Content and Spatial Distribution Analysis of PD-L1 Positive Tumor Cells and CD8 Positive T-Cells within Mulitplex IHC Stained NSCLC Tumor Tissue

L. Dawson[1], J. Stevens[1], M. Cowan[2], N. Stillie[2] and L. Sherry[2]
[1]Asterand Bioscience, Royston, SG8 5HD, UK
[2]OracleBio, BioCity Scotland, ML1 5UH, UK

Introduction & Aim

Quantifying the number, type and distribution of immune cells in oncology tissue is key to obtaining a greater understanding of the actions of immuno-therapies as part of immuno-oncology research. Programmed death-ligand 1 (PD-L1) and its receptor, programmed cell death protein 1 (PD-1), are two important immune checkpoint proteins that negatively regulate the anti-tumor immune response. CD8 positive (CD8+) T-cells form part of the immune response to tumor progression while immunotherapies directed at inhibiting the PD-1/PD-L1 checkpoint pathway aim to enhance the immune response to target and destroy cancer cells.

The aim of this study was to quantify the content and spatial distribution of PD-L1 positive (PD-L1+) tumor cells and CD8+ cells within non-small cell lung cancer (NSCLC) tumor tissue stained by multiplex chromogenic immuno-histochemistry (IHC).

Methods & Materials

An automated, multiplex IHC assay was developed using the Ventana Discovery ULTRA platform and applied to FFPE sections of NSCLC tumor tissue from 4 individual donors (Table 1) to stain epithelial tumor cells (Pan-CK, yellow), CD8+ cells (purple) and PD-L1+ cells (brown), counterstained with hematoxylin (blue). See Table 2 for antibody details.

Immunostained whole slide images were generated on the Aperio ScanScope AT Turbo for image analysis. A customized algorithm, which included a 4-chromogen color deconvolution process, was utilized within the Indica Labs HALO platform to separate the 3 IHC chromogens plus counterstain. A classifier was developed to automatically segment tumor from stroma regions of interest (ROI) (Figure 1). Cell objects were formed by applying weighted optical density values for the individual chromogens. Each positive cell type was identified using defined size, shape and subcellular compartment staining parameters. CD8+ cells were quantified within the tumor and stroma ROI, while Pan-CK or dual Pan-CK/PD-L1+ cells were quantified within the tumor ROI.

Table 1. Donor details

Donor ID	Tissue type	Age	Sex	Significant Clinical Diagnosis
20483	Lung tumor	61	Male	Squamous cell carcinoma of lung
20491	Lung tumor	60	Male	Squamous cell carcinoma of lung
20504	Lung tumor	61	Male	Adenocarcinoma of lung
20619	Lung tumor	75	Male	Adenocarcinoma of lung

Table 2. Antibody details

Antibody	Supplier and Description
Anti-PD-L1 antibody [28-8]	Abcam #ab205921, monoclonal rabbit IgG
Anti-CD8 antibody (SP239)	SpringBioscience #M5394, monoclonal rabbit IgG
Anti-Cytokeratin, pan (mixture) antibody	Sigma-Aldrich #C2562, monoclonal mouse IgG1

