Introduction to in vitro Immunogenicity

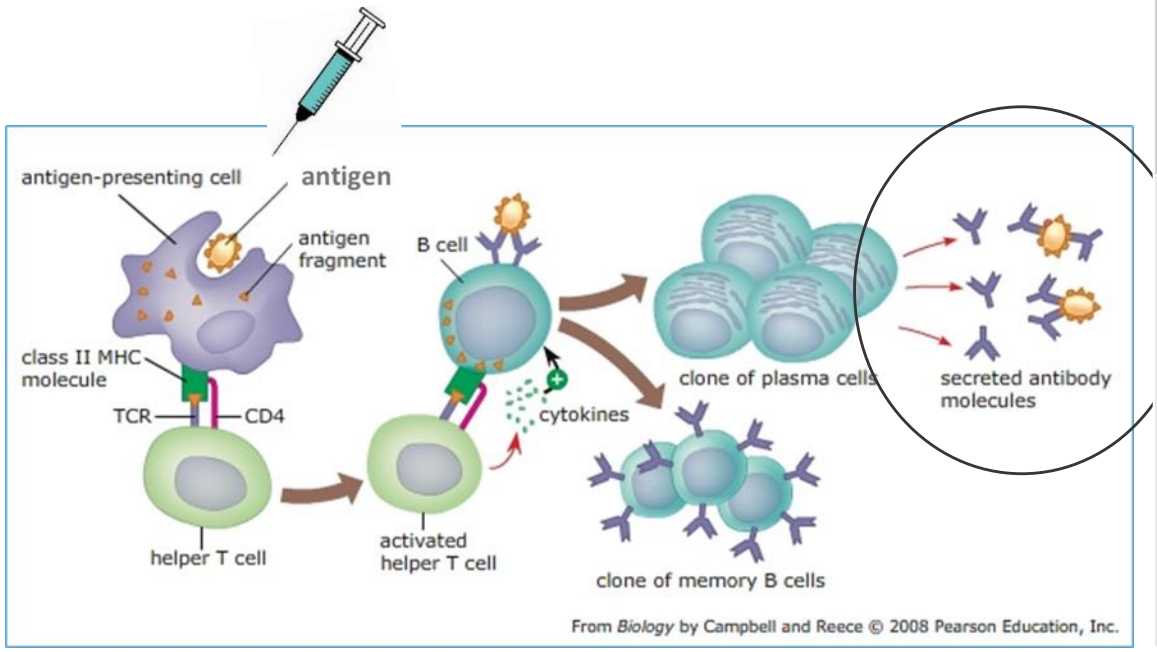
SOFIE PATTIJN, IMMUNXPERTS

EIP WORKSHOP

ImmunXperts
a Q² Solutions Company

Immunogenicity

"The ability of a particular substance, such as an antigen or epitope, to induce an immune response"

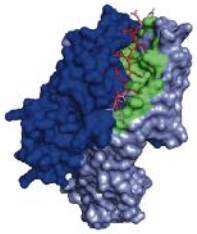


WANTED	UNWANTED	
Vaccines	**Therapeutic protein**	**Stem Cells and Gene Therapy products**
Immune response against the pathogen (virus, bacteria) aiming at protecting the organism	Production of antidrug- antibodies (ADAs) possibly neutralising the therapeutic effects of the treatment and, in rare cases, inducing adverse effects	Cellular and humoral responses Anti HLA antibodies Immune rejections Potential safety effect

ImmunXperts
a Q² Solutions Company

Unwanted Immunogenicity

Early Immunogenicity Assessment Tools



In silico Assays

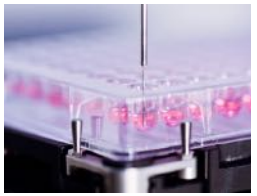
1. Computer based assessment of T cell epitopes
   De-immunization

In vitro early/Innate Assays

3. DC Activation/Maturation Assay
4. Reporter cell line Assay
5. Cytokine Release Assay

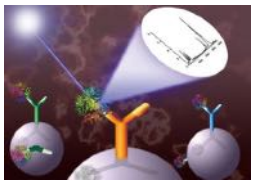
In vitro T cell Assays

6. CD8-depleted PBMC Assay
7. DC-T cell Assay
8. Peptide Assay



MAPPS Assay

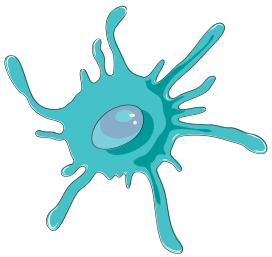
9. Identification processed and presented epitopes



