

Crack-IT : Cytokine release assay

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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	++	++

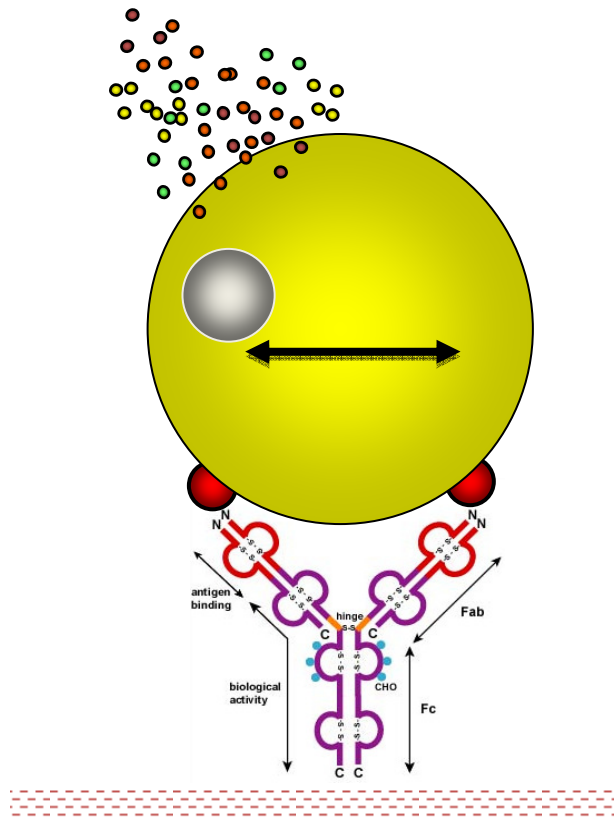
Prediction - *In vitro* vs *In vivo*

	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

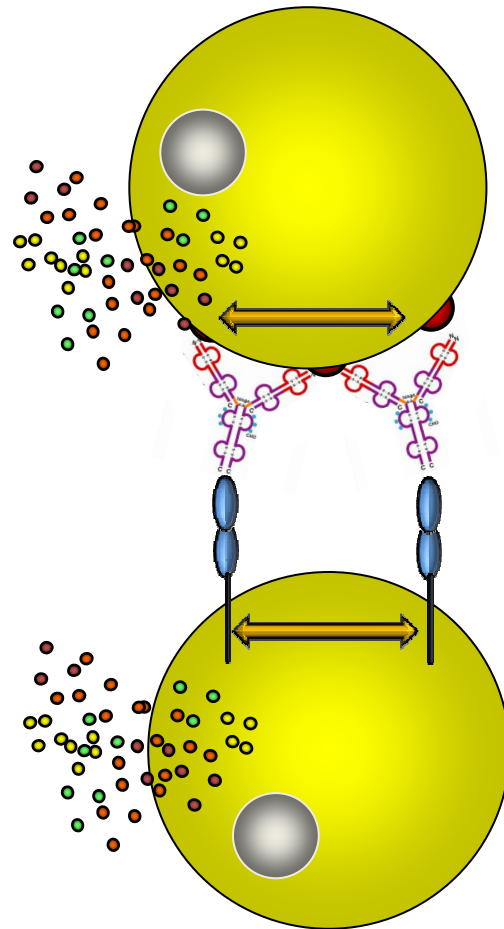
¹CLL Patients with <50⁹ cells/L, ²patients with >50⁹ cells/L – Blood 94 : 2217-24 1999

