Development of High Sensitivity and High Throughput Immunoassays for PD-1 Cancer Immunotherapy

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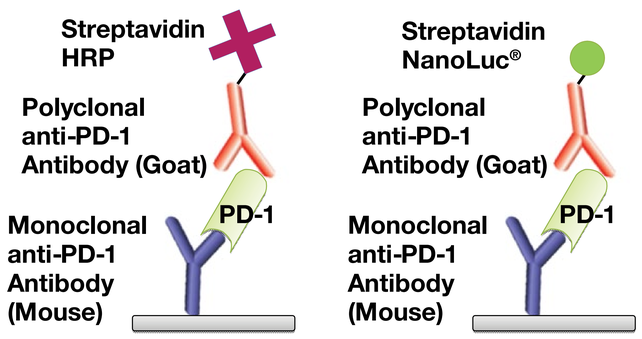
Introduction

Programmed death-1 (PD-1) is an inhibitory receptor on T cells and overexpression of PD-1 leads to suppression of anti-tumor activity. Clinical trials inhibiting PD-1 have shown remarkable success and advances in understanding the upstream regulation of PD-1 may lead to new therapeutics. Thus measurement of this biomarker for diagnosis, disease progression, and therapeutic efficacy is crucial for cancer immunotherapy. However, biopsy based immunohistochemistry is the current standard of care for assessment of PD-1. We developed a non-invasive ELISA for soluble PD-1. We further aim to increase the sensitivity of the ELISA utilizing the NanoLuc® luciferase reporter, an ultra-bright luciferase with low background.

Here we report a sensitive, robust ELISA for the measurement of PD-1 in human plasma using antibodies from Somru BioScience. In addition, we also developed a comparable method using streptavidin conjugated NanoLuc® from Promega Corporation. NanoLuc® is a small (19kDa) and extremely bright luciferase with stable luminescence signal that can be used as a reporter in ELISAs.

Methods

Figure 1. Assay formats for detection of PD-1 with HRP ELISA and NanoLuc®



The method is based on the ELISA kit developed by Somru BioScience for the measurement of soluble PD-1 in human plasma. Briefly, anti-PD-1 antibody is coated to a 96-well plate overnight. After incubation with plasma samples, biotinylated detection antibody is added to the immune complex. Streptavidin HRP or streptavidin NanoLuc® is then bound and the plate is analyzed for absorbance (for HRP) or luminescence (for NanoLuc®) signal.

Results

Figure 2. Standard Curve HRP ELISA Platform

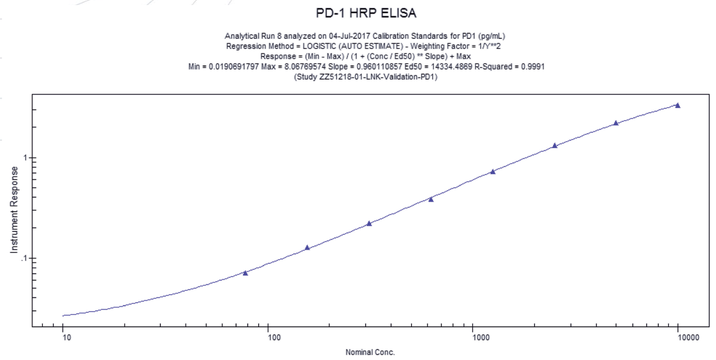


Table 1. Intra- & Inter-Batch Precision and Accuracy of QC Samples with HRP ELISA Platform

Run #	Concentration of PD1 (pg/mL)				
	2500	2000	1000	150	100
Run 1	2948	1638	994	172	116
Run 2	2685	2194	954	139	107
Run 3	2661	1981	1076	143	77
Run 4	2601	1878	1022	146	81
Run 5	2101	1539	1067	167	97
Run 6	2476	1241	1093	167	112
Mean	2579	1745	1034	156	98
SD	256	312	49	13	15
% CV	10	18	5	9	15
% Bias	3	-13	3	4	-2
n	6	6	6	6	5

Table 2. Matrix Effect Selectivity of HRP ELISA and Endogenous PD-1 Concentration in Normal vs. Melanoma Human Plasma

Lot #	Spiked Concentration (pg/mL)	Unspiked Concentration (pg/mL)	Observed Concentration (pg/mL)	Expected Concentration (pg/mL)	Percent Recovery
Normal 1	200	123	282	323	-13
Normal 2	200	77	274	277	-1
Normal 3	200	88	286	288	-1
Normal 4	200	147	296	347	-15
Normal 5	200	134	355	334	6
Normal 6	200	180	333	380	-12
Normal 7	200	211	382	411	-7
Normal 8	200	167	379	367	3
Normal 10	200	69	252	269	-6
Melanoma 11	400	346	688	746	-8
Melanoma 12	400	233	545	633	-14
Melanoma 13	400	366	701	766	-8
Melanoma 14	400	482	1032	882	17
Melanoma 15	400	290	704	690	2

Figure 3. Standard Curve NanoLuc® Platform

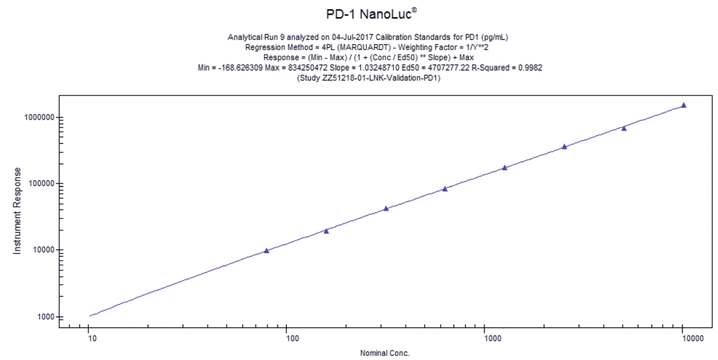


Table 3. Precision and Accuracy of QC Samples with NanoLuc® Platform

Run Date	Run #	LLOQ (78.1 pg/mL)	QC A (156 pg/mL)	QC B (1000 pg/mL)	QC C (7500 pg/mL)	ULOQ (10000 pg/mL)
04-Jul -2017	9	82.4	138	854	7900	7760
		85.6	134	1040	6740	7800
		87.7	142	999	6350	
Intrarun Mean		85.2	138	964	7000	7780
Intrarun SD		2.67	4.00	97.7	806	28.3
Intrarun % CV		3.1	2.9	10.1	11.5	0.4
Intrarun % Bias		9.1	-11.5	-3.6	-6.7	-22.2
n		3	3	3	3	2

Conclusions

Here we demonstrate the development of a high throughput and non-invasive ELISA for soluble PD-1 and the use of a novel reporter, NanoLuc®, to support immuno-oncology studies. The ELISA detected increased endogenous PD-1 in plasma from melanoma patients as expected.

The ELISA meets FDA Bioanalytical Guidance with no cross reactivity against PD-L1, CD28, CTLA-4, and ICOS up to 10 ng/mL.

We also demonstrated that the transition from routine ELISA to NanoLuc® is simple, cost effective, easy to execute, and has a wider dynamic range than standard ELISAs.

Further experiments to optimize the sensitivity of the PD-1 assay are underway.

Acknowledgements

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