High Throughput Combinatorial Profiling of Checkpoint Inhibitor Antibodies on the iQue® Screener PLUS

Stephen Kosteski, Zhaoping Liu, and Tom Duensing, IntelliCyt Corporation

Abstract

Checkpoint inhibitors have become valuable immuno-modulatory targets in the advancement of cancer treatment. Looking for the synergy between new checkpoint inhibitor antibodies and known inhibitors is an important aspect of this research. The iQue Screener PLUS platform is a powerful tool to simultaneously assess these interactions in a single well of a microtiter plate. ForeCyt® software provides plate-level analytics and high content visualization to generate deep insight rapidly. Using a mixed lymphocyte reaction (MLR) model, we profiled potential synergies of several known checkpoint inhibitors antibodies. Responses of PD-1, PD-L1, and CD73 inhibitors both individually, and in combination with CTLA4 inhibitors, were assessed for proliferation, viability and cytokine secretion simultaneously in the same well. MultiCyt® cell-based and bead-based reagents were used for this analysis. Synergies ranging from 2-10 fold increase over CTLA-4 alone were observed in the secretion of TNF-α and IL-1β. Results were obtained and analysis completed in a 384-well plate in 30 minutes. In conclusion, this study highlights the power of the iQue Screener PLUS platform to rapidly characterize multiple endpoints and the ForeCyt software to provide high content visualization that reveals actionable insights.

Materials and Methods

Eight combinations of donor PBMCs were mixed together and distributed in 384-well plates. The cells were treated for varying combinations and concentrations of checkpoint modulating antibodies (anti-PD1, anti-PD-L1, anti-CTLA4, and anti-CD73) and MultiCyt® Cell Proliferation Dye was added. After incubation for 3 days, reagents for measuring cell viability (MultiCyt Cell Membrane Integrity Dye) and the secretion levels of 3 different cytokines (MultiCyt® OBeads®) were added to the wells of the plate. Plates were sampled on the iQue Screener PLUS and data was analyzed with ForeCyt® Software (See Figure 1).

Results

Combinations of donor PBMC pairs were treated with varying concentrations of anti-CTLA-4 and then cytokine secretion, viability, and proliferation were measured. Cytokine secretion variations were seen between different donor pairs treated with anti-CTLA-4, especially IFN-γ. The anti-CTLA-4 antibodies had no effect on cell viability or cell proliferation (Figure 2).

Summary and Conclusions

In this study, the iQue Screener PLUS platform, along with ForeCyt® Software and MultiCyt® reagent kits, enabled comprehensive profiling of checkpoint modulating antibodies on primary cells of the immune system. The iQue Screener PLUS platform enables the:

- Rapid sampling and analysis of a 384-well plate in 30 minutes and allows many samples and conditions to be measured in a single experiment.
- Combination of cell and bead-based measurements in each well to evaluate complex immuno-modulatory profiles.
- Revelation of deep insights into the interactions between components of combinatorial therapies.

Multiplexing cells and beads together on the iQue Screener PLUS enables a deeper characterization of checkpoint inhibitor antibodies in a shorter amount of time, providing valuable information to make decisions on therapeutic candidates.