Current design



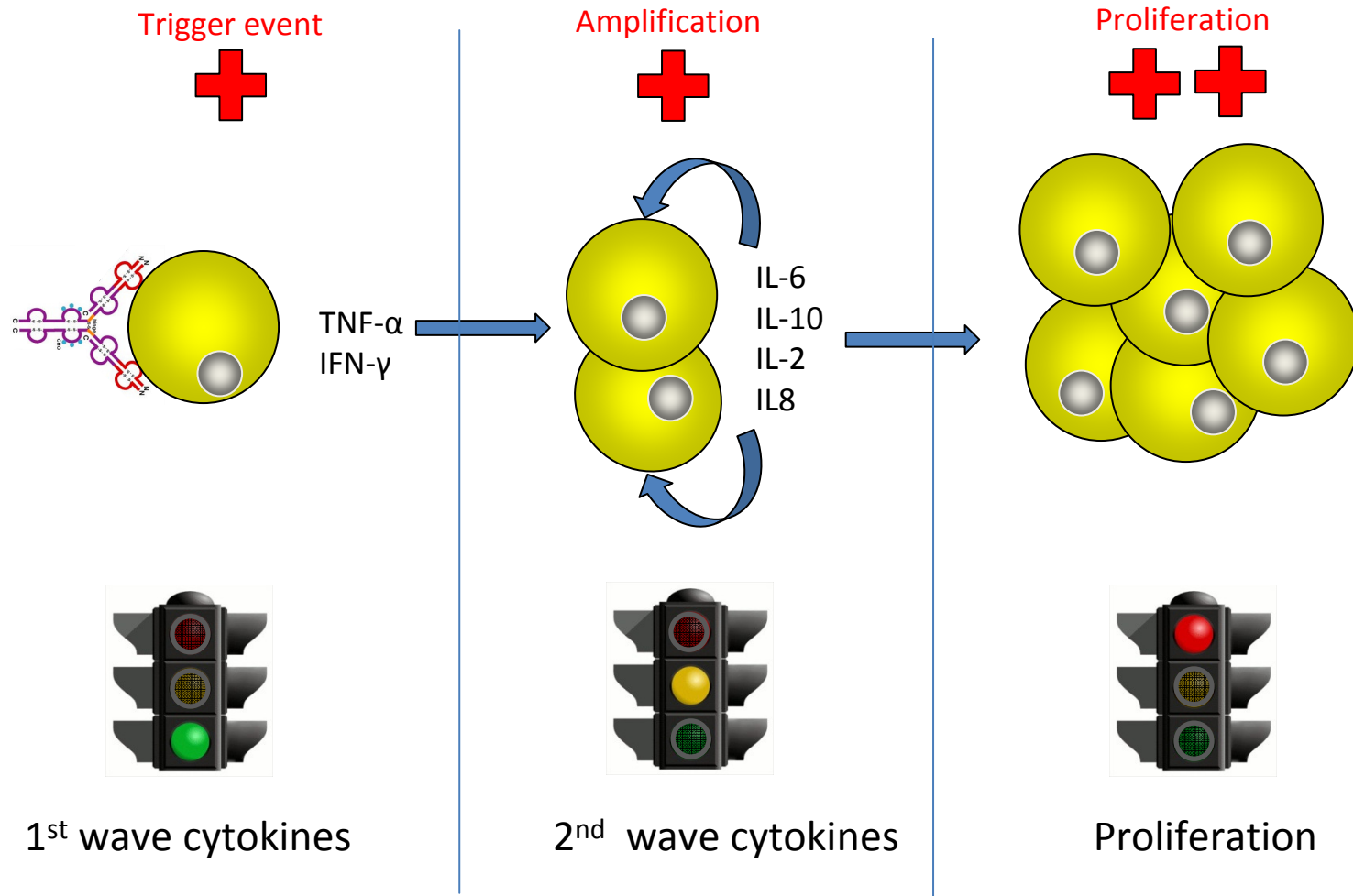
Air dried / Protein G/ Beads

Type I



Type II

