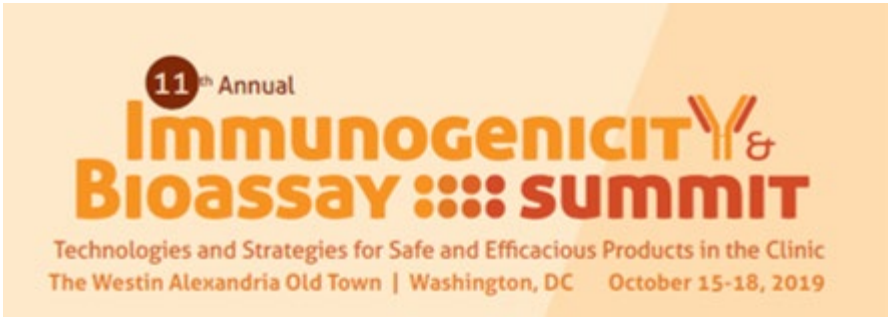
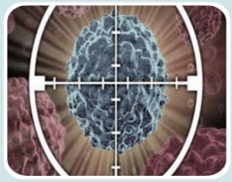


Functional Bioassays for Immune Checkpoint Inhibitor screening and I/O lead selection

SOFIE PATTIJN, CTO, IMMUNXPERTS

The therapeutic development cycle







Target selection

Genomics
Proteonomics
Bioinformatics

Lead Discovery

Synthesis/isolation
Assay development
High-throughput screening

Medicinal Chemistry

Library development
Structure based drug design
Medical chemistry
In silico screening

In vitro screening

Functional screening
Cellular disease models
Drug mechanism of action

In vivo screening

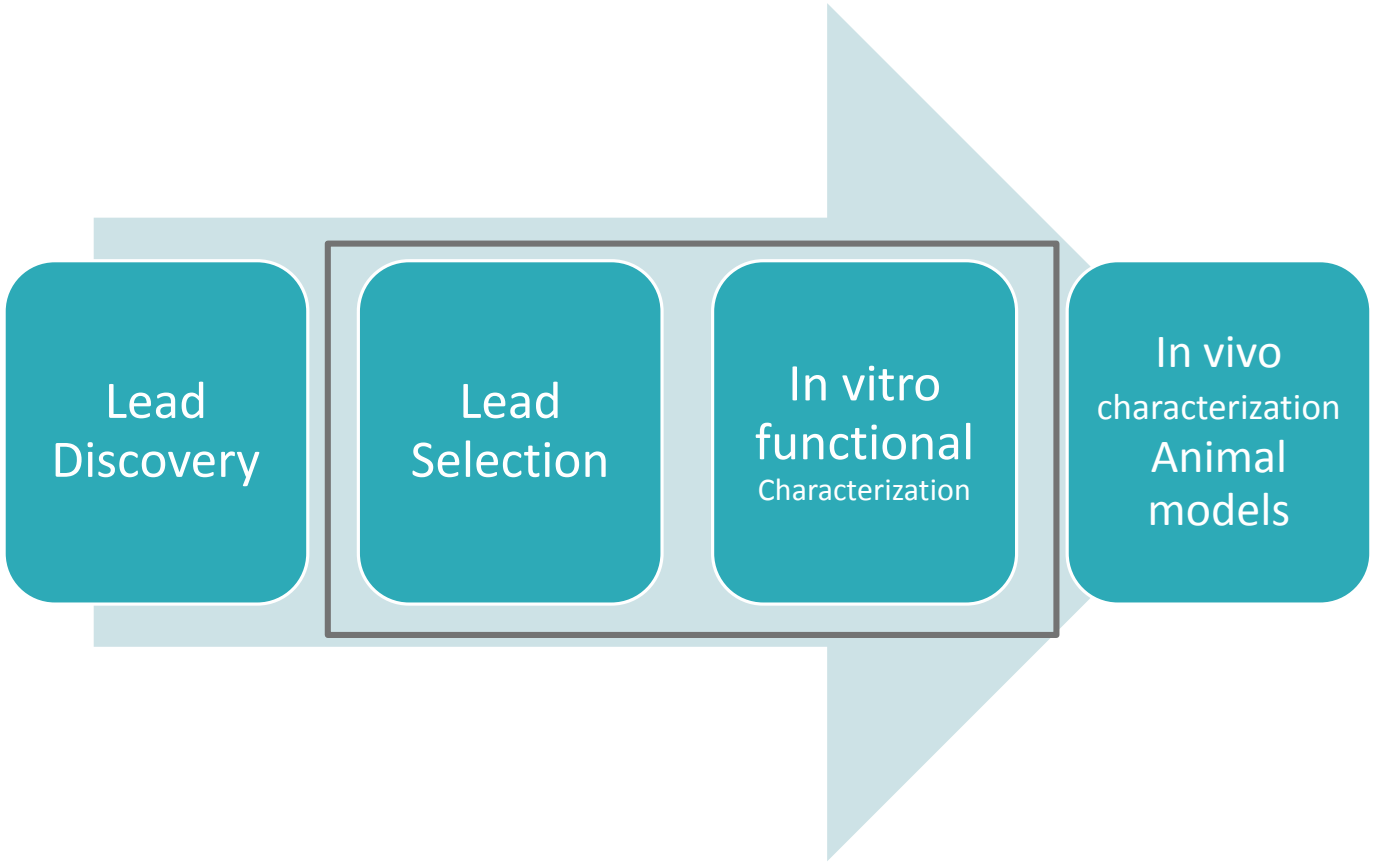
Animal models disease state
Ex vivo studies

Clinical trials

Phase I
Phase II
Phase III
Phase IV

Commercial product

The therapeutic development cycle



- Lead candidate selection based on functional parameters
- Comparison with historic/benchmark molecules
- Select best candidates to move forward
- Pre-selection on functionality prior to animal studies (3R principle)
- R&D stage

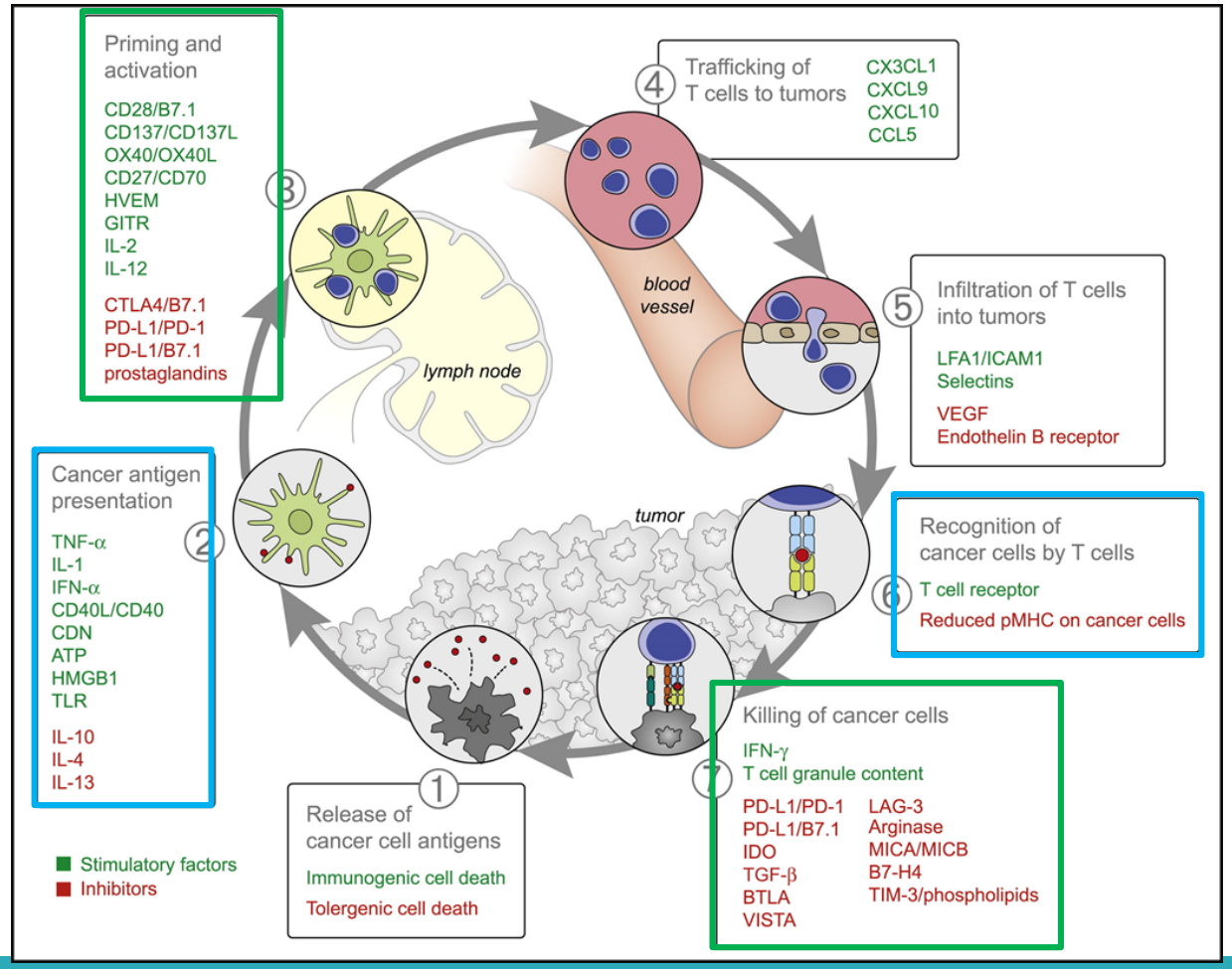
Tools to accelerate immuno-oncology therapy development

In vitro screening

Functional screening

Cellular disease models

Drug mechanism of action



T cell assays

Myeloid cell assays

ImmunXperts

Functional screening of immune checkpoint inhibitors

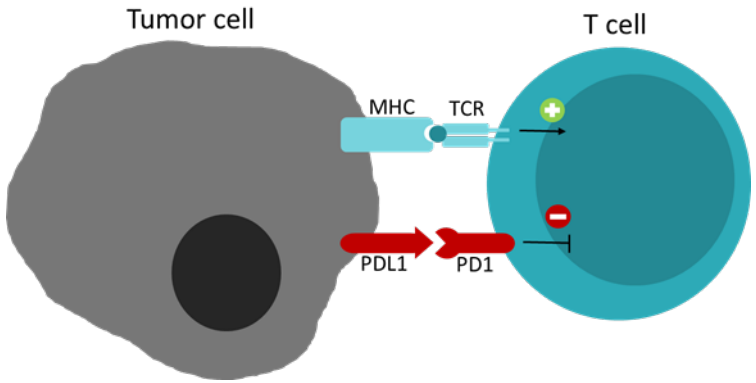
	Cell-based reporter assays	Functional in vitro bioassays
Cells	Genetically engineered cell lines	Primary Immune cells
Easiness to use	Thaw and use	Some experience required
Representative for MOA	(Yes)	Yes
Representative population	No	Yes
Robustness/variability	Robust	Donor to donor variability
Read out parameters	TCR signaling and NFAT-mediated luciferase activity	Multiple immune functions
Limitations	Not all ligand/receptors available	Natural representation ligand/receptors

T cell activation assays

1. **Mixed Lymphocyte Reaction (MLR)**
2. **Mouse Mixed Lymphocyte Reaction (MLR)**
3. **CMV reactivation assays**
4. **T cell exhaustion assays**

Functional screening of immune checkpoint inhibitors: T cell activation assays

- The functional screening of immune checkpoint inhibitors can be done by the evaluation of the ability to promote T cell responses



