ABSTRACT

KCAS, LLC optimized a primary and secondary stimulation assay of Peripheral Blood Mononuclear Cell (PBMC) samples from a clinical trial in order to characterize the immune response to a tri-valent peptide vaccine. In partnership with our pharmaceutical sponsor, an agreed upon set of samples were tested by KCAS, LLC to optimize a culture method, culture time course, stimulation, and re-stimulation. These samples were also used to optimize surface and intracellular staining of the following panel: CD45, CD3, CD8, PD1, CCR7, IFNγ, TNFα, IL-2 and viability dye. Upon review of the initial optimized method, the full cohort of patient samples were cultured, treated, stained, and analyzed.

Subsequent data analysis provided strong correlation between PBMC response to stimulation/re-stimulation, measured by production of TNFα and IFNγ, and clinical outcome. Additional testing continues in order to further assess individual peptide responses.

METHOD (cont’d):

Once optimal spectral signals and signal to noise ratios were optimized for the 10 markers, a pilot experiment allowed for determination of best in vitro stimulation of cytokine production.

Patient samples were analyzed for viable cell counts, diluted and plated for culture. Treatment conditions tested include:
- PMA + Ionomycin stimulation (positive control)
- Co-administered with immunostimulants
- Peptide pool (negative control)

Four hour stimulation in the presence of Brefeldin-A was sufficient for IFNγ, TNFα and IL-2 production in the presence of either PMA + Ionomycin or the peptide pool.

Cells were harvested following stimulation surface staining, followed by fixation and permeabilization for intracellular staining for IFNγ, TNFα, IL-2.

CONCLUSION

- Well planned and executed experiments supported clinical outcomes
- Having successfully demonstrated in vitro stimulation results which support clinical observations allowed the sponsor to move forward with development
- Working together the Pharmaceutical Sponsor and KCAS were able to complete a complicated set of experiments to meet the sponsors timeline

KCAS takes pride in its work:
- Thoughtful design and well planned experiments
- Optimizing experimental conditions
- Data integrity at the forefront