In situ multiplex analysis of regulatory and effector T cells in multiple tumor types

Elena Baranova, Maroua Tliba, Manon Motte, Naomi Defort, Amanda Finan-Marchi, Domenico Lazzaro, Fanny Estermann, Jean-Philippe Coton, Renaud Burrer
HISTALIM, 126 rue Emile Baudot, 34090 Montpellier, France.

Background : T cells are the most important immune effector cells and are therefore preferred targets for immunomodulation. T cells can be broadly classified as either T effector (Teff) or T regulatory (Treg) cells. Teff cells ensure optimal immune responses against invading microbes and tumor antigens. Under homeostatic conditions, Tregs promote peripheral tolerance. However, within tumors, Tregs can suppress Teff cell functions. The complex interplay of Teff and Treg dictate the outcome of tumor-specific immune responses. Favorable survival in numerous types of cancer as been associated with high Teff to Treg cells ratios as determined by flow cytometry or immunohistochemistry on serial sections. Multiplex immunofluorescence offers a technical advantage by allowing for the detection of co-expression and spatial organization of multiple targets within a preserved tissue architecture on a single slide.

Objective : Demonstrate the utility of Histalim's HISTOPROFILE® -Treg human and mouse multiplex immunofluorescence panel to identify Treg and Teff cells in situ

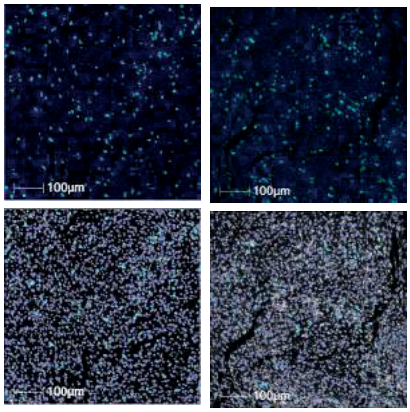
Methods :

- Panel Design and Validation
- Targets – CD3/CD4/CD8/FoxP3/CD25
- Sequential multiplex protocol with Opal® (Akoya Biosciences) fluorophores on the BOND RX® (Leica) slide stainer
  - Subcutaneous Murine Patient-Derived Xenograft (PDX) Model
  - Human tissue microarrays
- Non small cell lung carcinoma (NSCLC) tumors
- Multispectral images acquired with the VECTRA® PolarisTM (Akoya Biosciences) slide scanner.
- Images were spectrally deconvoluted with INFORM® (Akoya Biosciences) software.
- Image Analysis with the HALO® (Indica Labs) Highplex module on NSCLC

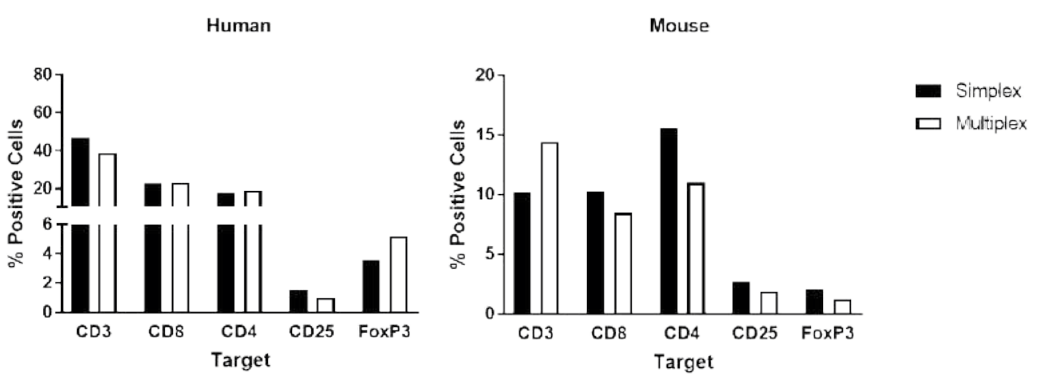
Panel Validation : Comparison of Simplex and Multiplex staining

Simplex slides were stained for each individual biomarker in a simplex protocol and compared to a serial section stained with the multiplex IHC. Staining concordance between the multiplex slide and simplex slides was determined by analysis with HALO® after multispectral deconvolution. A) Example images (top) from a simplex slide for target A (left) and the multiplex slide (right) and the corresponding HALO® masks identifying positive cells (bottom). B) Results from the human and mouse Treg panels analysis. The percentage of positive cells from the simplex slide (black bar) is compared to the multiplex slide (white bar). Satisfactory results were obtained for both HISTOPROFILE® -Treg panels.