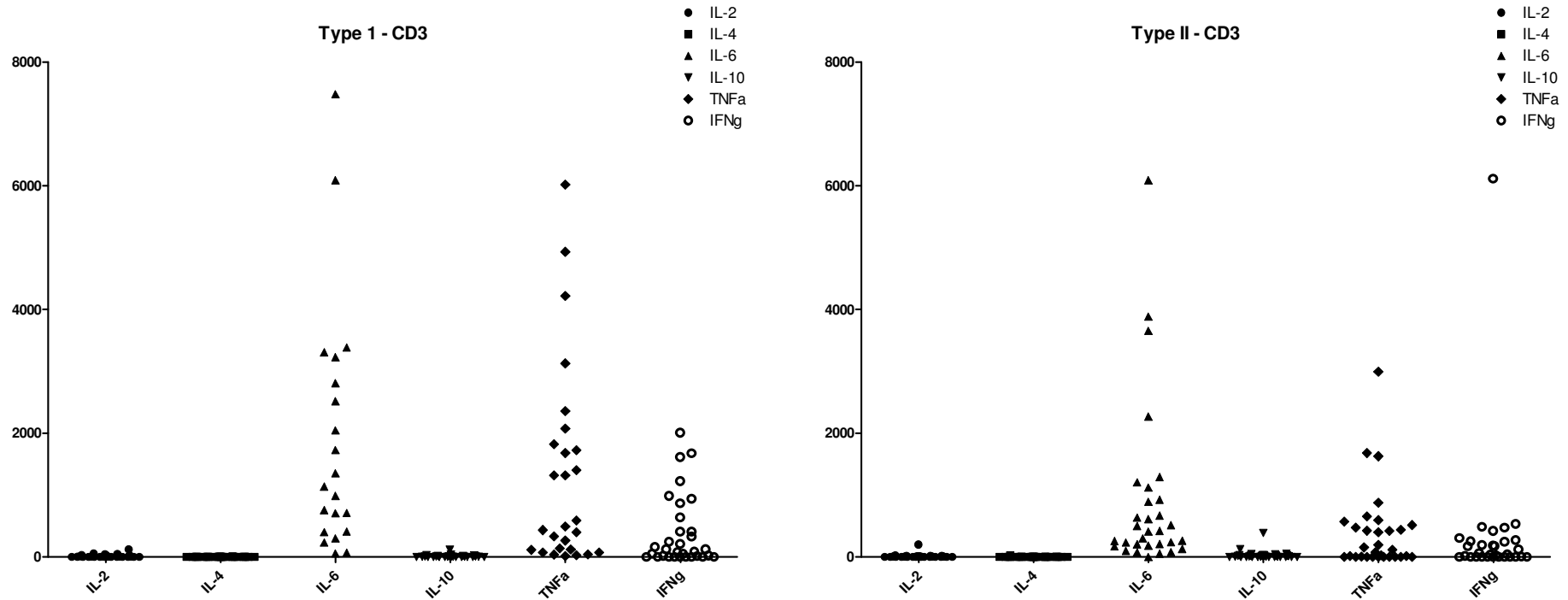
Cytokines involved in CRS



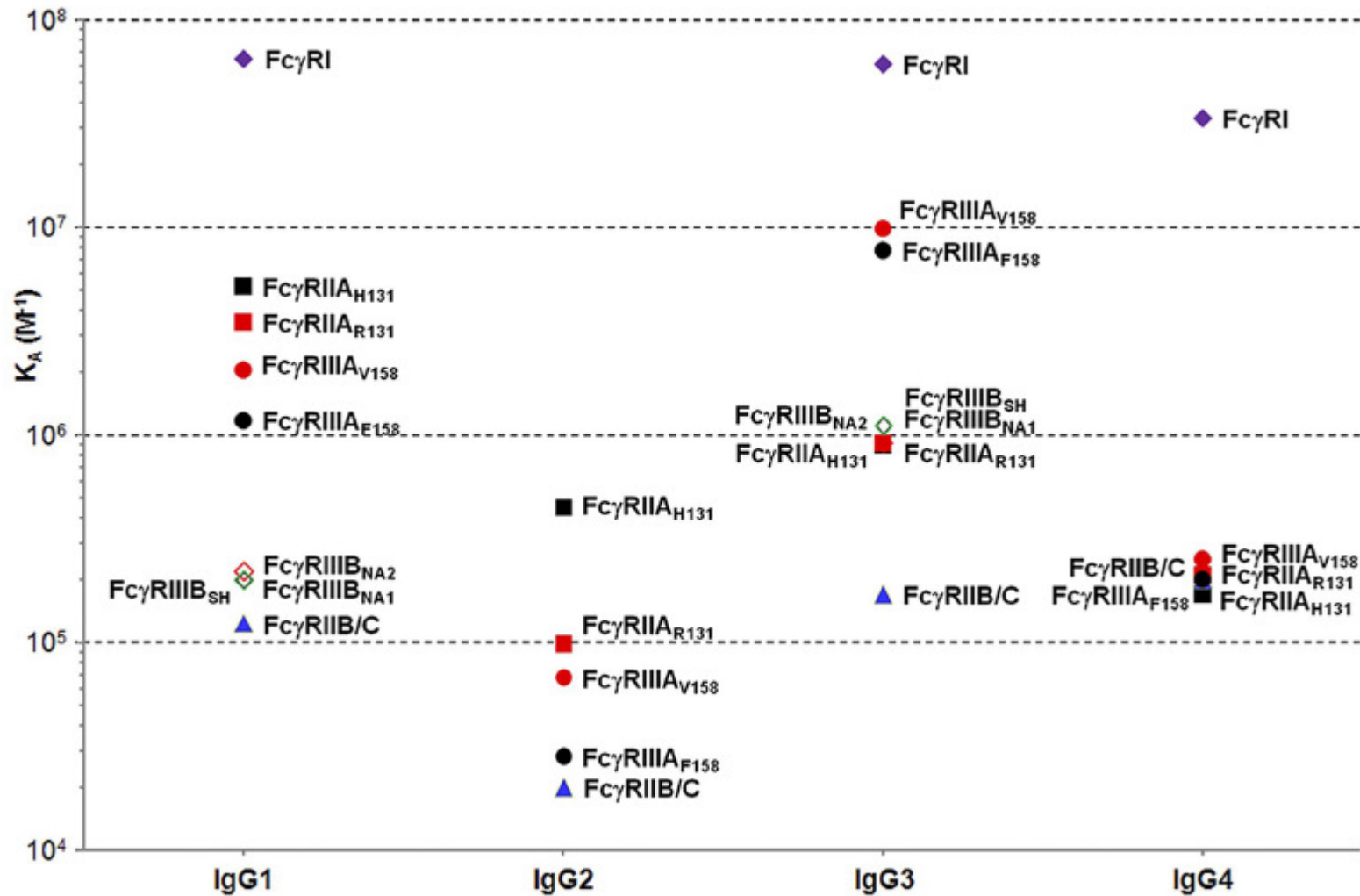
Problems with current models

- 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 severe reactions.