ImmunXperts
a Q² Solutions Company

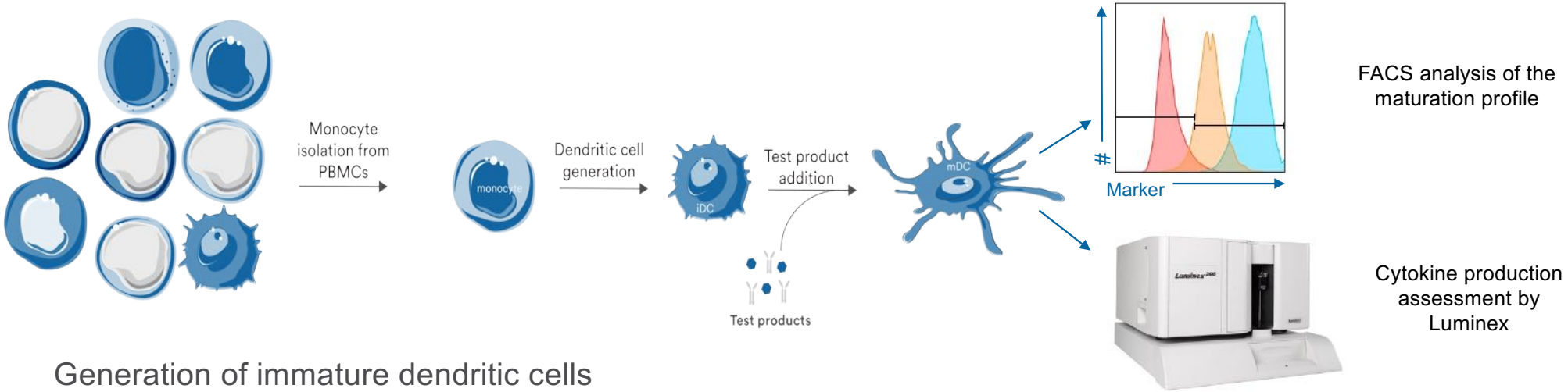
In vitro tools: Early/Innate Assays

3. DC Activation/Maturation Assay
4. Cytokine Release Assay
5. Reporter cell line Assay



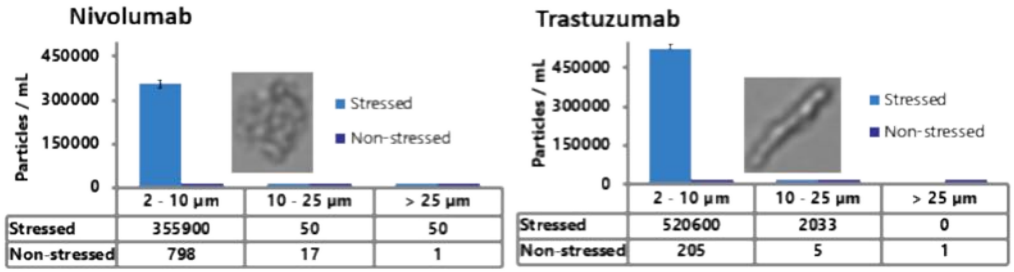
ImmunXperts
a Q² Solutions Company

3. DC Activation/Maturation Assay



Generation of immature dendritic cells
Incubation with aggregated and reference therapeutics or test products
RO:

- Measurement of cytokines/chemokines in the supernatant (Elisa/Luminex/HTRF)
- Evaluation of maturation markers (Flow cytometry)

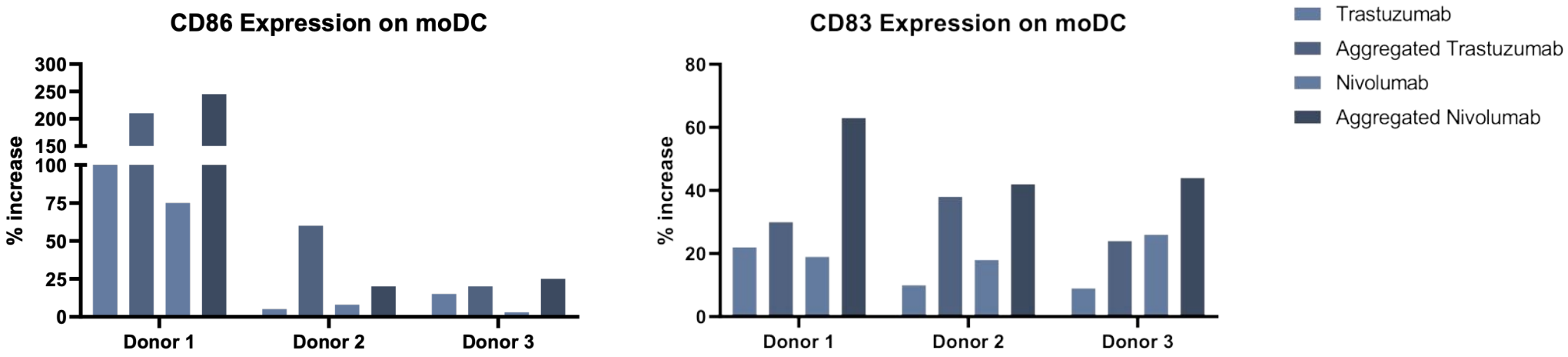


Nivolumab	2 - 10 µm	10 - 25 µm	> 25 µm
Stressed	355900	50	50
Non-stressed	798	17	1

Trastuzumab	2 - 10 µm	10 - 25 µm	> 25 µm
Stressed	520600	2033	0
Non-stressed	205	5	1

ImmunXperts
a Q² Solutions Company

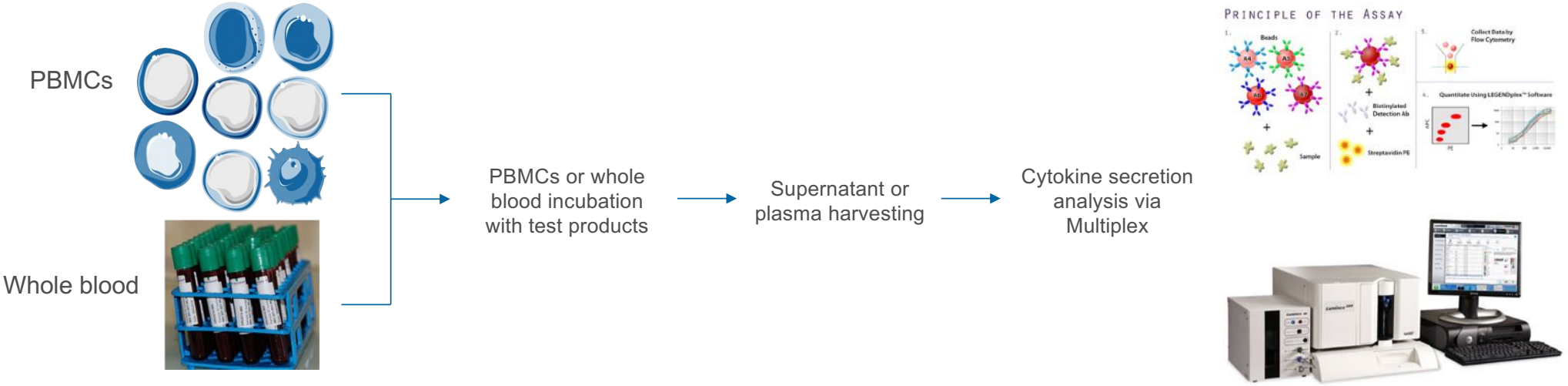
3. DC Activation/Maturation Assay



Increased expression of CD86 and CD83 upon incubation with aggregated therapeutics

ImmunXperts
a Q² Solutions Company

4. Cytokine Release Assay



Test molecules' potential to induce a cytokine release response assessment using:

