5641. Single cell spatial molecular imaging of 119-plex proteins in clinical cancer samples in response to personalized treatment

Zachary R. Lewis[1], Giang Ong[1], Tiên Phan-Everson[1], Evelyn Metzger[1], Brian Birditt[1], Emily Brown[1], Kan Chantranuvatana[1], Chris Corless[2], Brian Filanoski[1], Gary Geiss[1], Evie Hobbs[2], Brett Johnson[2], Taylor Kelley[2], Mithra Korukonda[1], Charles Lopez[2], Rhonda Meredith[1], Anastasiya Olson[2], Erin Piazza[1], Jason Reeves[1], Alyssa Rosenbloom[1], Kiara Siex[2], Hye Son Yi[1], Edward Zhao[1], Joseph M. Beechem[1], Gordon Mills[2]

1. NanoString® Technologies, Seattle WA. 2. OHSU, Knight Cancer Institute, Portland OR

OHSU KNIGHT CANCER Institute

nanoString

Nanostring Technologies
530 Fairview Avenue North, Seattle, WA 98109

Abstract

The power of spatial biology lies in the integration of multiple scales of information from subcellular to tissue scale. Until recently, spatial analysis of protein biomarkers in tissues was limited to a few markers at a time using traditional IHC colorimetric or fluorescent readout. Here, we demonstrate the ability of NanoString's CosMx™ Spatial Molecular Imager (SMI) platform to quantify more than100 proteins encompassing key targets in immuno-oncology and tumor biology, localize the proteins and analyze protein expression at a single cell level. Key to the technology is the use of fully automated fluidics and imaging systems, short turnaround time, and high sensitivity. The CosMx protein assay has been optimized for FFPE samples, which represent the largest collection of biospecimens available for clinical investigation. To that end, we explored the utility of CosMx spatial proteomics on a series of clinical samples from cancer patients, including the Serial Measurements of Molecular and Architectural Response to Therapy (SMMART) Clinical Trials Program, across multiple cancer types.

CosMx SMI's high-level protein multiplexing capabilities enabled spatial analysis of metastatic tumors in response to personalized treatment for a single patient over time. Detection of phosphoproteins also allows for analysis of the impact of kinase inhibitor treatment on the spatial environment in longitudinal biopsies. The highly-multiplexed spatial analysis of proteins in longitudinal metastatic breast cancer biopsies under therapeutic pressure provides a unique opportunity to understand evolution of tumors and develop and implement therapeutic approaches that can directly target mutations arising in the tumor cells while effectively engaging the immune system.

Highlights

- Spatial profiling of **14 breast cancer patients** undergoing personalized treatment
- **33 biopsies** (FFPE core needle) across 19 slides
- **119-plex** CosMx protein assay
- **Post-translational** modifications captured
- Immune **cell composition and expression** characterized over treatment

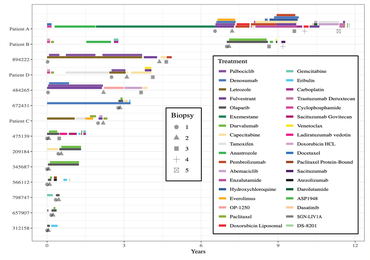
Longitudinal study of patients undergoing personalized treatment for breast cancer

Serial Measurements of Molecular and Architectural Responses to Therapy (SMMART®) Clinical Trials Program

The patients are followed longitudinally, which in this case means that they are monitored before, during and after being given study treatments.

The treatments are tailored to each individual patient in the study according to the characteristics of the individual's cancer and genetics.

Adaptive treatment in real time. Therapy for each individual patient is tailored, then re-evaluated, and changed during the trial according to changes observed in the patient's response.