Diversity in response – CD3

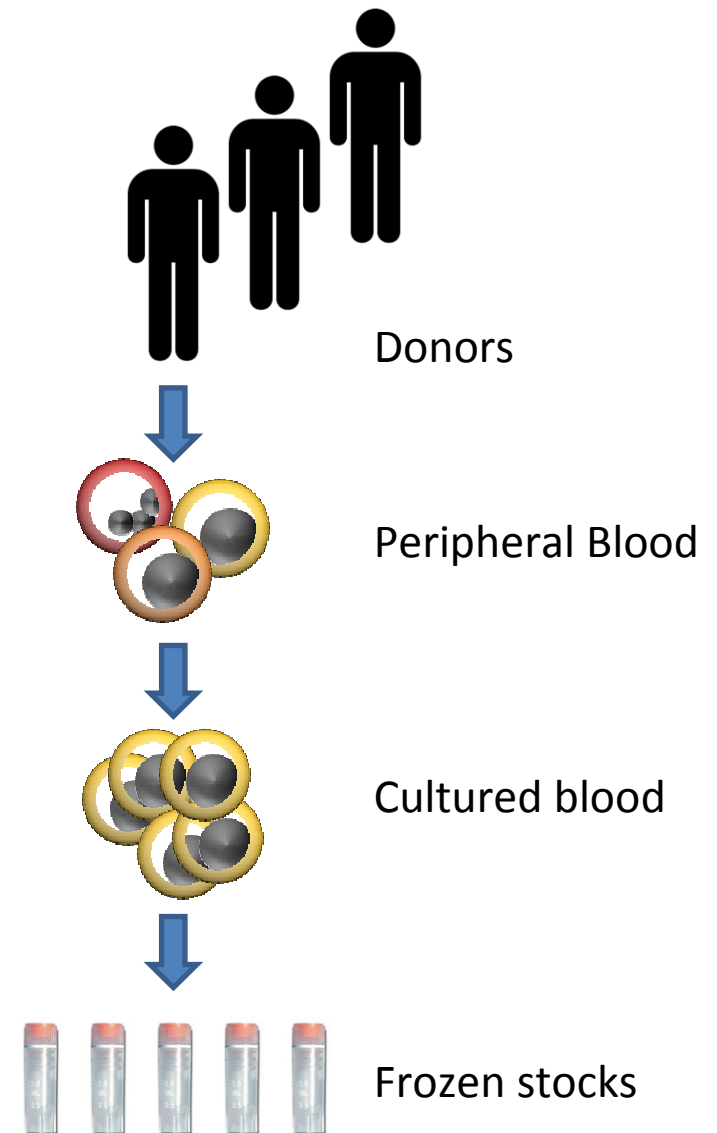


FcR as sources of variation

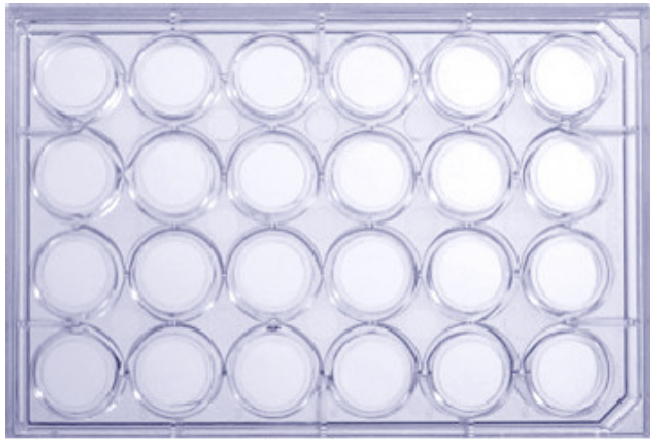


Generation of cell bank

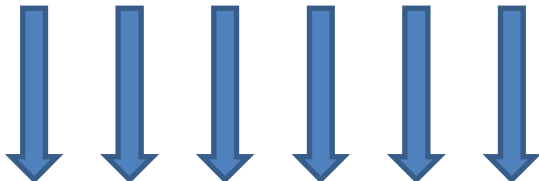
- One source of assay variability is person-person differences.
- FcR phenotyping of stocks may allow the selection of donors to give a more predictable distribution.
- Culturing of cells will potentially give a more LN like phenotype.



