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

S. Kar¹, J. Knight², W. Adamowicz¹, S. Johnson¹ and R. Islam¹
¹Celerion Inc. and ²Somru BioScience Inc.

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

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

Figure 2. Standard Curve HRP ELISA Platform

Figure 3. Standard Curve NanoLuc® Platform

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

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

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

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

The authors thank Dr. Nidhi Nath and Promega Corporation for providing the streptavidin NanoLuc® reporter.