Immune cell checkpoint profiling of solid tumors by multiplex immunofluorescence

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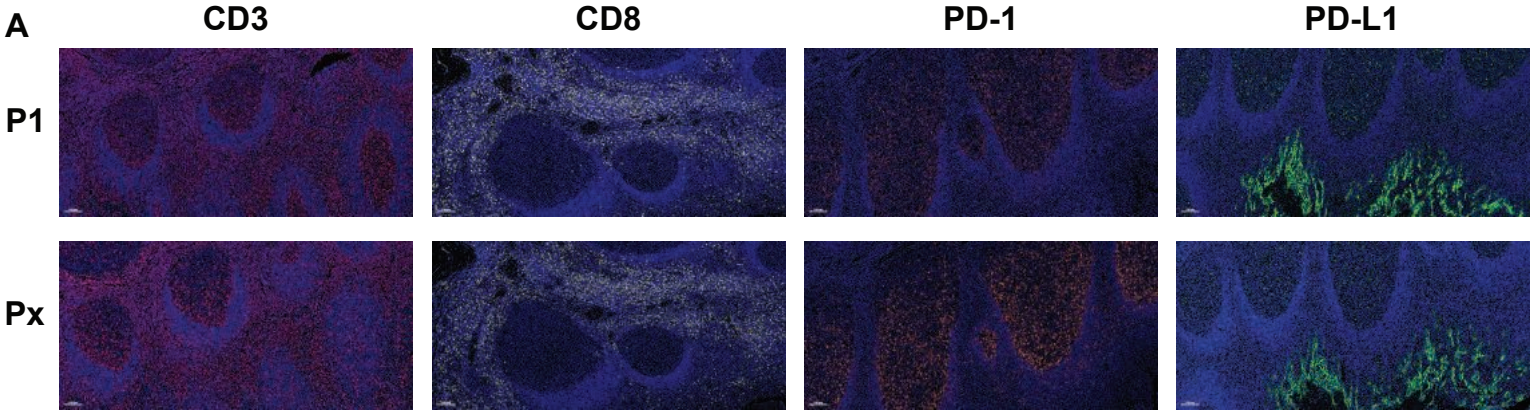
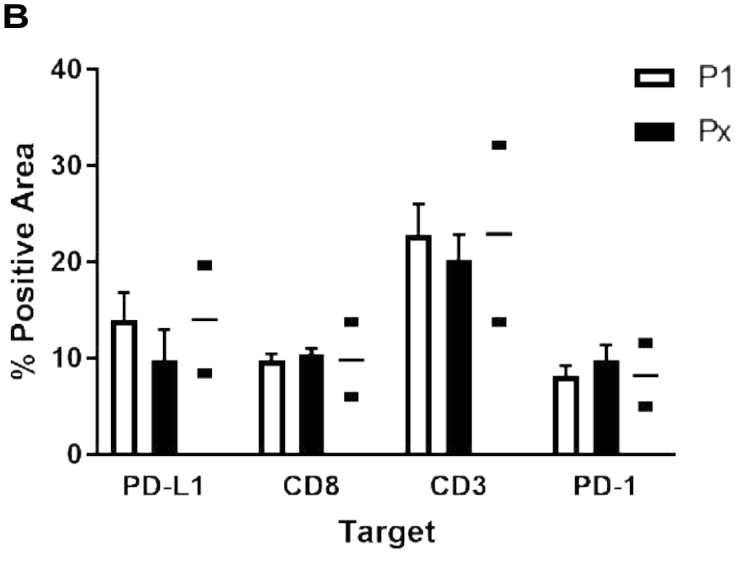
Background : Immune checkpoint proteins are important regulators in self-tolerance but also allow cancer cells to evade immune destruction. Checkpoint inhibitor (CKI) blockade therapies can help restore antitumoral immunity. Combination blockade has demonstrated the potential to result in greater tumor growth inhibition than monotherapies in preclinical studies. 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. We have developed the HISTOPROFILE®-CKI multiplex immunohistochemistry panel to offer personalized immune cell checkpoint profiling.

