Development of biochemical assays for immunotherapy drug discovery and development

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ABSTRACT

Immunotherapy has become an important approach for the treatment of numerous diseases including cancer. A number of immunotherapies target one or more co-stimulatory or co-inhibitory pathways regulating immune activation such as cell surface receptors and enzymes like IDO1 and TDO. Reliable high throughput screening (HTS) methods are needed to successfully screen and identify small molecules, antibodies, or antibody fragments (Fab) that modulate these pathways. Here, we report the development of a toolbox of HTS biochemical assays to screen for inhibitors of immune receptor-ligand interaction and for screening of inhibitors of IDO1 and TDO activity. These assays have been internally validated using known inhibitors. Future work will focus on establishing novel biochemical and cell-based assays for immunoregulatory pathways.

RESULTS

A. Wells are coated with receptor

**Figure 2. Immunotherapy biochemical assay design.** BPS ELISA-based immunotherapy assays take advantage of our biotin-labeled proteins. One binding partner is allowed to adhere to a plate overnight and the next day the cognate binding partner along with test inhibitor is added. Binding is detected by addition of Strep-HRP along with chemiluminescent or colorimetric substrate.

B. Biotin-labeled ligand is added with or without inhibitor

**Figure 3. Validation of BPS’ Immunotherapy Receptor Inhibitor Screening Assay Kits.** BPS Bioscience’s assay kits are validated by inhibiting receptor-ligand interaction with known inhibitors, when available, or with unlabeled ligand. Figure 3 depicts inhibition of (A) PD-1:PD-L1 (B) PD-1:PD-L2 (C) CD137:CD137L, (D) CD28:B7-1 (E) CTLA4:B7-1 (F) CTLA4:B7-2, and (G) BTLA-HVEM interaction by PD-1 neutralizing antibody, BPS Cat. #71120, CTLA4 neutralizing antibody BPS Cat. #71121, or unlabeled ligand as indicated.

C. Step HRP is added, diluted in chemiluminescent or colorimetric substrate

**Figure 4. The role of IDO1 & TDO in the tumor microenvironment.** (A) PD-1 inhibitors have shown clinical efficacy, but many tumors upregulate expression of IDO1 and TDO, resulting in Tryp depletion in the tumor microenvironment and increased levels of the Tryp metabolite, KYNA, which may inhibit T cell function. (B) Inhibition of IDO1 and TDO has the potential to halt T cell inhibition caused by Tryp catabolism.

**Figure 5. Validation of BPS’ IDO1 and TDO inhibitor screening assay kits.** Both the IDO1 (BPS Cat. #72021) and TDO (BPS Cat. #72023) inhibitor Screening Assay Kits measure enzyme activity by measuring the absorption of product (kynurenine (Kyn)) at A = 320-325 nm. (A) IDO1 activity in the presence of the IDO1-specific inhibitor INCB024360, the IC50 was determined to be 60 nM, demonstrating the ability of the kit to serve as a high throughput screening tool for IDO1 inhibitors. (B) TDO activity in the presence of the TDO-specific inhibitor 680C91, the IC50 was determined to be 338 nM, demonstrating the ability of the kit to serve as a high throughput screening tool for TDO inhibitors.

SUMMARY

Immunotherapy is a rapidly growing field with a number of currently approved therapies that are showing excellent efficacy in the clinic, especially in cancer. However, these success stories also have their shortcomings as not all cancers have been responsive, emphasizing the need for novel therapeutics.

In an effort to increase the rate of immunotherapy drug discovery and development, we have developed and validated biochemical high throughput screens for established drug targets as well as potential new targets. These assays are straightforward, easy-to-use, and come in different formats such as chemiluminescent, colorimetric, TR-FRET, and AlphaLISA™. In addition to these assay kits, BPS also provides screening services for its entire immunotherapy portfolio, allowing researchers to take advantage of our assay expertise. Future work will focus on developing assays for novel therapeutic targets in addition to cell-based assays that complement our portfolio of biochemical-based assays.

REFERENCES/CONTACT INFO


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