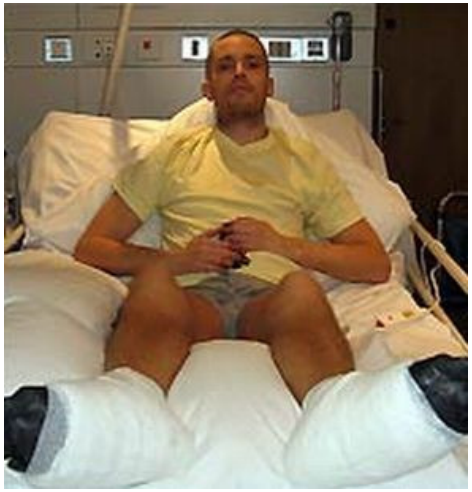
Crack-IT : Cytokine release assay

Chris Kirton

Cytokine Storms

A **cytokine storm** (hypercytokinemia).
A potentially fatal immune reaction consisting of a positive feedback loop between cytokines and immune cells, with highly elevated levels of various cytokines.



Symptoms
Angiodema, fever, chills, nausea, hypotension.
Selectin upregulation, margination, lymphopenia.

Predictive value of animal models for CRS

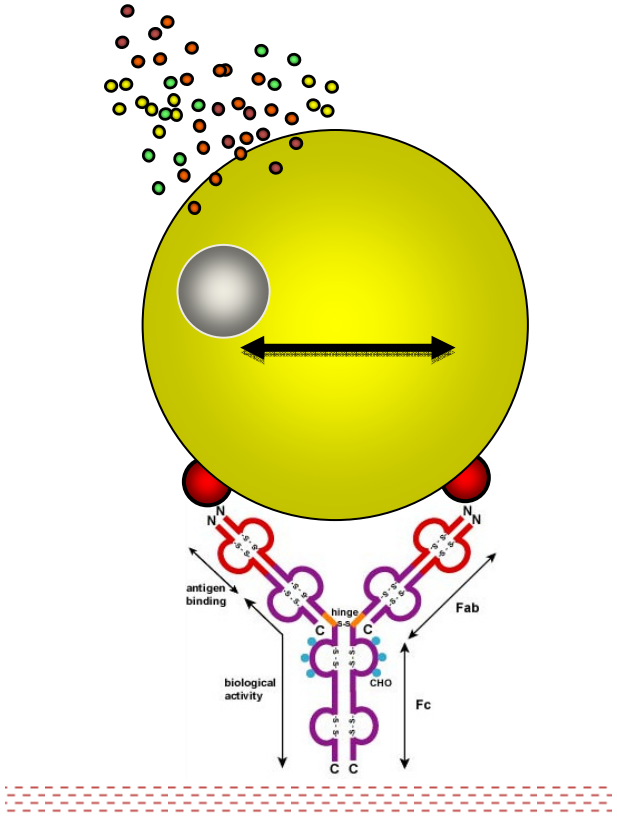
Antigen	Antibody	Species	Animal	Human
CD3	Muromonab	Mice	+++	+++
CD3/CD19	MT103	Chimp	++	++