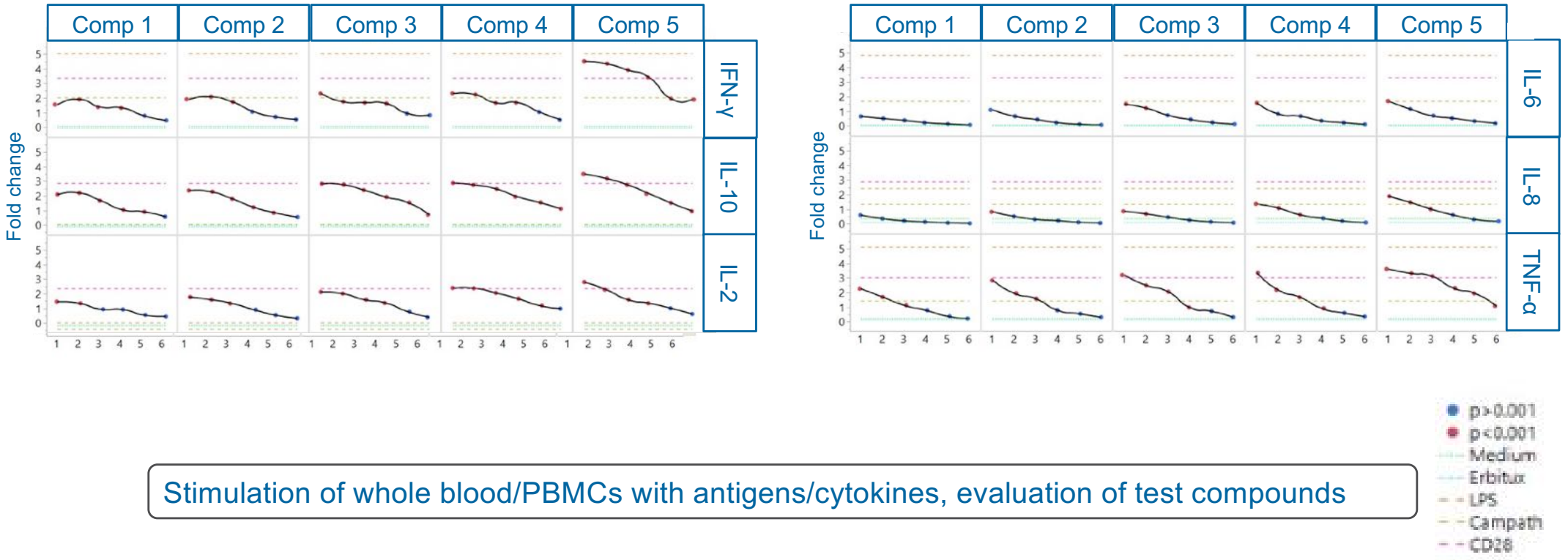
- Whole Blood Cytokine Release Assay
- PBMC Cytokine Release Assay

RO: Measurement of cytokines/chemokines in supernatant or plasma (Luminex, LegendPLex)

- Early phase cytokines: TNF-α, IL-2, IL-8
- Late phase cytokines: IFN-γ, IL-6, IL- 10

ImmunXperts
a Q² Solutions Company

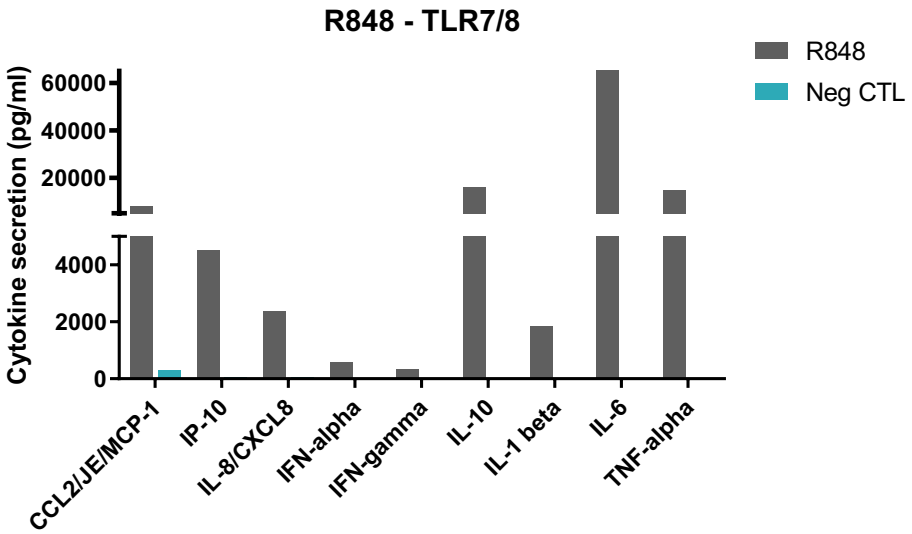
4. Cytokine Release Assay

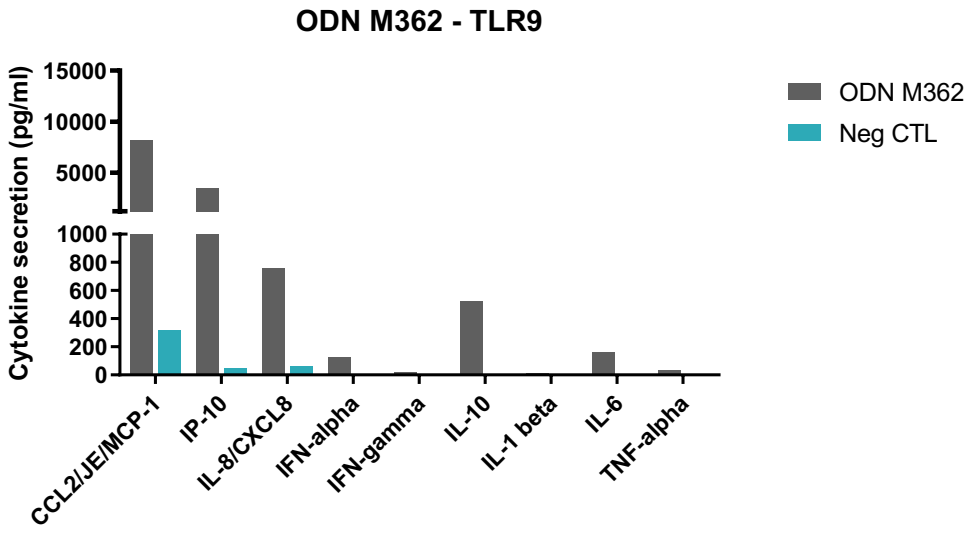


Stimulation of whole blood/PBMCs with antigens/cytokines, evaluation of test compounds

ImmunXperts
a Q² Solutions Company

4. Cytokine Release Assay

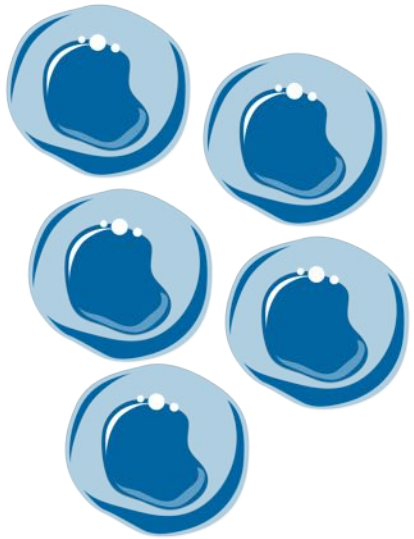




Stimulation of whole blood/PBMCs with antigens/cytokines, evaluation of TLR stimulating test compounds

