Multiparameter Quantification of PD-1 and PD-L1 on Tumor and Immune Cells in Non-small Cell Lung Cancer

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Background

The programmed death 1 protein (PD-1) on activated T and B cells has been shown to play a key role in the ability of cancer cells to evade the immune system. In conjunction with the primary associated ligand (PD-L1), immune responses are negatively regulated. PD-L1 is up-regulated in certain tumors to faciilitate this immune evasion. IncellDx's IncellPREP™ liquid-biopsy technology has the ability to create single cell suspensions through tissue disruption. This technology was utilized on fresh lung tissue biopsies to create single cell suspensions for protein and DNA quantification by flow cytometry. The analysis of these single cell suspensions holds potential for greater quantification than that of immunohistochemistry.

Materials & Methods

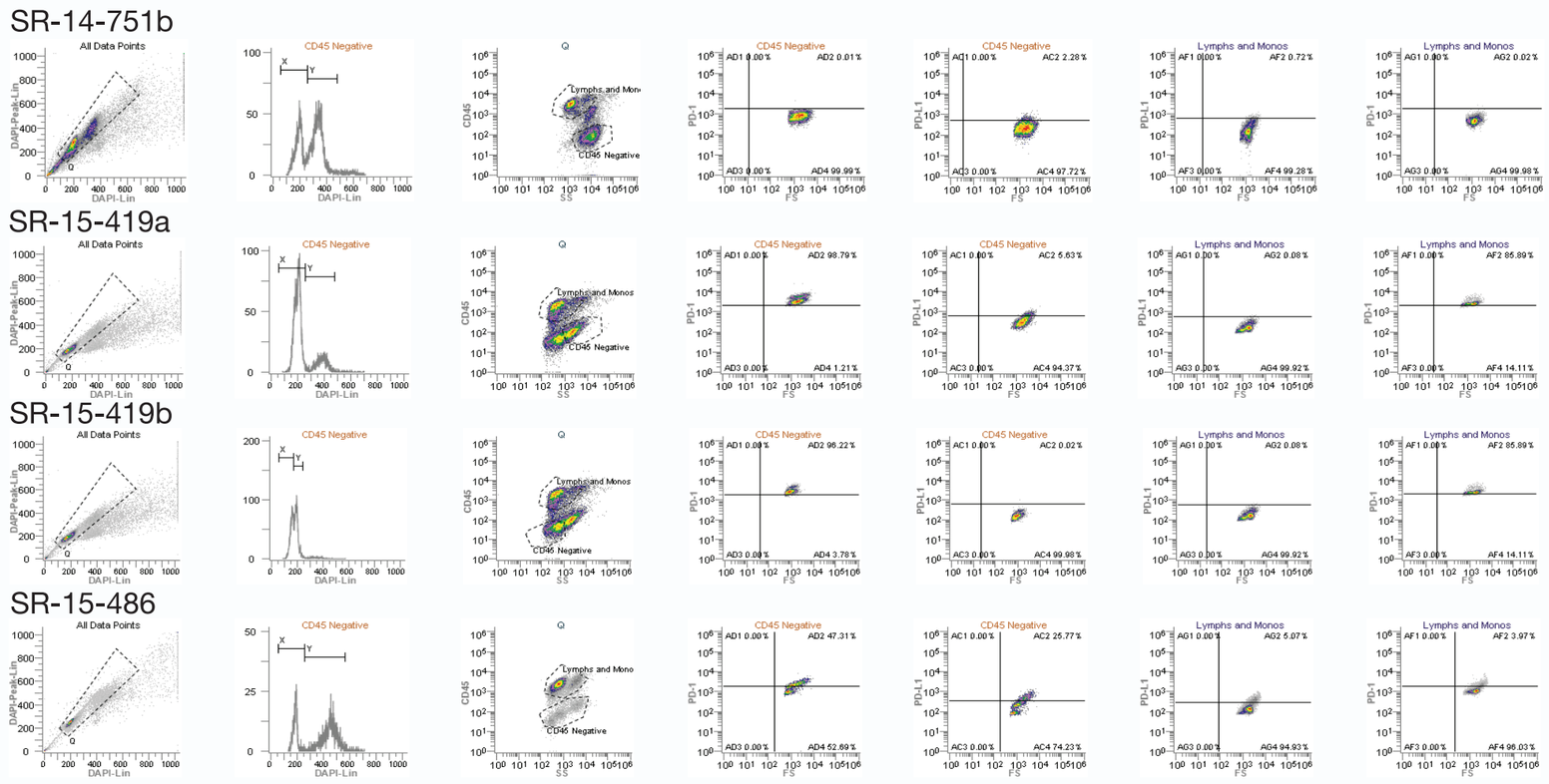
Fresh tissues were obtained from 11 non-small cell lung cancer (NSCLC) cases. 4 mm punches were taken from each tissue opon receipt at IncellDx. Punches were processed into single cell suspensions using non-enzymatic homogenization (IncellPREP™) in D-PBS. Suspensions were then counted on a Cellometer cell counter for total cells isolated. Cells were pelleted and fixed and permeabilized using IncellPREP Reagent in preparation for staining with anti-human PD-1 and PD-L1 antibodies (Biolegend), a CD45 antibody (Biolegend), and DNA staining by DAPI (Sigma). Total nucleated cells, CD45+ percentages, and percent expression of PD-1 and PD-L1 on CD45+ and CD45- cells were recorded on a Sony EC800 flow cytometer.

