P01.03 VOC PATTERN RECOGNITION OF LUNG CANCER: A COMPARATIVE EVALUATION OF DIFFERENT DOG- AND ENOSE-BASED STRATEGIES USING DIFFERENT SAMPLING MATERIALS

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Background It has been reported that canine scent tests offer the possibility to screen for cancer. Assuming that breath samples can be collected with carrier materials, we tested the practicability of different carrier materials to be presented to dogs, and validated and compared results with an eNose. Moreover, we hypothesised that cancer detection ability of dogs differs according to their working experience.

Materials and Methods In a methodological approach two dog teams participated, one using experienced working dogs and the other ordinary household dogs to find discover which dogs were better qualified and the best training method. To find best carrier material for breath sampling we compared charcoal containing glass tubes with fleece masks. In a second validating part, experienced working dogs were trained with improved training strategies. For breath sampling two different, previously successfully tested fleece-based carrier materials were used: one was used with the dog team and both materials were compared with eNose.

Results In the first part of the study it was shown overall that experienced working dogs performed better to family dogs and the dogs achieved a sensitivity of 45–59% and a specificity of 45–69%. Charcoal based breath sample carrier materials did not qualify for detection of VOC by dogs. In the second part of the study, the dogs achieved a specificity of 83% and a sensitivity of 56%, but with considerable differences between individual dogs. The eNose provided a specificity of 97% for both fleece based carrier materials and a sensitivity of 89% for fleece filled glass tubes and 100% for earloop masks. Measurements of breath samples collected directly in respiratory bags as reference measurements achieved a sensitivity and specificity of 100%.

Conclusions Our data confirmed that diagnostic accuracy of dogs depended on the type of dog training and on the carrier materials. A comparison of breath samples analysis with an eNose achieved better results for both, sensitivity and specificity, than for dogs. The use of fleece masks or fleeces in glass tubes as a sampling material can be recommended as successful VOC carriers, encouraging their use for clinical screenings.

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P01.04 A SPATIALLY RESOLVED, HIGHLY MULTIPLEXED BIOMARKER ANALYSIS PIPELINE THAT BRIDGES THE DIVIDE BETWEEN DISCOVERY AND CLINICAL RESEARCH

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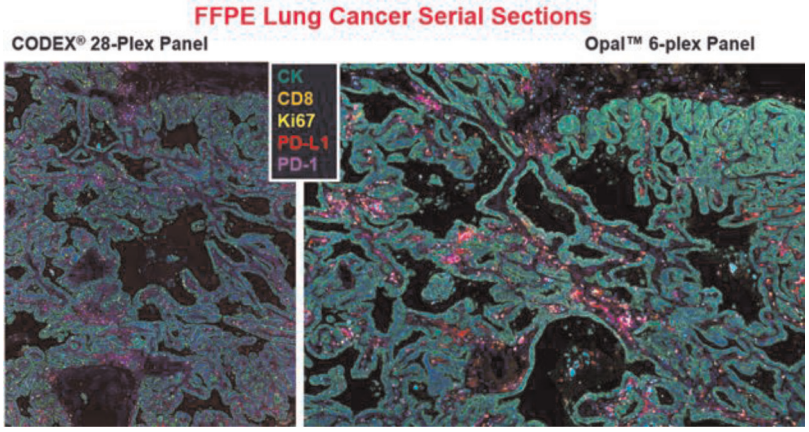
Background Multiplexed immunofluorescence (mIF) allows the visualization of multiple biomarkers in a single tumor tissue section, while at the same time preserving the spatial biology of the tumor microenvironment (TMI). CO-Detection by indEXing (CODEX®) and Phenoptics™ platforms are complementary mIF technologies that span the full spectrum of cancer research, from discovery to translational and clinical research. CODEX® is ultra-high plex and allows imaging of up to 40 antigens on a single tissue section with single-cell resolution. Phenoptics™ is an established mIF platform that enables high-throughput whole slide multispectral image acquisition and tissue interrogation with up to 8 markers plus DAPI. Here we present a study that compares shared sets of immune and tumor markers between the CODEX® and Phenoptics™ platforms. This cross-platform comparison provides a conceptual framework for researchers to translate biomarker signatures from discovery to high-throughput translational studies.

Materials and Methods Serial sections of human formalin-fixed paraffin embedded non-small cell lung cancer (NSCLC) and tonsils were analyzed. An initial screen with a 28-plex CODEX® antibody panel revealed multiple biomarkers of



Abstract P01.04 Figure 1

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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™ Phenotpics™ 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

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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.

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

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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

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