Optimization of an Exploratory Oncology Panel for Immunoprofiling Tissues in the Tumor Microenvironment using CO-Detection by indEXing (CODEX) methodology

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Multiplex Immunofluorescence and Digital Pathology Laboratory

Background

- Cancer immunotherapy is revolutionizing cancer treatment by aiding the natural ability of the immune system to identify and defend against tumors.
- In order to better design cancer therapeutics and advance clinical care, the immunoregulatory proteins involved in immune checkpoints and immunosuppression must be immunoprofiled.
- We used highly multiplexed tissue imaging technologies, such as CODEX (CO-Detection by indEXing), which allows more than 40 antibody markers to be placed and analyzed on tissues.
- Herein, we describe the optimization, validation and application of a 27-antibody panel used for multiplexed immunophenotyping.

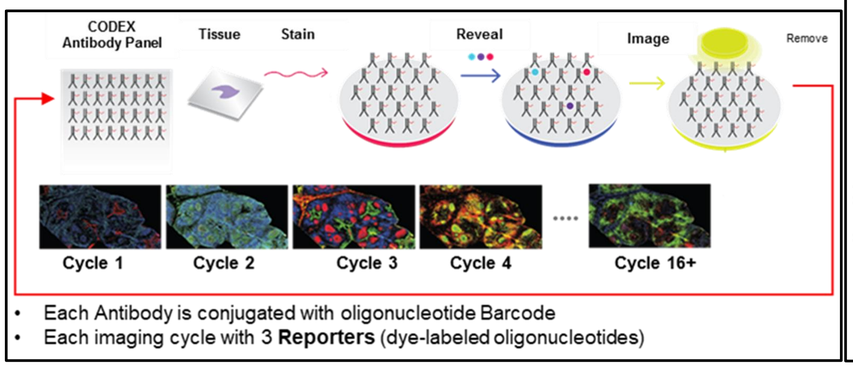
Hypothesis

- We hypothesize that the various markers will stain in similar patterns with both chromogenic IHC and CODEX, and that the antibodies will correctly recognize various lymphoid, myeloid, structural and immune cells in order to effectively depict the immunoprofile.

Methods

- Antibody selection
- Panel design
- Individual antibody optimization by Immunohistochemistry
- Antibody conjugation with their oligonucleotide
- Gel electrophoresis testing to validate proper conjugation
- Individual antibody staining in CODEX
- Group the antibodies in cycles and staining

Figure 1. Workflow of the application of the CODEX panel.



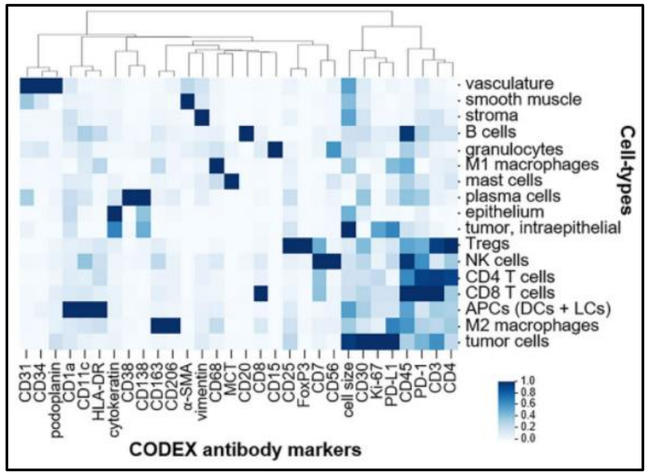
Results

Figure 2. Antibodies included in the CODEX panel.

Target	Cycle	Vendor
CD3E	1	Akoya
CD8	1	Akoya
Granzyme B	1	CST
CD20	2	Akoya
CD19	2	CST
CD21	2	Akoya
CD4	3	Akoya
FOXP3	3	Biolegend
CD56	3	Abcam
Arg-1	4	CST
CD206	4	Abcam
CD68	4	Akoya
CD73	5	CST
CD39	5	Abcam

Target	Cycle	Vendor
CD31	5	Akoya
PD-1	6	Abcam
PD-L1	6	CST
CTLA-4	6	Abcam
pCK	7	Akoya
Ki67	7	Akoya
HLA-DR	7	Akoya
CD45RO	8	Akoya
CD33	8	Abcam
OX40	8	BD
CD45	9	Biolegend
MHC-1	9	Abcam
GFAP	9	Abcam

Figure 3. Heat map showing the presence of various markers in different cell-types.