Prediction - *In vitro* vs *In vivo*

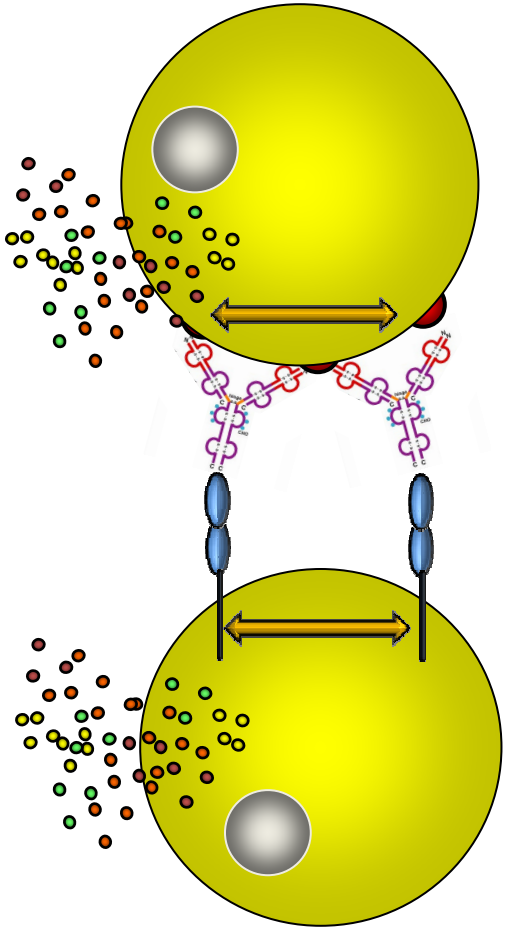
	In vitro pg/ml	In vivo pg/ml	First Dose Symptoms in Man
CD4 IgG1	BLQ	BLQ	-
Campath IgG4	39	35 (19-296)	+
Rituximab	30*	84[1]	+
Rituximab		550[2]	++
Alemtuzumab Campath IgG1	204	246 (153-264)	++
TGN1412	Yes	4800	+++

***Normal healthy donors**
[1]CLL Patients with <50[9] cells/L, [2]patients with >50[9] cells/L – Blood 94 : 2217-24 1999