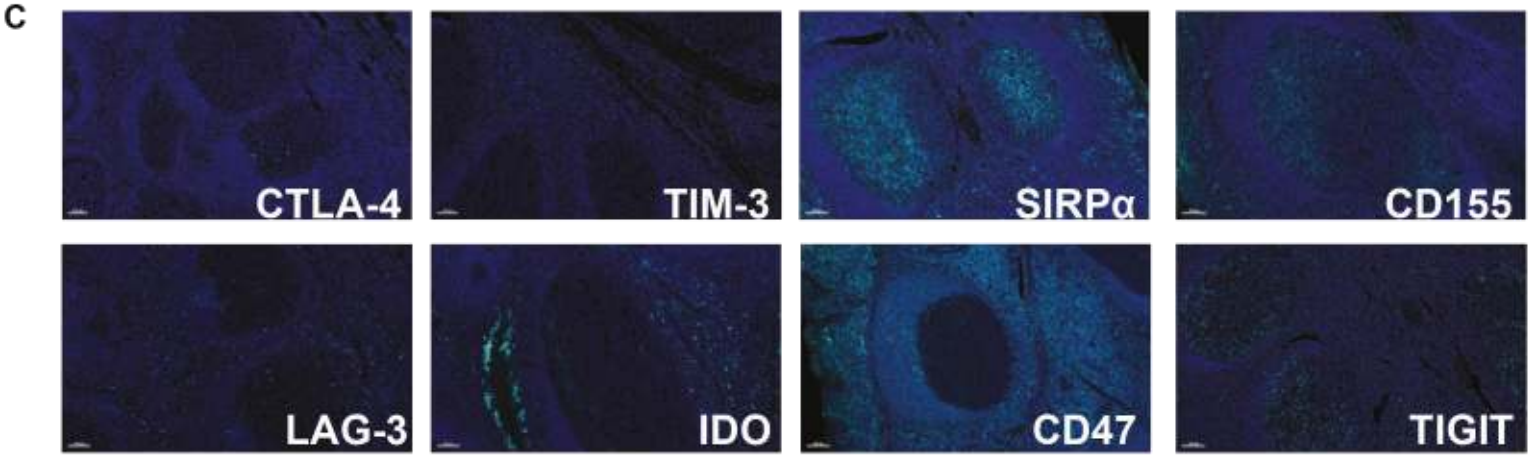
Multiplex Design and Validation

Methods : Sequential multiplex protocol with Opal® (Akoya Biosciences) fluorophores was performed on the BOND RX (Leica) slide stainer.
The multiplex panel was tested on human healthy tonsil and prostate, liver, lung, and skin tumors.
Whole slide multispectral images were acquired with the VECTRA ® PolarisTM (Akoya Biosciences) slide scanner.
Images were analyzed with INFORM® (Akoya Biosciences) or with HALO® (Indica Labs) Highplex module.