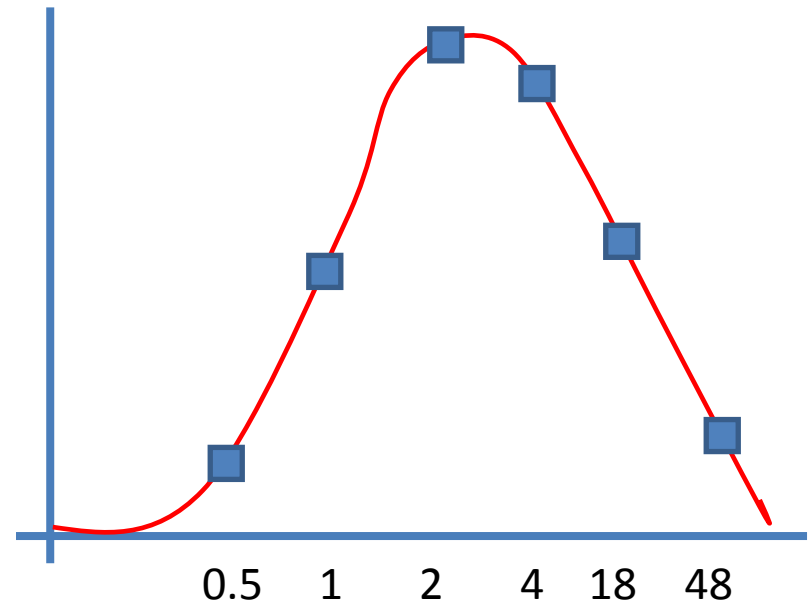
Time course



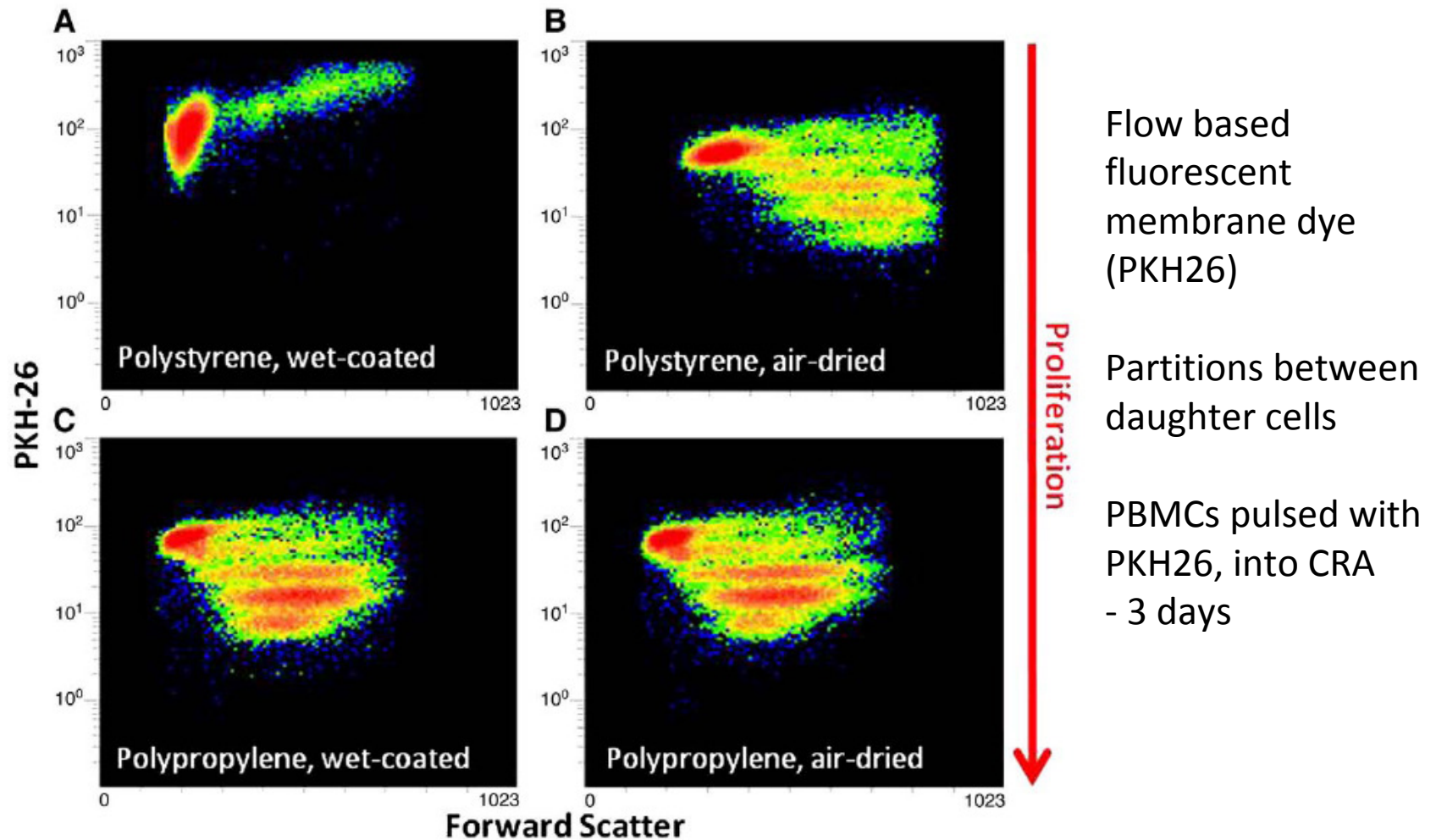
0.5 1 2 4 18 48



Collect samples for analysis



In vitro cytokine release assays to predict cytokine release syndrome



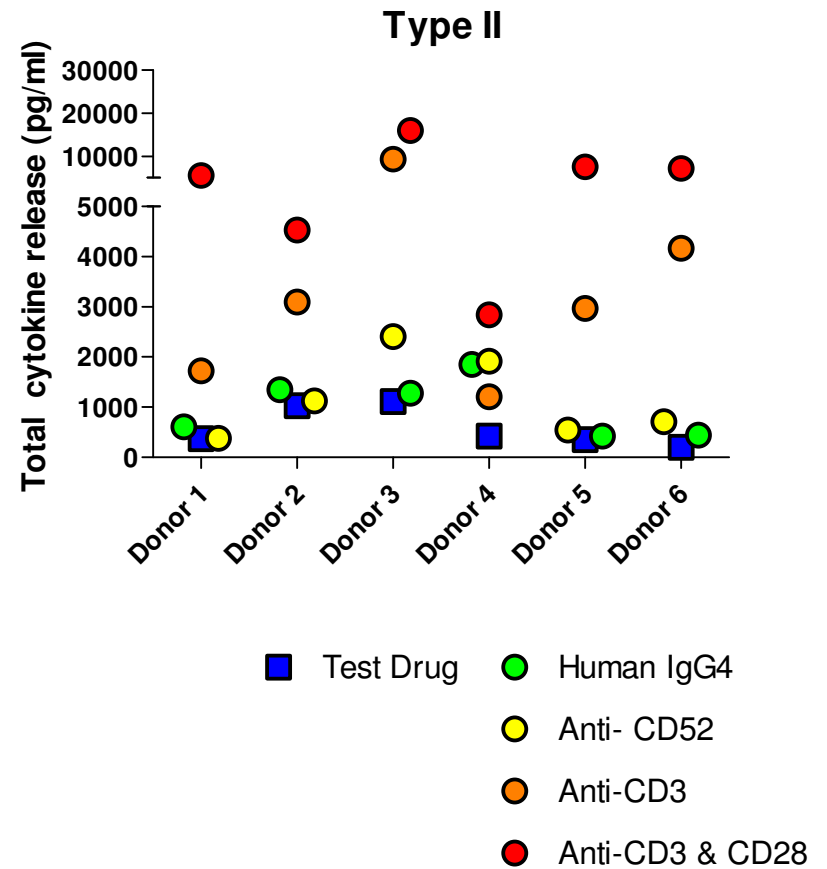