Current design



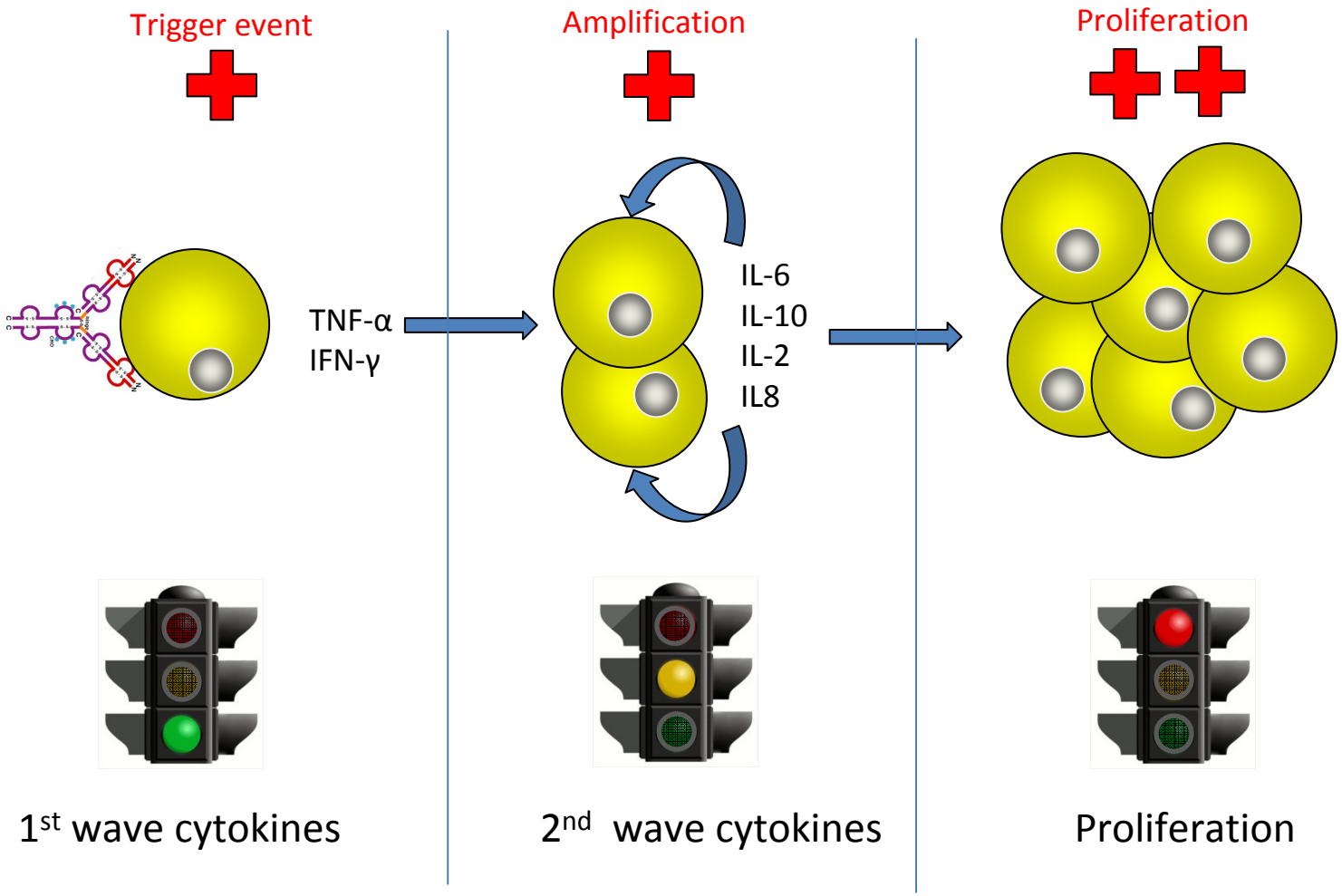
Air dried / Protein G/ Beads



Type I

Type II

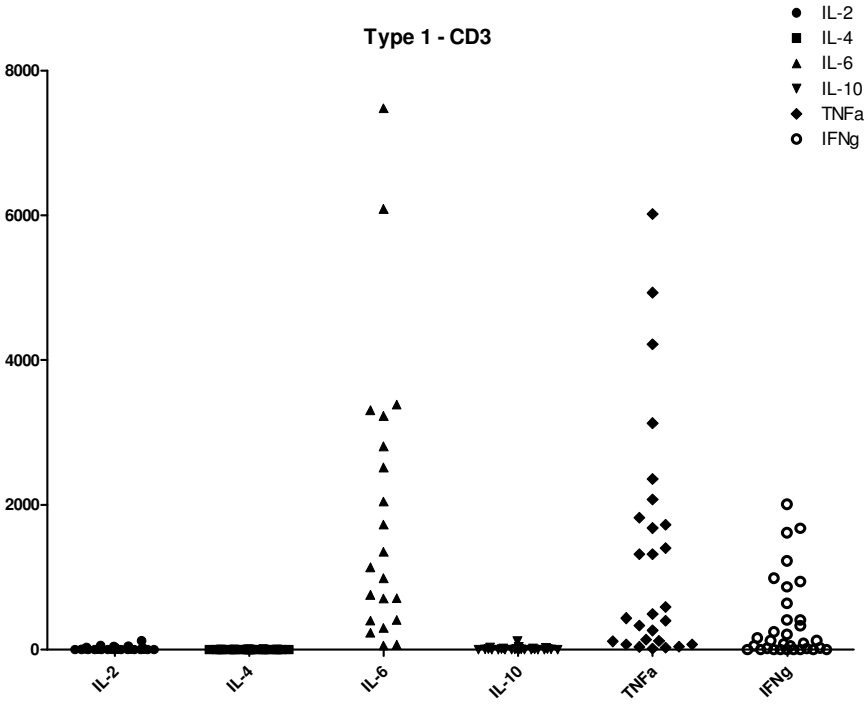
