



# Exploring pharmacodynamics in early drug development:

## Measuring pharmacologic perturbations of human physiology

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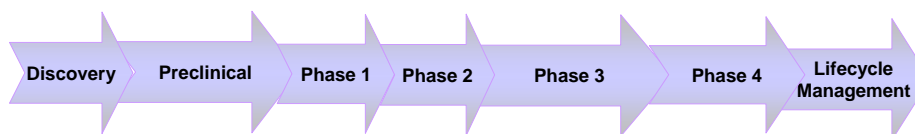
## Outline

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- Biomarkers in early drug development
- Discovery to PD assay
  - Multiplexed assays
  - *Ex vivo* whole blood challenge:response assay
- Proximal biomarker assays – Phosflow™
- Conclusions

## Drug Development: Traditional Approach vs. New Paradigm

### Traditional Approach:



### New Paradigm (FDA Critical Path Initiative):



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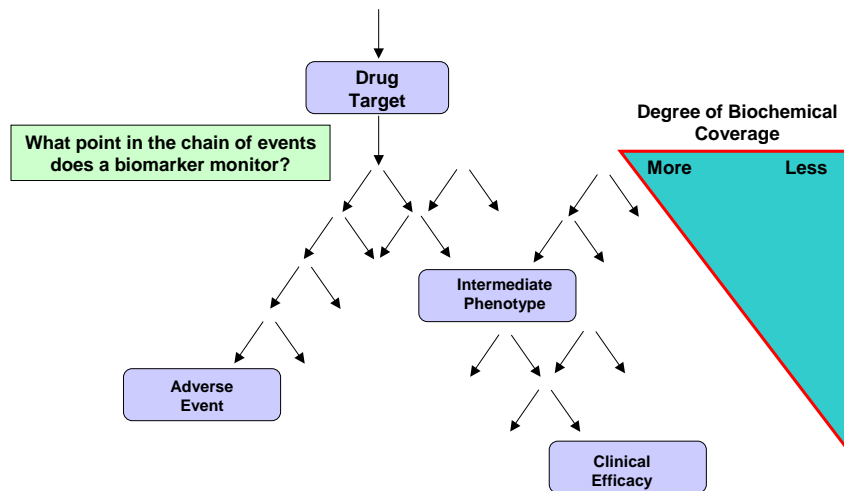
## Pharmacodynamic (PD) biomarkers in drug development

- PD: Measuring what the drug does to the body
  - Pharmacokinetics (PK): what the body does to the drug
- Requirements
  - Results must be reliable: Need to understand the performance characteristics of the assay
  - Unlike diagnostics we have pre-treatment samples, so comparison is before and after treatment
    - Individual/genetic variation decreases statistical significance in diagnostic discovery
- Biochemical Coverage
  - Measured analyte must inform us as to whether desired pathway is affected (or not) and would like to see an exposure-response relationship

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## Hypothetical Relationship between Target Modulation and Downstream Effects



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## Molecular PD biomarkers currently used

CLINIC

- ✓
  - Cytokine/chemokine/other protein levels in blood/other fluids (including *ex vivo* stimulation) by MAP (multi-analyte profiling: beads/planar arrays)
- ✓
  - Signaling proteins in cell lysates by MAP (p & tGene)
- ✓
  - Signaling proteins in permeabilized cells by flow cytometry (Phosflow™)
- ✓
  - Cell surface proteins by flow cytometry
- ✓
  - Transcript levels in cell lysates
- ✓
  - Gene sequence and copy number in cell lysates
- ✓
  - Specific DNA levels in fluids
  - Mass spectrometry based discovery

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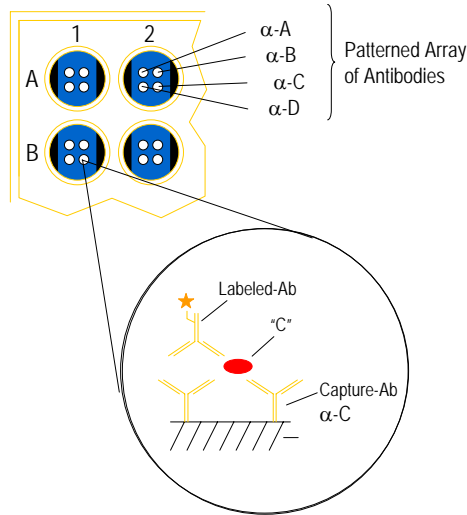
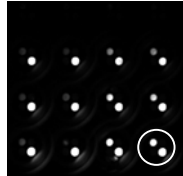
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## Meso Scale Discovery (MSD) multiplex assay