ImmunXperts
a Q² Solutions Company

5. Reporter Cell line Assay



Cell line
(*e.g.,* HEK, THP-1, RAW)

Test product addition

Luciferase reporter

Luminescence analysis

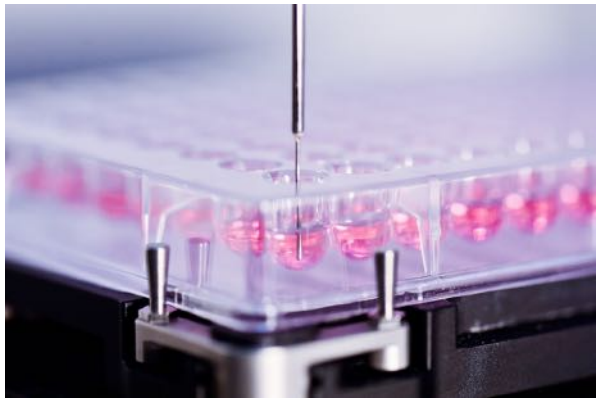
SEAP reporter

Absorbance analysis

TLRs (TLR2, TLR3, TLR4, TLR5, TLR7, TLR8, TLR9)
RIG-1
NF-κB
IL-8
TNF-α
IRF
MDA5

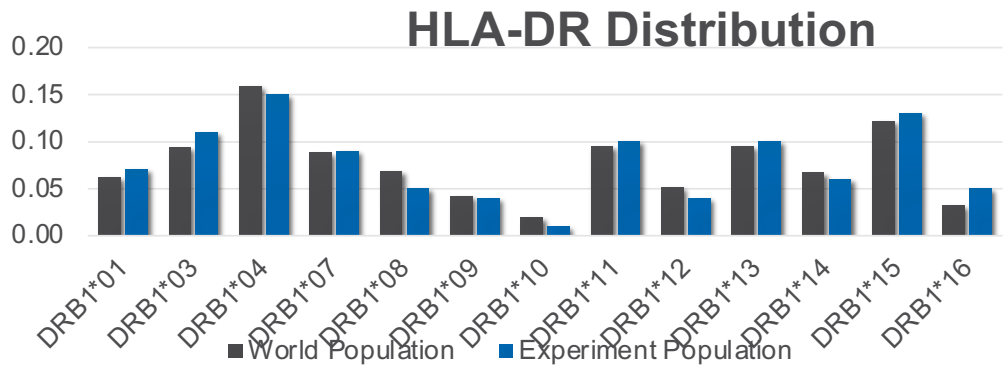
In vitro tools: T Cell Assays

6. CD8-depleted PBMC Assay
7. DC-T Cell Assay
8. Peptide Assay



ImmunXperts
a Q² Solutions Company

Importance of Donor Selection



PBMCs = critical reagent

Need for high quality and functionality

4-digit HLA-typed donor samples

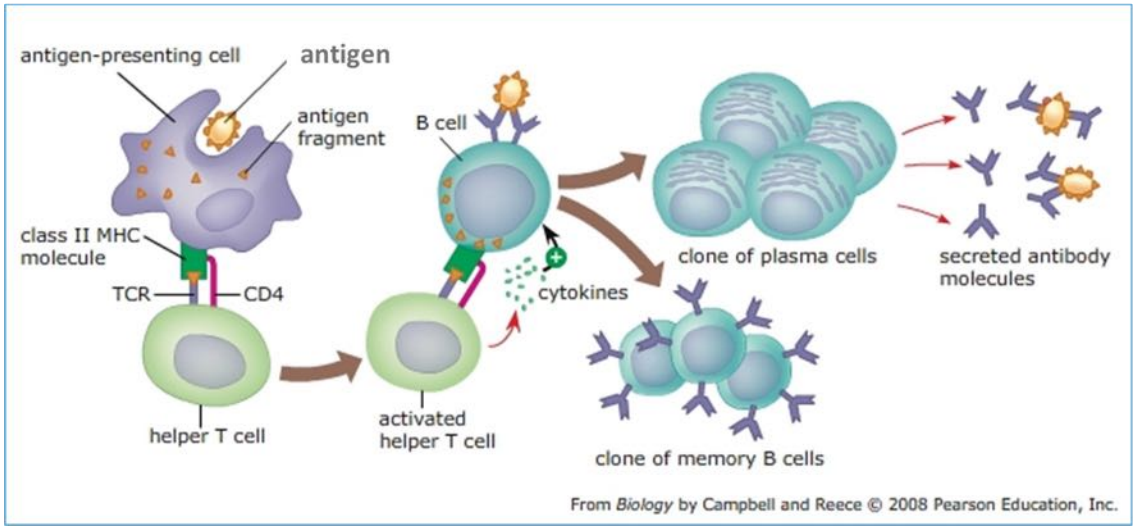
Plus 1000 cell preparations

ImmunXperts
a Q² Solutions Company

Immunogenicity

"The ability of a particular substance, such as an antigen or epitope, to induce an immune response"

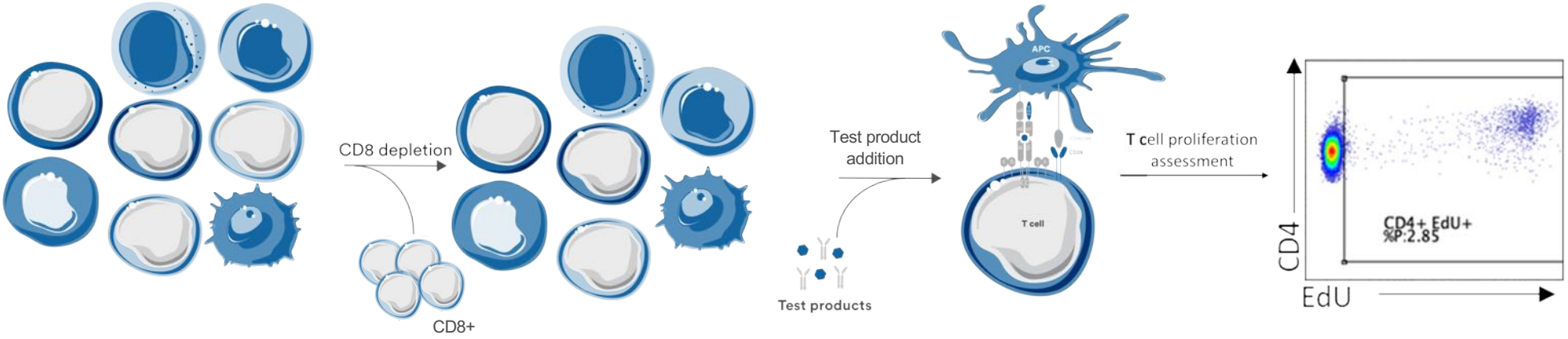
T cell activation/proliferation assays using human PBMC can be used as a **surrogate marker** for antibody responses: good correlation between **T cell activation assays** and reported **ADA responses** (when clinical products are tested in T cell activation/proliferation assays).