Cytokines involved in CRS

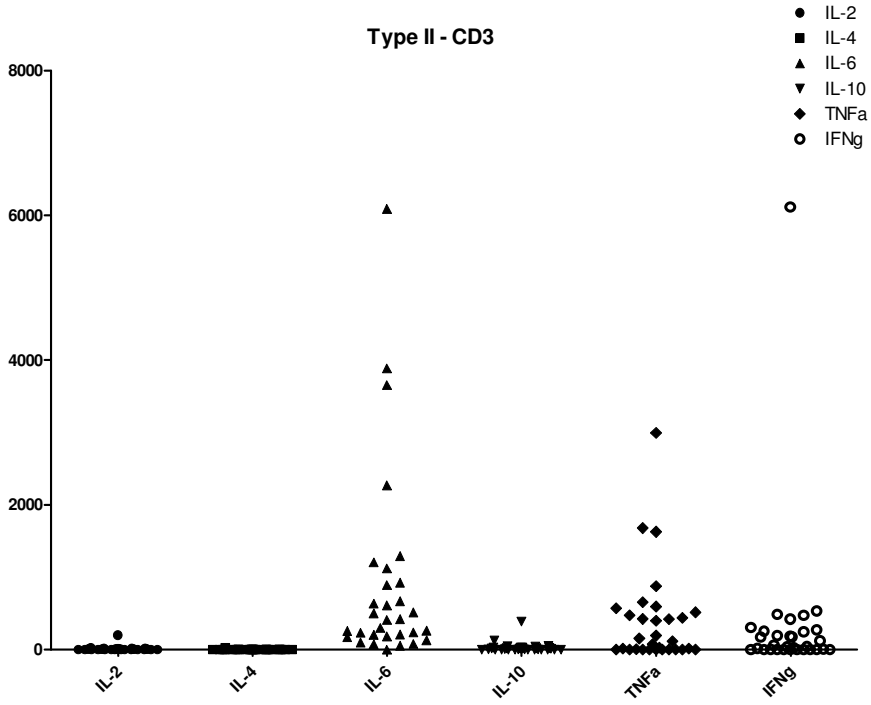


Problems with current models

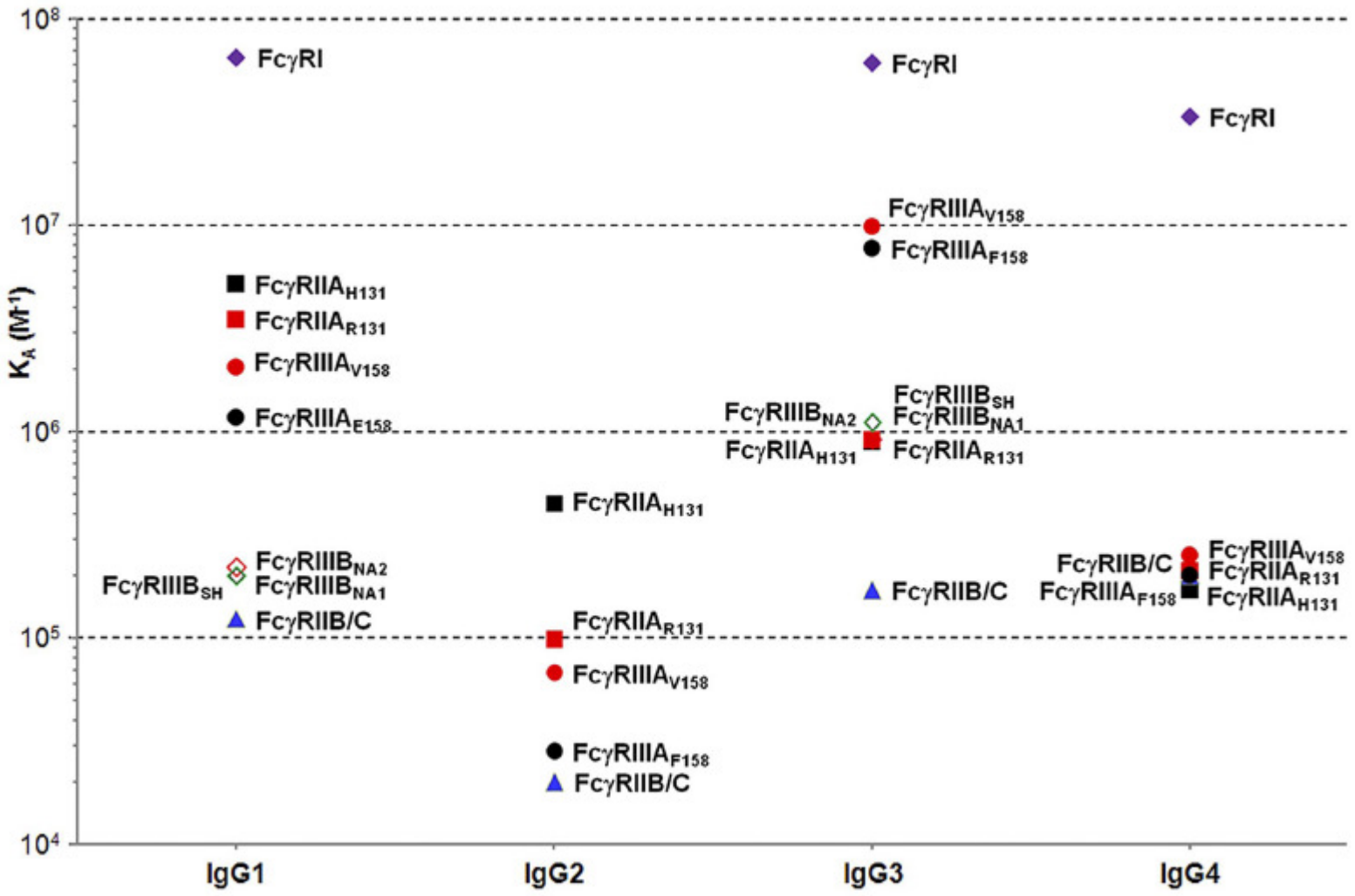
- Assay data is very variable (donor:donor)
- Cytokine release is dynamic, we only use one time point.
- No measurement of cellular proliferation, which is common in sever reactions.

