Development of a Platform T Cell Exhaustion Assay Using an iQue® Screener PLUS

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Introduction

T cell exhaustion occurs during prolonged exposure of the immune system to an antigen such as a virus or cancer cell. Persistent antigen exposure results in several physiological changes in the T cell population such as an increased expression of inhibitory receptors (IRs) and alterations in cytokine production including a decrease in IFNγ secretion. Overall, these changes inhibit the T cell response to the persistent antigen. Immuno-oncology mAbs, binding IRs such as PD-1, are able to reverse T cell exhaustion, for example by increasing IFNγ secretion, thereby enhancing the immune response to promote the clearance of persistent antigens such as cancer cells. The platform format was tested with a number of immuno-oncology mAbs including nivolumab, pembrolizumab, atezolizumab and durvalumab all of which demonstrate the ability to reverse T cell exhaustion as measured by an increased IFNγ secretion of exhausted T cells.

1. Experimental approach

This in vitro primary cell-based platform assay approach utilizes exhausted T cells generated in house from fresh blood provided by a pool of six donors. PBMCs isolated from fresh blood are treated with multiple rounds of stimulation with a CEF peptide pool. The CEF peptides pool is a group of 38 peptides, 8-12 amino acids in length with sequences derived from the human Cytomegalovirus (Epstein-Barr virus) and Influenza virus. The peptide pool is specific to HLA-class I molecules on the surface of all selected positive cells in the PBMCs. They will be recognized by peptide-specific CD8 cells without the need for additional antigen processing. PBMCs are put through multiple rounds of stimulation with the CEF peptide pool to generate exhausted cells that are frozen into single-use vials.

The exhausting effect of the peptide pool is dependent on the dose concentration used. Lower doses are more effective than higher doses. Viable cells from this pool are then tested in the platform assay kit. The exhaustion assay can be performed on exhausted cells generated under different conditions. PBMCs isolated from fresh blood are treated with multiple rounds of stimulation with a CEF peptide pool and are then frozen into single-use vials to allow ease of use.

2. Gating strategy

The platform assay uses a Sartorius developed T cell exhaustion kit (iQue® Screener PLUS). The multiplexed assay generates readouts for cell viability, T cell characterization, expression of IRs and cytokine release of IFNγ and TNFα. The assay allows a small sample from an assay plate to be analyzed, the readout and methodology are summarized in Figure 1. The gating strategy shown in Figure 2, the assay design incorporates positive and negative controls to fine-tune gate placement.

3. Results – CEF peptide pool treatment exhausts T cell treatments

To demonstrate T cell exhaustion had occurred, exhausted T cells (no molecule) were compared to fresh PBMCs (NC) from the same donor. The data obtained is shown in Figure 3. A 2-way ANOVA and Bonferroni post test was used to test for a significant increase in IFNγ no molecule control vs yes assay response. Fold increase calculated from mean test material response at the high concentration/mean no molecule control.

4. Results – anti-PD-L1 T cell exhaustion assay

A therapeutic mAb against PD-L1 can enhance T cell activation by disrupting the inhibitory signals from PD-L1:PD-L1 binding. There are many PD-L1 mAbs on the market including Opdivo®, Keytruda®, Avastin® and Durvalumab® (Imfinzi®). We tested two of these mAbs in six sample positions across seven assay runs using our platform T cell exhaustion assay. Example data for the reportable IFNγ MFI responses is shown in Figure 5 and a summary of responses across the runs is available in Table 1.

5. Results – anti-PD-1 T cell exhaustion assay

A therapeutic mAb against PD-1 can enhance T cell activation by disrupting the inhibitory signals from PD-L1:PD-L1 binding. There are several PD-1 inhibitors on the market including Opdivo® (Nivolumab), and Keytruda® (Pembrolizumab). Here we test these mAbs in 3 sample positions across 5 assay runs using our platform T cell exhaustion assay. Example data for the reportable IFNγ MFI responses is shown in Figure 6 and a summary of responses across the runs is available in Table 2.

6. Conclusion

The data presented demonstrates that the platform T cell exhaustion assay is highly sensitive and specific for detecting exhaustion. The readouts shown were robust across different molecules, donors and assay runs. A workflow of how we can support your molecule testing is available in Figure 7.