Results

A)

Sample ID	Diagnosis	Total Count	% Nucleated single cells	% CD45+	% CD45- (tumor)	CD45+ PD-L1%	CD45- PD-L1%	CD45- PD-L1%	CD45+ PD-1%	CD45- PD-1%
SR-14-751a	Squamous cell carcinoma	24870	59.04	74.07	12.17	2.63	3.97	3.97	0.56	0
SR-14-751b	Squamous cell carcinoma	30000	64.63	41.41	40.72	0.72	2.28	2.28	0.02	0.01
SR-14-772a	Adenocarcinoma	30011	15.35	0.96	83.11	9.09	7.83	7.83	0	0.81
SR-15-42	Adenocarcinoma	13940	53.82	22.61	65.58	30.69	63.98	63.98	2.04	6.39
SR-15-56	Adenocarcinoma	30000	85.94	59.45	30.06	26.18	69.81	69.81	1.24	12.53
SR-15-88	Squamous cell carcinoma	30006	64.26	46.54	45.53	23.65	89.44	89.44	1.71	0.88
SR-15-251	Squamous cell carcinoma	30005	12.23	15.07	28.75	0.18	13.74	13.74	0	0
SR-15-343	Squamous cell carcinoma	19016	58.89	25.72	47.84	0.97	0.52	0.52	0	0
SR-15-419a	Acinar Adenocarcinoma	30007	68.8	32.03	30.53	0.8	5.63	5.63	0	0
SR-15-419b	Acinar Adenocarcinoma	30007	68.9	32.03	27.71	0.8	0.02	0.02	0	0
SR-15-478	Adenocarcinoma	30002	70.16	20.8	70.8	0.47	0	0	0.54	0.86
SR-15-486	Squamous cell carcinoma	30010	85.73	88.52	8.81	5.07	25.77	25.77	3.97	47.31
SR-15-496	Adenocarcinoma	30005	12.42	37.25	44.74	0	0.06	0.06	0	0.06
Matched Normal Samples										
Sample ID	Diagnosis	Total Count	% Nucleated single cells	% CD45+	% CD45- (tumor)	CD45+ PD-L1%	CD45- PD-L1%	CD45- PD-L1%	CD45+ PD-1%	CD45- PD-1%
SR-15-486	NA	21891	83.81	67.43	25.51	2.04	0.02	0.02	5.56	0.21
SR-15-496	NA	3987	45.02	14.71	58.94	1.89	0.09	0.09	9.85	1.89

B)



A) 11 non-small cell lung cancer samples and two matched normal samples with CD45, PD-1 and PD-L1 percent positivity.

B) Histograms from samples included in table A with DAPI, CD45, PD-1 and PD-L1 staining

Conclusion

The ability to disrupt soild tumors without enzyamtic digestion offers the promise of studying multiple protein sites for both the presence as well as quantity of protein expression. As new drug entities are tested for efficacy, the ability to quantify the targets on the desired cells is imperative. The combination of both the IncellPREP™ system with the capabilities of the Cellular Multplex™ offers the promise of fulfilling both needs.