A feasibility study utilizing DNA-barcoded multiplex fluorescence IHC for immune profiling in the tumor microenvironment of bladder and lung samples

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Background

- Ultivue multiplex IHC technology uses antibody DNA-barcoding with signal amplification in tissues (**Fig. 1**)
- Ultivue ULT30102 MTO assay includes 7 markers: CD3, CD8, CD68, GZMB, PD-L1, Ki67 and PanCK
- The staining is automated with 2 Rounds of iterative staining & imaging (**Table 1**)

Aim

- To establish a multiplex IHC method to study the tumor cellular microenvironment in biopsies
- The long-term goal is to qualify and/or validate this multiplex IHC method for immunophenotyping (**Table 2**)

Methods

- We tested the kit ULT30102 in 20 bladder and 20 lung whole tumor resections using the Bond RX automated stainer
- Digital images from Round 1 and Round 2 were acquired with the fluorescence scanner Vectra Polaris (additional images were acquired with Axio Slide Scanner)
- Image processing and cell analysis was performed using HALO software (**Fig. 2**)
- Data analysis was completed with GraphPad and R

Results

- Ultivue ULT30102 assay was able to detect CD3, CD8, CD68, GZMB, PD-L1, Ki67 and PanCK in bladder and lung samples
- We classified tumors as Hot or Cold based on pathology and image analysis cellular data (**Fig. 3**)

Fig. 1. Ultivue DNA-barcoded multiplex IHC

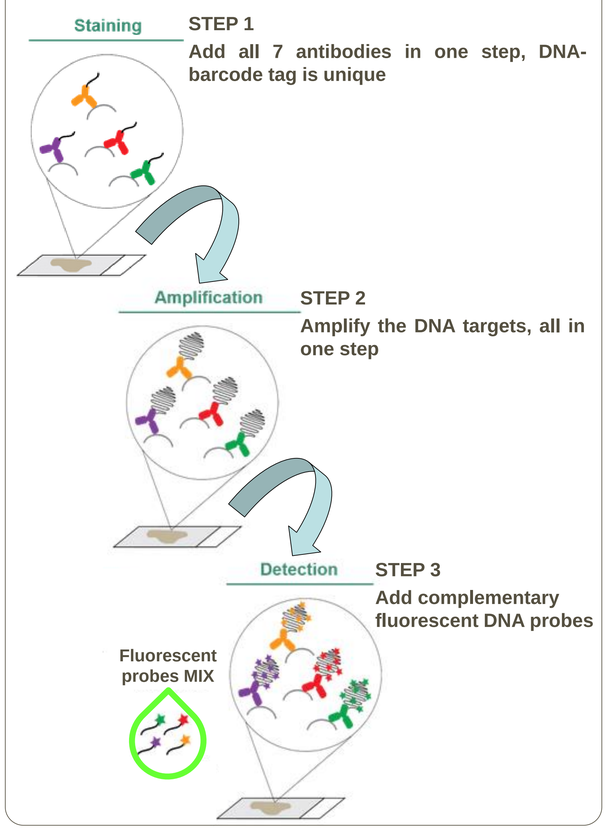
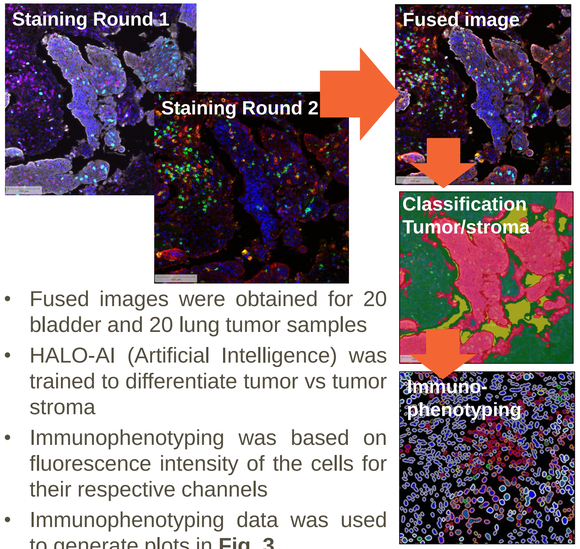


Table 1. Ultivue 7-plex kit markers are stained and imaged in 2 iterative rounds

Ultivue 7-plex Kit, ULT30102		
	Round 1	*Round 2*
FITC	CD8	Ki67
TRITC	CD68	GZMB
Cy5	PD-L1	CD3
Cy7	----	PanCK

All antibodies are applied in staining STEP 1 (see **Fig. 1**). *Round 1* fluorescent DNA probes are applied and imaged first. Then, *Round 2* DNA probes are applied and imaged.

Fig 2. Image processing and cell analysis workflow



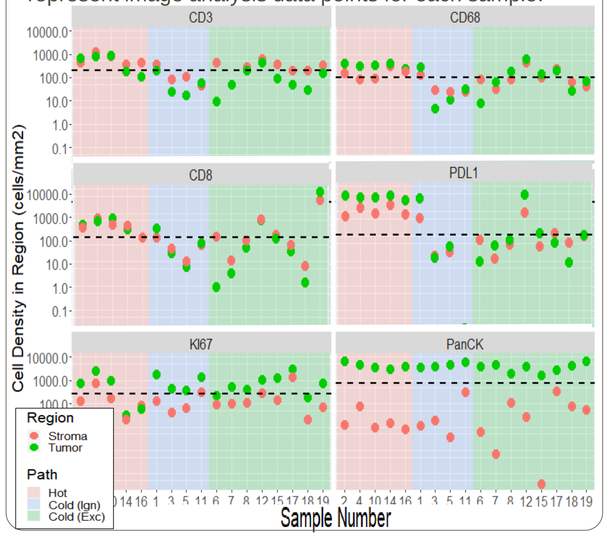
- Fused images were obtained for 20 bladder and 20 lung tumor samples
- HALO-AI (Artificial Intelligence) was trained to differentiate tumor vs tumor stroma
- Immunophenotyping was based on fluorescence intensity of the cells for their respective channels
- Immunophenotyping data was used to generate plots in **Fig. 3**

Table 2. List of potential immunophenotypes and classification segments for these studies

• **CD3+/CD8+** • **PanCK+/Ki-67+** • **CD3+/CD8+/Ki-67+** • **PanCK+/PD-L1+ ….etc**	• Normal tissue • Tumor nest (epithelial) • Tumor stroma

Fig 3. Hot & cold classification of bladder tumors based on histopathology and HALO image analysis

Tumor histopathologic classification is highlighted in the background: Hot (pink), Cold-ignored (blue), or Cold-excluded (green). Red (stroma) & green (tumor) dots represent image analysis data points for each sample.



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The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol."