

Development of PD-L1 screening platform using Circulating Tumor Cells

Cham Han Lee¹, Young Hun Kim¹, Jae Hyuk Lee¹, Young Woong Sohn¹, Jeong Mi Moon¹, Myoung Shin Kim¹,
Byung Hee Jeon¹

¹Cytogen, Inc., Seoul, Korea

<http://www.cytogenlab.com/>

SMART BIOPSY™ SYSTEM

Abstract

The programmed death-ligand 1 (PD-L1) and programmed cell death protein 1 (PD-1) are major targets of cancer therapy, and the targeted drugs are developed and commercialized. In order to prescribe the PD-L1/PD-1 drugs for cancer patients, PD-L1 should be evaluated via tissue biopsy. Tumor biopsies in cancer patients are not always possible, which can be a limiting factor for PD-L1/PD-1 immunotherapy. It has been known that circulating tumor cells (CTCs) are found rarely in the peripheral blood of cancer patients, which can provide genetic information of cancer tissue. CTCs are non-invasively and repeatedly obtained from cancer patient, therefore liquid biopsy using CTC can be easily used as a replacement of tissue biopsy.

We have developed immunofluorescence staining platform of CTCs in order to screen the biomarkers of tumor cells. Based on our CTC staining platform, PD-L1 staining platform was developed and optimized. We used lung (PC9, A549, NCI-H1299, NCI-H1975, NCI-H2228, and NCI-H3122) and prostate cancer cells (LnCAP-LN3 and 22RV1) for optimization of the PD-L1 staining platform. As the results, we confirmed that PD-L1 expression levels of A549 and NCI-H1299 were relatively low (average intensity; 15.7 and 15.0), and PD-L1 expression in PC9, NCI-H1975, NCI-H2228 and NCI-H3122 were relatively high (average intensity; 22.0, 21.9, 25.2, and 28.9). Additionally, PD-L1 in 22RV1 and LnCAP-LN3 were also analyzed (average intensity; 19.4 and 18.1). The results of PD-L1 expressed level in each cell lines were similar with previous report, which indicated that the platform can be utilized as the cell based PD-L1 screening.

Although additional verification with clinical samples is required, we confirmed that the platform can be used for PD-L1 expression screening of CTCs. Furthermore the application and the scalability of the platform will be confirmed by the PD-L1 as well as other biomarkers of CTCs from cancer patients.

SMART BIOPSY™ SYSTEM

Smart Biopsy™ Cell Image Analyzer

- Automated system for high-resolution scanning images capture and analysis.
- Resolution (pixel size : 3.02um) ; 1,936 x 1,456 to 9,680 x 8,736 pixels.



Automatic Sample Loading

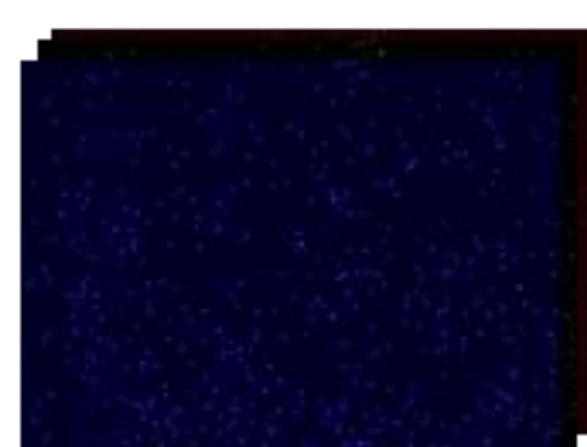


Image Capture

Image Analysis Module (Software)

- Optical image-based automatic analysis software.
- Fluorescence intensity display (objective value)