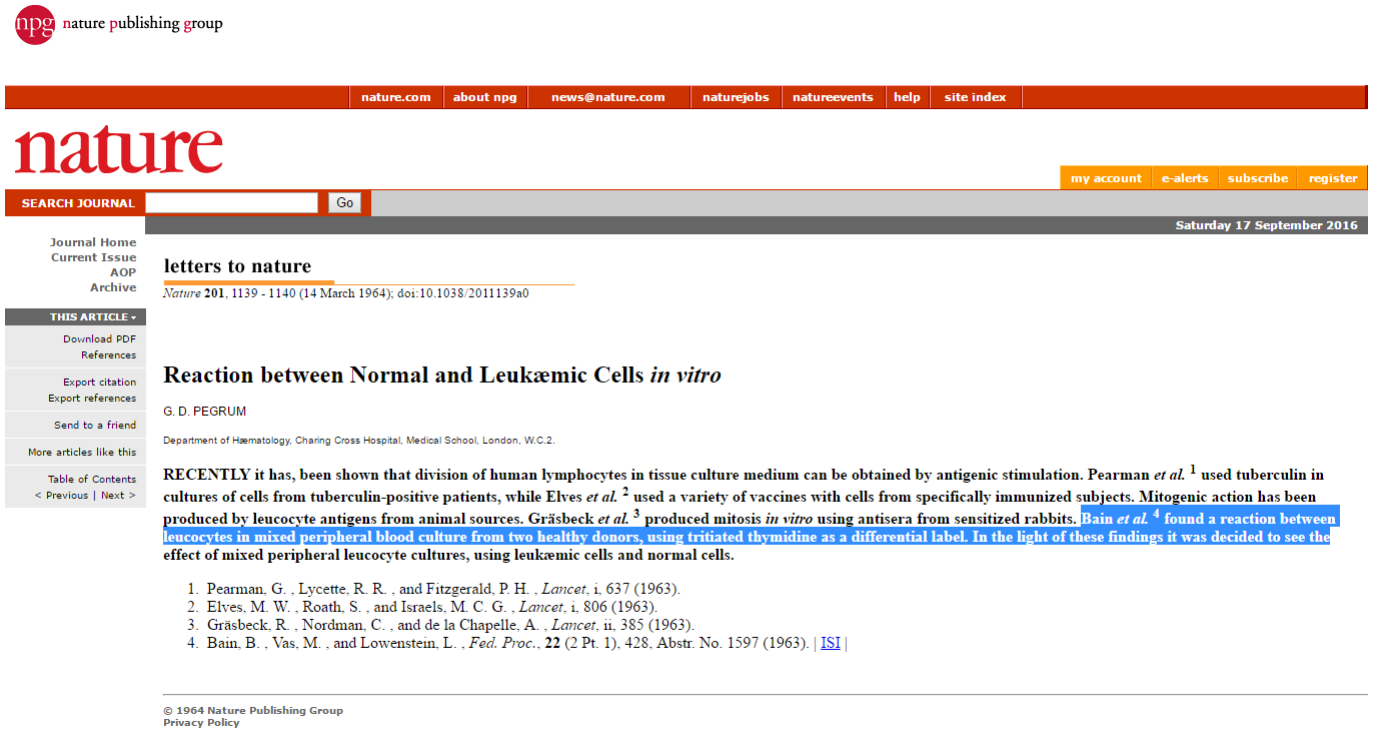
Biological Activity

Condition 1: immune cells expressing receptor/ligand

Condition 2: T cells need to be activated

Condition 3: Immune Checkpoint Inhibitor (= sample)

Mixed Lymphocyte Reaction Assay



nature publishing group

nature.com | about npg | news@nature.com | naturejobs | natureevents | help | site index

nature

my account | e-alerts | subscribe | register

SEARCH JOURNAL | Go

Saturday 17 September 2016

Journal Home
Current Issue
AOP
Archive

THIS ARTICLE
Download PDF
References
Export citation
Export references
Send to a friend
More articles like this
Table of Contents
< Previous | Next >

letters to nature

Nature **201**, 1139 - 1140 (14 March 1964); doi:10.1038/2011139a0

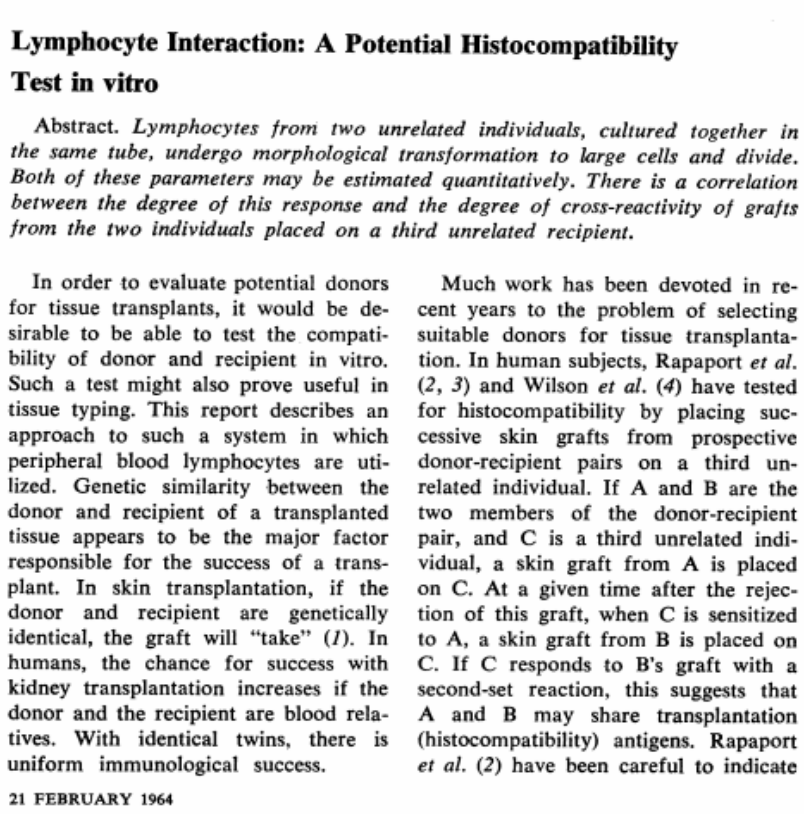
Reaction between Normal and Leukæmic Cells *in vitro*

G. D. PEGRUM

Department of Hæmatology, Charing Cross Hospital, Medical School, London, W.C.2.

RECENTLY it has, been shown that division of human lymphocytes in tissue culture medium can be obtained by antigenic stimulation. Pearman *et al.* [1] used tuberculin in cultures of cells from tuberculin-positive patients, while Elves *et al.* [2] used a variety of vaccines with cells from specifically immunized subjects. Mitogenic action has been produced by leucocyte antigens from animal sources. Gräsbeck *et al.* [3] produced mitosis *in vitro* using antisera from sensitized rabbits. Bain *et al.* [4] found a reaction between leucocytes in mixed peripheral blood culture from two healthy donors, using tritiated thymidine as a differential label. In the light of these findings it was decided to see the effect of mixed peripheral leucocyte cultures, using leukæmic cells and normal cells.

1. Pearman, G. , Lycette, R. R. , and Fitzgerald, P. H. , *Lancet*, i, 637 (1963).
2. Elves, M. W. , Roath, S. , and Israels, M. C. G. , *Lancet*, i, 806 (1963).
3. Gräsbeck, R. , Nordman, C. , and de la Chapelle, A. , *Lancet*, ii, 385 (1963).
4. Bain, B. , Vas, M. , and Lowenstein, L. , *Fed. Proc.*, **22** (2 Pt. 1), 428, Abstr. No. 1597 (1963). | ISI |



Lymphocyte Interaction: A Potential Histocompatibility Test in vitro

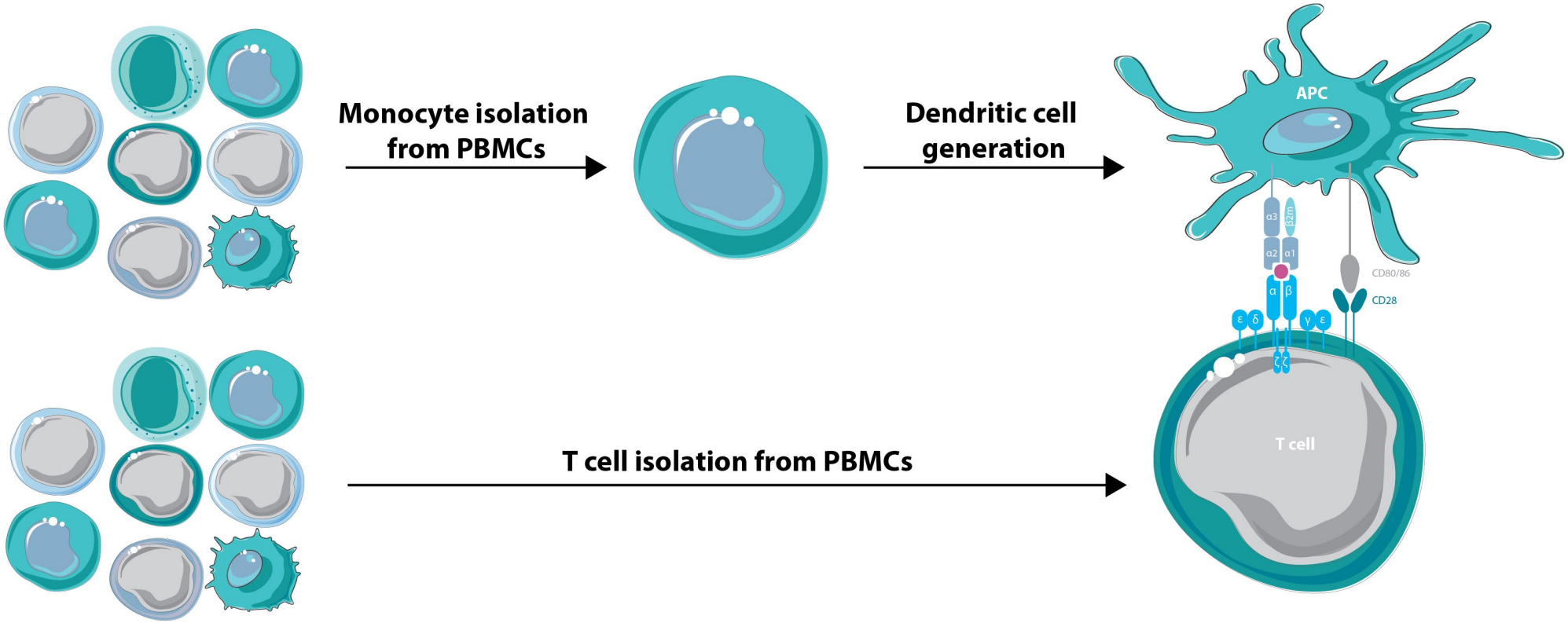
Abstract. *Lymphocytes from two unrelated individuals, cultured together in the same tube, undergo morphological transformation to large cells and divide. Both of these parameters may be estimated quantitatively. There is a correlation between the degree of this response and the degree of cross-reactivity of grafts from the two individuals placed on a third unrelated recipient.*

In order to evaluate potential donors for tissue transplants, it would be desirable to be able to test the compatibility of donor and recipient in vitro. Such a test might also prove useful in tissue typing. This report describes an approach to such a system in which peripheral blood lymphocytes are utilized. Genetic similarity between the donor and recipient of a transplanted tissue appears to be the major factor responsible for the success of a transplant. In skin transplantation, if the donor and recipient are genetically identical, the graft will "take" (*1*). In humans, the chance for success with kidney transplantation increases if the donor and the recipient are blood relatives. With identical twins, there is uniform immunological success.