### Features:

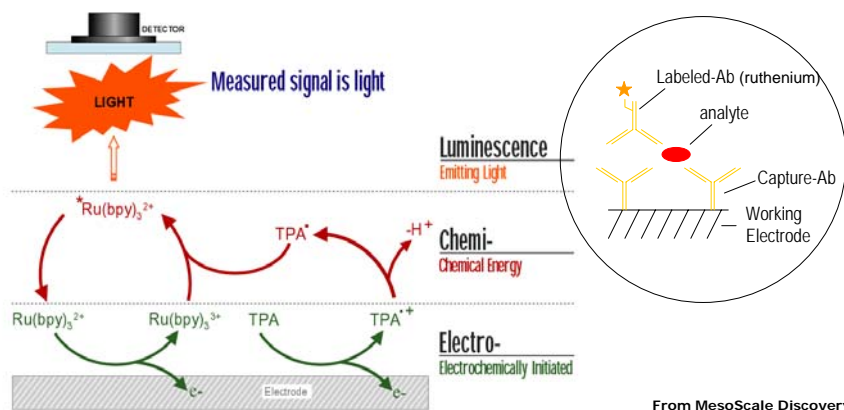
- Multiple spots within each well
- Each spot is an independent assay
- Biospecific reagents are deposited on each spot
- Homogeneous assay; simple, no-wash protocols save time and labor
- Multiplexing resulting in lower cost / data point



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## Electrochemiluminescence detection



From MesoScale Discovery

Light emission is the reduction of Ru(III) by deprotonated TPA radicals to electronically excited Ru(II), which emits light when it decays to the ground state

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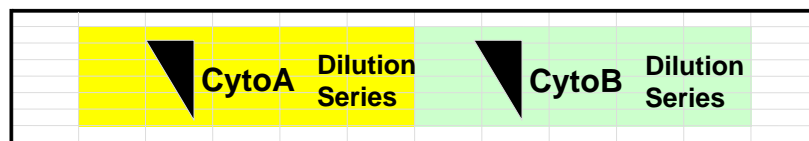
## Discovery → PD Assay *Ex Vivo* Whole Blood Culture

- Discovery approach for readout of cytokine stimulation
  - Whole blood from healthy volunteers stimulated with each of two cytokines followed by microarray analysis of PBLs & Rules Based Medicine analysis of serum/supernatant
- Experimental aim
  - Determine PD effect of increasing doses of a therapeutic antibody directed against a cytokine receptor through stimulation of receptor/pathway with two different cytokines using a multiplexed readout of 4 chemokines
- *Ex vivo* whole blood challenge: response assay
  - Dilute whole blood 1:1 with RPMI/10%FBS
  - *Ex vivo* stimulation with CytoA and CytoB
  - Incubate for 48hr @ 37C
  - Spin, transfer supernatant. Freeze & ship for readout assay
  - Readout assay: multiplexed MSD plate

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## The assay



8 Readouts/patient/timepoint: Dilution curves for (4 analytes x 2 stimuli)

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## MSD Validation Requirements

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- Optimization of Assay Protocol
- Reagent Stability
- Sample Short Term Stability
- Sample Stability: Freeze/ Thaw
- Linear Dilution
- Independent Spike-in Recovery
- Spike Dilution
- Determine potential interference of
  - HAMA, lipemic sample, hemolysed samples, excess therapeutic

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## Generation of Validation and Screening controls

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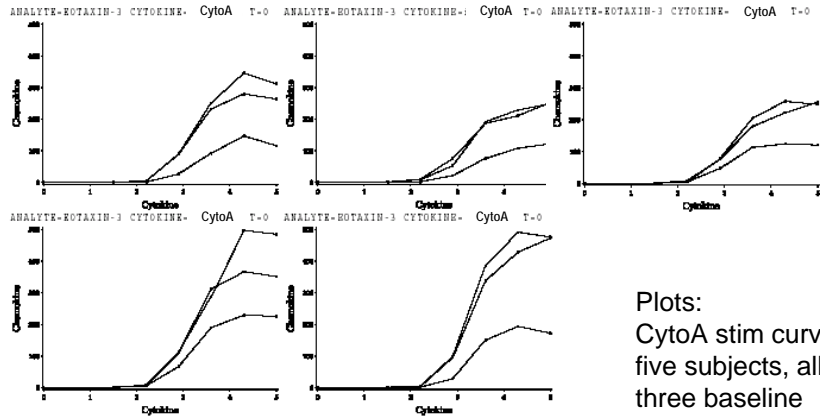
- Five donors
  - High (CytoA@ 100ng/ml)
  - Medium (800pg/ml)
  - Low (10pg/ml)
  - CTRL(control) Matrix (no induction)