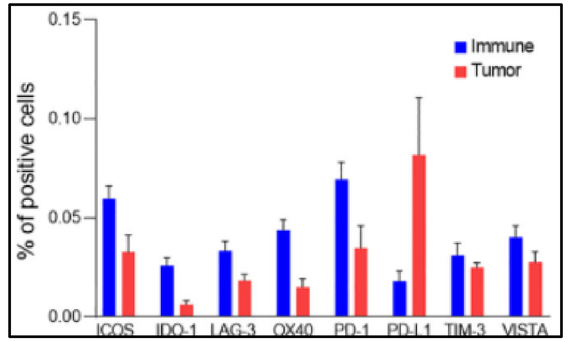
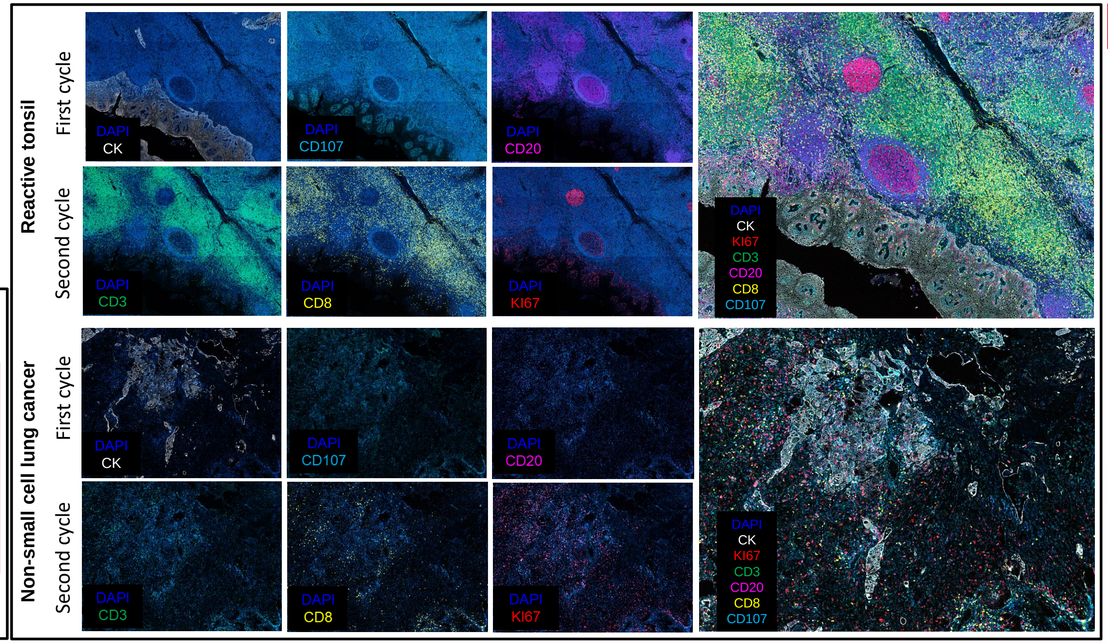


Figure 4. Percent of immune and tumor cells that express various immunoregulatory proteins.

Figure 5. Example of cycles staining in human reactive tonsil and non-small cell lung cancer case .



Discussion and Conclusion

- We successfully optimized the various markers to obtain similar staining patterns with both chromogenic IHC and CODEX. PD-L1 was predominantly expressed on the cell membranes of epithelial cells on tonsil tissue. Other immune checkpoint proteins were observed in various immune cells. In control tonsil, CK was expressed by epithelial cells, and among cells surrounding the germinal centers, the T cell marker CD3 was most abundant, followed by CD8, Granzyme B, CD45RO, FOXP3. PD-1 was predominantly distributed within the germinal center of the tonsil tissue. We are continuing to test the other markers in the panel.
- Multiplexed immunophenotyping approaches reveal key insight into identifying therapeutic mechanisms. A comprehensive immunoprofile will advance our understanding of how various factors can determine disease progression, resistance and response to immunotherapies and facilitate the development of new treatment approaches.

References

- Phillips D, Schürch CM, Khodadoust MS, Kim YH, Nolan GP, Jiang S. Highly Multiplexed Phenotyping of Immunoregulatory Proteins in the Tumor Microenvironment by CODEX Tissue Imaging. Front Immunol. 2021 May 19;12:687673. doi: 10.3389/fimmu.2021.687673. PMID: 34093591; PMCID: PMC8170307.

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