Much work has been devoted in recent years to the problem of selecting suitable donors for tissue transplantation. In human subjects, Rapaport *et al.* (*2*, *3*) and Wilson *et al.* (*4*) have tested for histocompatibility by placing successive skin grafts from prospective donor-recipient pairs on a third unrelated individual. If A and B are the two members of the donor-recipient pair, and C is a third unrelated individual, a skin graft from A is placed on C. At a given time after the rejection of this graft, when C is sensitized to A, a skin graft from B is placed on C. If C responds to B's graft with a second-set reaction, this suggests that A and B may share transplantation (histocompatibility) antigens. Rapaport *et al.* (*2*) have been careful to indicate

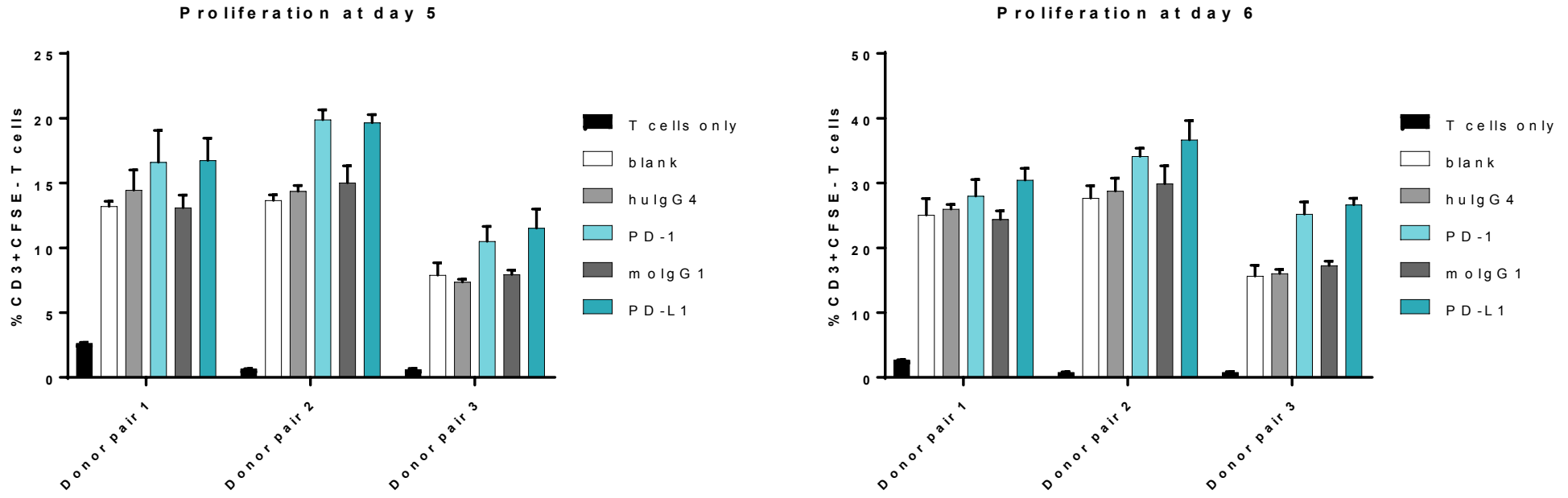
MLR Design

Functional Screening Immune Check point inhibitors



Mixed Lymphocyte Reaction Assay

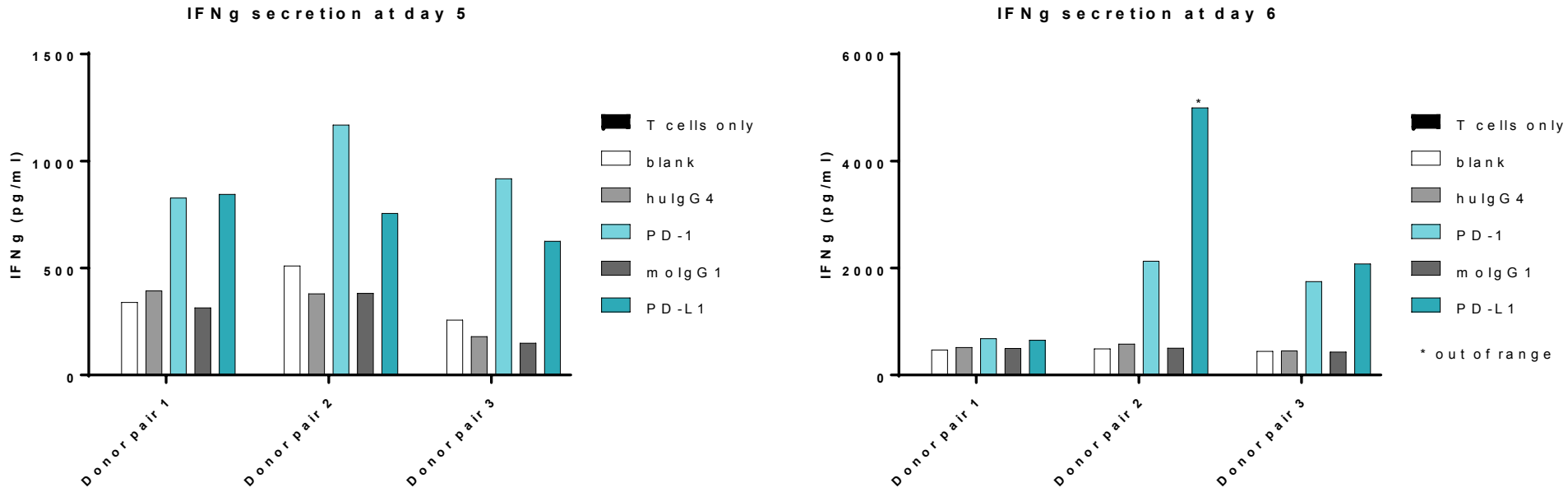
Results proliferation with Nivolumab, PD-L1 and isotype controls



Enhanced proliferation upon in vitro incubation with nivolumab (PD-1) and PD-L1

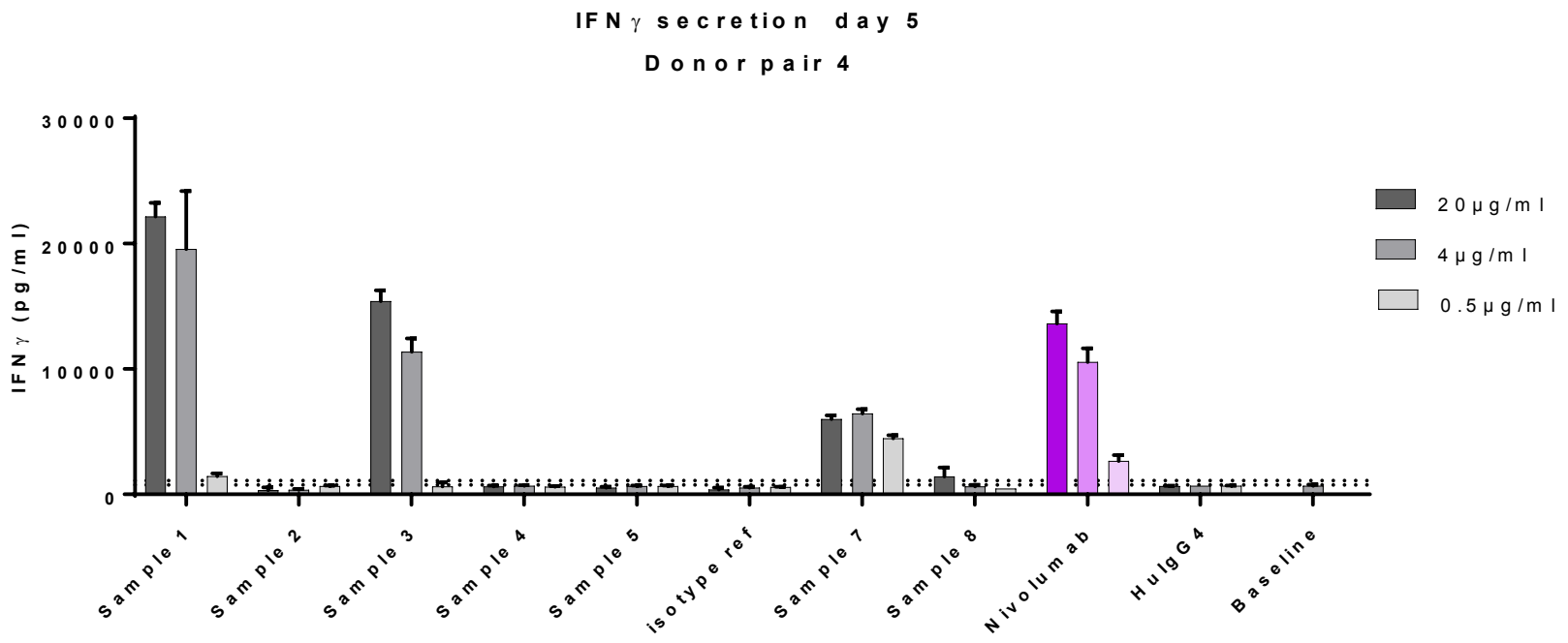
Mixed Lymphocyte Reaction Assay

Results IFNg production with Nivolumab, PD-L1 and isotype controls



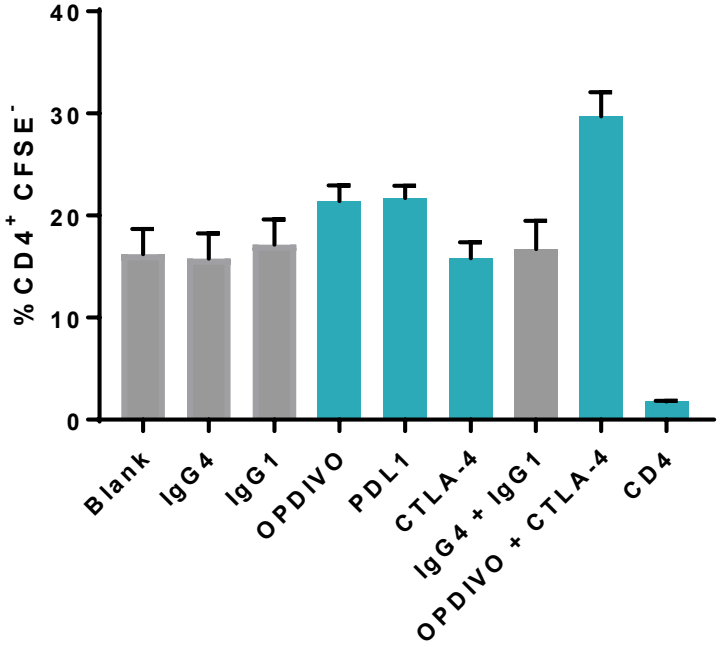
Enhanced IFNg secretion upon in vitro incubation with nivolumab (PD-1) and PD-L1

