T cell exhaustion assessment using an automated sequential chromogenic multiplex assay (Brightplex)

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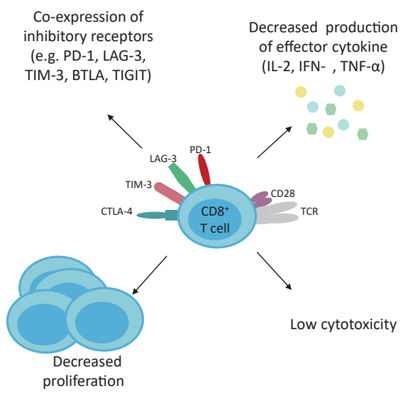
Background

- Non-small cell lung cancer (NSCLC) is a leading cause of cancer-related mortality.
- Anti-PD-1/PD-L1 antibodies are now established as an efficient approach to restore the cytotoxic activity of exhausted T cells. However 80% of patients do not respond to these treatments.
- Resistance to treatment could be due to the exhaustion of T cells expressing inhibitory receptors (immune checkpoints).
- Since exhausted T cells may co-express several checkpoints, targeting only PD-1 may not be sufficient to restore their activity.

➔ **To improve the prediction of response to immunotherapies, a novel multiplex IHC method was developed to detect and quantify exhausted T cells expressing PD-1, LAG-3 and TIM-3 checkpoints.**

T Cell Exhaustion (TCE)

A state of T cell dysfonction in infection and in cancer:



References

- In-depth tissue profiling using multiplexed immunohistochemical consecutive staining on single slide. Remark et al., Sci. Immunol., (2016).
- Quantitative Multiplex Immunohistochemistry Reveals Myeloid-Inflamed Tumor-Immune Complexity associated with Poor Prognosis? Tsujikawa et al., Cell reports (2017)
- Halo™ software: //www.indicalab.com/halo/

Multiplex IHC workflow

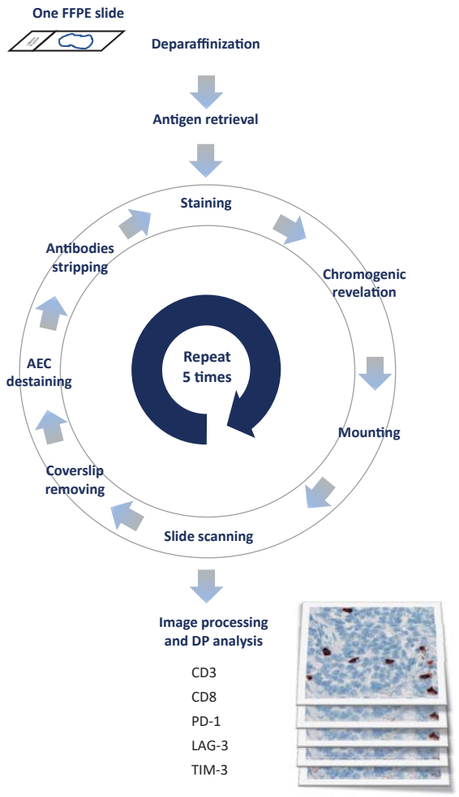


Figure 1: Multiplex IHC Worflow. Primary antibodies are applied and visualized with horseradish peroxidase conjugate followed by 3-amino-9-ethylcarbazole (AEC) chromogeny. Stained slides are digitized with a whole slide scanner before AEC removal and antibody stripping. All immunostainings were automated on Leica Bond RX.

In a few words

Brightplex is an integrated solution developed by HalioDx for sequential multiplex chromogenic IHC and whole slide digital pathology analysis allowing phenotyping of immune cells in FFPE tumor tissue.
Here we present a Brightplex panel to study exhausted T cells.

- Staining on 1 FFPE slide → conservation of precious material
- Identification of CD8⁺ T cell expressing 1, 2 or 3 checkpoints
- Calculation of cells density within any region of interest
- Display of exhausted T cells within the tumor landscape

Digital pathology workflow with Brightplex

Module 1 Brightplex

- Virtual slides alignment
- Color deconvolution for each biomarker
- Creation of peudo-color image

Module 2 HALO™ Indica Labs

- Detection of positive cells for each biomarker
- Detection of regions of interest (e.g. core tumor, invasive margin, tumor parenchyma/ stroma)
- Analysis of positive cells in all ROIs

Module 3 Brightplex

- Calculation of cell densities of phenotypes of interest
- Map reconstruction with complex phenotypes