Diversity in response – CD3



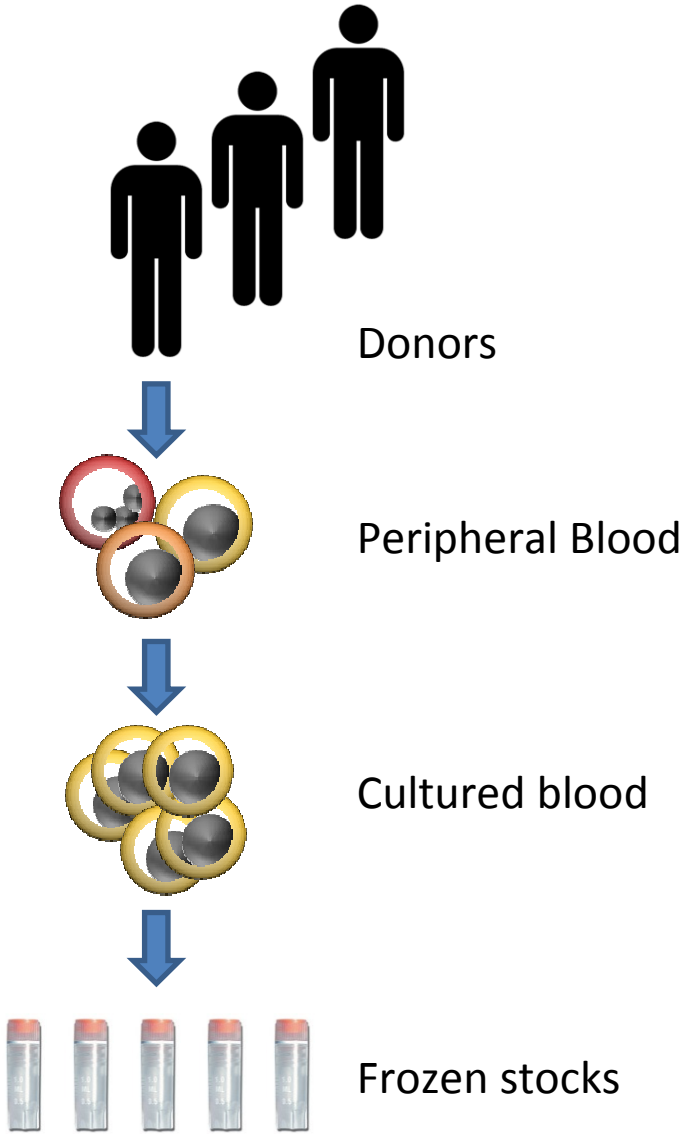


FcR as sources of variation

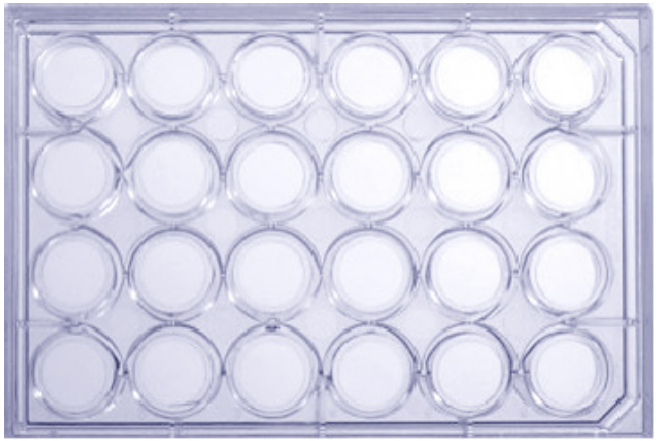


Generation of cell bank

- One source of assay variability is person-person differences.
- FcR phenotyping of stocks may allow the selection of donors to give a more predictable distribution.
- Culturing of cells will potentially give a more LN like phenotype.