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# Varying Baselines

## *CytoA stim. Eotaxin-3*

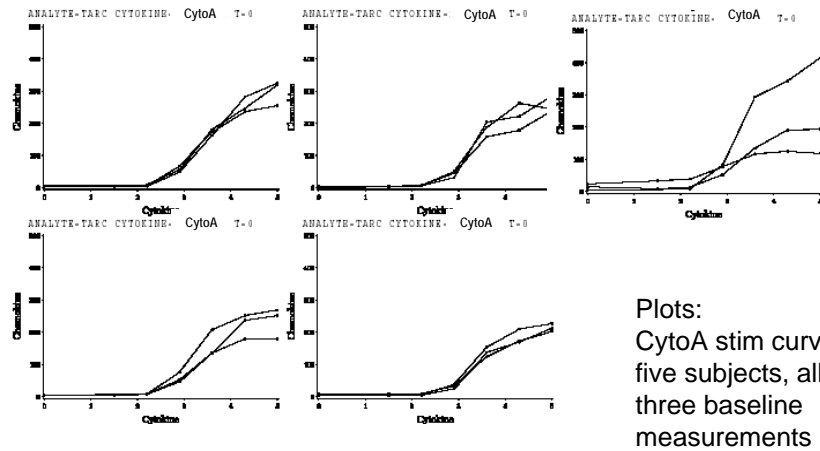


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# Varying Baselines

## *CytoA stim. TARC*



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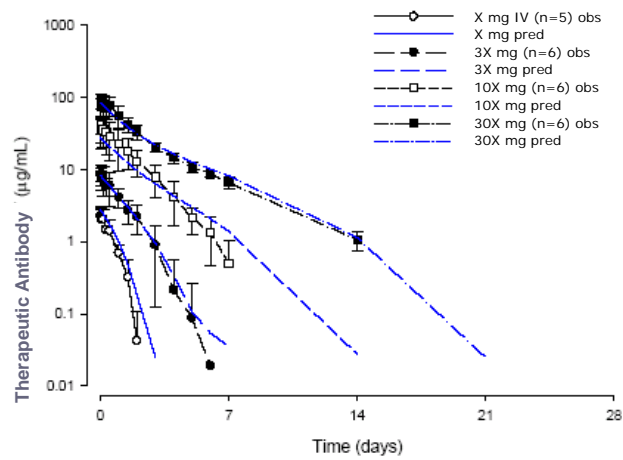
## Associating PD with PK

- Dose:Activity relationship depends upon knowing how much drug onboard.

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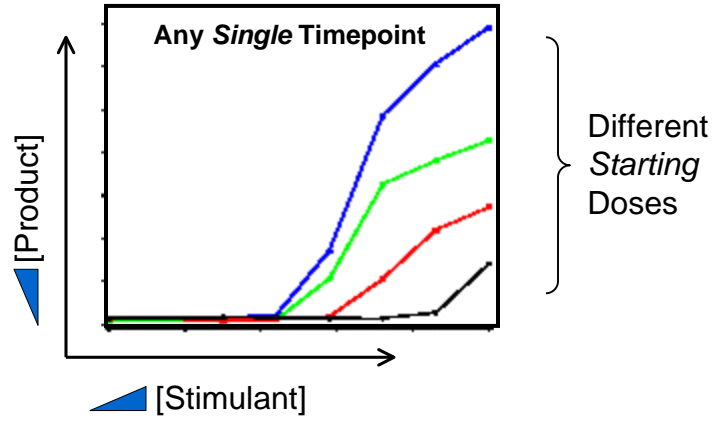
## Human Observed vs Predicted (Chimp Based)