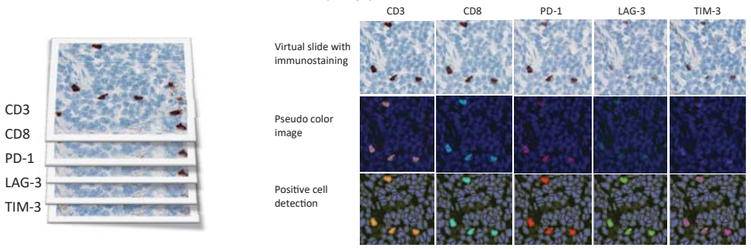


Figure 3: Digital pathology workflow. The 5 digitized slides obtained from the same FFPE tissue section were transformed with a proprietary software. It provides a set of tools for images alignment and color deconvolution. Positive cells detection of each biomarker was then processed with Halo™ software. Information for each cell were exported to extract cell densities and reconstruct a multiplex "staining" of the tissue section.

Exhausted CD8⁺ T cells analysis

Example of cell densities obtained on a NSCLC resection

Phenotype	Density by ROI (cells/mm2) Core Tumor	IM	Parenchyma	Stroma
CD3⁺	1400	1800	430	2400
CD3⁺ CD8⁺	690	870	260	1100
CD3⁺ CD8⁺ PD-1⁺ LAG-3⁻ TIM-3⁻	50	100	12	89
CD3⁺ CD8⁺ PD-1⁺ LAG-3⁻ TIM-3⁺	97	160	29	170
CD3⁺ CD8⁺ PD-1⁺ LAG-3⁺ TIM-3⁻	33	56	6,5	60
CD3⁺ CD8⁺ PD-1⁺ LAG-3⁺ TIM-3⁺	170	210	72	270
CD3⁺ CD8⁺ PD-1⁻ LAG-3⁺ TIM-3⁻	38	51	9	67
CD3⁺ CD8⁺ PD-1⁺ LAG-3⁺ TIM-3⁺	100	78	62	150

Table 1: Cell densities of CD8⁺ T cells expressing one or more checkpoints were evaluated in the core tumor, in invasive margin regions (720µm), in tumor parenchyma and in tumor stroma.

Exhausted T cell location on tumor landscape

Example of phenotypes identified with the TCE panel on a NSCLC resection

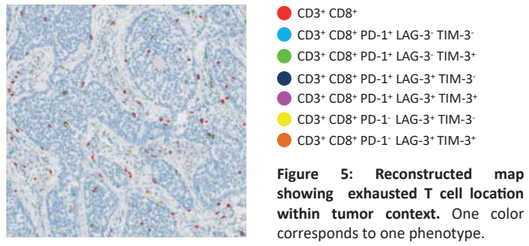


Figure 5: Reconstructed map showing exhausted T cell location within tumor context. One color corresponds to one phenotype.

TCE panel : CD3, CD8, PD-1, LAG-3 & TIM-3

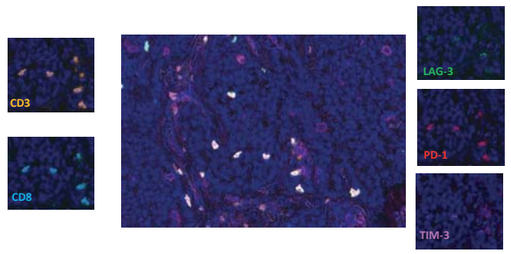


Figure 2: CD3, CD8, PD-1, LAG-3 and TIM-3 immunostainings with AEC chromogenic detection on NSCLC resection after alignment and color deconvolution with Brightplex software.

Analysis in various regions of interest of a NSCLC FFPE resection

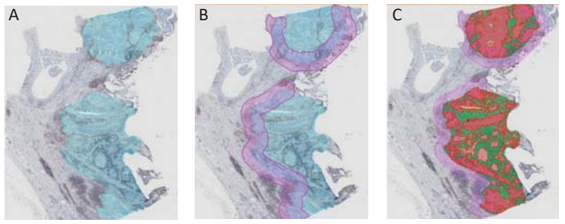


Figure 4: Different regions analyzed on a NSCLC resection. Detection of CD8⁺ T cells expressing checkpoints was assessed in core tumor (A, blue area), invasive margin (purple line, 720µM), in tumor parenchyma (red area) and in tumor stroma (green area).

Exhausted T cell densities in 9 tumor samples (NSCLC)

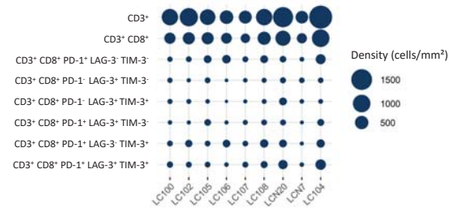


Figure 6: Cell densities of CD8⁺ T cells expressing one or more checkpoints. Only cell densities in core tumor are showed.