Results iDC x CD4 MLR PD-1 screening

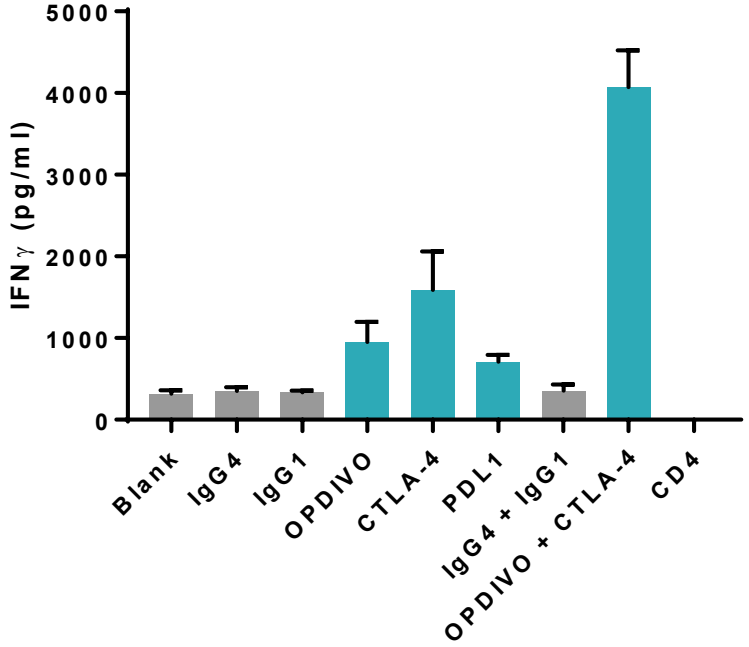


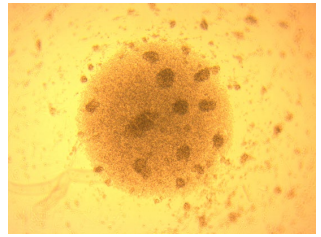
MLR Combination/Bispecifics

Proliferation

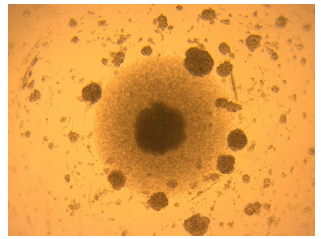


IFNγ secretion





MLR

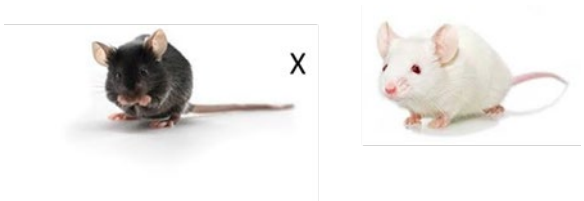


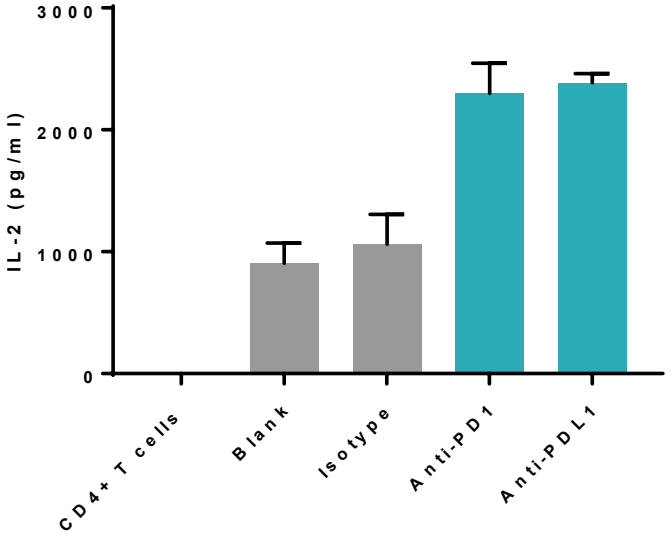
MLR+
anti-PD-1

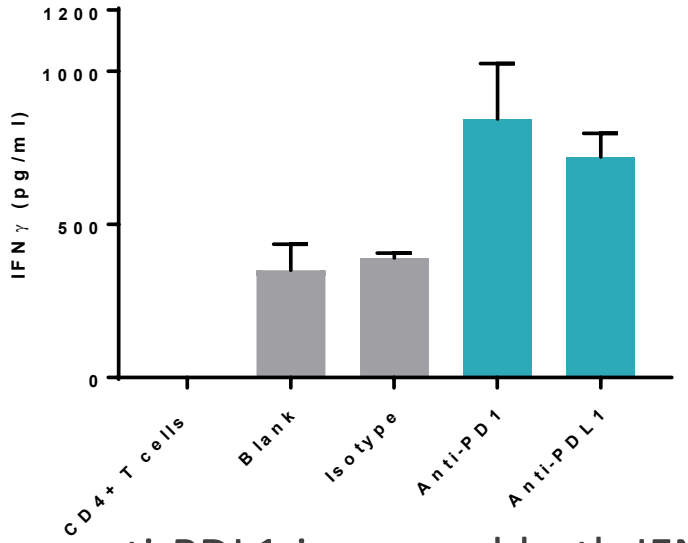
Enhanced proliferation/IFN-g production upon in vitro incubation with Opdivo + CTLA4

Mouse Mixed Lymphocyte Reaction Assay

- Screening test antibodies in a mouse MLR:
  BMDC (from Bone-Marrow of C57/Black 6 mice) x CD4+ T cells from Balb/C mice

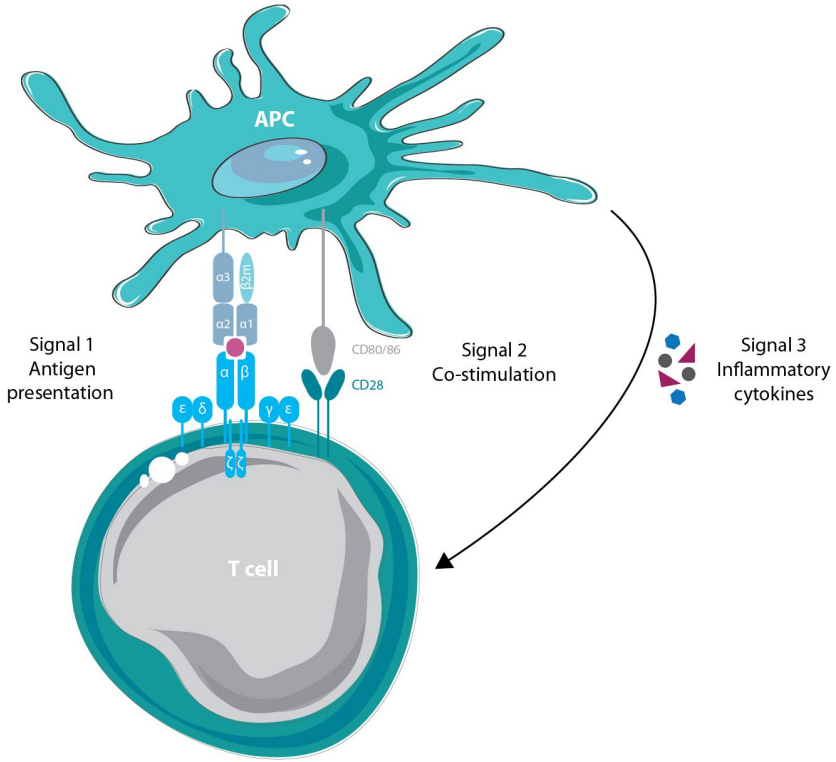


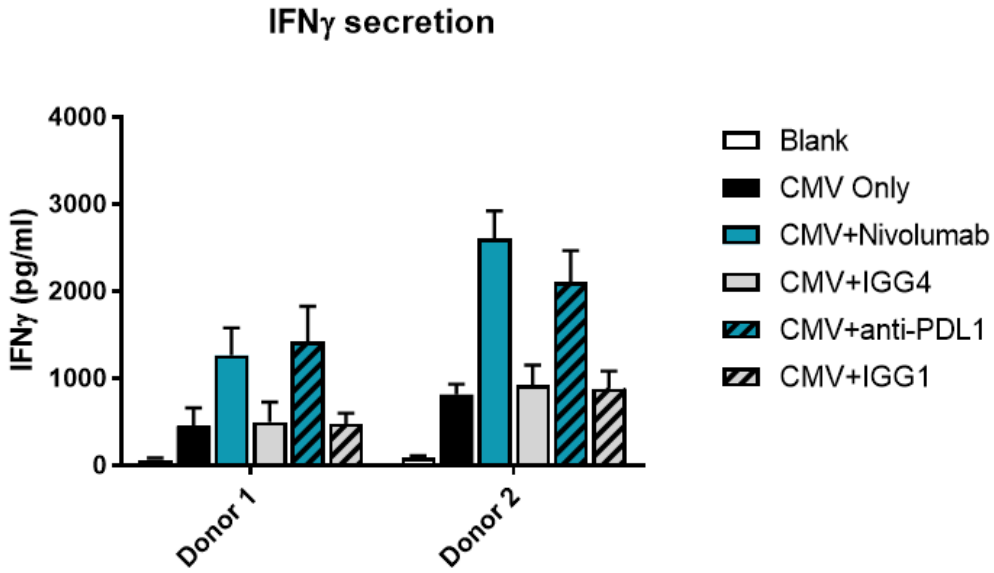




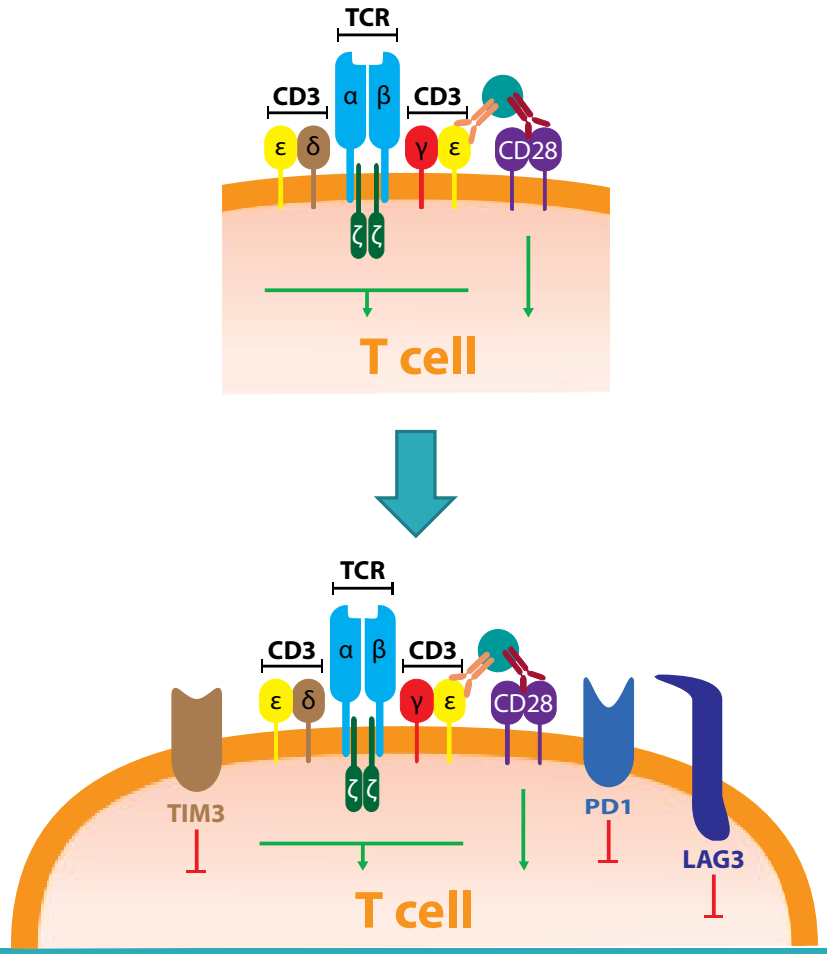
In vitro culture in the presence of anti-PD1 or anti-PDL1 increased both IFNγ and IL-2 production in Mouse MLR assay.

CMV re-activation assay





T cell exhaustion



Non exhausted

Exhausted

→ Chronic Stimulation of T cells

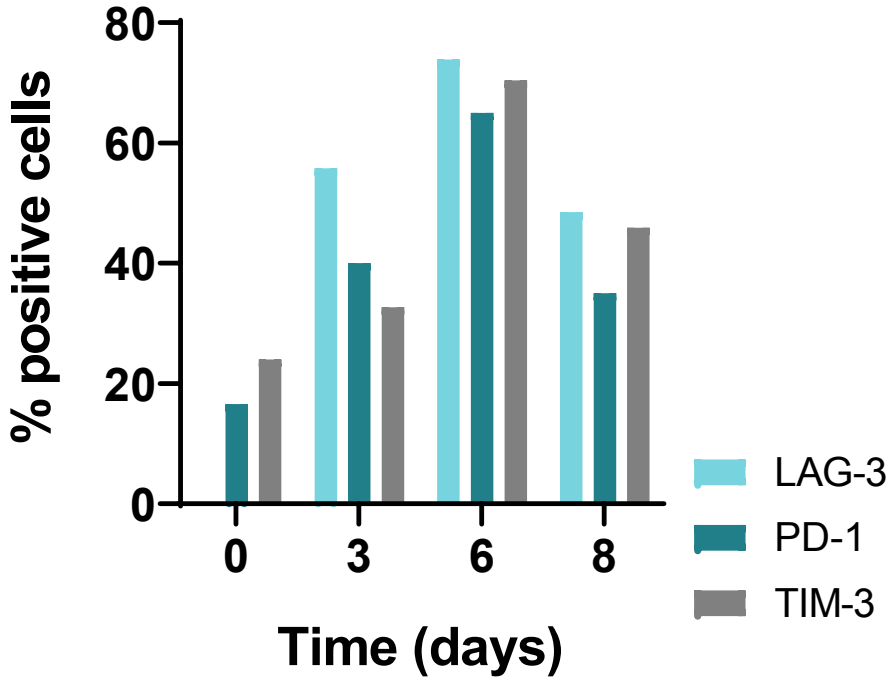
→ Tumor microenvironment

→ Expression of exhaustion marker
- → PD1
- → LAG-3
- → TIM-3
- → CTLA4
- → others