UNWANTED	
Therapeutic protein	**Stem Cells and Gene Therapy products**
Production of antidrug-antibodies (ADAs) possibly neutralising the therapeutic effects of the treatment and, in rare cases, inducing adverse effects	Cellular and humoral responses Anti HLA antibodies Immune rejections Potential safety effect

ImmunXperts
a Q² Solutions Company

6. CD8-depleted PBMC Assay

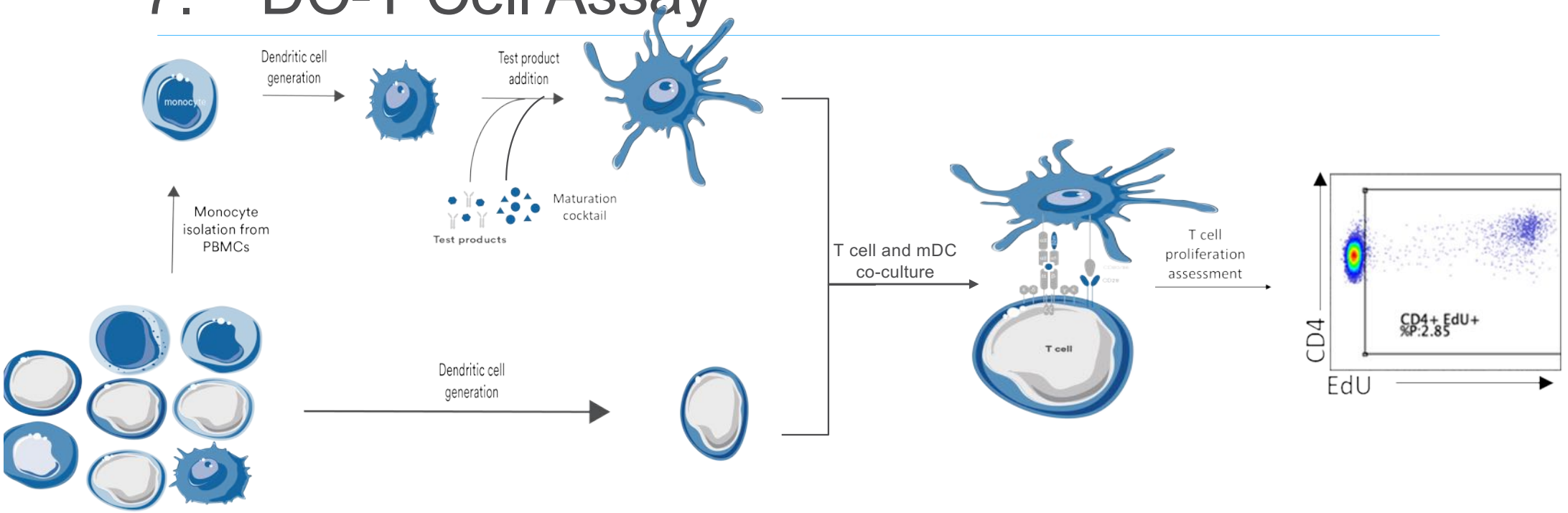


T cell activation and proliferation assays to assess and compare the immunogenicity potential of test molecules
Format depends on the nature and function of the test products:

CD8-depleted PBMC format is used for test products with non-immuno-modulatory functions.