A

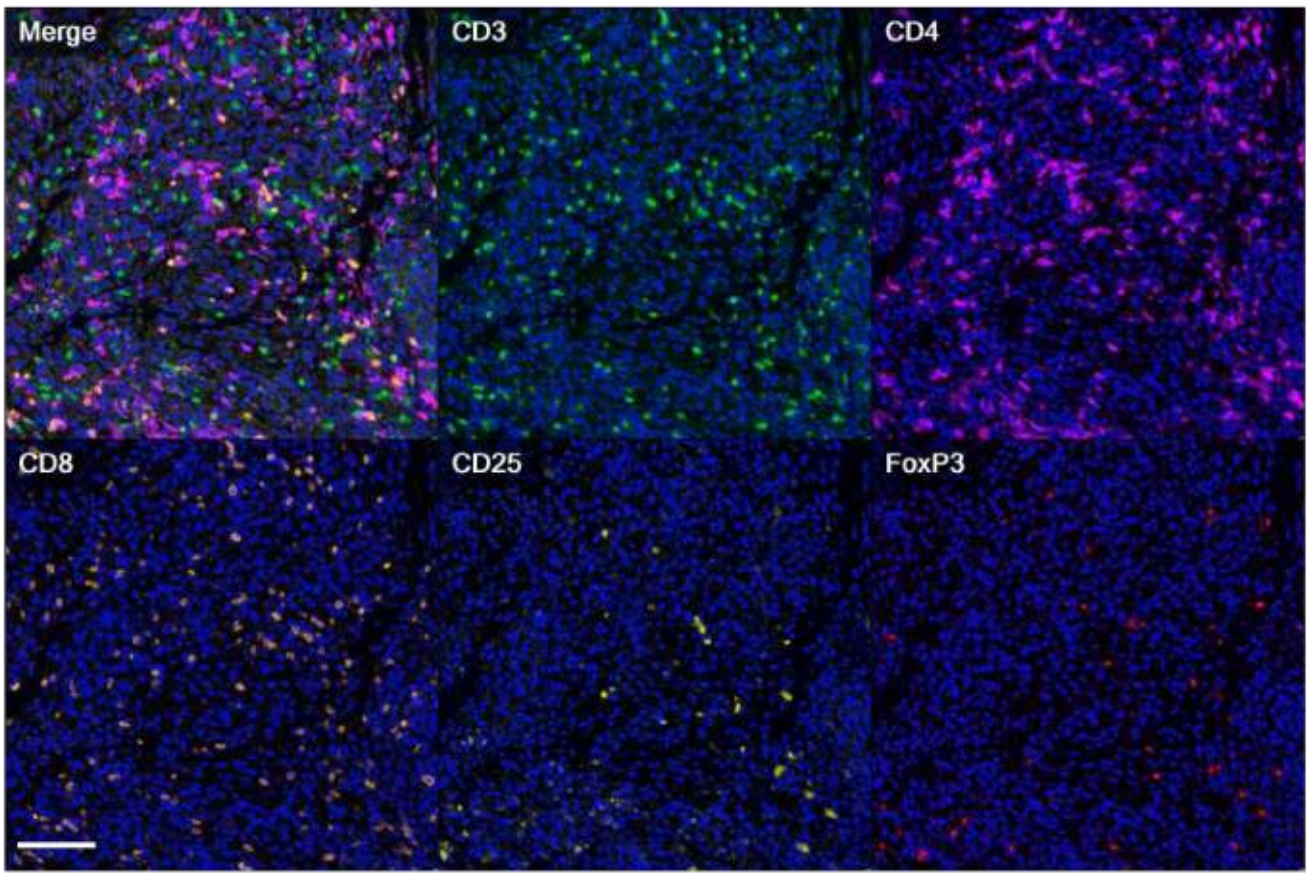


B



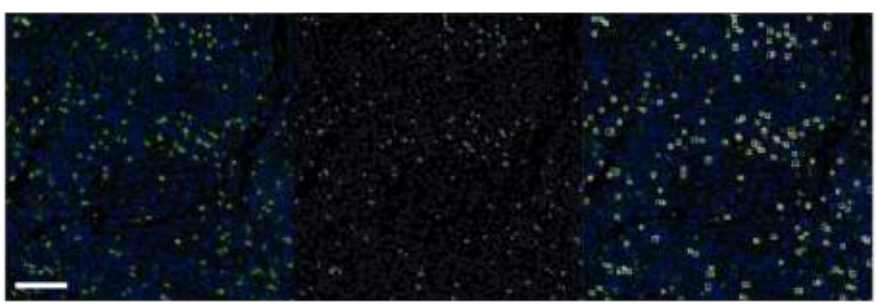
HISTOPROFILE® -Treg Mouse panel

A

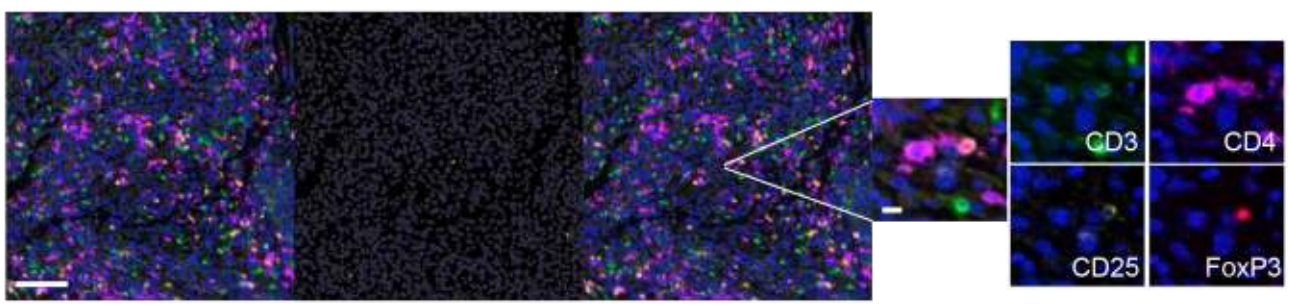


A) Deconvoluted Multispectral image of the full HISTOPROFILE®-Treg mouse panel on a subcutaneous PDX model – CD3 (green), CD4 (purple), CD8 (orange), CD25 (yellow), and FoxP3 (red).
B) Example image of the CD3 and CD8 cells (left). The CD3+/CD8+ population was detected with the HALO® Highplex module. Cell masks (blue masks) or object detection (white squares) allow for localization of the double positive cells.