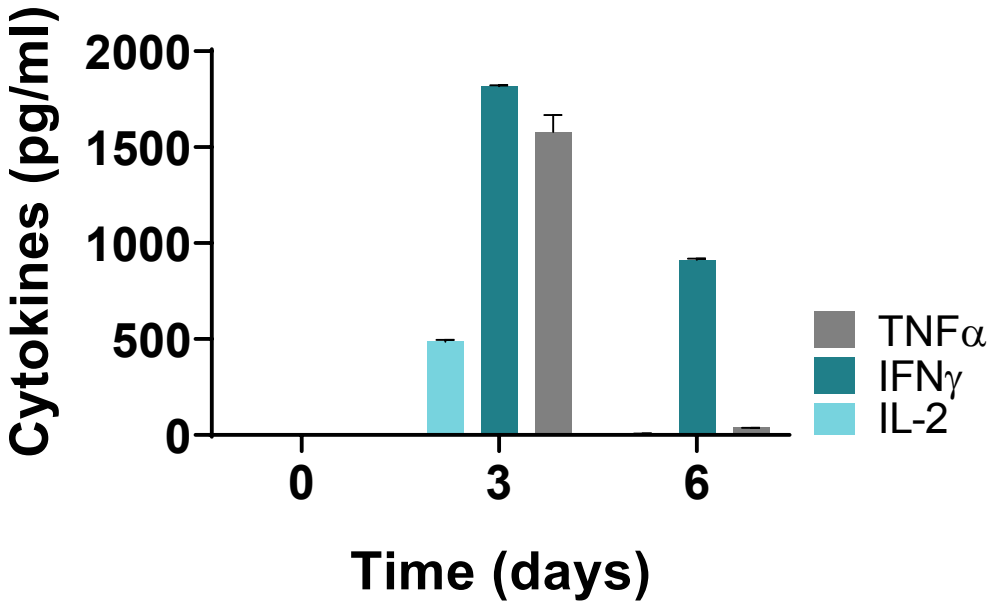
→ Inhibition of T cell function

In vitro T cells exhaustion

Exhaustion marker expression



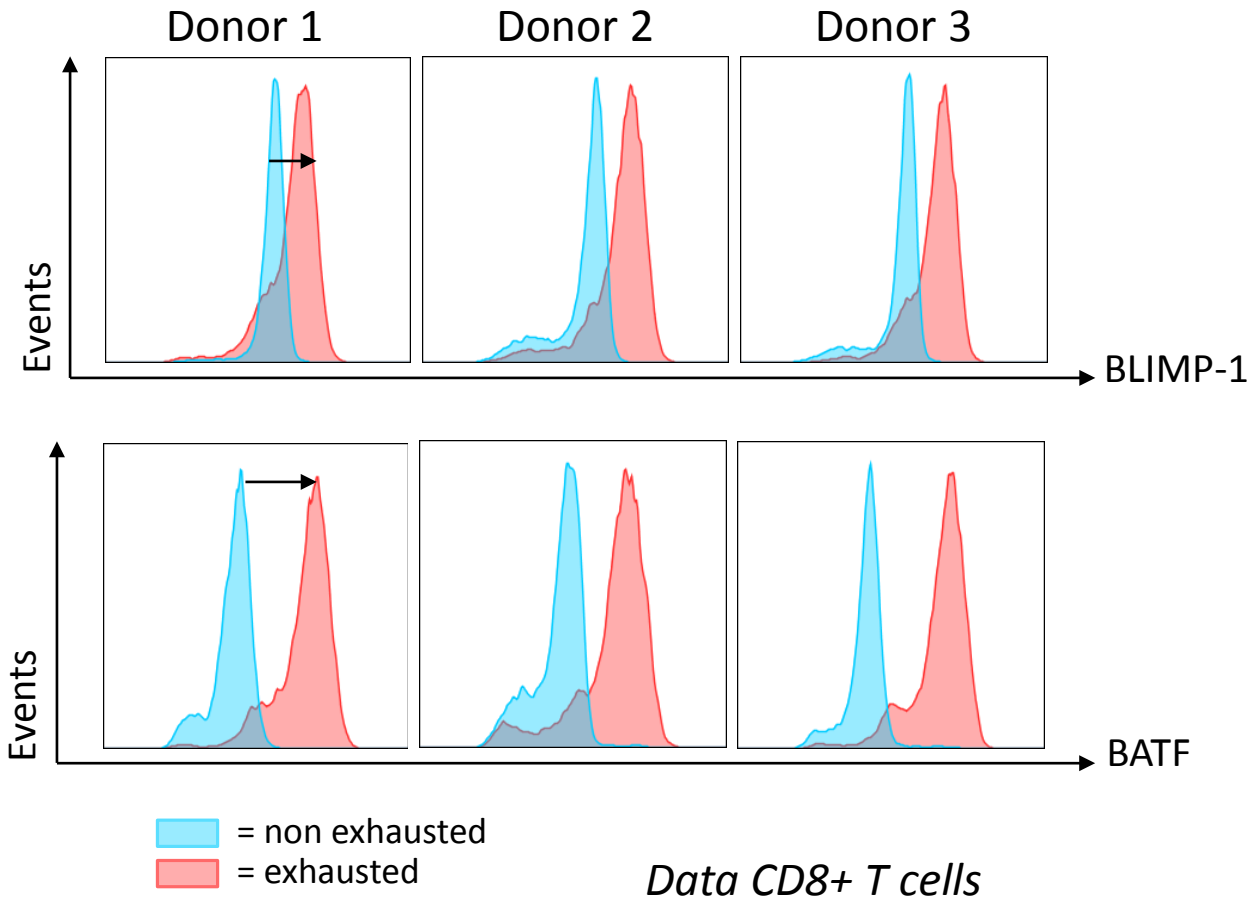
Loss of function (cytokines secretion)



T cells exhaustion

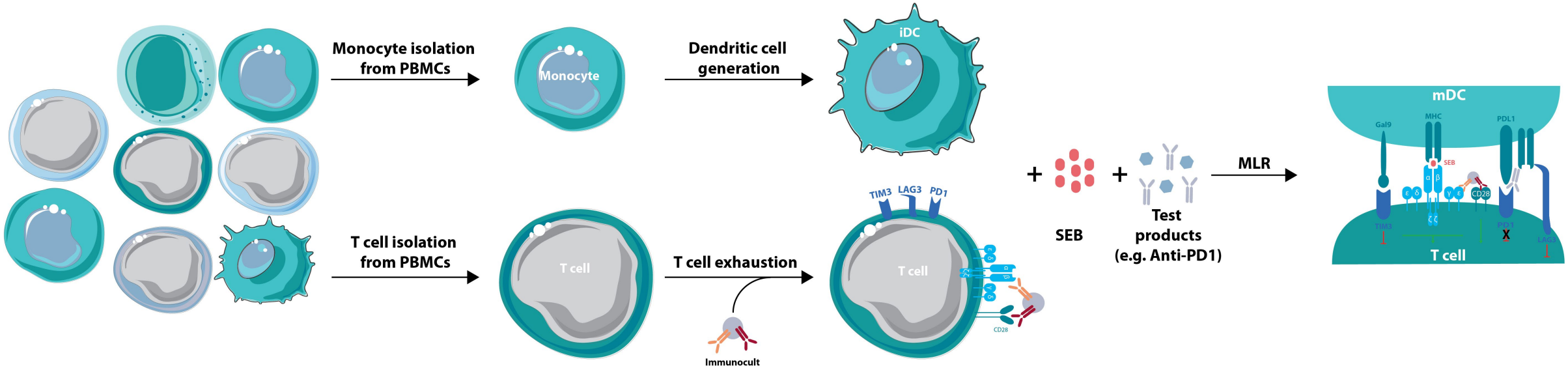
Transcription factor expression

- BATF (high)
- EOMES (high)
- T-Bet (low)
- BLIMP-1 (high)



Data CD8+ T cells

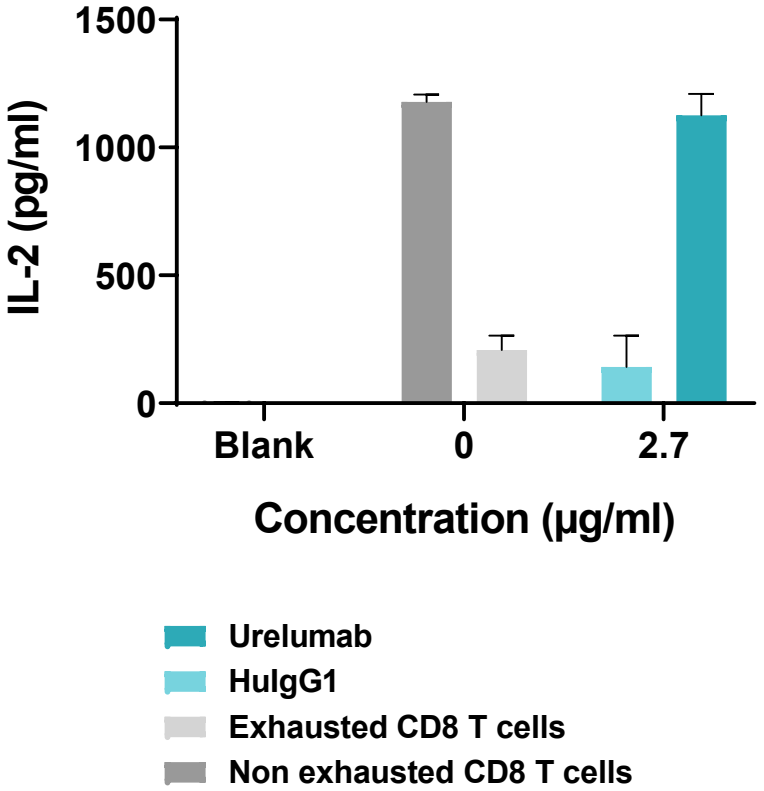
T cell exhaustion assay



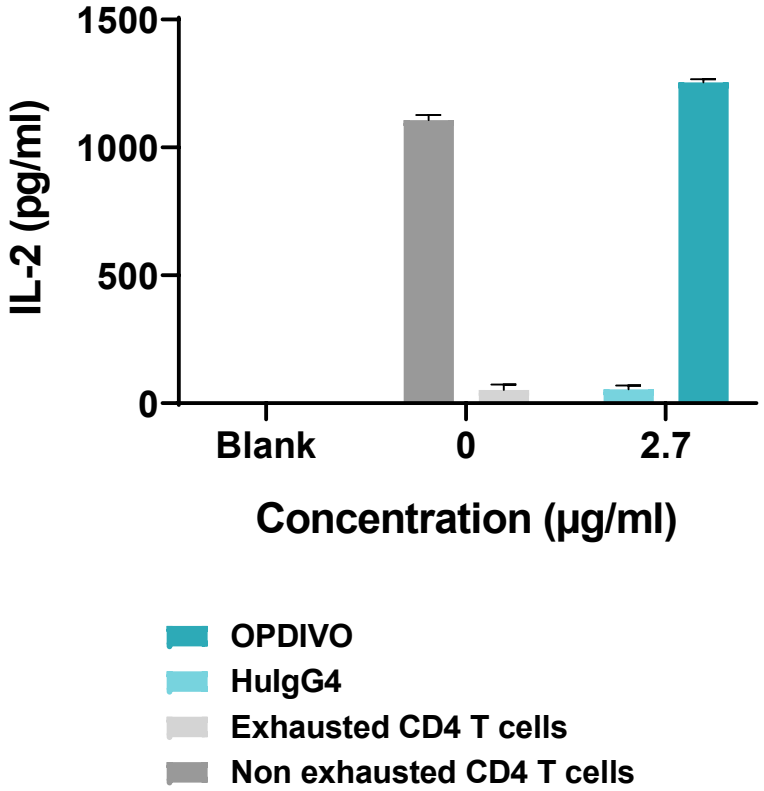
- **CD4+** T cells or **CD8+** T cells isolation from **PBMCs**
- **CD3/CD28** stimulation
- Autologous **CD4+** T cells or **CD8+** T cells and iDCs MLR in the presence of SEB superantigen
- Read-out: IL-2, IFNγ and TNFα