ImmunXperts
a Q² Solutions Company

7. DC-T Cell Assay

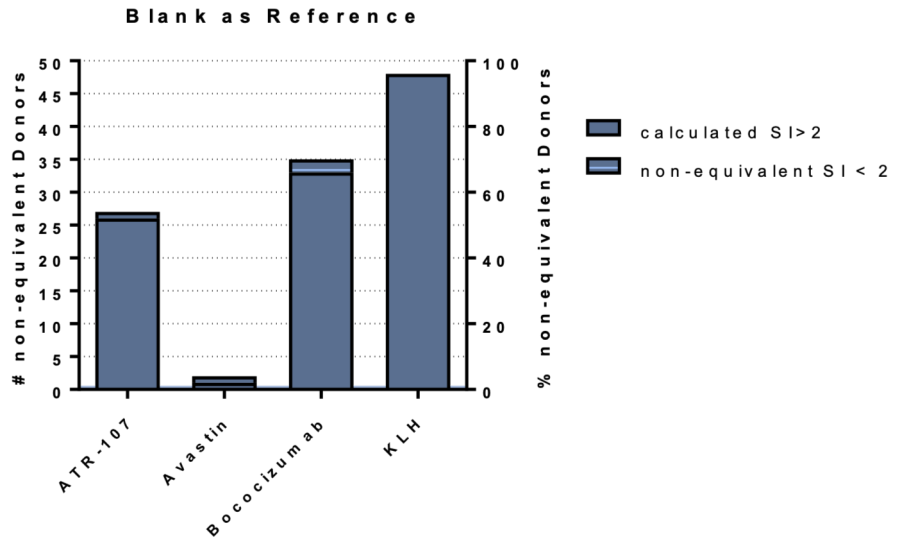


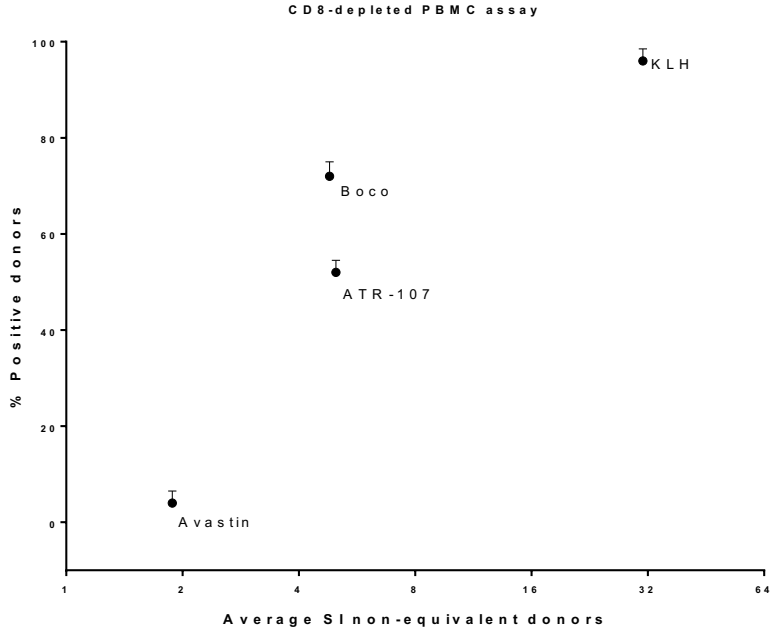
T cell activation and proliferation assays to assess and compare the immunogenicity potential of test molecules
Format depends on the nature and function of the test products:

DC-T Cell format is used for test products with immuno-modulatory functions.

ImmunXperts
a Q² Solutions Company

In vitro tools – T Cell Assays: Outcome



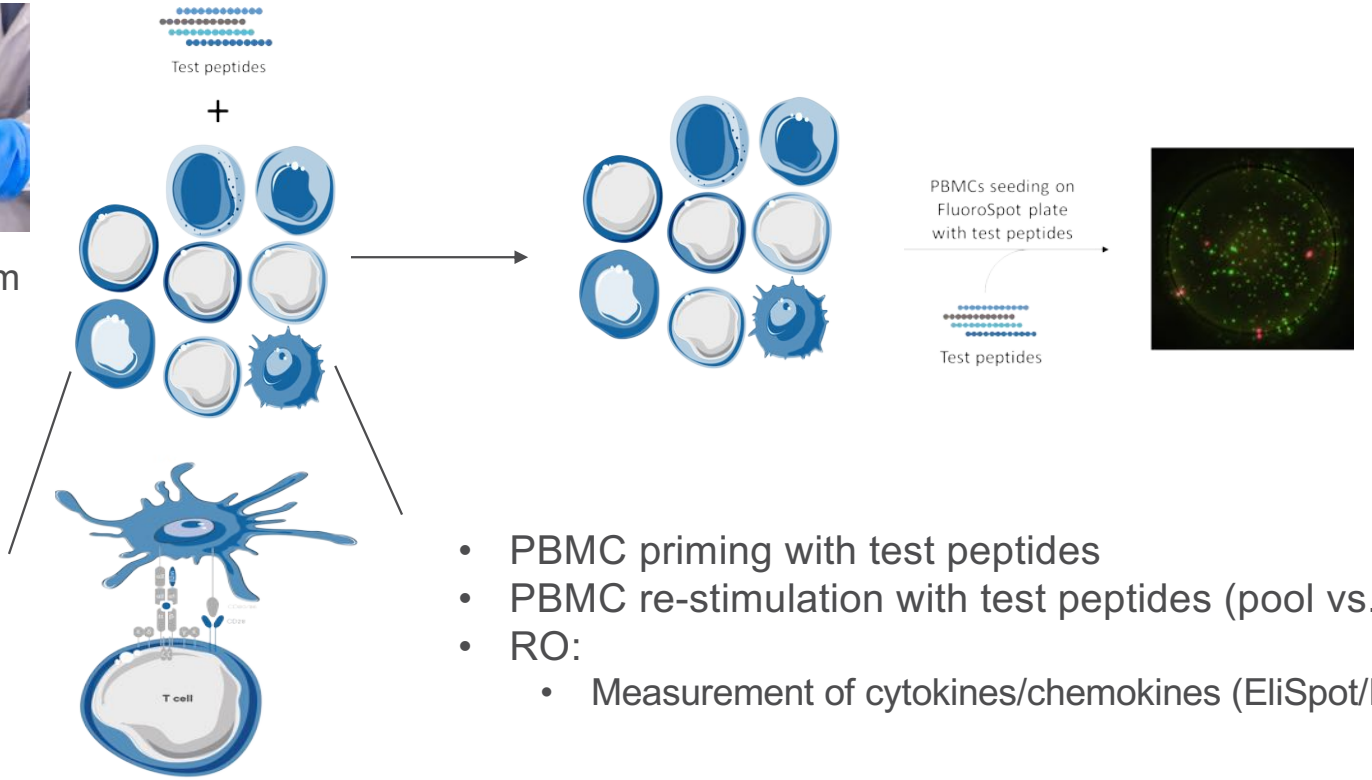


Per donor, a stimulation index (mean response in test condition/mean response in blank condition) is calculated. All reactions with a calculated SI > 2 are considered positive.

ImmunXperts
a Q² Solutions Company

8. Peptide Assay

Sample PBMC from test population

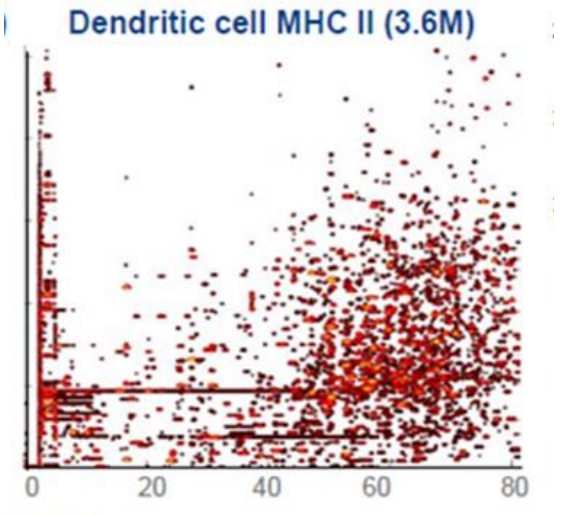


- PBMC priming with test peptides
- PBMC re-stimulation with test peptides (pool vs. individual)
- RO:
  - Measurement of cytokines/chemokines (EliSpot/FluoroSpot)