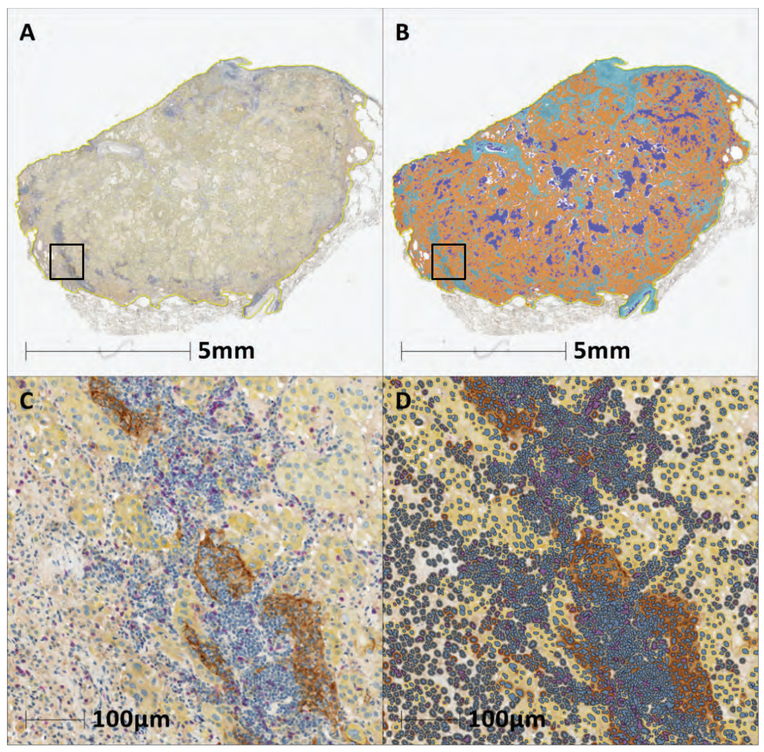

Figure 1. Example whole tissue classification and cell detection showing (A) multiplex stained IHC whole section, (B) classification overlay showing tumor (orange), stroma (light blue), necrosis (dark blue) and white space (white), (C) magnified area showing positive IHC staining for PD-L1 (brown), Pan-CK (yellow), CD8 (purple) and hematoxylin (blue) and (D) the corresponding image analysis cell detection.

Results & Discussion

The % tumor and stroma content across the 4 samples ranged from 26.53% to 62.62% and 23.92% to 71.71%, respectively (Figure 2). The % of PD-L1+ tumor cells ranged from 0.04% to 2.63%, while the number of CD8+ cells per mm2 of tumor plus stroma ranged from 92 to 475 cells per mm2 (Table 3). In tissues showing higher PD-L1 staining (Table 4, Figure 3), PD-L1+ tumor cells were predominantly distributed around the periphery of the tumor with less cells detected closer to the core. Similarly, the highest density of CD8+ cells were found within tumor and stroma regions adjacent to the tumor periphery with lower numbers towards the tumor core. Across the whole tissue, the average distance of a CD8+ cell to its nearest PD-L1+ tumor cell was 199.35µm while the % of total CD8+ cells (located in tumor and stroma) within 100µm of a PD-L1+ tumor cell was 39.09% (Figure 4).

Table 3. Quantitative analysis of ROI composition and IHC positive cell content within tumor and combined tumor/stroma ROI

PARAMETER	Donor 20483	Donor 20491	Donor 20504	Donor 20619
ROI QUANTIFICATION				
Whole section area (mm²)	61.86	47.40	58.50	23.65
Tumor area (mm²)	38.74	12.57	34.24	14.70
Stroma area (mm²)	17.31	33.98	13.99	6.73
Necrosis area (mm²)	1.72	0	7.72	0
White space area(mm²)	4.10	0.83	2.55	2.22
TUMOR ROI DATA				
Total cells	364,868	86,990	177,952	116,314
CD8+ cells	1,956	861	5,534	2,377
Pan-CK cells	268,620	74,563	143,538	92,616
PD-L1+ cells	11,574	797	5,622	185
Dual PD-L1+ and Pan-CK cells	7,057	543	1,663	33
COMBINED TUMOR STROMA DATA				
Total cells	473,888	178,808	287,235	152,954
CD8+ cells	5,152	5,620	22,912	7,027
CALCULATED DATA				
% Pan-CK cells positive for PD-L1	2.63	0.73	1.16	0.04
CD8+ cells/mm²of tumor & stroma	92	121	475	328
% Tumor content	62.62	26.53	58.53	62.16
% Stroma content	27.98	71.71	23.92	28.44