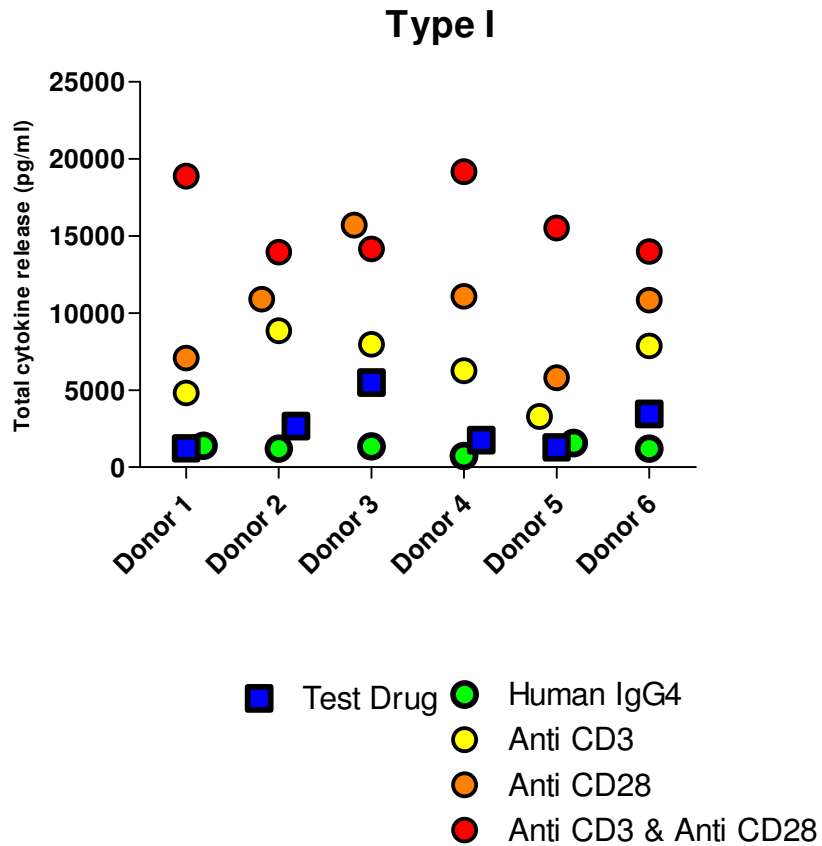
Flow based
fluorescent
membrane dye
(PKH26)

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](https://doi.org/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 appropriately and is therefore amenable to statistical analysis