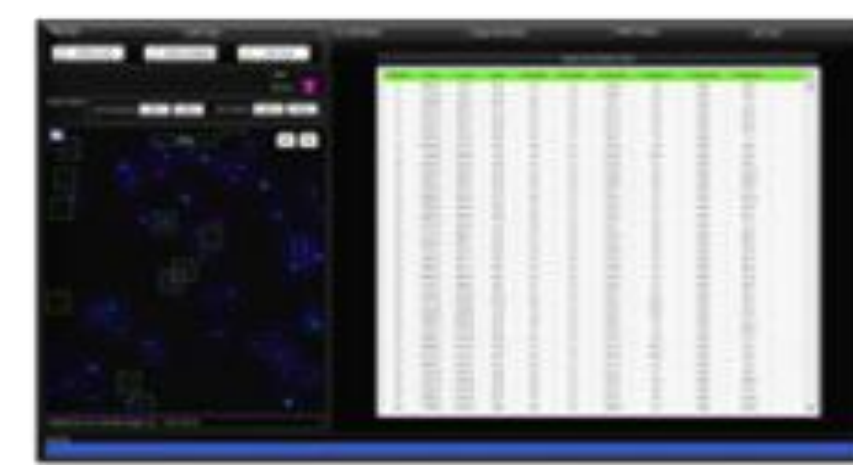
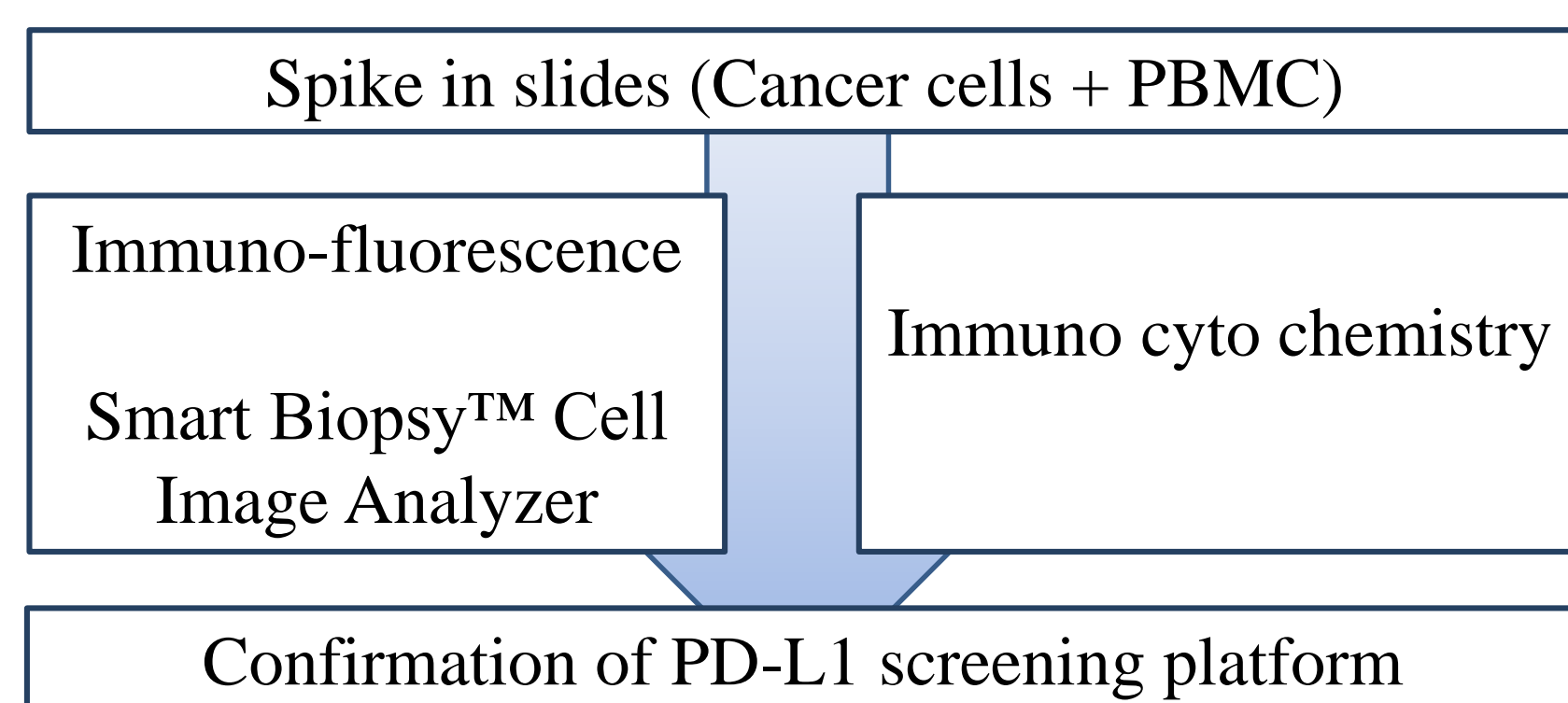


Image Loading / Merge / Analysis

Materials & Methods

Research Flow-Chart



Study Design

1. PD-L1 IF staining vs PD-L1 ICC staining
2. PD-L1 IF staining screening platform test
3. Various cancer cells are tested by PD-L1 screening platform

Conclusion

Immuno-fluorescence staining based PD-L1 automatic screening platform are developed, and the platform is confirmed using various cell lines and PBMC.