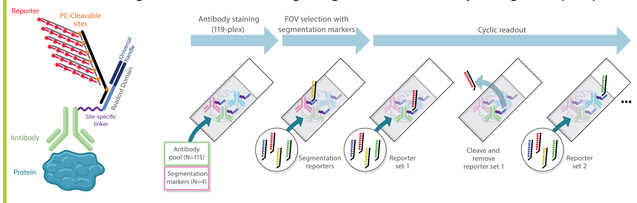


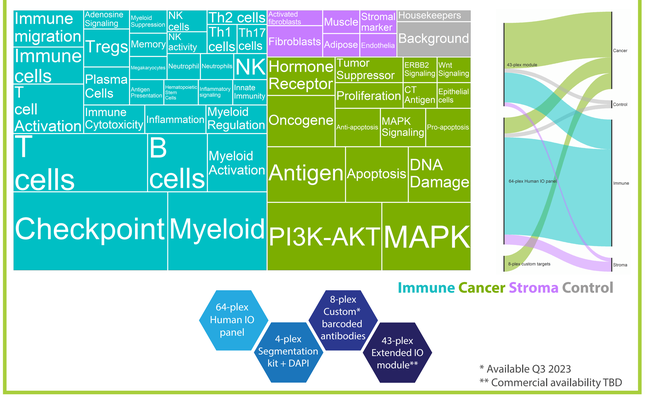
Objectives:

- Identify new treatments for cancer that last longer (are more durable)
- Allow better quality of life (are more tolerable) for patients with advanced disease
- Understand why therapies often stop working

CosMx Spatial Molecular Imaging

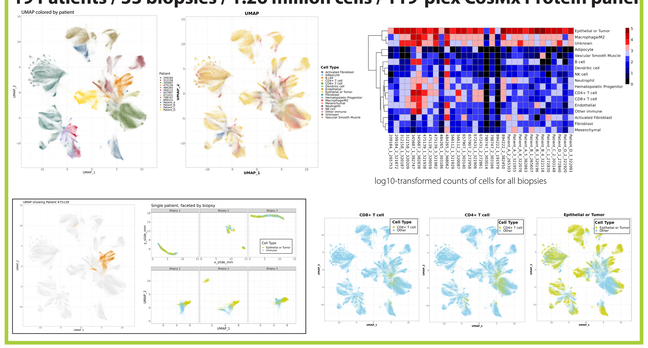
CosMx protein assays detect antibody localization by hybridization of a bright, photocleavable reporter construct. The reporter construct can be gently removed by photocleavage. The commercial 64-plex Human Immuno-oncology panel was combined with conjugated antibodies for an additional 51 targets, as well as a four-target segmentation cocktail, yielding a 119-plex panel.



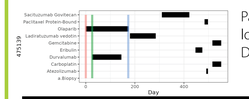


* Available Q3 2023
** Commercial availability TBD

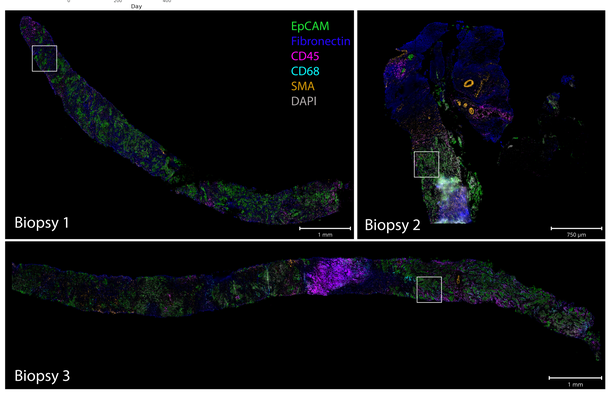
19 Patients / 33 biopsies / 1.26 million cells / 119-plex CosMx Protein panel



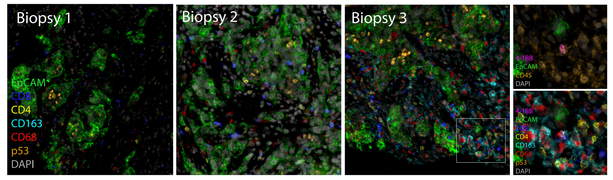
Characterization of immune response over the course of treatment

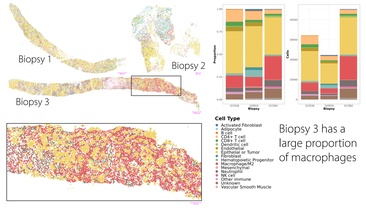


Patient 475139 was treated with PARP inhibitor Olaparib, followed by treatment with PD1/PD-L1 checkpoint inhibitor Durvalumab.