B

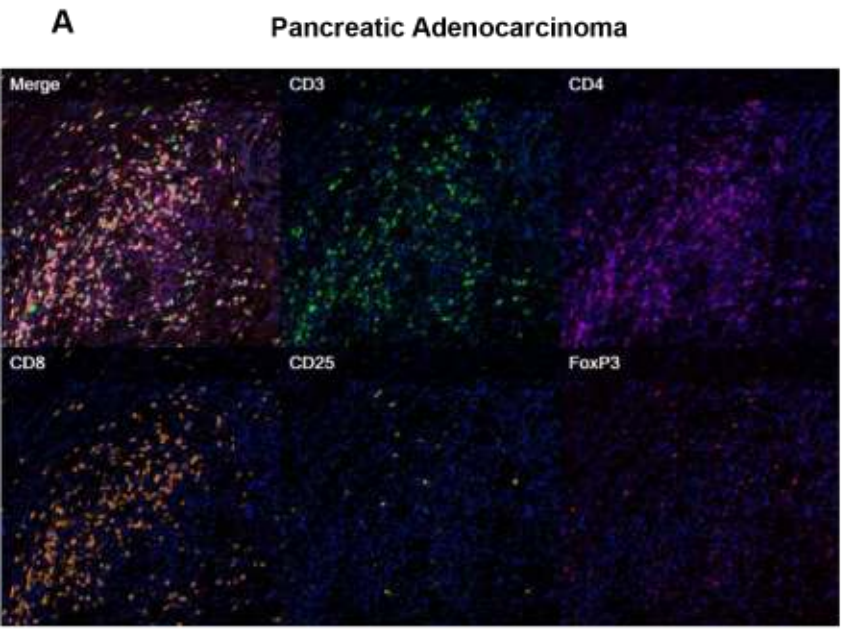


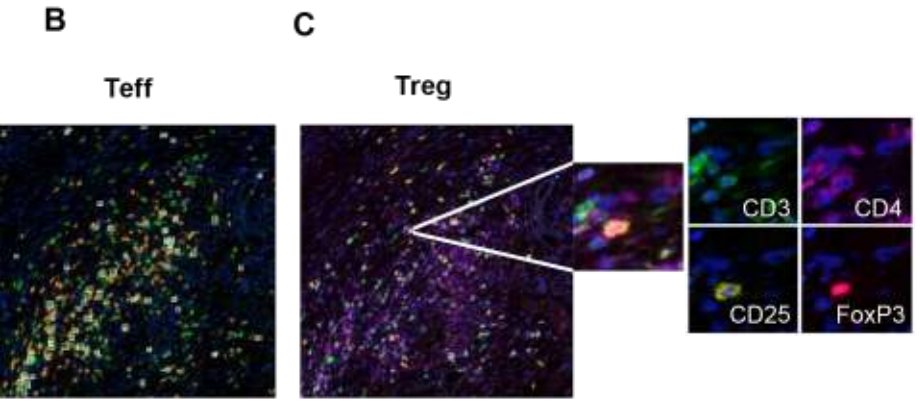
C



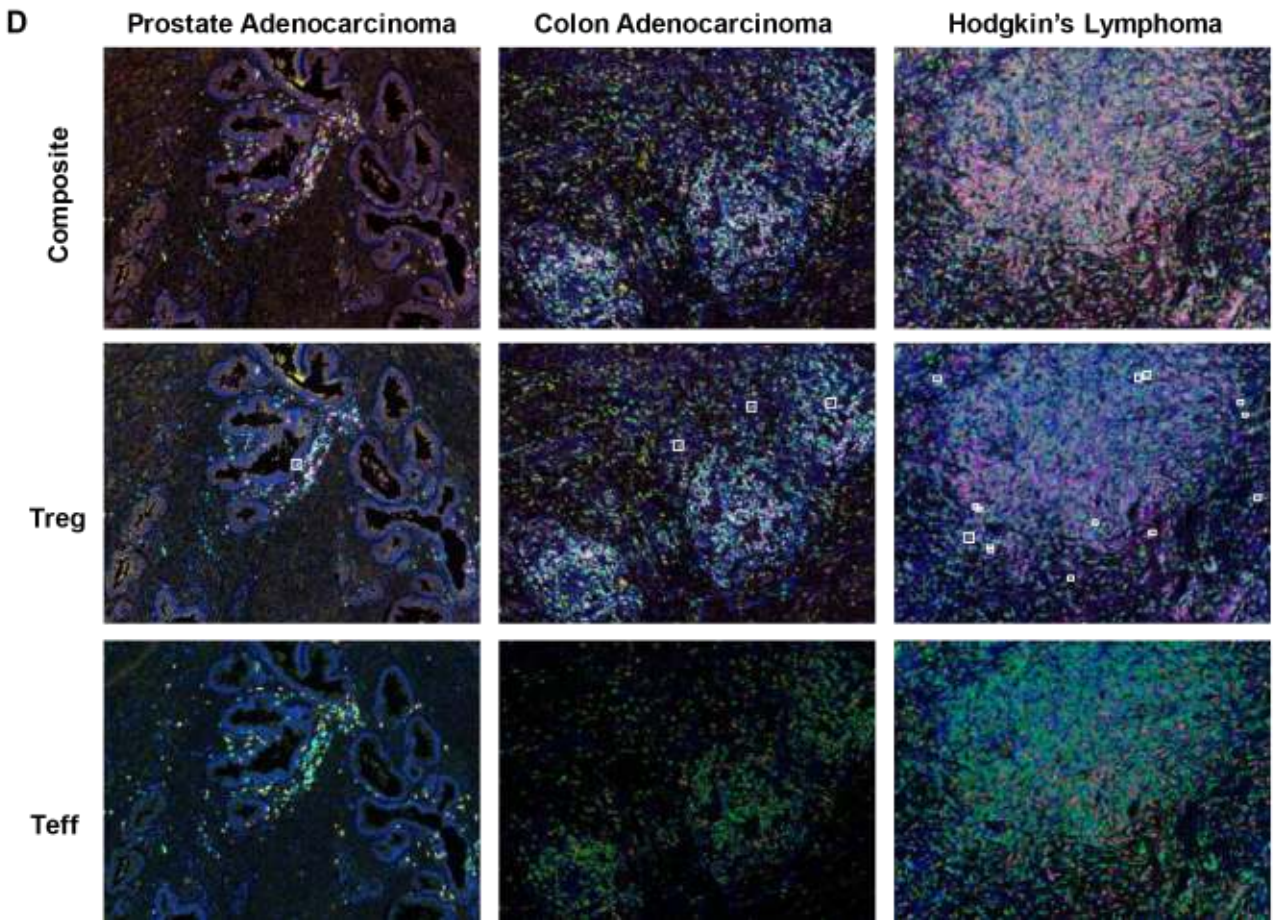
C) Example image of Tregs (CD3+CD4+CD25+FoxP3+) detection with the HALO® Highplex module. Cell masks (orange masks) or object detection (white squares) allow for easy visualization of the Tregs in the field. A higher magnification of a Treg cell is displayed on the right. The white scale bar represents 100 µm except in the zoomed image, where it represents 10 µm.

