The success of immune checkpoint inhibitors in the treatment of diverse types of cancers and their continued growth in the market have driven burgeoning interests in developing more drugs in this category. However, the intrinsic complexity of the immunological models and the variable drug responses among different cancer types have become the most prominent challenges.

To facilitate large-scale research projects and drug discovery of immune checkpoint inhibitors, we conducted a comprehensive protein profiling of ATCC® cell lines to provide researchers with high expression of endogenous immune checkpoint molecule ligands (PD-L1, CD155, and B7-H3) for future experiments.

**Abstract**

The clones that yielded the highest luciferase signal upon IFN-γ stimulation were selected for future experiments. Created with BioRender.com.

**Results**

Immune checkpoint protein profiling of cancer cell lines. T cell lines, and primary T cells

**Figure 1:** Schematic of immune checkpoint molecule-expressing GAS-Luc2 reporter system. (A) Disruption of immune checkpoint bindings, such as PD-1/PD-L1, results in luciferase expression. To block antibody activity in CD8+ T cells, which in turn, release IFN-γ, Phc activates JAK-STAT signaling in cancer reporter cells, prompting GAS-induced transcription of the luciferase gene, producing an easily detectable immunoluminescent signal. Created with BioRender.com. (B) Selected cell lines with high endogenous expression of PD-L1, CD155, and B7-H3 were transduced with GAS-Luc2 reporter cell line in response to IFN-γ stimulation. T cells in the absence of an anti-CD3/CD80/CD28 antibody treatment were isolated by automatic cell sorting (Sony SH800). Expanded single cell clones were evaluated by IFN-γ stimulation and (C) HCC827 GAS-Luc2 cell line with high endogenous expression of PD-L1 and (D) H460 GAS-Luc2 cell line with high endogenous expression of CD155. The cloned cell lines were treated overnight with IFN-γ at the concentrations ranging 0.01 to 1000 ng/mL (n = 4). The same immunoluminescence checkpoint reporter cancer cell lines were administered with either non-activated or activated human primary CD8+ cytotoxic T cell-conditioned media and incubated overnight. The activated T cells were prepared with anti-CD3/CD80/CD28 beads, NCTD and others experiment. *P < 0.05.

**Luciferase expression upon JAK-STAT signaling pathway activation**

**Figure 2:** Heat maps based on protein profiling data of selected cancer cell lines. T cell lines, and primary T cells for immune checkpoint molecule expression for basophylometry. To evaluate the efficacy, potency, and dynamics of the inhibitor.

**Figure 3:** IFN-γ stimulation of GAS-Luc2 reporter candidate cell lines. Candidate cell lines were selected for GAS-Luc2 modification based on high expression of selected immune checkpoint markers and were assessed via IFN-γ cytostatic assay following viral transcription. GAS-Luc2-modified cells that endogenously expressed a high level of (A-D) PD-L1, (B) CD155, or (C-D) B7-H3 checkpoint molecules in a dose-dependent manner in response to IFN-γ stimulation. These results highlight the robustness and reproducibility of the reporter system for the assessment of T cell-mediated immune responses triggered by checkpoint inhibitors. As compared to immune checkpoint assays that use an artificial checkpoint ligand overexpression system, these reporter cell lines provide high signal sensitivity and reproducibility while simplifying the complex immunological model by providing physiologically relevant expression of immune checkpoint molecules.

**Figure 4:** Evaluation of monoclonal GAS-Luc2 cell lines as immune checkpoint inhibitors. Candidate cell lines were selected for the assessment of T cell-mediated immune responses triggered by checkpoint inhibitors. When comparing the physiological relevance and stable expression of the checkpoint ligand owing to the endogenous expression, these reporter cell lines provide high signal sensitivity and reproducibility, effectively eliminating the donor variability issue commonly experienced by using primary cell models.

**Conclusion**

- Despite the recent success of immune checkpoint inhibitors as cancer treatments, the built-in complexity of the immunological models and the variable drug responses among different cancer types are currently the most formidable challenges in this area of immunology.

**References**