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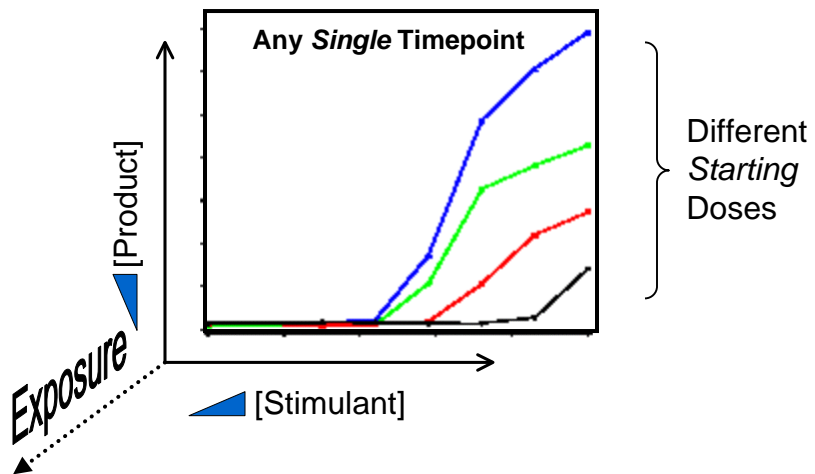
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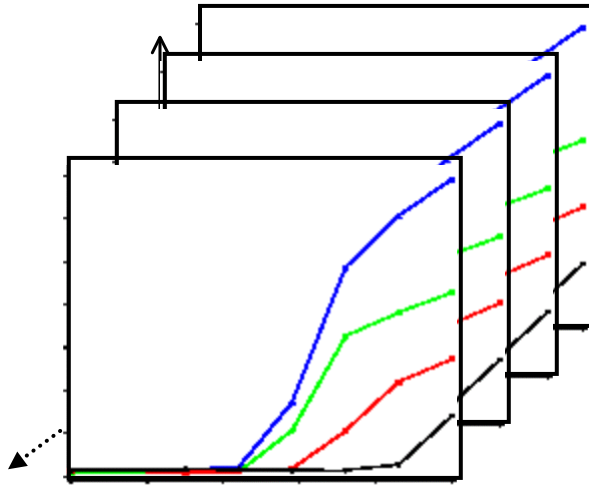
## Associating PD with PK



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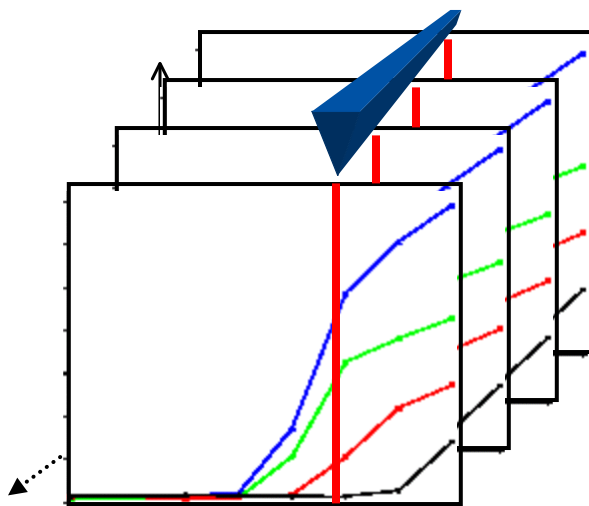
Time post-dosing is no longer a parameter:  
look at all timepoints at once



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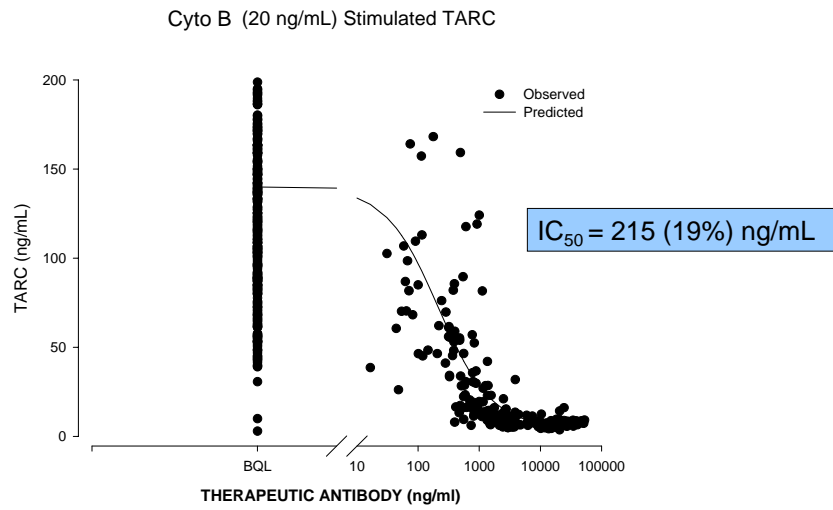
Reflecting biological reality:  
What's a reasonable estimate of stimulus *in vivo*?



