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 CTLA-4 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-a 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 QBeads®) 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).

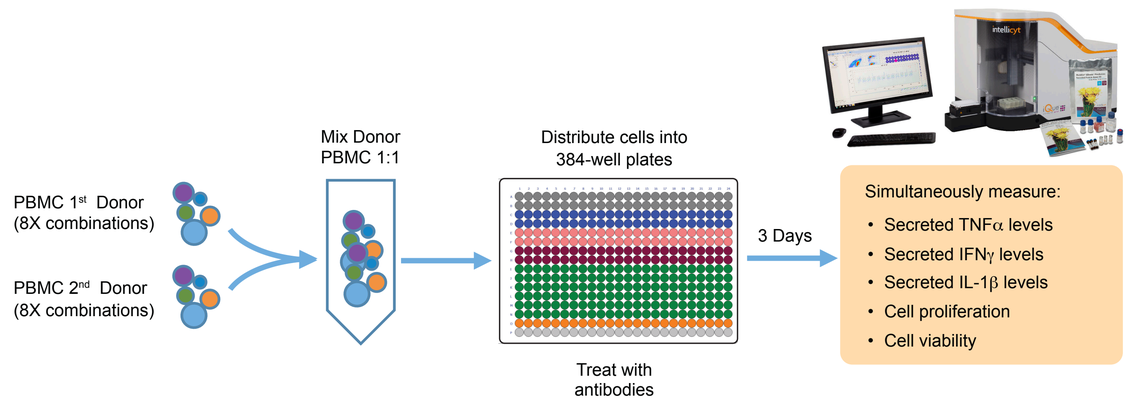

Figure 1: Outline of Experiment Design for the measurement of checkpoint inhibitor functionality in a Mixed Lymphocyte Reaction (MLR). A pictorial representation of the protocol as described in the materials and methods section. Several donor PBMCs were combined together in varying permutations to create eight donor pairs.

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 IFNγ. The anti-CTLA-4 antibodies had no effect on cell viability or cell proliferation (Figure 2).

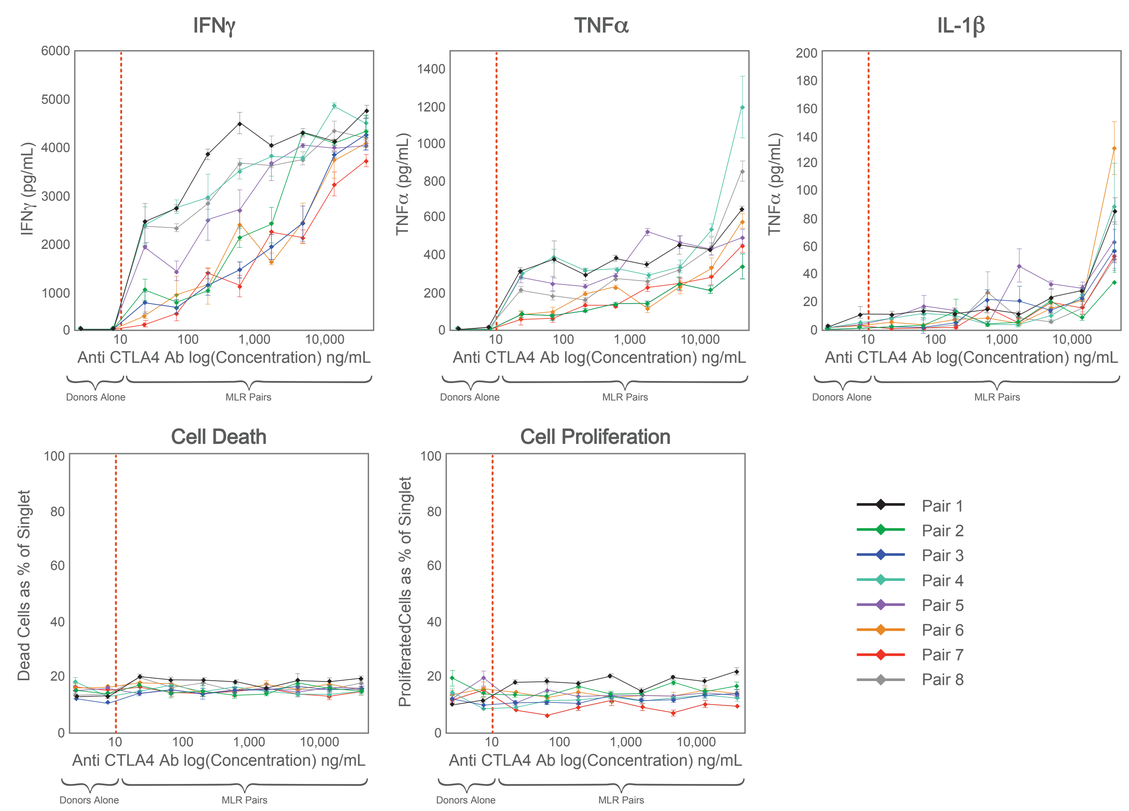

Figure 2. Rapid Testing of Anti-CTLA-4 Antibodies in Mixed Lymphocyte Reactions with Multiple Donor Pairs Eight pairs of donor PBMCs from different donors were treated with increasing concentrations of antibodies specific for CTLA-4 in triplicate. Data from control compounds, also included on the plate, are not shown. The levels of secreted cytokines IFNγ, TNFα, IL-1β and cell viability and proliferation were assessed.