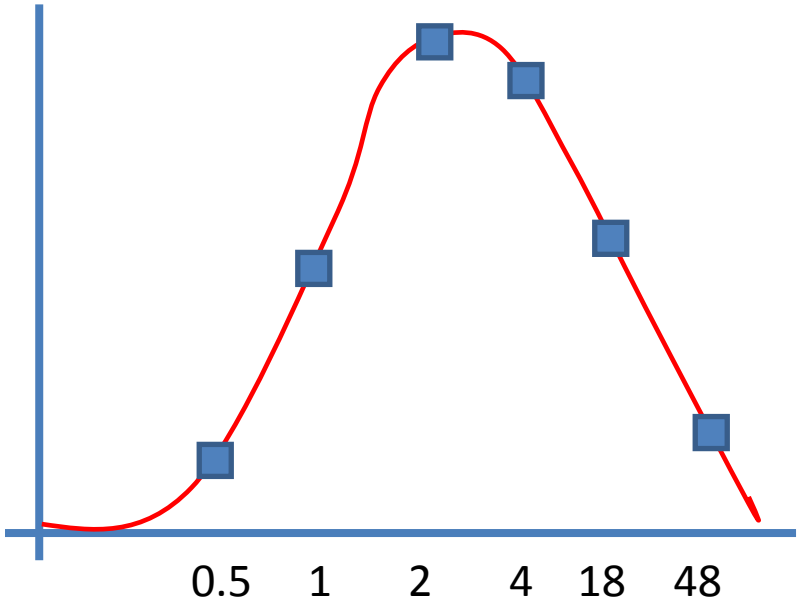


Time course

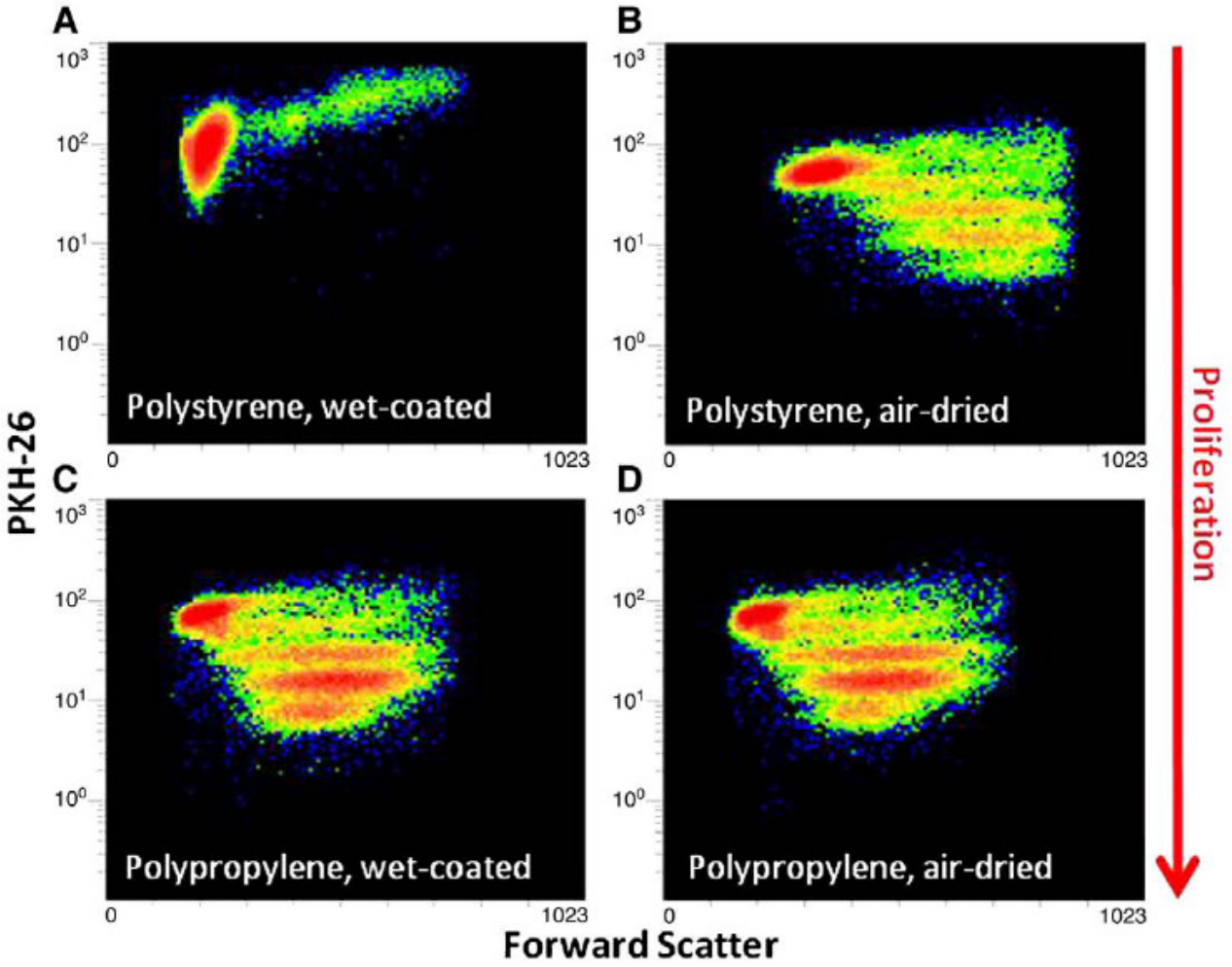


0.5 1 2 4 18 48

Collect samples for analysis



In vitro cytokine release assays to predict cytokine release syndrome



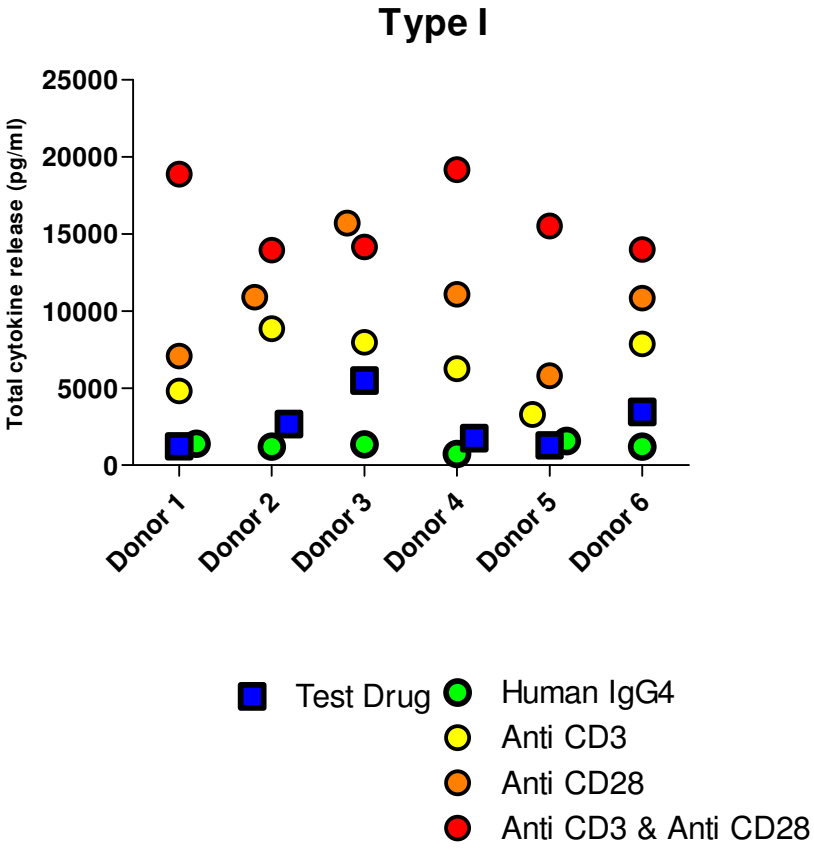
Flow based fluorescent membrane dye (PKH26)