MLR Post exhaustion - case study

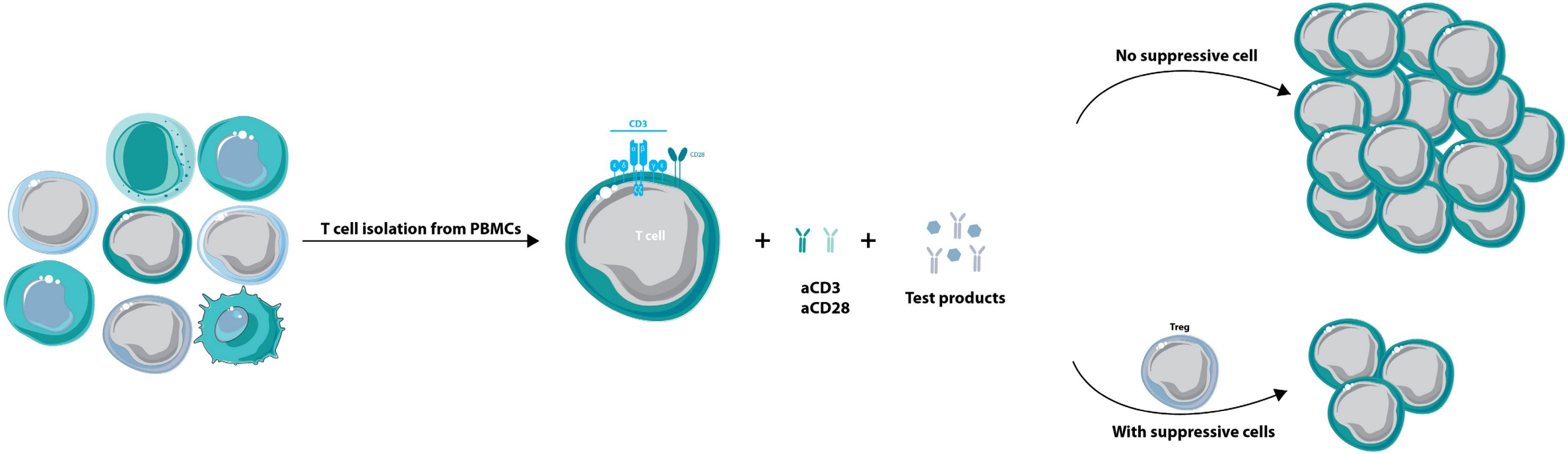
Exhaustion MLR :
CD8+ T cells + anti-CD137



Exhaustion MLR :
CD4+ T cells + anti-PD1

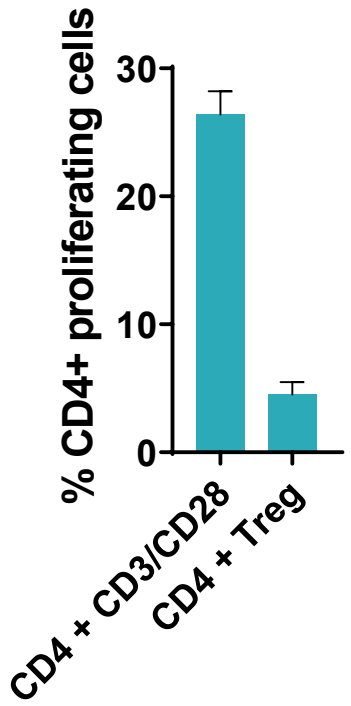


Treg Suppressive Assay Design

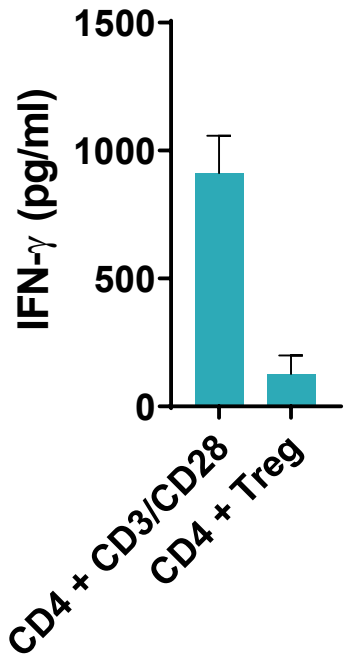


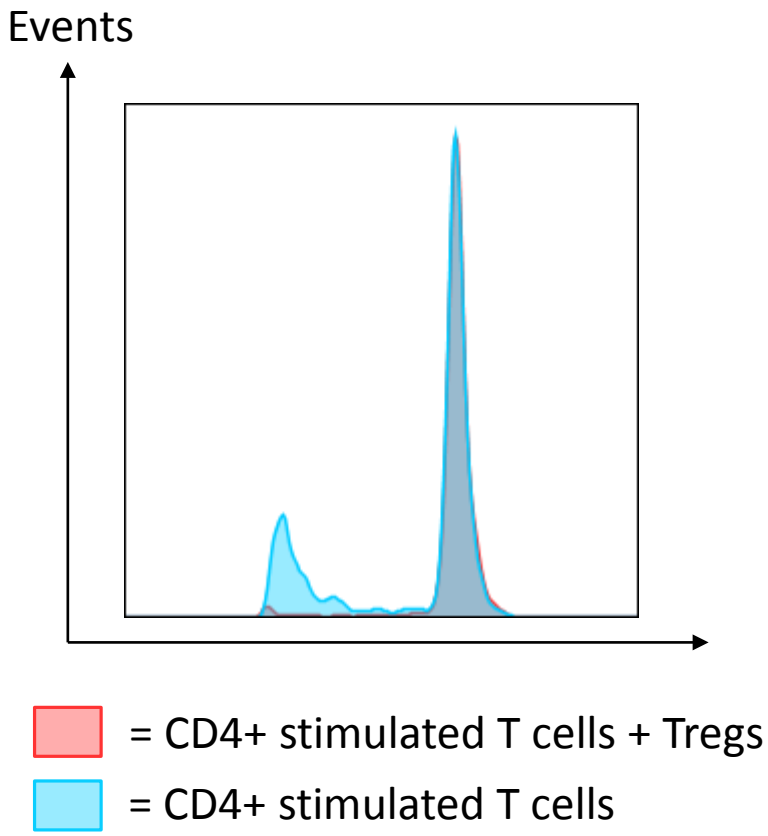
Treg Suppressive Assay Results

Proliferation



Cytokines secretion

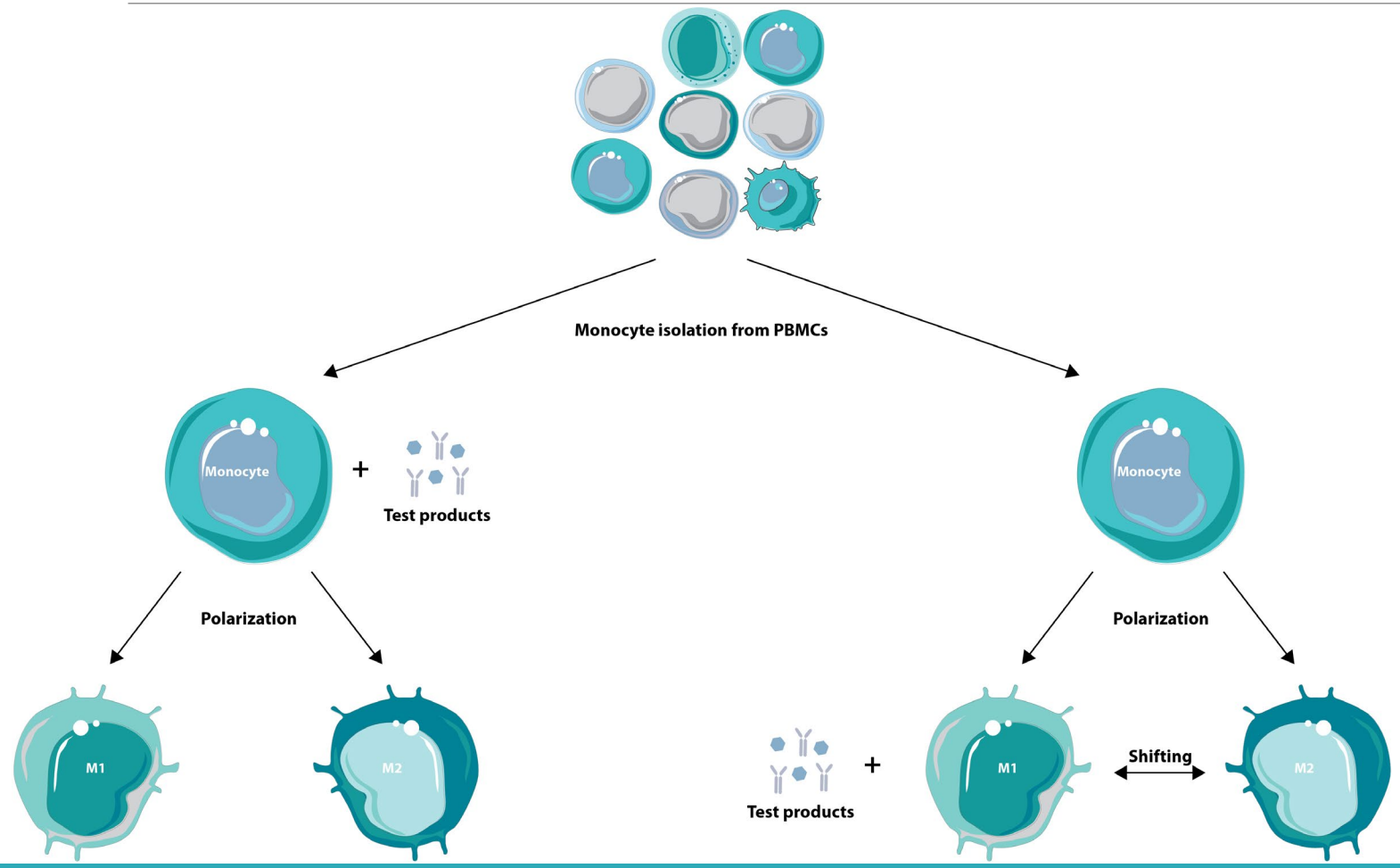


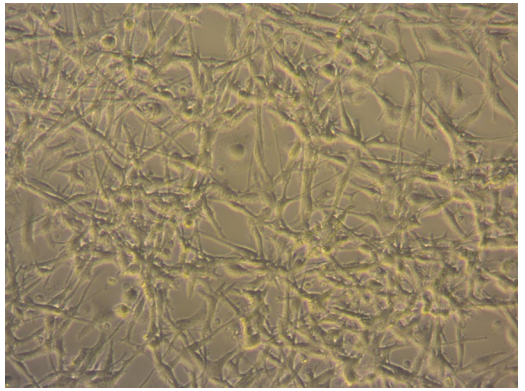


Myeloid cell assays

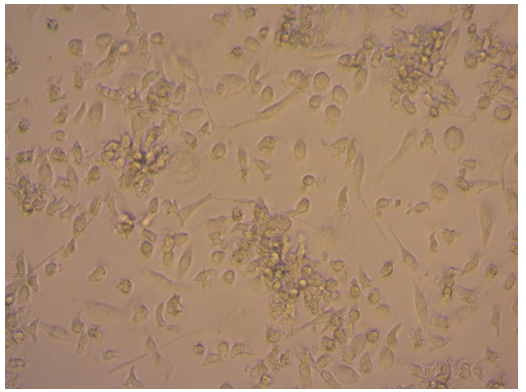
1. **Macrophage (M1/M2) Polarization Assay**

