## 139 IDENTIFICATION OF DISTINCT TISSUE PHENOTYPES IN LUNG AND COLORECTAL CANCERS BY IMAGING MASS CYTOMETRY IS CONSISTENT ACROSS SERIAL SECTIONS

[1]Smriti Kala*, [2]Rasmus Lyngby, [2]Rasmus Sorensen, [1]Andrew Quong, [1]Sam Lim, [1]Clinton Hupple, [1]Nina Lane, [2]Jeppe Thagaard, [2]Johan Dore-Hansen, [2]James Mansfield, [2]Fabian Schneider, [1]Michelle Macpherson. [1]*Standard BioTools, San Francisco, CA, USA*; [2]*Visiopharm, Hørsholm, Denmark*

**Background** The growth in cancer immunotherapy agents requires an understanding of the immune contexture of the tumor microenvironment (TME). Imaging Mass Cytometry™ (IMC™) is a powerful tool for the study of complex cellular interactions in the TME and in the discovery of biomarkers that can predict disease outcome or response to therapy. The Hyperion Imaging System (Standard BioTools) utilizes CyTOF® technology to simultaneously assess 40+ protein markers at subcellular resolution without spectral overlap or background autofluorescence, thus providing unprecedented insight into the organization and function of the TME. However, despite the advances in staining and imaging methods, developments in analysis software had not kept pace, as a complete, user-defined workflow in a single software package for the analysis of highplex imaging data was lacking.

**Methods** This study demonstrates a tissue phenotyping workflow in highly autofluorescent lung and colorectal cancer tissues using highplex IMC, which offers the advantage of zero autofluorescence, and hence more reliable results. The data analysis pipeline uses Oncotopix® Discovery (Visiopharm) software for easy, accurate and quantifiable phenotyping. Serial sections of both tissue types were stained with a 40-marker panel comprised of structural, tumor, stroma, and immune cell markers, including immunoregulatory proteins that are targets of immunotherapy. The IMC cell segmentation kit was included in the panel for improved nucleus and plasma membrane demarcation. The analysis pipeline consisted of tissue segmentation (tumor, stroma, necrosis, etc), nuclear detection using a deep-learning algorithm pre-trained on IMC DNA channels, a threshold-based cellular phenotyping step, and spatial analyses.

**Results** In this work, we have shown that analysis of IMC images from lung and colorectal cancer tissues can uncover tissue phenotypic signatures of the TME through the determination of immune cell types found in the vicinity of cancerous cells and their numbers. Moreover, cell counts and tissue phenotypes were highly consistent across the serial sections, demonstrating the power of IMC in generating robust data.

**Conclusions** Overall, this work demonstrates that even for highly autofluorescent tissues, IMC can generate high-quality data, consistent across serial sections, which can be easily and accurately analyzed using a single software package, thus empowering IMC users to be confident in biological interpretation of high-dimensional proteomic data. This study showcases the capability of IMC technology combined with Oncotopix Discovery analysis in classification of cellular components within the TME, which is important for development of systematic digital profiling of the spatial TME.

http://dx.doi.org/10.1136/jitc-2022-SITC2022.0139

J Immunother Cancer: first published as 10.1136/jitc-2022-SITC2022.0139 on 7 November 2022. Downloaded from http://jitc.bmj.com/ on November 10, 2024 by guest. Protected by copyright.