HISTOPROFILE® -Treg Human panel

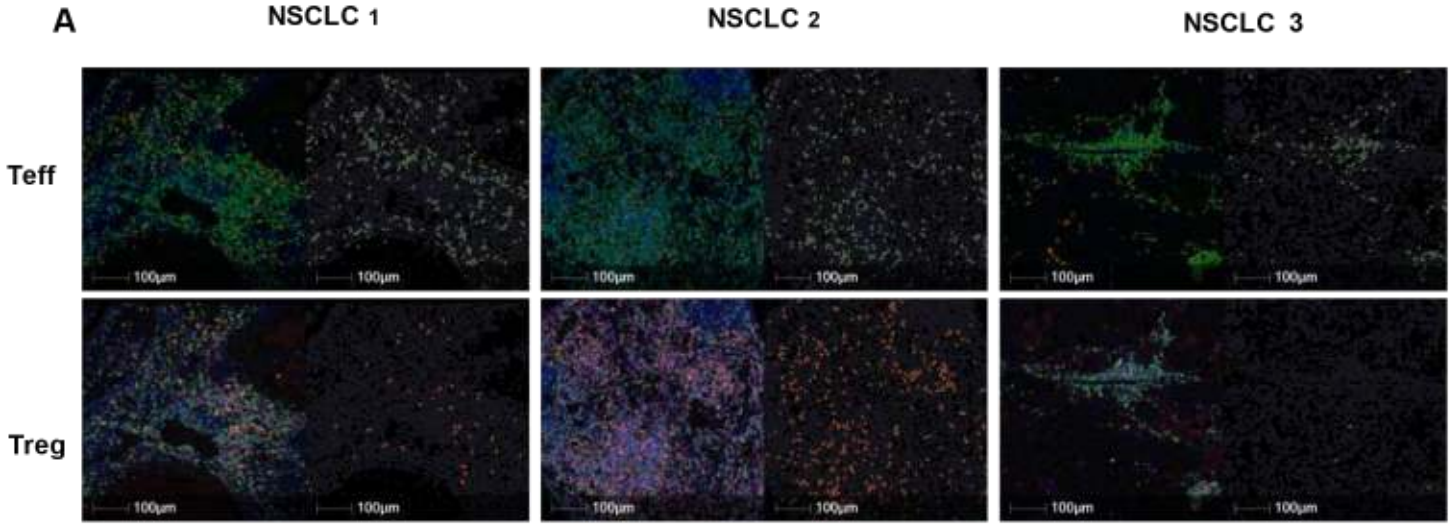




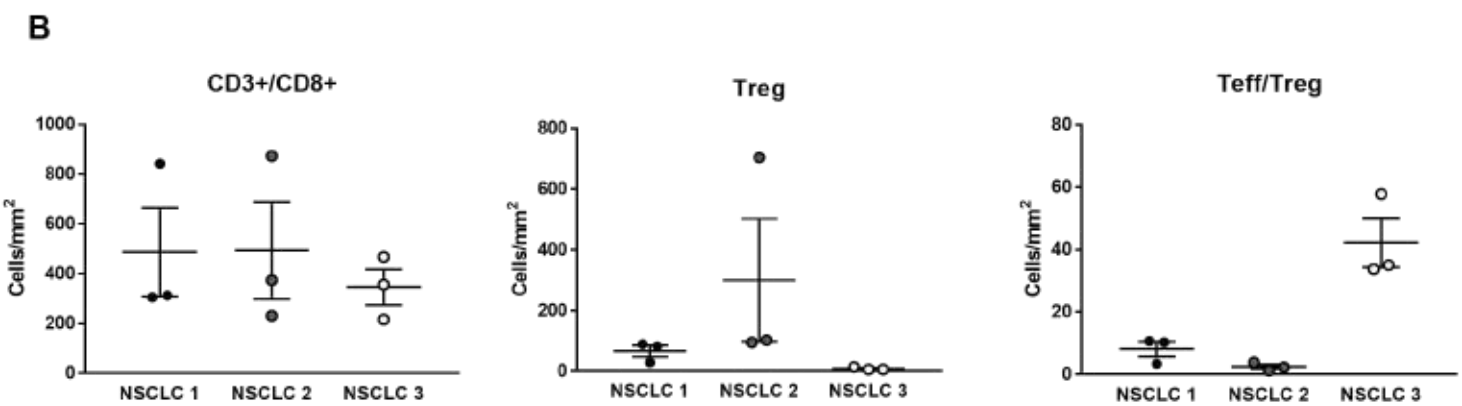
A) Multispectral image of the HISTOPROFILE®-Treg human panel after deconvolution on pancreatic adenocarcinoma : CD3 (green), CD4 (purple), CD8 (orange), CD25 (yellow), and FoxP3 (red). CD3+CD8+ cells and Tregs (CD3+CD4+CD25+FoxP3+) were detected with the HALO® Highplex module. B) Example image of the CD3 and CD8 staining with cell detection (white squares) identifying the double positive cells. C) Example image of the CD3, CD4, CD25, and FoxP3 staining used to detect Tregs. Cell detection (white squares) allow for easy visualization of a few Tregs in a large image field. A higher magnification of a Treg cell is demonstrated on the right. The white scale bar represents 100 µm except in the zoomed Treg image where it represents 10 µm. D) The robustness of the panel was tested on a tissue microarray. An example composite image of all cells, Treg, and Teff targets illustrated for prostate and colon adenocarcinoma and Hodgkin's lymphoma. White squares localize the Tregs (CD3+CD4+CD25+FoxP3+) in the image fields. The difference in Treg presence can be appreciated between the different tumor types.



Analysis of Treg and CD3+/CD8+ cells on NSCLC tissue