M1-M2 Polarization Assay Design



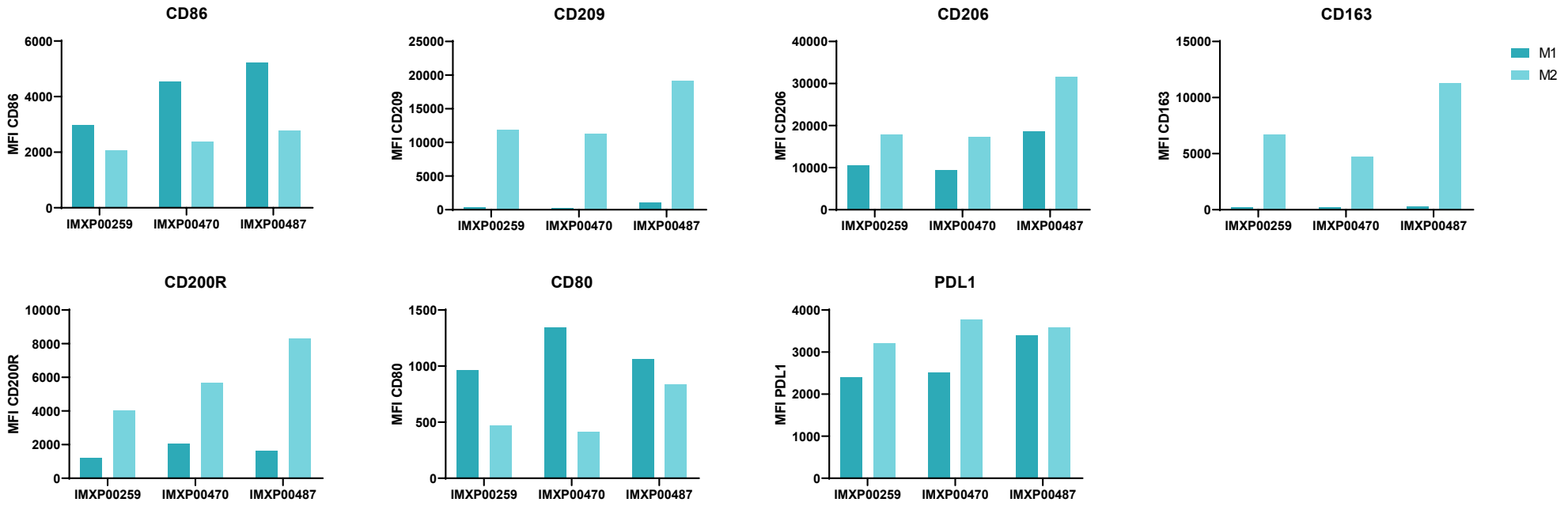


M1



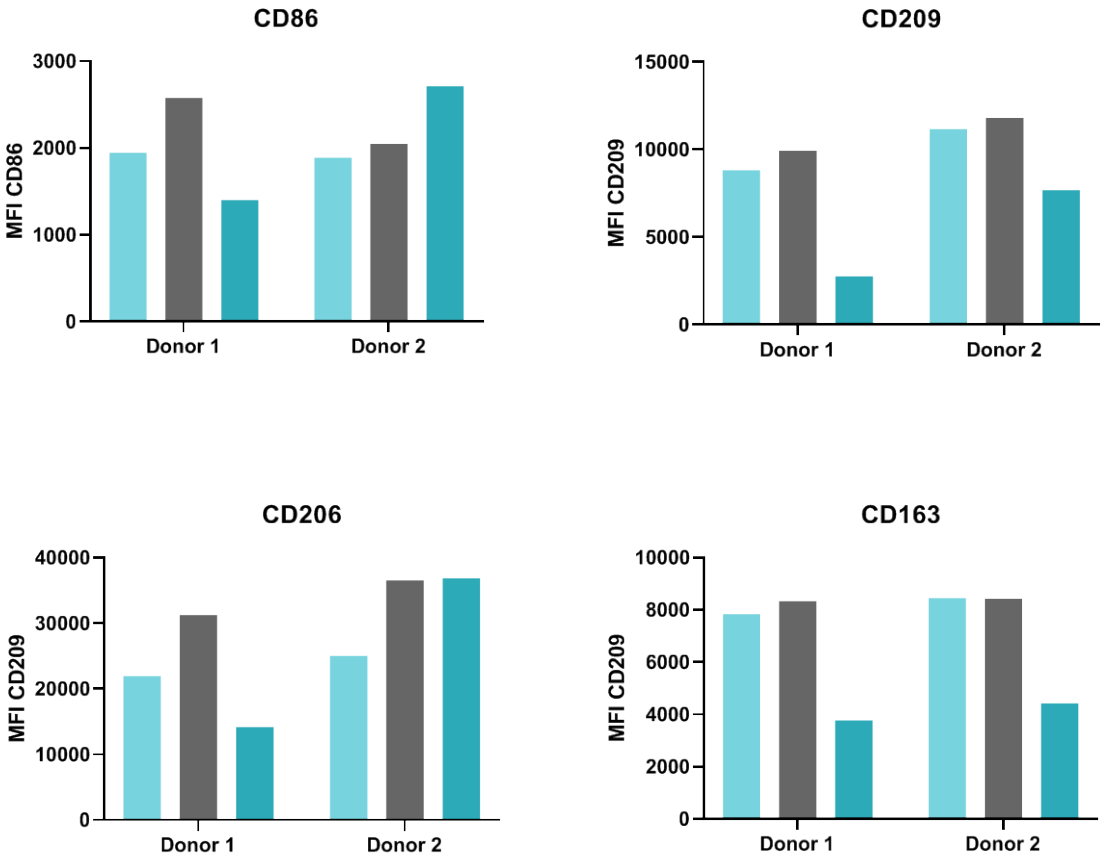
M2

Cell surface marker analysis-Macrophages



- Polarization expected
  - For M1-like : High CD86 and CD80
  - For M2-like : High CD209, CD206, CD163 and CD200R

M2 Polarization assay-case study



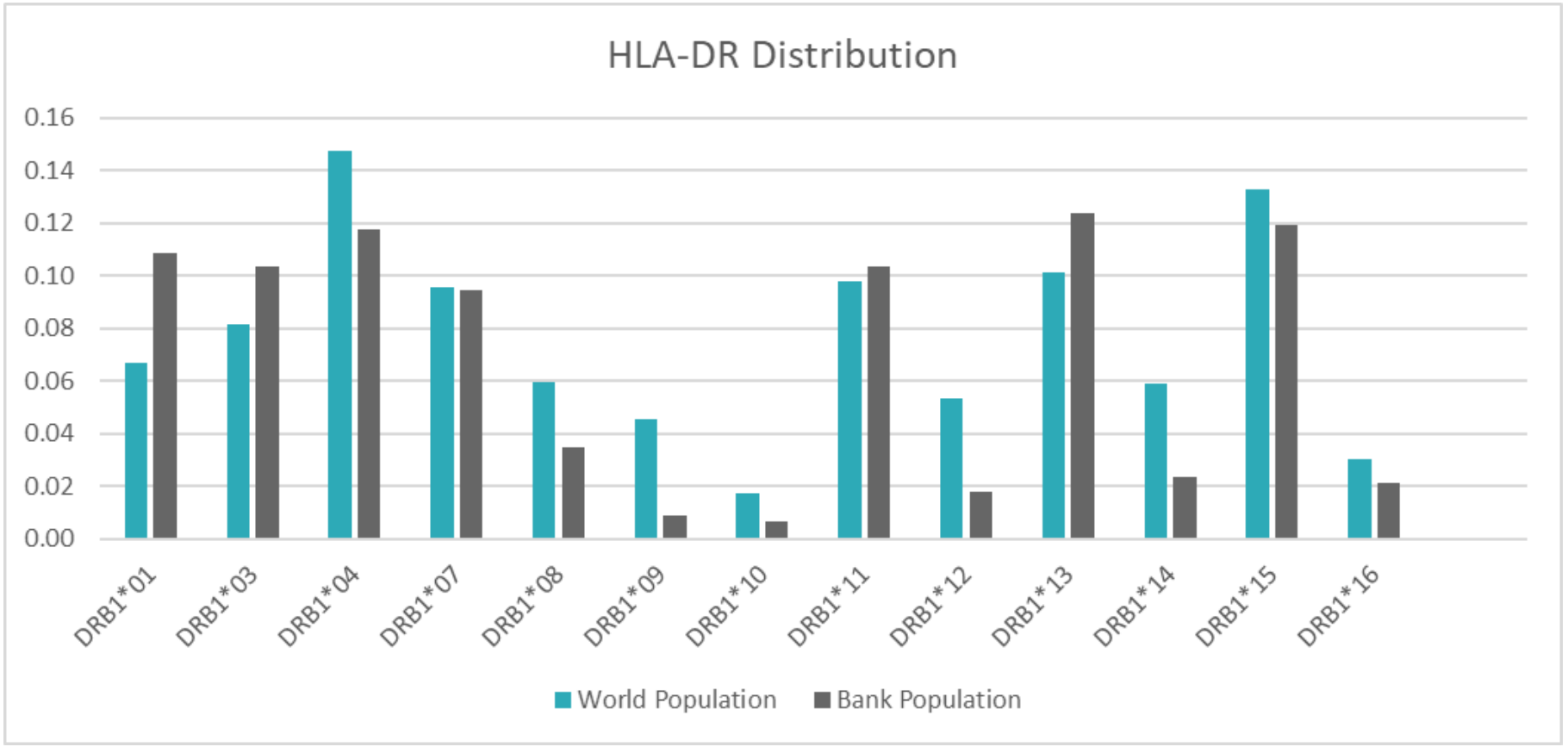
The addition of a neutralizing antibody during the entire differentiation protocol of M2 macrophages affects their phenotype and induced a decreased expression of both CD163 and CD209 markers.

In vitro assay using primary cells

Quality of the primary cells:

- Variability and reproducibility of the results highly depends on the initial quality
- Quality = viability and functionality
- Most critical reagent
- Standardized procedures for sampling, shipping, isolation, cryopreservation, thawing, handling, …
- Need for a large number of HLA-typed donors in order to represent the wide range of responders (strong-responders versus medium-low responders)

Donor diversity



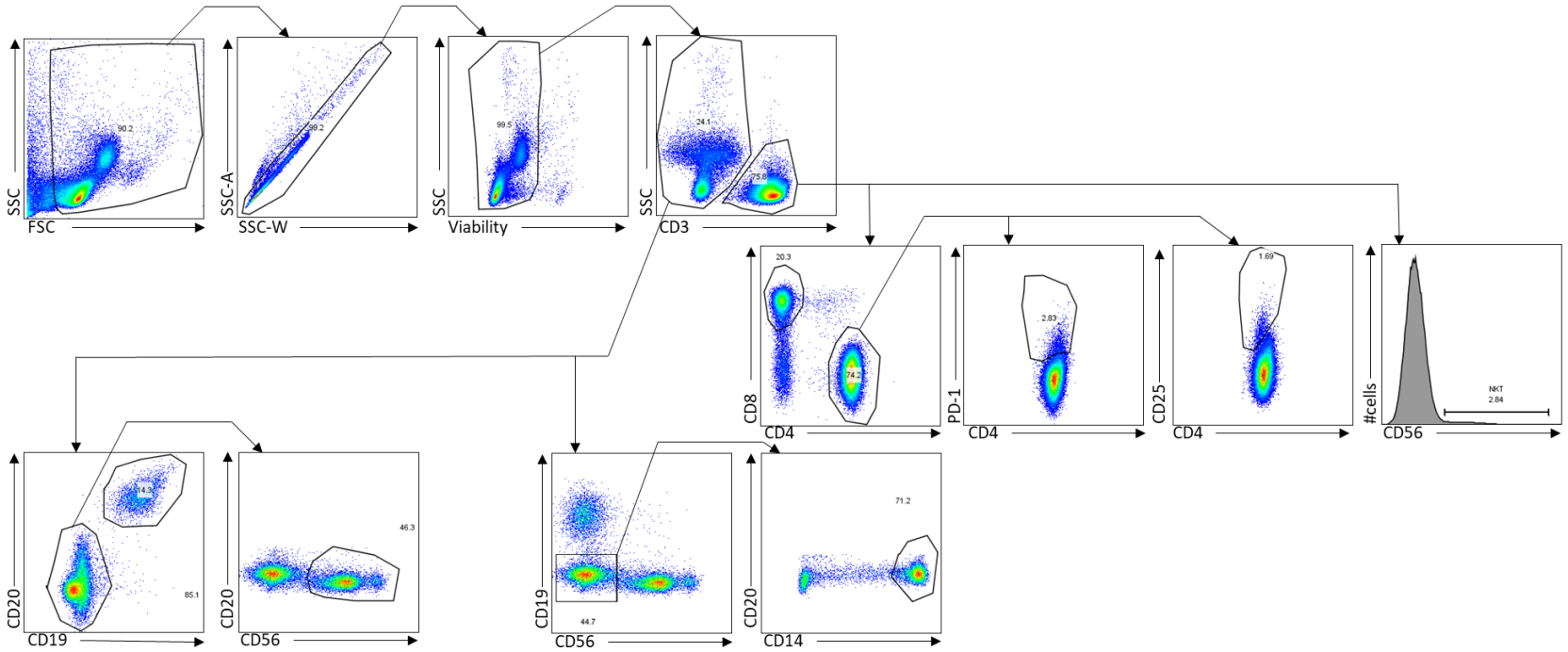
Subpopulation analysis

Classic Surface marker staining for:

