00362 Comparison of Two Multiplex IHC Methods to Study the Tumour Immune Microenvironment: Introducing the Imaging Mass Cytometry

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Aims: Background: Tissue immune microenvironment is a critical bridge for the bed-to-bench and bench-to-bed approach in the era of immunotherapy. It is key to predicting the responsiveness of immunotherapy and contributes to therapy personalisation.
Aim: Like CyTOF, Imaging Mass Cytometry (IMC) enables simultaneous measurement of 37 biomarkers with single-cell and spatial resolution on tissues, particularly on formalin-fixed paraffin embedded (FFPE) clinical samples. Using human hepatocellular carcinoma tissues, we aim to compare of this new imaging technology with a flourochrome-based multiplex IHC (m-IHC) technology that we and others have optimized and reported.

Methodology: We adopted 12 markers, including basic lineage markers as well as immune-checkpoint molecules, and performed staining on two consecutive tissue sections, followed by imaging-analysis with IMC and m-IHC standard workflows.
To accurately evaluate the quantitative power of these pipelines, two teams of pathologists and immunologists analyzed the imaging data independently. Then, comparison analysis such as scoring, colocalization, staining time usage, resolution and imaging-throughput were scrutinized.

Result: The key advantage of IMC is the high staining throughput, where 37 markers can be labelled simultaneously, taking less than an hour per slide. m-IHC, with sequentially staining protocol, takes 11 hours per slide with manual preparation for only 6 markers.
However, our group recently published an optimized fully automated staining protocol (PATHOLOGY, 2018) with diagnostic autostainer, which minimised m-IHC staining time to 0.25 hour per slide. Also, within 2 hours, IMC can acquire a 1mm2 region of interest (ROI) while m-IHC can acquire 90X1mm2 of ROI.
Lastly, our preliminary data demonstrated high correlation between the two aforementioned platforms for markers such as CD8 (R2=0.77) and CD45RO (R2=0.90). Further studies are ongoing.

Conclusion: A strategic integration of IMC, m-IHC and CyTOF provides a revolutionary strategy to empower validated single cell data containing spatial and morphological information to harness new discoveries in cancer immunotherapy.