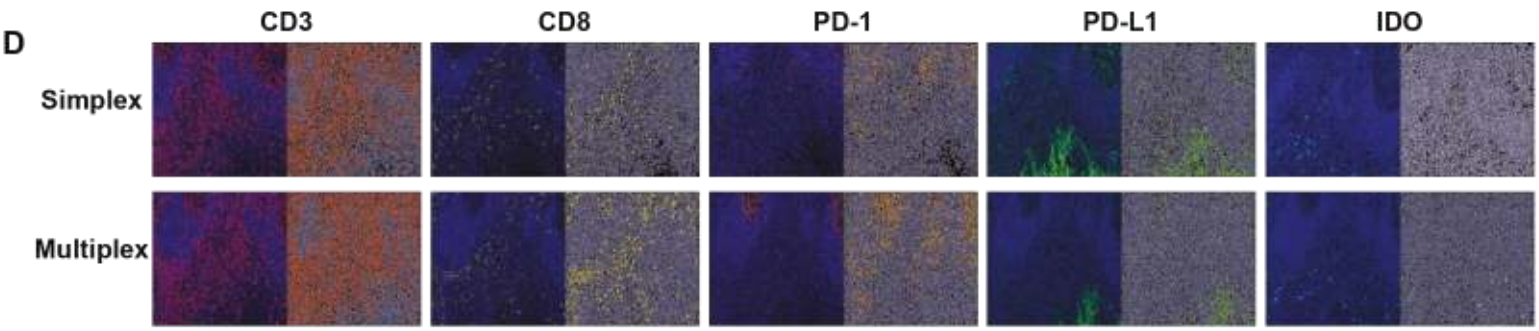


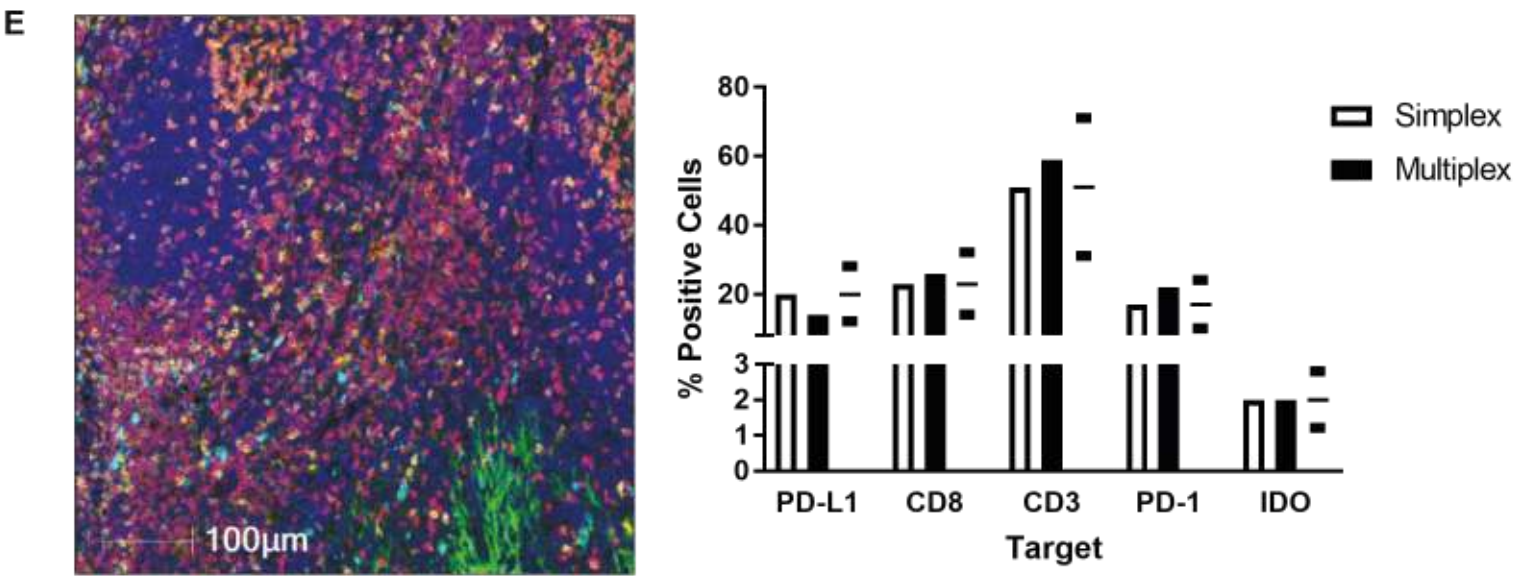


Base HISTOPROFILE®-CKI Panel : A) We have validated a base panel, comprised of CD3 (red), CD8 (yellow), programmed cell death 1 (PD-1, orange), programmed death-ligand 1 (PD-L1, green), and DAPI (blue) (human tonsil, scale bar = 100 µm). For each marker, the position of interest (Px) in the multiplex was compared to the reference (simplex IHC) protocol (P1). B) The % of positive area was compared between the two protocols to validate the panel.