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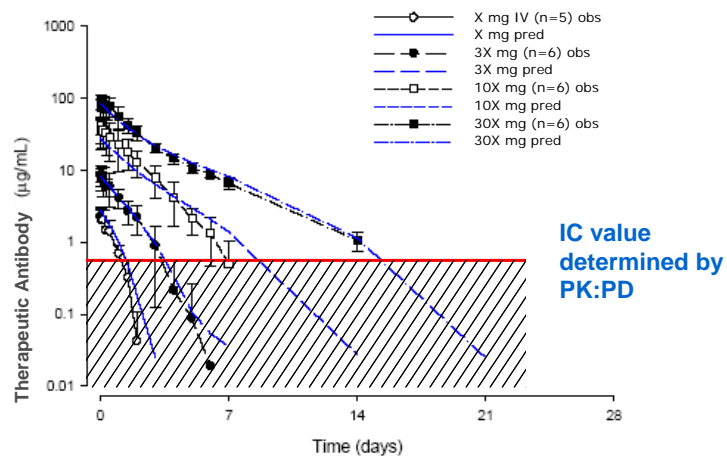
## PK:PD modeling



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## Establishing Relationship: Minimal PD coverage *vs* Time *vs* Dose



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## Discovery → PD Assay Conclusions

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- Broad-based discovery can be translated into specific assays
- Multiplexed assays can be used successfully for *ex vivo* whole blood assays
- Effective PK:PD modeling can be conducted using this data
- As with any assay format, thorough validation is critical to success

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## Biomarker identification in *ex vivo* stimulations of whole blood

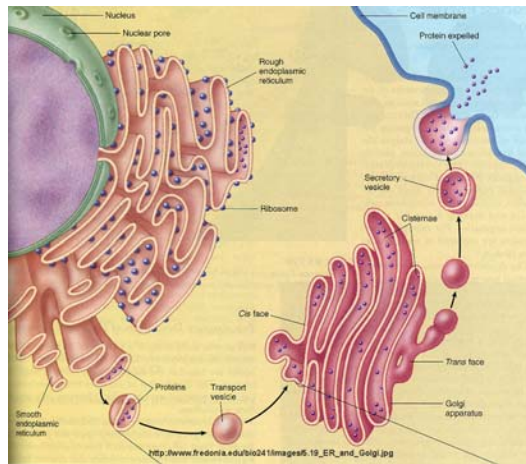
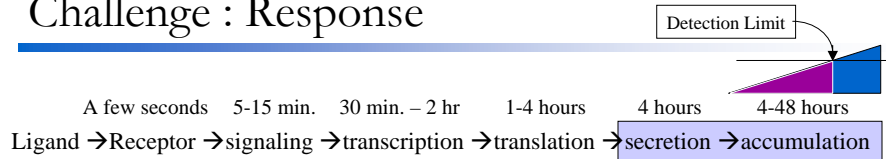
### Challenge : Response Assays

- Whole blood is the most readily available tissue for biochemical coverage analysis if the pathway of interest exists in blood cells.
- Detection of secreted proteins (cytokines/chemokines) following a biochemical challenge is one of the most commonly used assays for measurement of therapeutics which block inflammatory processes.
- While secreted proteins have proven useful, we have tried to develop methods which would allow the identification of biomarkers which are more receptor proximal.

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## Challenge : Response



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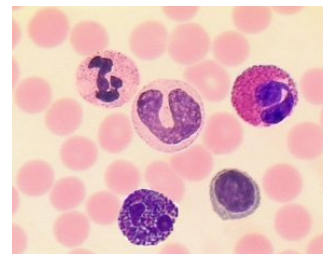
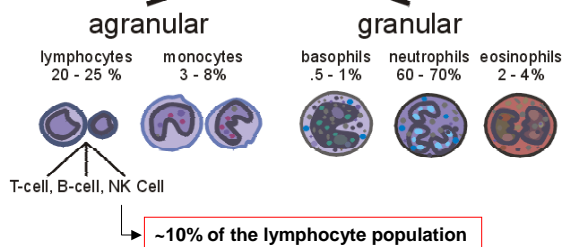
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## Challenge : Response

- Analyte assays measure only the responding cells in whole blood.
- A given analyte may be difficult to detect if it is produced in rare cell types
  - We aim to be more specific through flow cytometry.