The HISTOPROFILE®-Treg human panel was applied to three non small cell lung carcinoma (NSCLC) tissues on the Leica Bond RX. Multispectral images were acquired on Vectra Polaris and the resulting images were spectrally deconvoluted with INFORM software. CD3/CD8 double positive cells and Tregs (CD3+/CD4+/CD25+/FoxP3+) were detected with the HALO highplex module. A) Example images from each of the three NSCLC tissues showing the CD3 and CD8 cells (top) and the CD3, CD4, CD25, and FoxP3 cells (bottom). HALO cell masks localize the CD3/CD8 double positive (blue encircled with green) and Treg cells (red encircled by yellow). B) Quantification of the CD3+/CD8+ Teff cells, the Treg cells, and the Teff/Treg ratio. For each sample, three regions of interest were analyzed. The results represent an average cells/mm². The variability of Teff/Treg ratio can be easily appreciated between the three samples.



Conclusion : The approach presented here demonstrates the power of Histalim's Histoprofile® Treg multiplex immunohistochemistry in the identification and quantification of multiple immune cell populations on a single tissue section and the potential application of this method on a range of preclinical and clinical tissues.

Contact :
Renaud Burrer, R&D Manager
Phone : +33 (0)430 969 828
E-mail : rburrer@histalim.com
HISTALIM
Address : 126 rue Emile Baudot,
34090 Montpellier, France

This work was supported by the French Government and BPI France in the Programme d'investissements d'avenir with the project EFFI-CLIN.