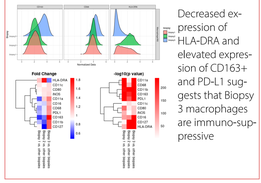
Influx of suppressive macrophages in biopsy 3





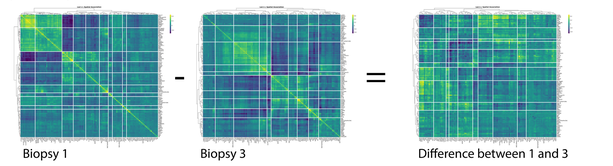
Biopsy 3 has a large proportion of macrophages

Differential expression in macrophages

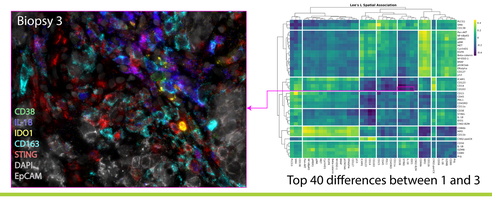


Decreased expression of HLA-DRA and elevated expression of CD163+ and PD-L1 suggests that Biopsy 3 macrophages are immuno-suppressive

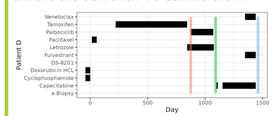
Spatial expression correlation shifts over treatment



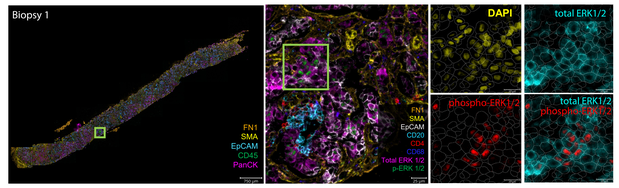
By subtracting the spatial correlation of proteins in Biopsy 3 from Biopsy 1, a measure of difference between spatial expression patterns emerges. Small values indicate strong spatial associations in Biopsy 3 that were not present in Biopsy 1.



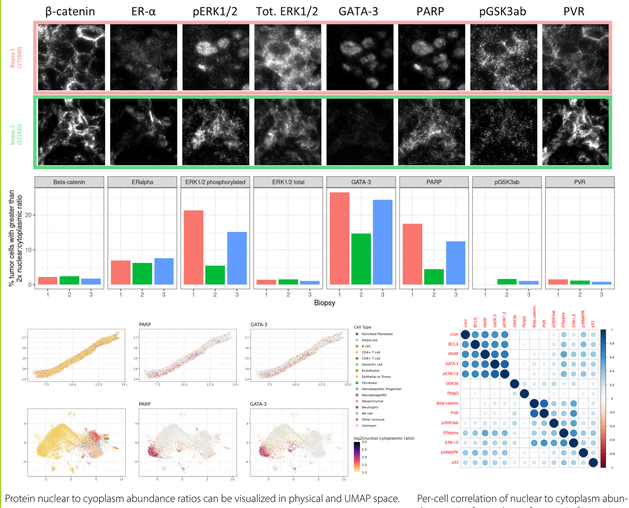
High content investigation of protein post-translational modification and subcellular localization



Patient D was treated with CDK4/6 inhibitor Palbociclib, aromatase inhibitor Letrozole and BCL-2 blocker Venetoclax, among other medications.



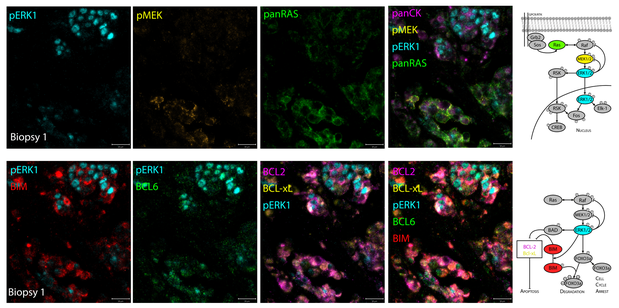
The fraction of tumor cells with nuclear-localized phospho-ERK1/2, GATA-3 and PARP declines in biopsy 2.