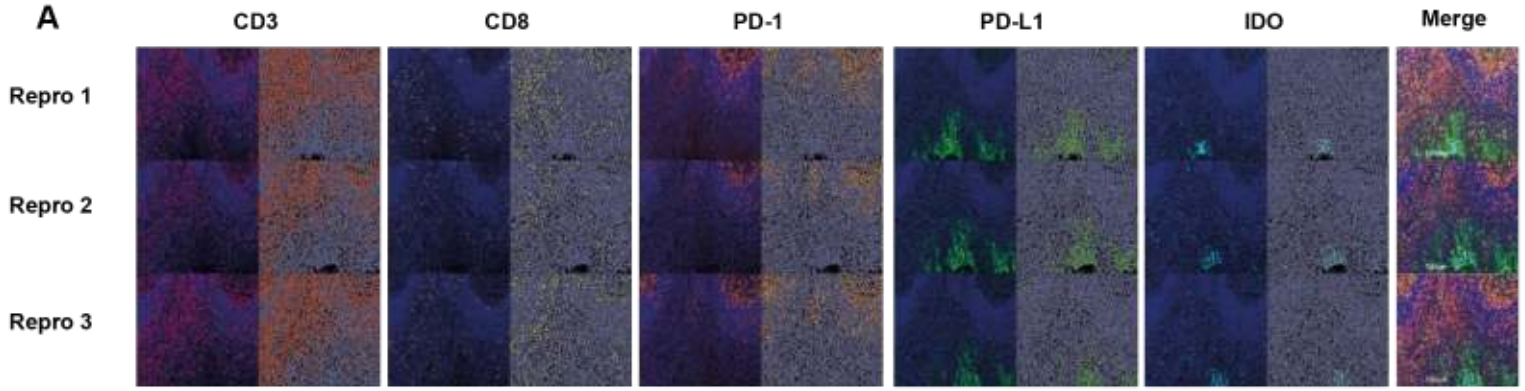
Simplex CKI protocols : C) We have validated protocols to detect numerous immune cell checkpoint proteins, that can be adapted to the HISTOPROFILE®-CKI multiplex panel. Representative images of the simplex staining for cytotoxic T-lymphocyte-associated protein 4 (CTLA-4/CD152), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), signal regulatory protein alpha (SIRPα), CD155, Lymphocyte activation gene 3 (LAG-3), Indoleamine-pyrrole 2,3-dioxygenase (IDO), CD47, T cell immunoreceptor with Ig and ITIM domains (TIGIT) are visible in cyan (Scale bar = 100 µm).

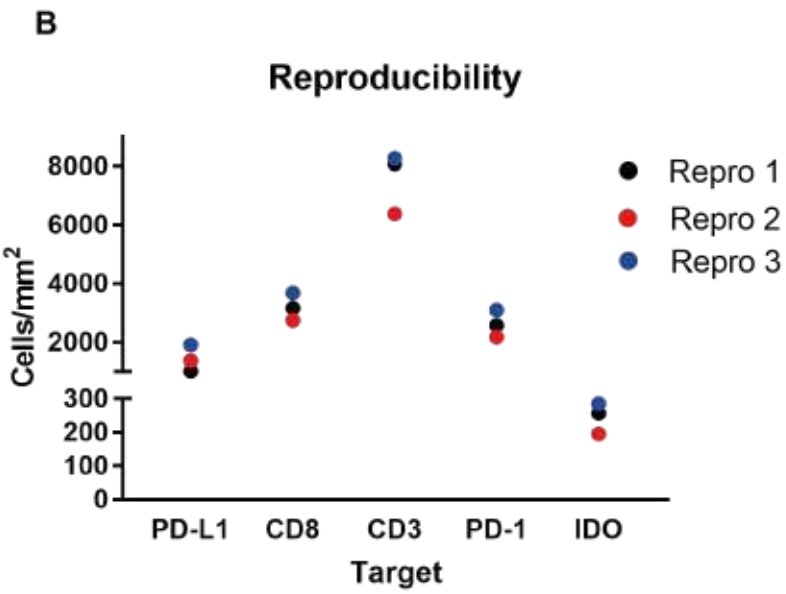




Multiplex validation : D) Example of the final validation step of HISTOPROFILE®-CKI base multiplex panel with the target Indoleamine-pyrrole 2,3-dioxygenase (IDO). Serial simplex stained slides with the individual biomarkers were compared with a multiplex slide. The concordance was evaluated with HALO. An example fluorescent image for each marker and its corresponding cell mask are shown for both the simplex and multiplex slides. E) A representative image from a whole scan showing staining for CD3 (red), CD8 (yellow), PD-1 (orange), PD-L1 (green), IDO (cyan), and DAPI (blue) (Scale bar = 100 µm). F) Image analysis results of the percentage of positive cells for each target on the simplex (white bar) and the multiplex (black bar) whole scans. The results show consistent results between the two slides, allowing for validation of the complete multiplex panel.

