Background The spatial interactions between the immune system and tumor cells greatly influence antitumoral immunity, patient prognosis, and therapeutic efficacy. However, few methods exist to query large numbers of immune biomarkers at subcellular spatial resolution. Launched earlier this year, the CosMx™ Spatial Molecular Imager (SMI) platform captures high-plex single cell and subcellular detection of proteins from FFPE tissues. To detect these key drivers of cancer progression and immune cell activation states and functions, we designed and validated a high-plex protein panel for the CosMx SMI platform. This panel contains 4 markers for cell segmentation and pre-experimental imaging and 64 barcoded antibodies emphasizing immuno-oncology focused targets.

Methods The CosMx protein assay uses antibodies conjugated with oligonucleotides, which are detected using universal, multi-analyte CosMx readout reagents. The fully automated CosMx instrument carries a widefield water immersion objective with 1.1 NA. The CosMx Human Immuno-oncology panel was optimized to comprehensively phenotype lymphoid and stromal lineages within the tumor microenvironment. The CosMx protein assay reagents were validated on multi-organ FFPE tissue microarrays covering prevalent solid tumor types and matched controls, and 52 human FFPE cell lines, including overexpression lines for key targets such as GITR, CD278, PD-L1, and PD-1.

Results We achieved 86% sensitivity and 90% specificity across well-characterized human cell lines compared to GeoMx spatial profiling, and further benchmarked to multiple orthogonal datasets (e.g., the Human Protein Atlas, low-plex IHC). Within the tissue sample profiled, we captured immune cell localization across and within the tumor, key signaling markers related to lymphoid and myeloid activation such as checkpoint engagement, and myeloid cell polarization and antigen cross-presentation markers. We made the data from the study profiling cancer tissue freely available online. This includes the protein images, AI-based cell segmentation, and the per-cell protein abundance profiles.

Conclusions CosMx SMI is a high-plex spatial multiomics platform that enables detection of more than 64 proteins at subcellular resolution in real-world FFPE tissues. Our 64-plex human immuno-oncology protein panel enables in-depth study of the tumor microenvironment, including markers covering cell typing and lineage, immune activation, and checkpoints. This new platform will enable researchers to collect high-plex protein immunophenotyping data and understand immune molecular mechanisms with full spatial context.

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