Multiplex immunofluorescence detection of resident memory T cells in solid tumors

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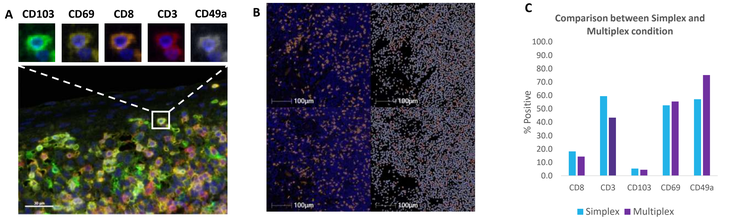
Background : Tissue-resident memory T cells (TRM) represent a lineage of T cells localized in peripheral tissues that do not reenter the circulation. Their establishment in the tissue makes them the initial T cell response in local infections and inflammatory conditions. TRM are characterized by the expression of CD103, CD69, and CD49a. TRM play a role in the efficacy of cancer vaccines and have been correlated with improved prognosis and/or survival in a number of cancers. Comparisons of TRM and cytotoxic CD8 T cells in the tumor microenvironment are limited.

Objective: Develop the HISTOPROFILE®-TRM multiplex panel to phenotype memory resident T cells in solid tumors and demonstrate the applicability of the panel to study TRM subpopulations in NSCLC FFPE tissue resections.

Methods:

- Panel Design and Validation
  - Targets – CD3/CD8/CD49a/CD69/CD103
    - Memory resident T cells – $CD8^+/CD103^+$
    - Subtypes of memory resident T cells – $CD8^+/CD49a^+$, $CD8^+/CD69^+$, $CD8^+/CD103^+/CD69^+$, $CD8^+/CD103^+/CD69^+/CD49a^+$
- Three NSCLC tissue resections samples were sourced from the Cerba Reseach Montpellier Biobank
- A pathologist delineated the NSCLC tumor area on hematoxylin & eosin stained slides
- Sequential multiplex protocol with Opal® (Akoya Biosciences) fluorophores on the BOND RX® (Leica) slide stainer
  - Multispectral images acquired with the VECTRA® Polaris™ (Akoya Biosciences) slide scanner.
  - Whole slide image analysis with the HALO® (Indica Labs) Highplex module on unmixed NSCLC images

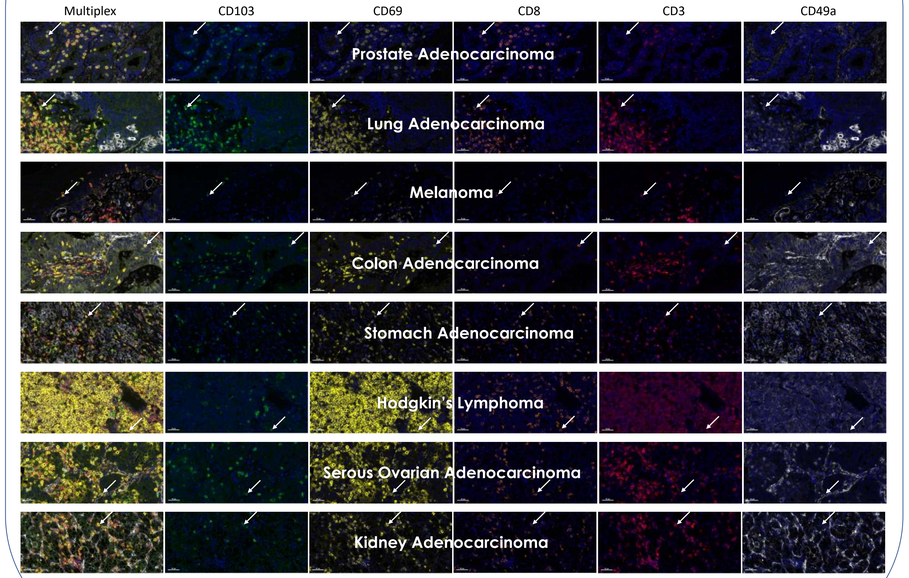
HISTOPROFILE®-TRM Panel Validation



Simplex slides were stained for each individual biomarker in a simplex protocol and compared to a serial section stained with the HISTOPROFILE®-TRM multiplex panel. Staining concordance between the multiplex slide and simplex slides was determined by analysis with HALO® without multispectral deconvolution. A) Example image of a multiplex stained healthy human tonsil. The co-expression of the five markers in the panel: CD103, CD69, CD8, CD3, CD49a can be appreciated in the zoom. B) Example images corresponding to the multiplex slide for CD8 (top) and the simplex slide (bottom) and the corresponding HALO® masks identifying positive cells. C) The percentage of positive cells from the simplex (blue bars) and multiplex panel (purple bars) slides showing comparable staining profiles between the slides.
The precision of the protocol was determined by intra-run repeatability analysis and inter-run reproducibility analysis (data not shown).