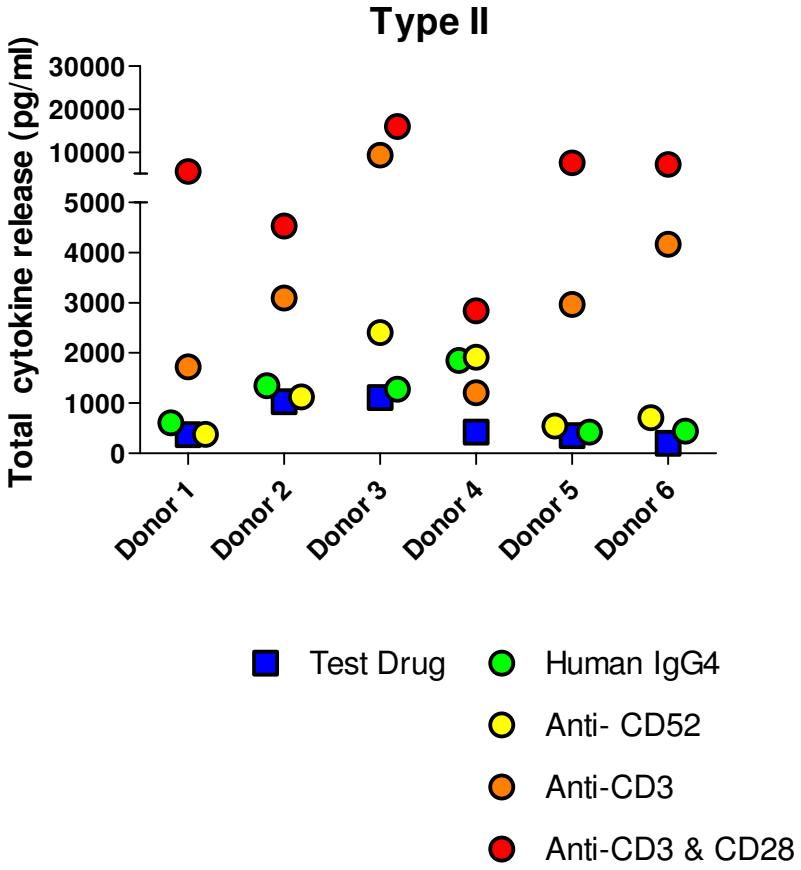
Partitions between daughter cells

PBMCs pulsed with PKH26, into CRA - 3 days

Please cite this article as: Findlay, L., et al., Improved in vitro methods to predict the in vivo toxicity in man of therapeutic monoclonal antibodies including TGN1412, J. Immunol. Methods (2009), doi:10.1016/j.jim.2009.10.013

Assay reporting





Challenge objectives

- To produce an in vitro cytokine release assay which:
  - Is predictive of clinical outcome.
  - Measures cytokine release dynamically in multiplex format.
  - Measures cellular proliferation.
  - Is able to define the FcR phenotype of donor cells.
  - Is powered appropriatly and is therefore amenable to statistical analysis