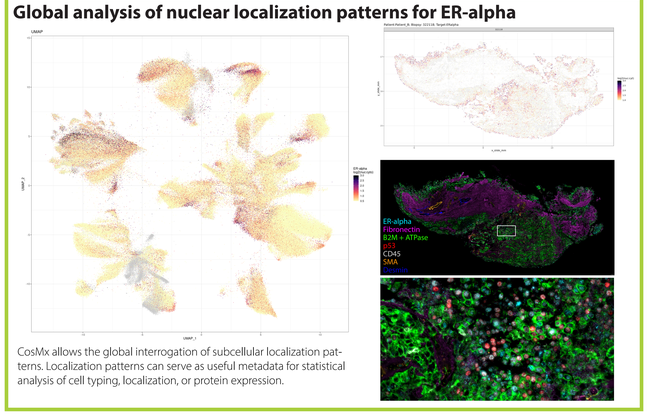
Protein nuclear to cyoplasm abundance ratios can be visualized in physical and UMAP space.

Per-cell correlation of nuclear to cytoplasm abundance ratios for a subset of targets in Biopsy 1.

119-plex panel allows the spatial characterization of cell signaling and apoptotic pathways

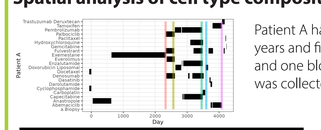


Global analysis of nuclear localization patterns for ER-alpha

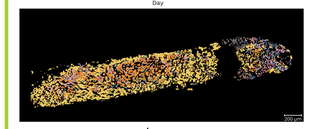


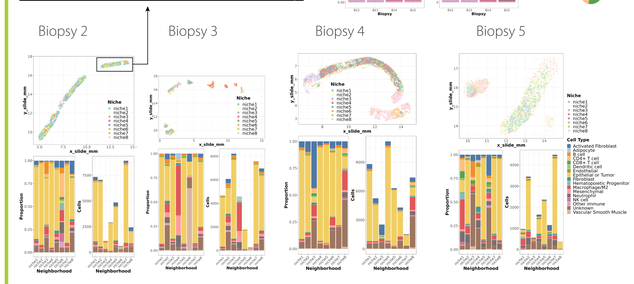
CosMx allows the global interrogation of subcellular localization patterns. Localization patterns can serve as useful metadata for statistical analysis of cell typing, localization, or protein expression.

Spatial analysis of cell type composition across longitudinal biopsies



Patient A had a complex set of treatments spanning multiple years and five biopsies. One block is exhausted (Biopsy 1) and one block has little remaining tissue (Biopsy 3). Biopsy 5 was collected *4 years* after Biopsy 2.





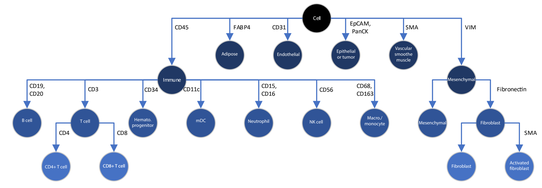
Conclusions

- Longitudinal studies are a valuable opportunity to assess response to treatments spanning months or years in individual patients. Biopsies from these studies are small and extremely valuable.
- CosMx protein assays offer a single solution to analyze **119 targets at subcellular resolution** in one experiment.
- CosMx protein assays allow the interrogation of **post-translational modifications** and subcellular localization patterns over large datasets.
- Spatial localization of protein expression is heterogenous across biopsies at both the tissue and subcellular levels.
- **High-plex protein** assays enable both robust cell-typing and interrogation of immuno-oncology target expression.
- Post-translational modifications, such as **ERK1/2 phosphorylation**, are key indicators of cellular state and signaling activity.
- Gentle and fast darkening chemistry through UV photocleavage permits fast turnaround time and low impact to sample stability.
- **8-plex customization** of CosMx protein assays adapts the assay to specific biological questions.

Methods

Slides, ranging in age from freshly-cut to over one year-old, were stained with a 119-plex antibody cocktail using the standard CosMx protein commercial protocol

Images were acquired on a CosMx instrument. Protein abundance was calculated as the pixel intensity normalized by cell or compartment area. Cell typing was performed using a modified version of CELESTA, tuning each marker based on probability thresholds.



Acknowledgements

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SMMART MMTERT Study: OHSU IRB 16113

AMTEC Trial: OHSU IRB 18504 (NCT03801369)

Zhang et al. 2022. Identification of cell types in multiplexed in situ images by combining protein expression and spatial information using CELESTA. **Nat. Methods.** https://doi.org/10.1038/s41592-022-01498-z