Figure 3 shows cytokine responses from MLR reactions treated with or without a single concentration of anti-CTLA-4 antibody (17 µg/mL). The addition of anti-CTLA-4 antibodies increased the secretion of IFNγ, TNFα, and IL-1β in the MLR reaction and donor specific variation was seen (Figure 3).

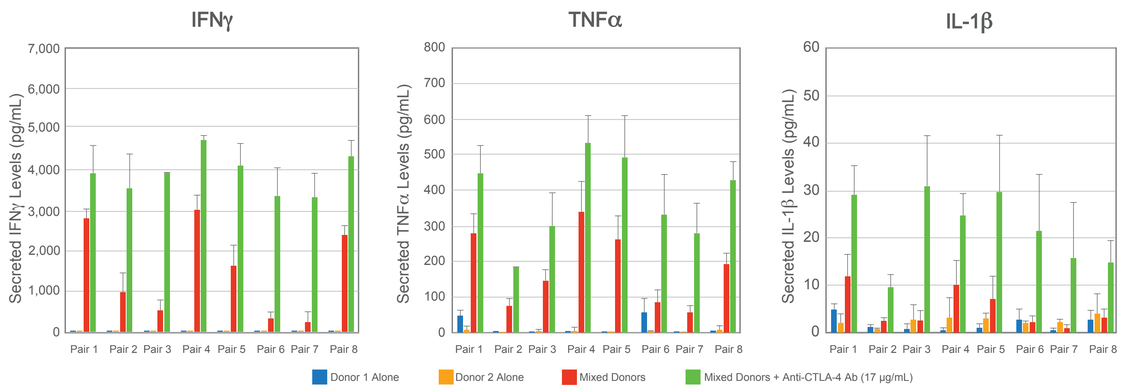

Figure 3. Differential Responses of Donor Pairs to Anti-CTLA-4 Antibodies in Mixed Lymphocyte Reactions. Cytokine responses of eight pairs of donor PBMCs at a single concentration of anti-CTLA-4 antibody (17 µg/mL) were evaluated.

In a second experiment, anti-CTLA-4, anti-CD73, anti-PD-1, and anti-PD-L1 were combined together in various permutations and added to a single donor pair. Figure 4 shows IL-1β was increased when cells were treated with anti-CD73 and anti-CTLA-4 and when treated with anti-PD-1 and anti-CTLA-4. INFγ was increased when cells were treated with a combination of anti-PD1 and anti-CTLA-4, a combination of anti-CD73 and anti-CTLA-4, and a combination of anti-CTLA-4 and anti-PD-L1. TNFα was increased when cells were treated with a combination of anti-PD1 and anti-CTLA-4, a combination of anti-CTLA-4 and anti-CD73, and a combination of anti-CTLA-4 and anti-PD-L1. Synergies ranging from 2-10 fold increase over anti-CTLA-4 alone were observed in the secretion of TNFα and IL-1β.

Large scale multiplexed experiments allowed extensive profiling of donor pair responses to treatment with checkpoint inhibitors in a MLR (Figure 3). The iQue Screener PLUS enables this kind of experiment to be run in 30 minutes. The ability to evaluate the secretion of multiple assay endpoints provided the construction of a comprehensive profile of checkpoint modulation with antibody combinations (Figure 4).

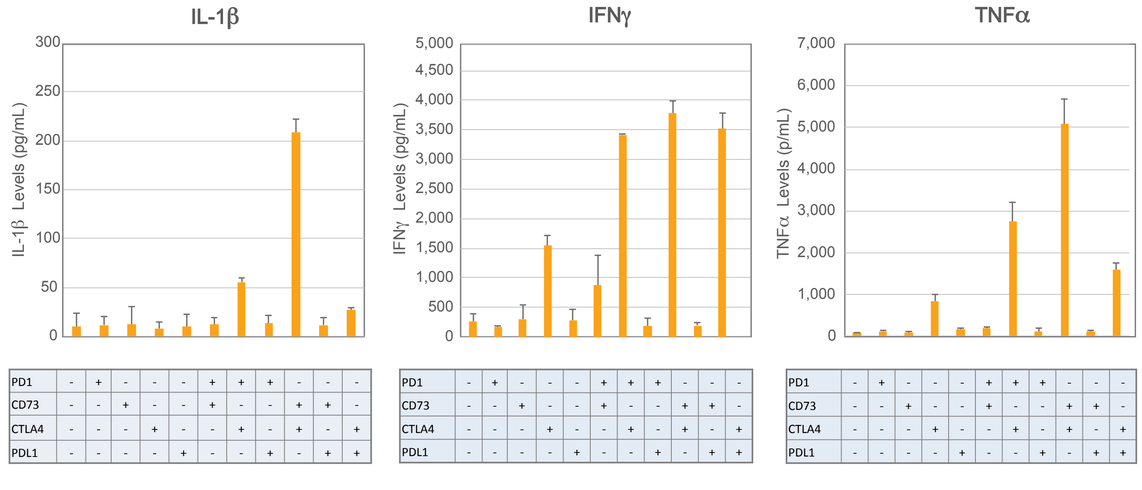

Figure 4. Evaluating Combinations of Checkpoint Inhibitor Antibodies in Mixed Lymphocyte Reactions. Cytokine secretion from a single donor pair was measured in response to four antibodies, alone and in combination, that target different immune checkpoints. Synergistic responses were observed for some combinations and there were no changes in cell viability or proliferation seen.

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.

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