### Leukocytes

white blood cells ~ WBC

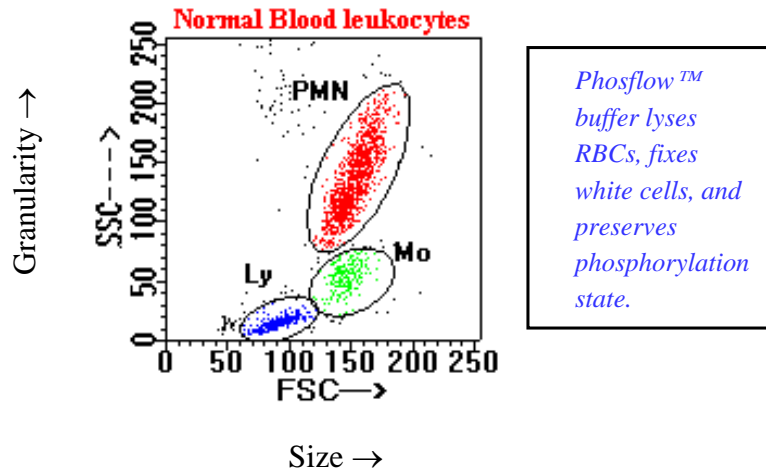


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## Flow Cytometry

-Can measure physical parameters as well as colors



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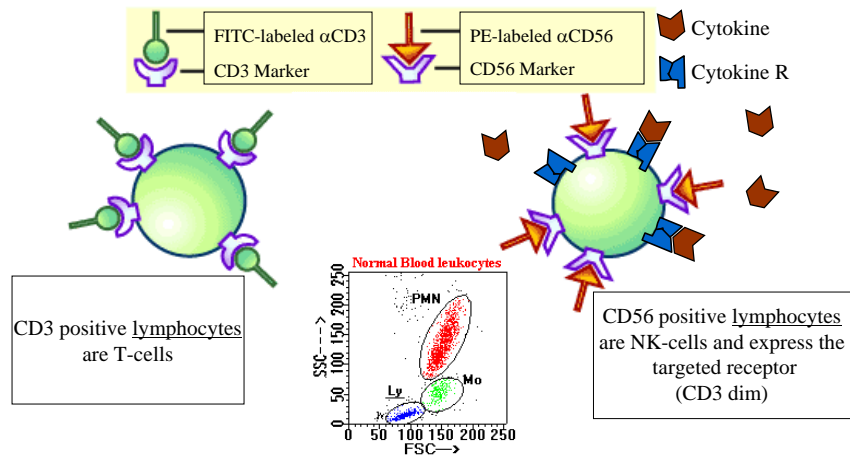
## Development of receptor proximal assays using multi-parametric FACS (+ Phosflow™)

1. Develop a validated, robust set of assays which quantitatively detect changes in phosphorylated proteins for biochemical coverage (PD biomarkers indicating on-target effects).
2. Assays should be seamlessly integrated into clinical trials.
  - Directly compare the sensitivity/ specificity/ reproducibility of receptor proximal assays to other platforms.
  - Determine the inter- and intra- patient variability (patient stratification?).
  - Determine the impact of sample handling/processing on the assay.
  - Minimize the manipulations required at the clinical site.
  - Validate the system in a Phase 0 clinical trial setting

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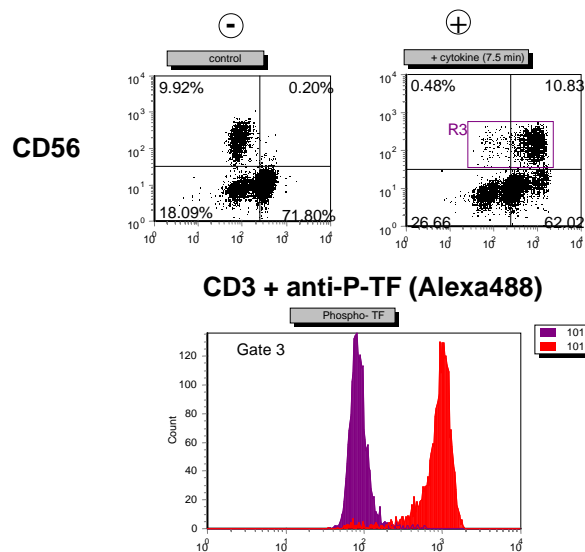
# Multi-parameter flow identification of cytokine responsive NK cells