- CD14: Monocytes
- CD3: T cells
- CD4: Helper T cells
- CD8: Cytotoxic T cells

Extended

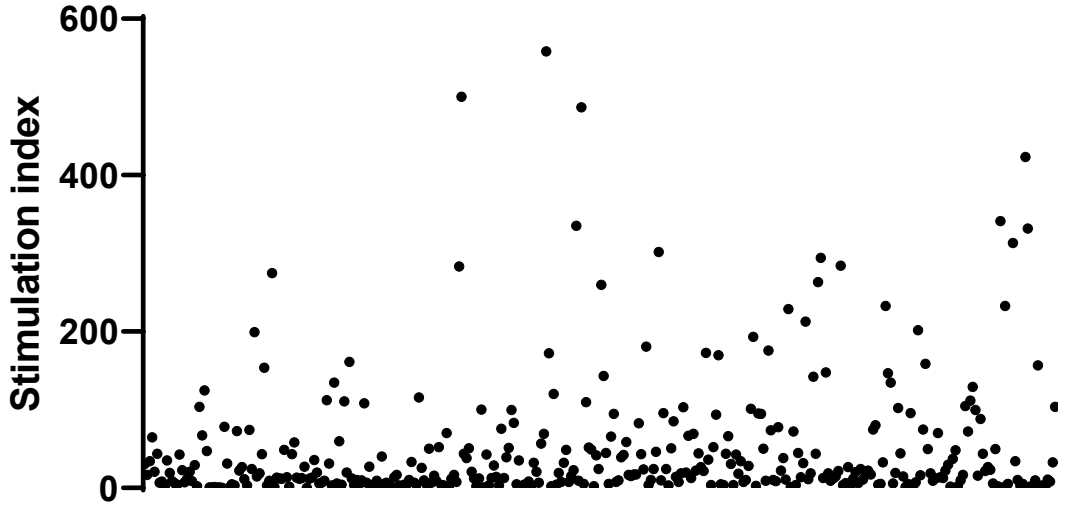
- CD14: Monocytes
- CD3: T cells
- CD4: Helper T cells
  - PD-1+
  - CD25+
- CD8: Cytotoxic T cells
- CD56: NK and NKT
- CD19/20: B cells



Donor diversity

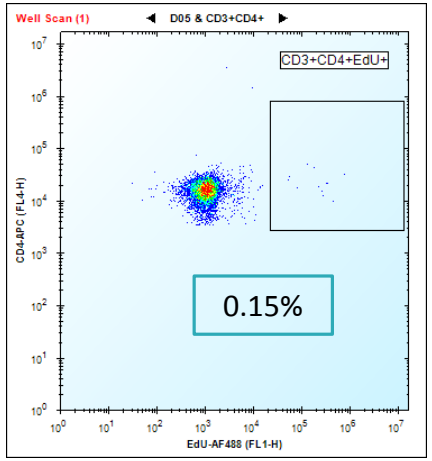
- Response to specific antigen: CMV, CEFT,…

CMV response

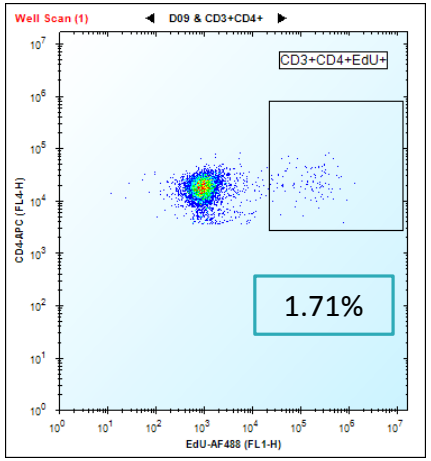


- Assessment of proliferative response towards polyclonal stimulation (anti-CD3 antibody)
- Assessment of proliferative response towards naïve antigen Keyhole Limpet Hemocyanin (KLH)

→ **Functionality Assessment**

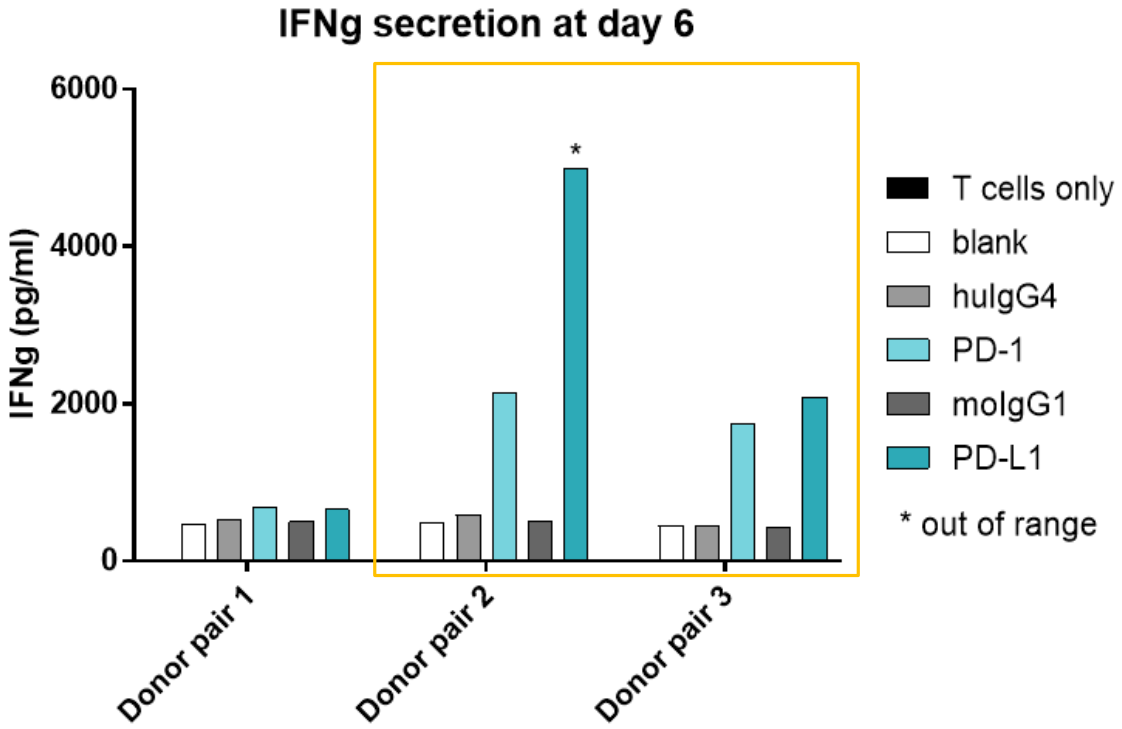


Blank



KLH

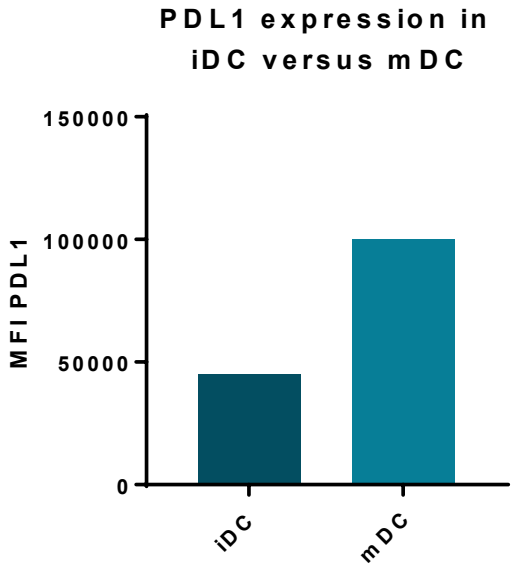
Donor diversity and pre-testing

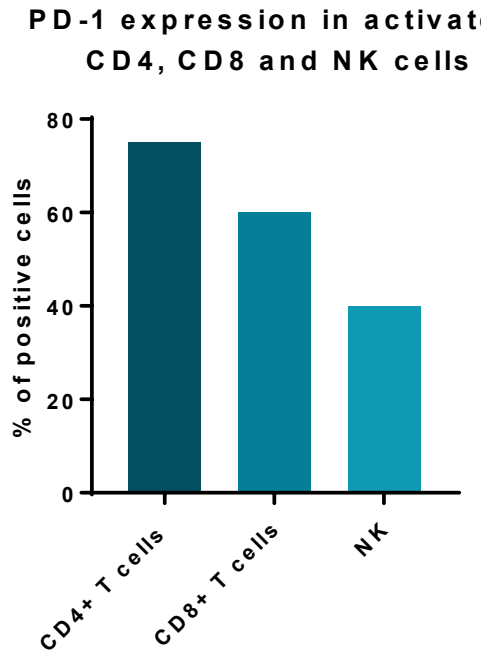


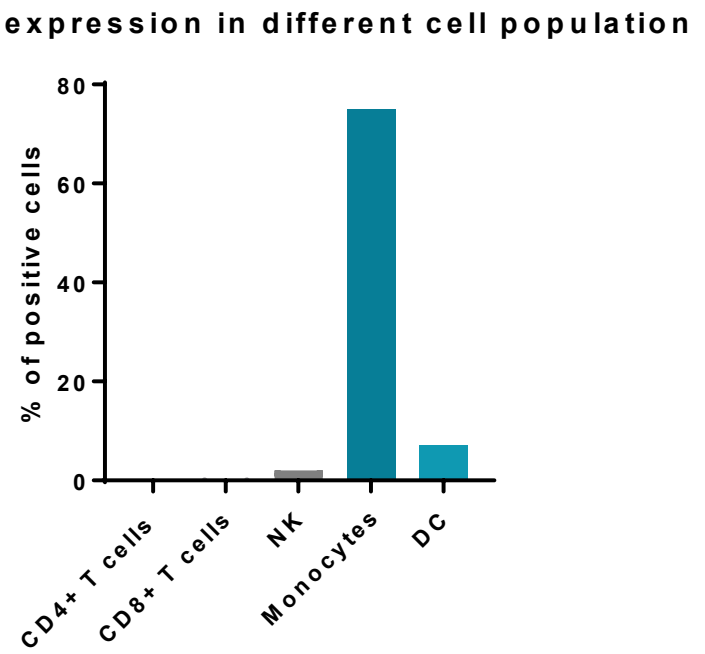
Selection of responder donors

Target expression

→ Selection of Donor/cell population with target expression







Primary cells

Good assays start with happy cells!

ImmunXperts: Your partner in Immunology projects – we think with you

ImmunXperts SA
Rue Auguste Piccard 48
6041 Gosselies
Belgium

www.immunxperts.com

info@immunxperts.com

BRUSSELS SOUTH CHARLEROI BIOPARK

ImmunXperts