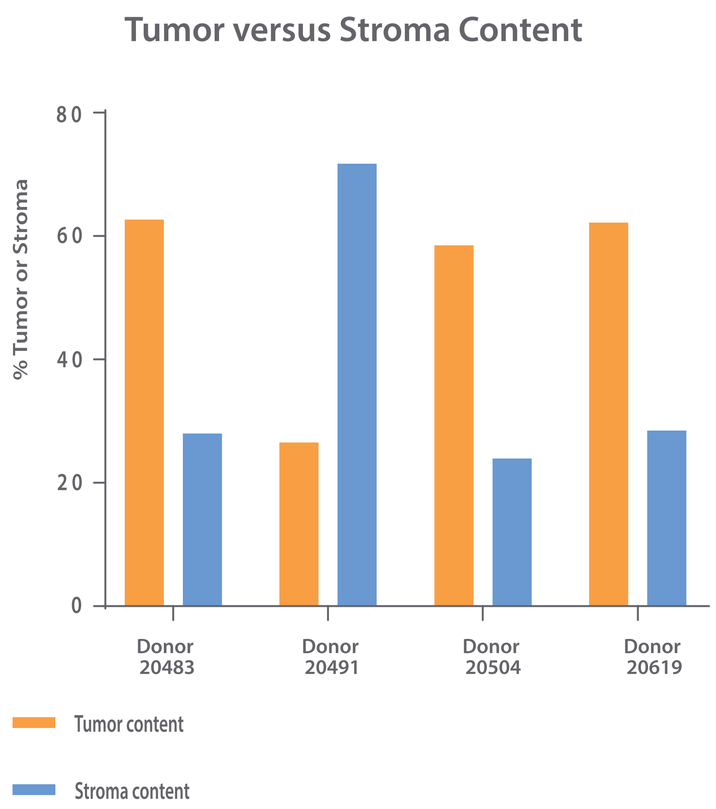

Figure 2. Tumor and stroma content across individual donors

Table 4. Quantitative juxtaposition of CD8+ cells to PD-L1+ tumor cells

PARAMETER	Donor 20483	Donor 20504	Average
CD8+to nearest PD-L1+ tumor cell			
Total CD8+ cell count	5,152	22,912	
Average neighbor distance (µm)	194.66	204.03	199.34
Number of unique neighbours	1,054	967	
CD8+ within 100µm of PD-L1+ tumor cell			
Total CD8+ cell count	5,152	22,912	
# CD8+ within 100 µm of PD-L1+ tumor cells	2,365	7,393	
% CD8+ within 100 µm of PD-L1+ tumor cells	45.90	32.27	39.09

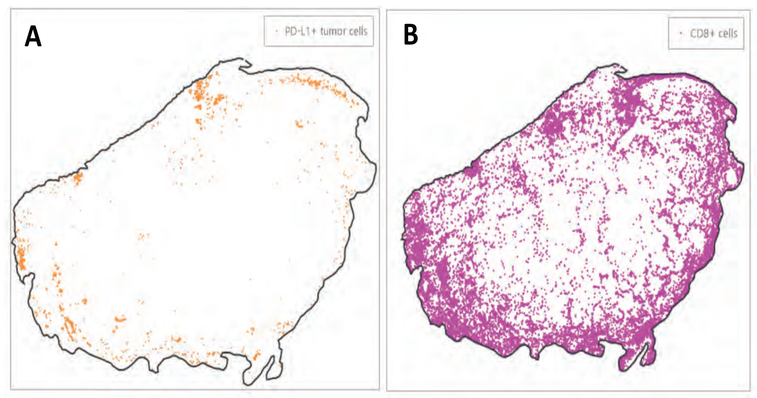

Figure 3. Example cell spatial distribution for (A) PD-L1+ tumor cells and (B) CD8+ cells across a whole section showing higher PD-L1 staining.

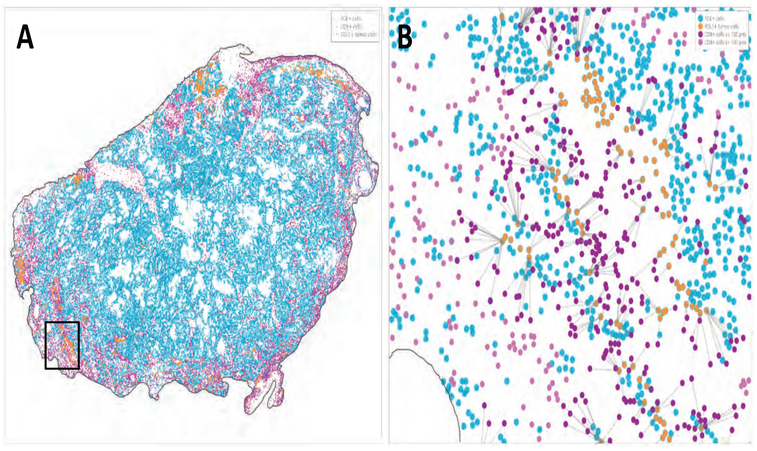

Figure 4. Example cell spatial distribution for (A) PD-L1+ tumor cells (orange), CD8+ cells (purple) and Pan-CK (blue) across a whole section and (B) proximity analysis depicting CD8+ cells within 100µm (dark purple) or more than 100µm (light purple) of PD-L1+ tumor cells (orange). Grey lines connect CD8+ cells within 100µm of PD-L1+ tumor cells (dark purple).

Conclusions

The use of multiplex IHC combined with digital image analysis can be used effectively to evaluate the content and spatial distribution of immune cell populations in relation to target positive tumor cells. This approach can help confirm the mechanism of action of therapies that are designed to enhance or reduce the interaction between specific cell types within tumor tissue.

Evaluating the functional status of detected immune cells, along with spatial relationship data to target positive tumor cells, may provide more in-depth evidence for proof of mechanism of immuno-therapies within the context of the tumor microenvironment.

Contact details:
www.asterandbio.com
advantage@asterandbio.com
www.oraclebio.com
info@oraclebio.com