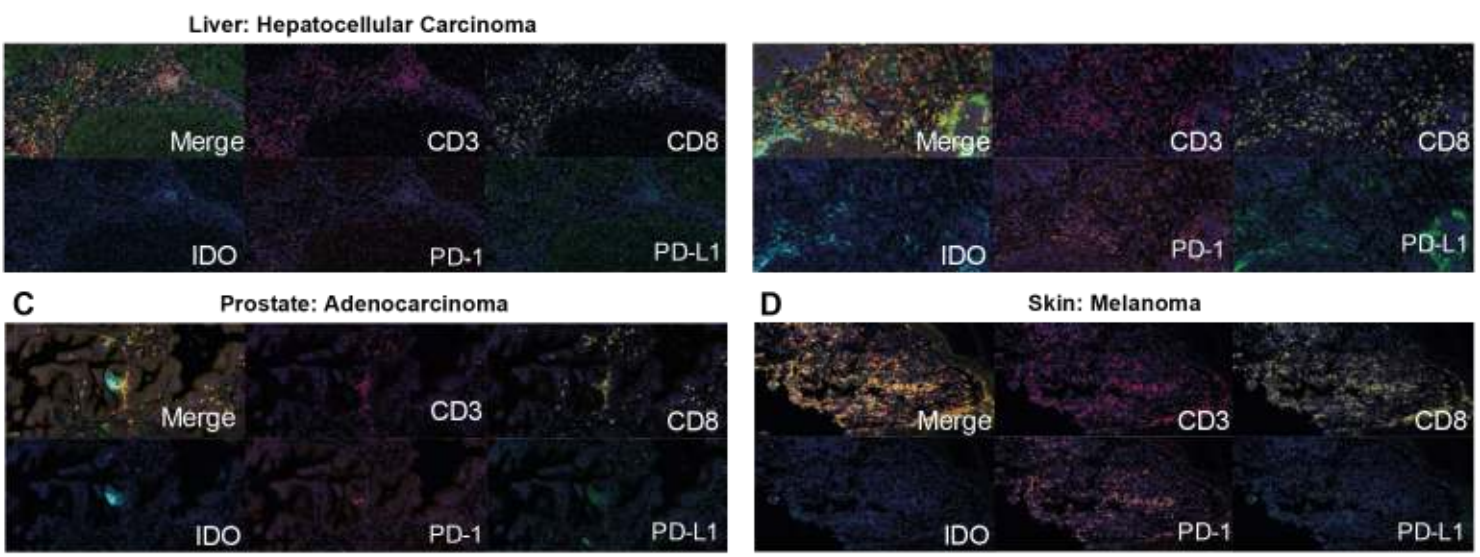
Results : Reproducibility





The reproducibility of the HISTOPROFILE®-CKI multiplex panel with IDO was evaluated over three different days, with the experiment performed by three different operators. A) Example fluorescent images and the corresponding HALO cell masks for each target for the three reproductions are shown.
A representative merge image from the whole scan for each reproduction demonstrates visually equivalent staining for all targets CD3 (red), CD8 (yellow), PD-1 (orange), PD-L1 (green), IDO (cyan), and DAPI (blue) (human tonsil, scale bar = 100 µm). B) Each target was analyzed on the three reproduction whole scans with HALO. The results demonstrate equivalent staining profiles for each target on reproduction slide 1 (black circle), 2 (red circle), and 3 (blue circle).