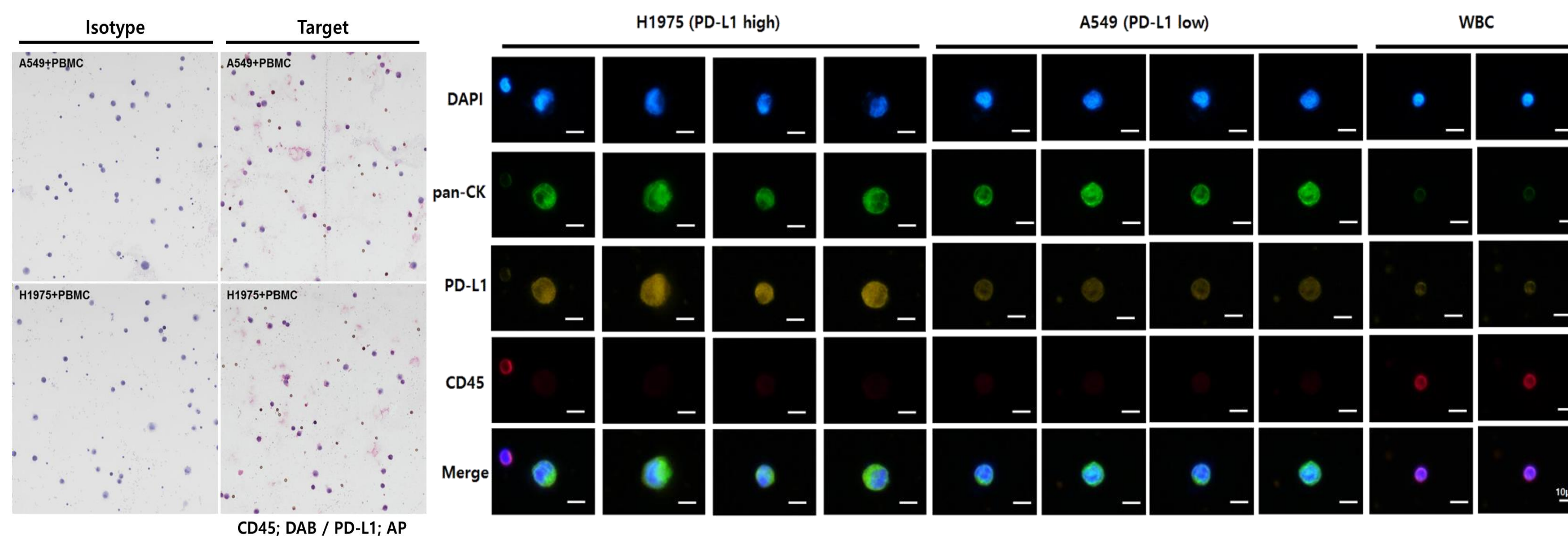
Before PD-L1 CTCs screening using the platform, spike-in test was performed using PC9+PBMC, A549+PBMC, and H1975+PBMC (data not shown).

1. Cancer cells in the spike-in samples can be identified with more than 95% confidence.
2. Reproducibility of analysis results are confirmed.
3. Accuracy of antibody reaction and IF staining are tested using each isotype antibody, negative, and positive control.

We confirmed that the platform can be used for PD-L1 expression screening of CTCs, and CTCs PD-L1 screening are performed using cancer patient samples.

Results

PD-L1 ICC & IF staining



Fluorescence intensity by Smart Biopsy™ Cell Image Analyzer

- Average fluorescence intensity

	22RV1	LnCaP-LN3	BT20	H1299	H1975	H2228	H3122	A549	PC9	WBC	isotype
CD45	4.28	4.52	4.51	4.36	4.73	5.85	5.21	4.27	4.83	22.68	3.99
pan-CK	7.75	7.95	27.57	6.73	8.64	10.36	40.55	21.14	42.95	3.98	3.38
PD-L1	19.38	18.14	17.67	15.03	21.89	25.22	28.86	15.71	22.07	13.16	5.80

- Fluorescence intensity range

