Optimization of Critical Steps for Measuring Immunomodulatory Therapeutic PD Effects by Innate and Adaptive T Lymphocyte Ex-Vivo **Stimulation and Flow Cytometry**



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Background

Assessing pharmacodynamic effect of immunomodulatory therapeutics often requires ex-vivo stimulation assays. Optimizing the ex-vivo stimulation conditions improves assay feasibility, precision, and sensitivity in measuring changes in critical biomarkers in phase I clinical trials.

Methods





Results

Cell Populations Analyzed



Critical Steps for Ex-Vivo Stimulation Assays Depend on Matrix and Target Biomarker

	From Clinic to Lab	Advantages	Critical Steps
Whole Blood	Less than 6 hrs	 High within-assay precision Physiological milieu Processing efficiency Low blood volume requirement Highest stimulation magnitude 	 Processing within blood stability window Efficient mixing of stimulant and blood Culture time of less than 48 hours
PBMC CD4+ T cells	Less than 48 hr	 Potentially less between-subject variability Long term stability/cryostorage Easy sample shipment Control over culture medium Adjust cell concentration for assay Long-term culture (days to weeks) 	 Processing within blood stability window Choice of media: +/- serum High viability of isolated and thawed sample Test if isolation steps increases background of analyte (pSTAT)

Ex-Vivo TCR Stimulation and Cell Surface Activation Marker Staining



Conclusions

- Whole blood matrix shows highest within-assay precision and greatest stimulation magnitude in ex-vivo stim assays
- PBMCs/isolated CD4+ T cells allow for more control of culture conditions and batching across time points (cryopreservation)
- Purification processes (PBMC/CD4+) could lead to elevated background in some analytes/donors
- allowed by operational constraints)

Ex-Vivo Cytokine Stimulation and Intracellular Phospho-STAT Staining



• Whole blood is the preferred matrix for ex-vivo stimulation assays when measuring the PD effect of TCR or cytokine signaling pathway inhibitors (as



Whole Blood TCR Stimulation Precision **Between Analysts**

Translating Science to Medicine