HISTOPROFILE®-TRM Panel Robustness

The robustness of the panel was demonstrated on a variety of human solid tumors including prostate, colon, lung and kidney adenocarcinomas, Hodgkin lymphoma, melanoma and serous ovarian adenocarcinoma. White arrows identify TRM expressing at least CD8 and CD103.



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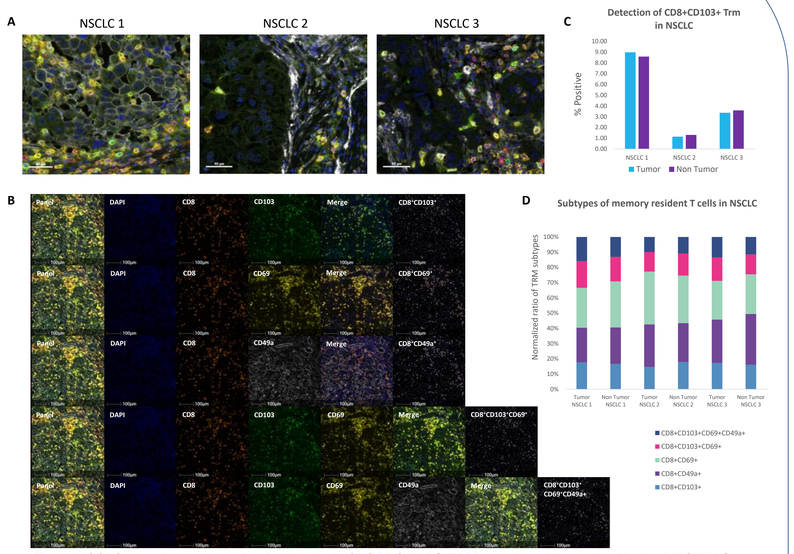
HISTOPROFILE®- TRM Panel NSCLC Analysis



Three NSCLC samples were stained with the HISTOPROFILE®-TRM panel and imaged with the VECTRA® Polaris™. Whole scan multispectral images were analyzed with HALO®. A) Example of the multiplex panel on each of three NSCLC samples: CD8 (orange), CD103 (green), CD49a (white), CD69 (yellow), CD3 (red). Scale bar 40 µm. In the literature, TRM have been phenotyped by different combinations of the markers used in the panel. The markers were combined to analyze different TRM subtypes: $CD8^+/CD103^+$, $CD8^+/CD49a^+$, $CD8^+/CD69^+$, $CD8^+/CD103^+/CD69^+$, $CD8^+/CD103^+/CD69^+/CD49a^+$ in tumoral and non tumoral areas of the three NSCLC human samples. B) Example images of the HISTOPROFILE®-TRM panel and the individual populations with the fluorescent image and the corresponding cell masks for the different subtypes of TRM cells. Scale bar 100 µm. C) The frequency of memory resident T cells ($CD8^+CD103^+$) in the tumor region (blue bars) and non tumoral area (purple bars) was analyzed. D) The normalized distribution of different subpopulations of resident T cells in the tumor and non-tumor regions of the three NSCLC samples: $CD8^+/CD103^+$ (blue), $CD8^+/CD49a^+$ (purple), $CD8^+/CD69^+$ (pale green), $CD8^+/CD103^+/CD69^+$ (pink), $CD8^+/CD103^+/CD69^+/CD49a^+$ (dark blue).

Conclusion: The HISTOPROFILE®-TRM Panel consisting of CD8, CD3, CD69, CD103 and CD49a markers was developed and used for the detection of resident T cells in human NSCLC samples. The panel shows a unique power to be able to phenotype the TRM by various combinations of the included targets. Different subpopulations of TRM in tumoral and non tumoral areas could be distinguished with image analysis. Robustness of this multiplex panel make it an useful tool to investigate TRM populations in various human solid tumors.