panel consisted of CD4 (clone SP35), CD8 (clone C8/144B), CD68 (clone PG-M1), FoxP3 (clone SP97), PD-L1 (clone SP263) and pan-Cytokeratin (clones AE1/AE3). Each marker was independently validated using single plex bright field immunohistochemistry and adequate stripping efficiency, of the previous applied primary and secondary antibody complex, was confirmed.

Automated image analysis was performed using a workflow of several custom Visiopharm applications, after optimization of segmentation in regions of interest, like tumor invasive margins, or surrounding microenvironment.

Results 6-plex mIF, followed by automated image analysis, was performed in controls and cancer tissue. The ability to multiplex allows for the detection of new immunophenotypes in the tumor and surrounding microenvironment. Such phenotypes, together with the validation of the markers and the image analysis workflow will be presented.

Conclusions Individual mIF immune marker panels and automated image analysis identify a broad number of different immune phenotypes, including rare double- or triple-positive cell subtypes, yielding new insights into the complexity of the tumor microenvironment. Such results could in the future improve cancer patient stratification in immunotherapy.

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