ImmunXperts
a Q² Solutions Company

In vitro MAPPs Assay

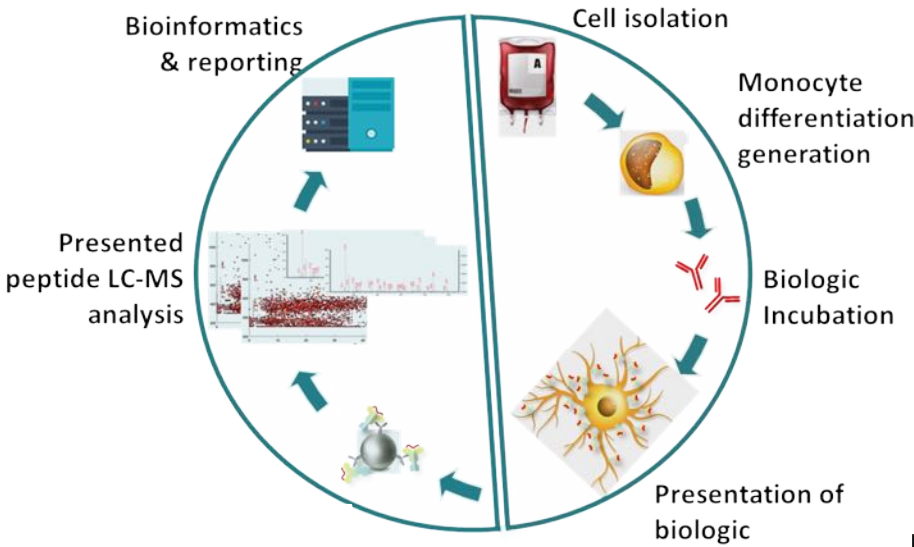
9. Identification processed and presented epitopes using MHC associated peptide proteomics



