interest, including CK, CD8, Ki67, PD-L1 and PD-1; all of these biomarkers showed abundant expression in the TMI. Building on this result, we next developed a 6-plex Opal™ Phenoptics™ panel. This panel was screened and analyzed via high-throughput whole slide scanning of sample tissues. Image processing and data analysis were conducted similarly for both datasets so that repeatability and consistency of measurements could be established.

Results Both CODEX® and Phenoptics™ detected the same cell phenotypes and displayed similar frequencies of cells expressing CK, CD8, Ki67, PD-L1 and PD-1 in serial sections of tonsil and NSCLC tissues. These observations were consistent and cross-validated in data from CODEX® and Phenoptics™ platforms. Crucially, this means that the two approaches can be made analytically equivalent, and hence, that they can be used in conjunction with each other as research progresses along the continuum from discovery to translational and clinical research.

Conclusions Our cross-platform comparison provides a conceptual framework for biomarkers discovered on the CODEX® platform to be translated to the Phenoptics™ platform for high-throughput translational studies. The resulting comprehensive phenotyping and quantification data retain spatial context and provide unprecedented insight into tumor biology.

Disclosure Information O. Braubach: A. Employment (full or part-time); Significant; Akoya Biosciences. M. Gallina: A. Employment (full or part-time); Significant; Akoya Biosciences. B. Remeniuk: A. Employment (full or part-time); Significant; Akoya Biosciences. C. Wang: A. Employment (full or part-time); Significant; Akoya Biosciences. N. Nikulina: A. Employment (full or part-time); Significant; Akoya Biosciences. R. Bashier: A. Employment (full or part-time); Significant; Akoya Biosciences. J. Kennedy-Darling: A. Employment (full or part-time); Significant; Akoya Biosciences. C. Hoyt: A. Employment (full or part-time); Significant; Akoya Biosciences.

P01.05 DEVELOPMENT OF SIGNAL AMPLIFICATION FOR SPATIALLY-RESOLVED, HIGHLY MULTIPLEXED BIOMARKER ANALYSIS OF HUMAN TUMOR TISSUES

O Braubach*, S Mistry, G Dakshinamoorthy, J Yuan, P Noordam, J Kim, W Lee, J Kennedy-Darling. Akoya Biosciences, Menlo Park, CA, USA

10.1136/jitc-2020-ITOC7.18

Background Characterizing the complexities of the tumor microenvironment is fundamental to understanding cancer. Spatial relationships between infiltrating immune cells and the existing cellular matrix are now recognized as key determinants of tumor heterogeneity. Nevertheless, most available technologies for studying cells within the context of their tissue microenvironment, like traditional immunofluorescence (IF) and immunohistochemistry (IHC), are limited—allowing the visualization of only a few markers at a time.

Materials and Methods CO-Detection by indEXing (CODEX®) technology has overcome this limitation through a DNA-based labeling strategy, involving the sequential addition and removal of dye-labeled oligonucleotide reporters to antibodies equipped with complementary oligonucleotide tags. In this manner, it is possible to visualize tens of antibodies in the same tissue, in situ and at cellular resolution. Additionally, CODEX® interfaces with existing inverted microscopes and provides a cost-

effective, fully automated platform for ultra-high plex immunofluorescence imaging. We have expanded the CODEX® platform to include Tyramide Signal Amplification of weak fluorescent signals, i.e. from low-expression biomarkers. This approach was tested with key biomarkers used in routine analyses of the tumor microenvironment, including PD-L1, PD-1 and FOXP3.

Results We demonstrate >50X amplification of PD-L1, PD-1 and FOXP3 signals when compared to control tissues. Moreover, we successfully included our amplification step in the CODEX® labeling/imaging workflow, so that it was possible to analyze amplified PD-L1, PD-1 and FOXP3 signals concurrently with a panel of 20+ additional antibodies. Analysis of our data also generated unique biological insights, including increased PD-L1 expression in T_{reg} cells and other tumor and stromal regions.

Conclusions Our findings demonstrate the feasibility of amplifying weak biomarker signals in the CODEX® workflow. Furthermore, our experiments were conducted on human formalin fixed paraffin embedded tumor tissues, thereby demonstrating the applicability of CODEX® analyses for clinical and translational research agendas.

Disclosure Information O. Braubach: A. Employment (full or part-time); Significant; Akoya Biosciences. S. Mistry: A. Employment (full or part-time); Significant; Akoya Biosciences. G. Dakshinamoorthy: A. Employment (full or part-time); Significant; Akoya Biosciences. J. Yuan: A. Employment (full or part-time); Significant; Akoya Biosciences. P. Noordam: A. Employment (full or part-time); Significant; Akoya Biosciences. J. Kim: A. Employment (full or part-time); Significant; Akoya Biosciences. W. Lee: A. Employment (full or part-time); Significant; Akoya Biosciences. J. Kennedy-Darling: A. Employment (full or part-time); Significant; Akoya Biosciences.

P01.06 SPATIALLY-RESOLVED, HIGHLY MULTIPLEXED BIOMARKER ANALYSIS OF CANCEROUS AND NORMAL HUMAN BREAST TISSUES

¹O Braubach*, ¹S Basak, ¹M Gallina, ¹W Lee, ¹J Kim, ¹C Hempel, ¹E Williams, ¹O Shang, ²B Cheung, ¹J Kennedy-Darling. ¹Akoya Biosciences, Menlo Park, CA, USA; ²Akoya Biosciences, Marlborough, MA, USA

10.1136/jitc-2020-ITOC7.19

Background Breast cancer has a high incidence rate and there is a need to develop new diagnostic tools and treatment regimens. Progress has, unfortunately, been slow and new technologies are urgently needed to generate a comprehensive understanding of breast cancer biology. Highly multiplexed imaging is an emerging tool that can help to unravel the complexities of the tumor microenvironment. This technology enables the detection of tens of biomarkers within a tissue specimen, and allows comprehensive cell phenotyping, biomarker quantification and spatial localization at cellular resolution. Such measurements can, in turn, provide insights into disease mechanisms and identify potential treatment targets. We demonstrate the development of a breast cancer specific CO-Detection by indEXing (CODEX®) panel that allows simultaneous in situ imaging of more than 30+ antibody markers.

Materials and Methods CODEX® relies on a DNA-based tagging approach, whereby antibodies are labeled with specific oligonucleotide tags (barcodes) and dye-oligonucleotides (reporters) are iteratively hybridized and dehybridized across