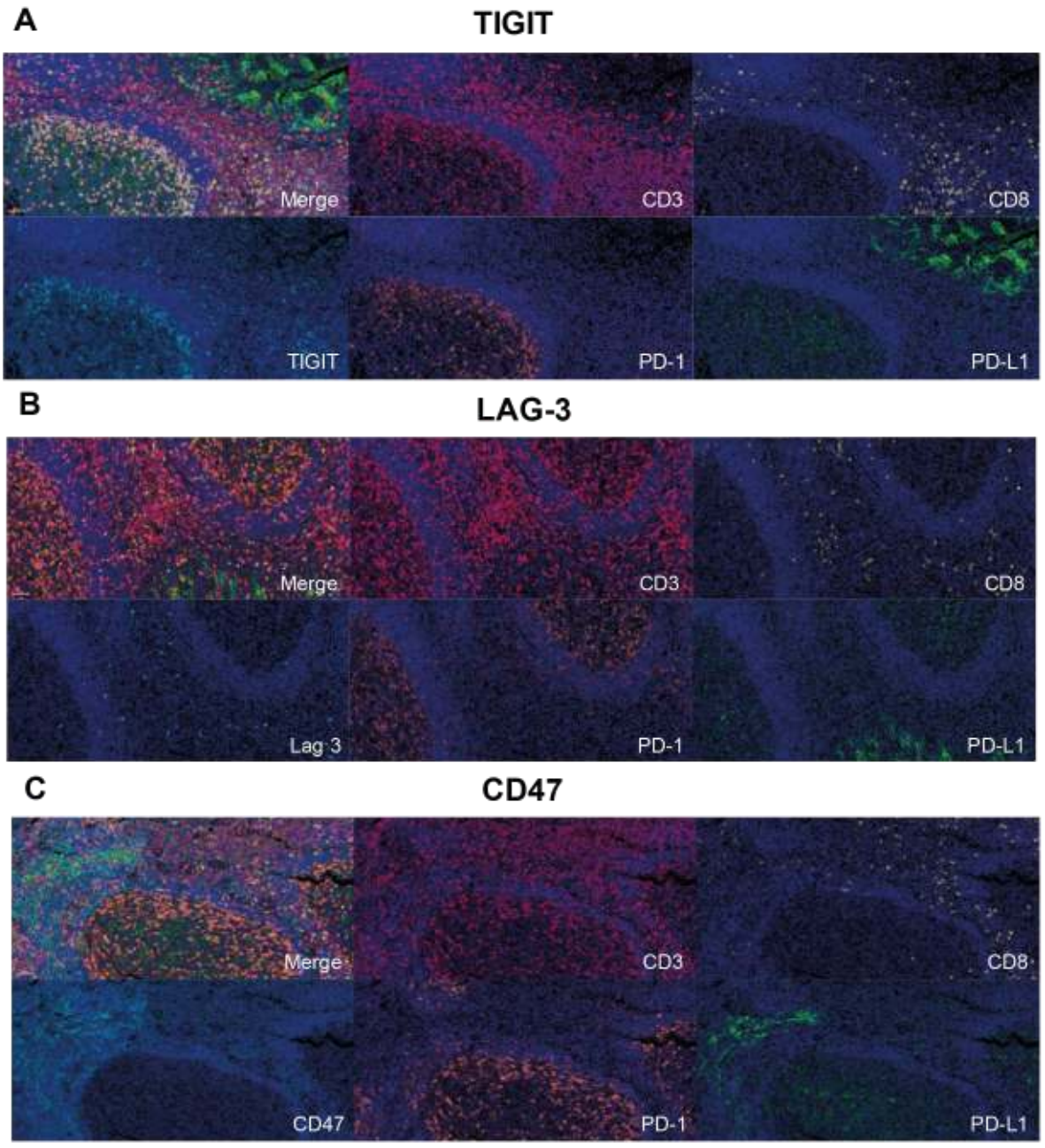
Results : Robustness



The robustness of the protocol of HISTOPROFILE ®-CKI multiplex panel with IDO was tested on numerous pathologic tissues. Each target CD3 (red), CD8 (yellow), PD-1 (orange), PD-L1 (green), IDO (cyan), and DAPI (blue) can be detected in A) hepatocellular carcinoma, B) lung adenocarcinoma, C) prostate adenocarcinoma, and D) melanoma. Representative images of whole scans for the four tissues (Scale bar = 100 µm).

Results : Modify Immune Checkpoint Protein Target

The CKI combination was varied in the base HISTOPROFILE ®-CKI multiplex panel on human tonsil. The targets A) TIGIT (cyan), B) LAG-3 (cyan), and C) CD47 (cyan) were easily applied to the base panel - CD3 (red), CD8 (yellow), PD-1 (orange), PD-L1 (green), and DAPI (blue). Representative images of whole scans for the three panels (Scale bar = 100 µm).



Conclusion :

The approach presented here demonstrates the flexibility of Histalim's Histoprofile®-CKI multiplex panel to interchange immune cell checkpoint targets and its application on multiple tissue types.

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