ImmunXperts
a Q² Solutions Company

9. MAPPS Assay

PARTNER

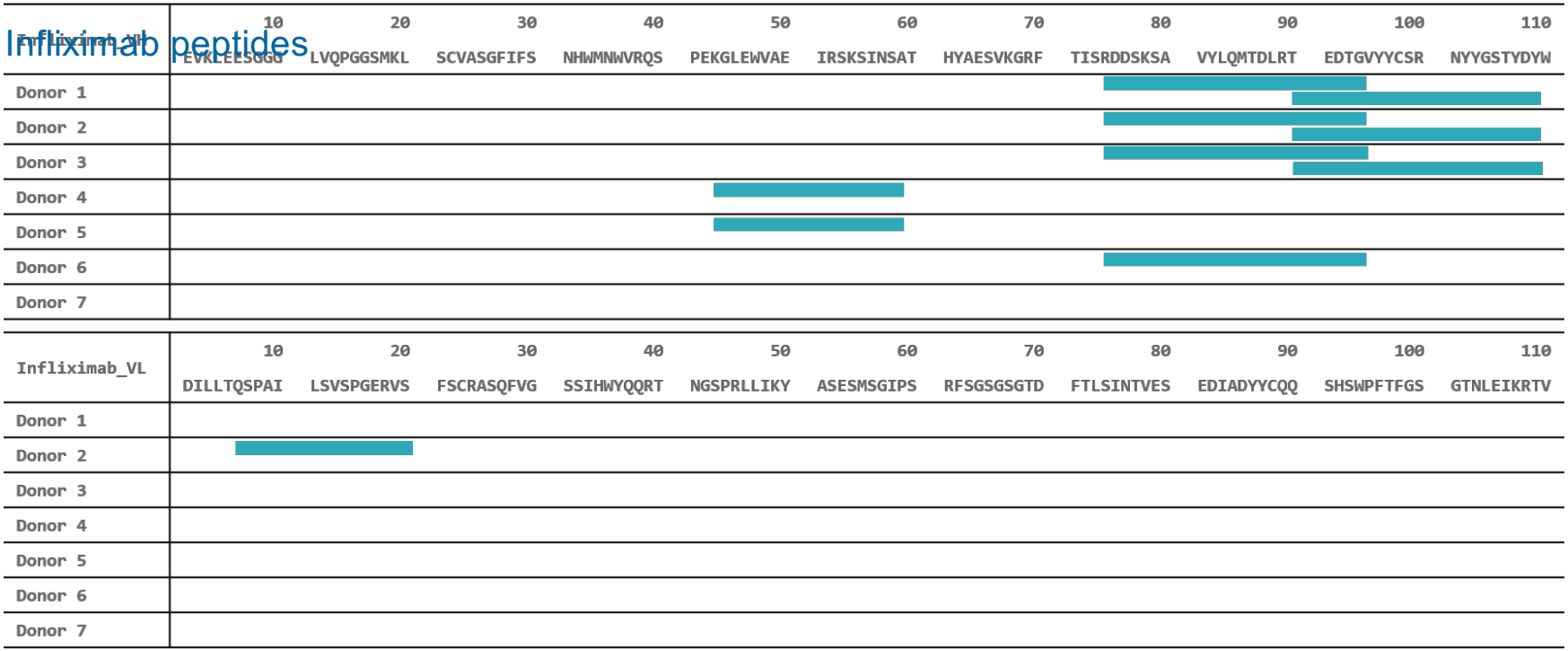


ImmunXperts
a Q² Solutions Company

ImmunXperts
a Q² Solutions Company

9. MAPPS Assay – Outcome

Infliximab peptides



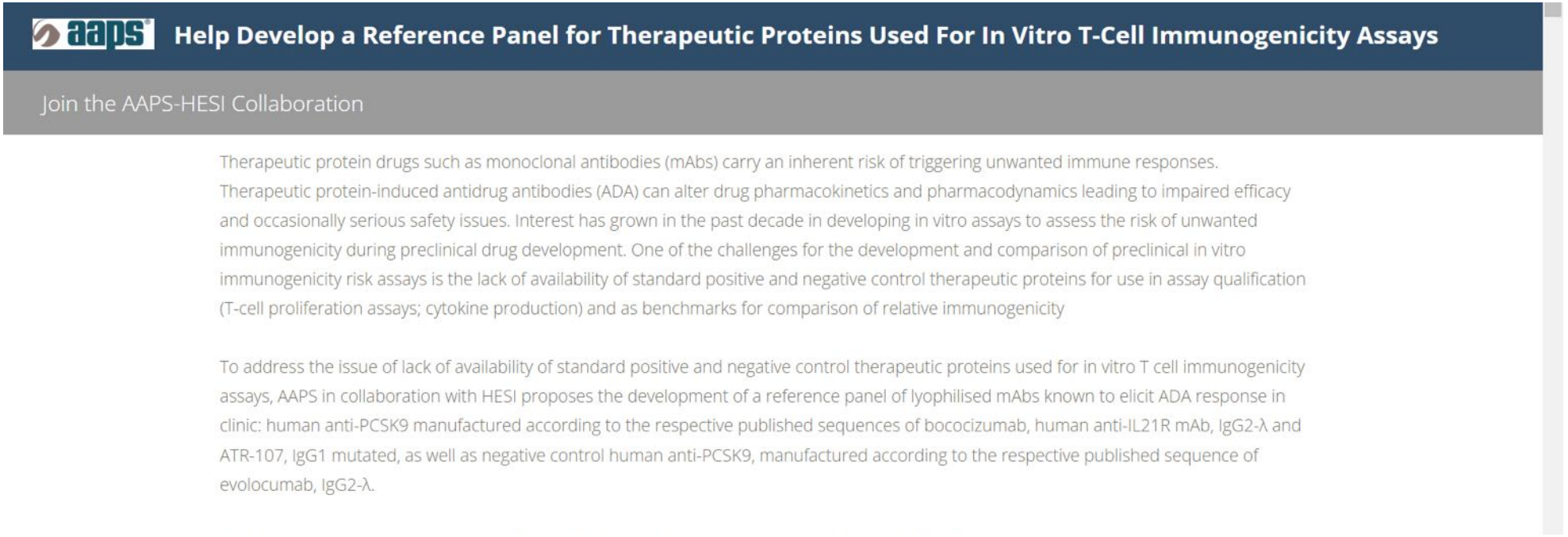
ImmunXperts
a Q² Solutions Company

In vitro Assays 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)

Reference Panel, controls



aaps

Help Develop a Reference Panel for Therapeutic Proteins Used For In Vitro T-Cell Immunogenicity Assays

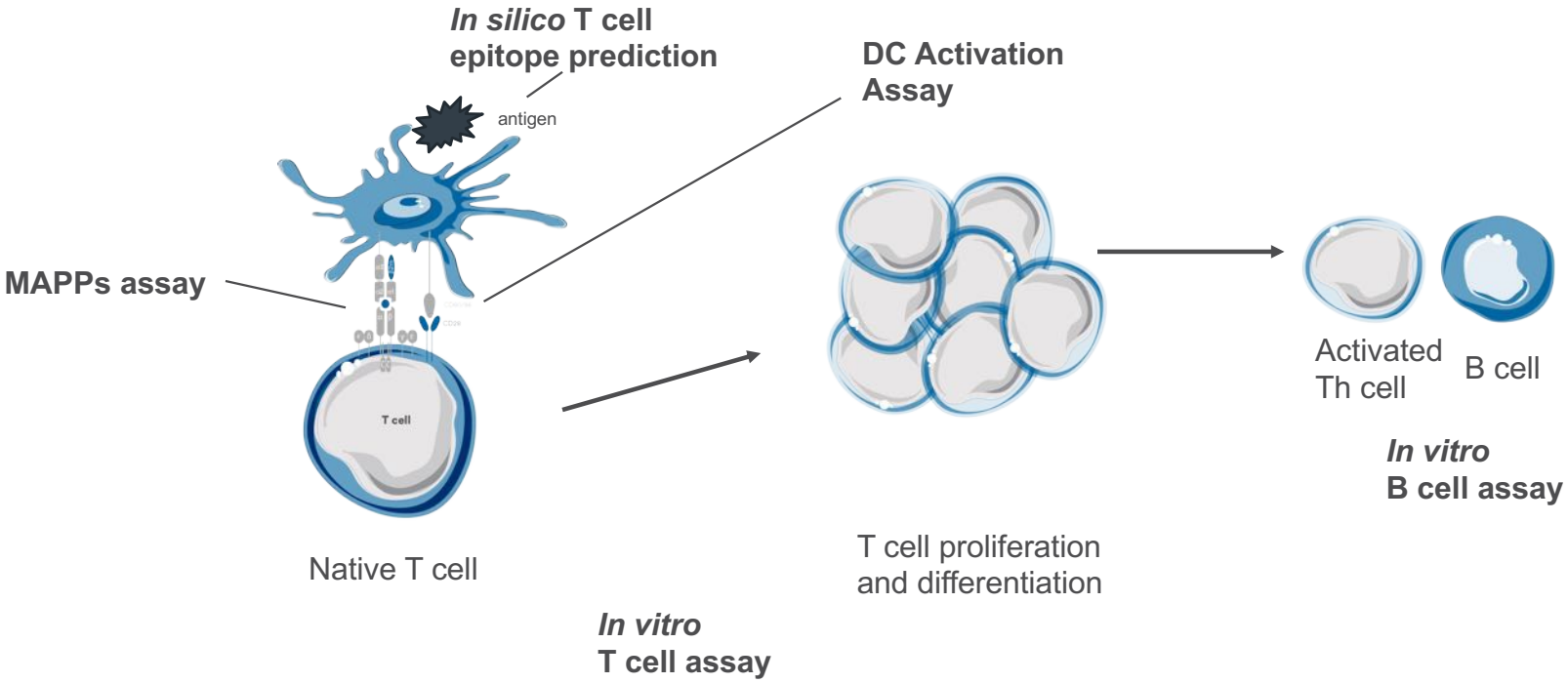
Join the AAPS-HESI Collaboration

Therapeutic protein drugs such as monoclonal antibodies (mAbs) carry an inherent risk of triggering unwanted immune responses. Therapeutic protein-induced antidrug antibodies (ADA) can alter drug pharmacokinetics and pharmacodynamics leading to impaired efficacy and occasionally serious safety issues. Interest has grown in the past decade in developing in vitro assays to assess the risk of unwanted immunogenicity during preclinical drug development. One of the challenges for the development and comparison of preclinical in vitro immunogenicity risk assays is the lack of availability of standard positive and negative control therapeutic proteins for use in assay qualification (T-cell proliferation assays; cytokine production) and as benchmarks for comparison of relative immunogenicity

To address the issue of lack of availability of standard positive and negative control therapeutic proteins used for in vitro T cell immunogenicity assays, AAPS in collaboration with HESI proposes the development of a reference panel of lyophilised mAbs known to elicit ADA response in clinic: human anti-PCSK9 manufactured according to the respective published sequences of bococizumab, human anti-IL21R mAb, IgG2-λ and ATR-107, IgG1 mutated, as well as negative control human anti-PCSK9, manufactured according to the respective published sequence of evolocumab, IgG2-λ.

ImmunXperts
a Q² Solutions Company

Early Immunogenicity Assessment Tools



ImmunXperts a Q² Solutions Company

Your partner in Immunology projects
"We think with You"

ImmunXperts SA, a Q² Solutions Company
Rue Auguste Piccard 48
6041 Gosselies
Belgium

www.immunxperts.com

info@immunxperts.com

BRUSSELS SOUTH CHARLEROI BIOPARK

ImmunXperts
a Q² Solutions Company