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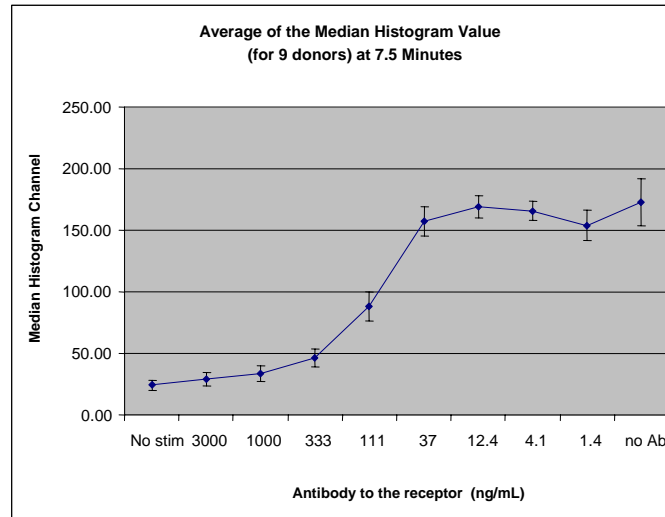
# $\alpha$ CD3 (FITC) and $\alpha$ CD56 (PE) identify NK cells responding to cytokine stimulation



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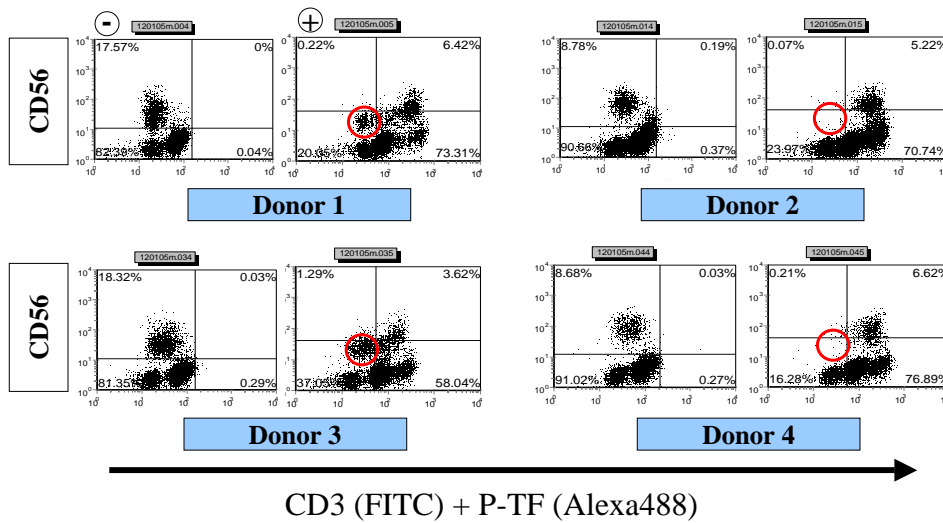
## Average Phosphoprotein Detected in 9 donors



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## Potential for patient stratification?



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## Proximal Biomarkers Conclusions

- Multi-color FACS allows the identification of specific subsets of cells from whole blood
- Phospho-specific monoclonal antibodies can be utilized to quantitatively assess the activation of a particular signal transduction pathway within a whole blood cell subset
- Phosflow™ technology can be used to replace/augment traditional whole blood assays with cytokine readouts

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## Acknowledgements

- Molecular Sciences, Thousand Oaks team
  - Dan Fitzpatrick, Sid Suggs, Andy Welcher, Ian McCaffery, Mike Damore, Tina Robson, Mike Davis, Yun Lan, Shen-Wu Wang, Brian Twomey, Kim Hamic, Alexia Parvex, Beate Sable, Jenny Wu, Paul Auger, Jen Claycamp, Roger Craveiro, Mara Campbell, Dan Baker, Pani Kiaei, Lisa Kivman, Hilary Chute, Karen King, Victor Barios, Nancy Shen-Chow
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- Early Development: Mike Vincent, Chris Banfield

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