aaps PharmSci 360

OCT 22–25 2023

Immunogenicity assessment and functional testing of candidate therapeutics

October 24, 2023

Chloé Ackaert

ImmunXperts

a Q² Solutions Company

Session Description and Objectives

- Biotherapeutics have revolutionized treatment options for several diseases and malignancies in the past few years. However, managing unwanted immunogenicity has become a challenge in the development cycle of these promising therapeutics as there is a trend towards higher unwanted immune responses with more complex molecules. In silico and in vitro tools can be used to assess this early on. Additionally, in vitro functional assays can identify the most promising candidates to take forward.

- 1) learn about potential causes and published examples of unwanted immunogenicity
- 2) define the best strategy for early immunogenicity testing and pipeline de-risking
- 3) learn about functional in vitro assays and other discovery tools to accelerate drug development

Biography and Contact Information

- Pharmacist by training, PhD in immunology
- Postdoc in immunogenicity of Nanobodies
- Senior scientist in the Immunogenicity team at ImmunXperts since 5 years

- Contact information :

chloe.ackaert@q2labsolutions.com

Rue Auguste Piccard 48

6041 Gosselies, Belgium

Office : +32 71 960 133

Direct : +32 71 49 95 11

Company History

Young company, rich history

2014

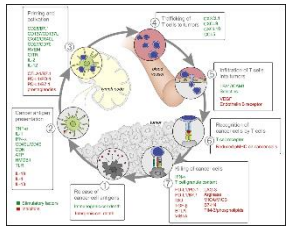
Founded with focus on *in vitro* immunogenicity assays

2015

Partnering with experts and investment in technologies

2016

Expansion to (IO) *in vitro* functional and potency assays



2017

Move to new dedicated facilities 400m²/4,300ft²

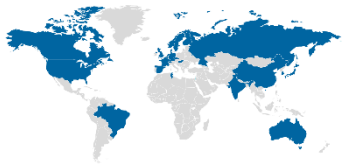
2019

17+ colleagues

200+ Projects

70+ Clients

18 Countries



2020

Acquisition Nexelis

2022

Acquisition Q^2 Solutions

2023

Expansion New facilities

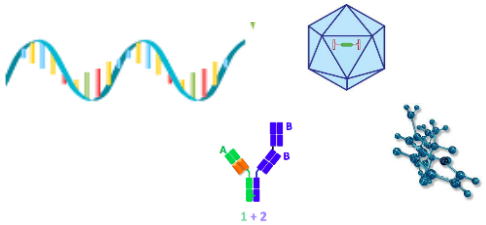
Expertise

- Focus: *In vitro* Immunology in a non-regulated, R&D context but at the highest level of quality
- When necessary, we partner with other experts for complementary knowledge
- Worked with all types of products (Ab, cell therapy, RNA/DNA vaccines, viruses, small molecules, nanoparticles) in all types of indications
- Recognized expertise - involved in various international R&D projects (H2020, Eurostars, ...)

Your mobile development team

In Vitro Assays

Sample
=
Drug in development

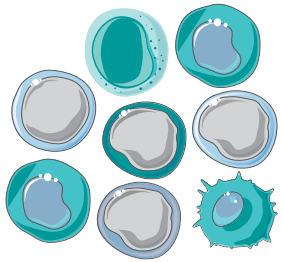


- ✓ Biologics: Abs, classical, new formats, scaffolds
- ✓ Generic peptides
- ✓ Vaccines (mRNA, DNA, …)
- ✓ Small Molecules
- ✓ Nanoparticles
- ✓ CGT Products
- ✓ …

+

Human Whole Blood

Human PBMCs

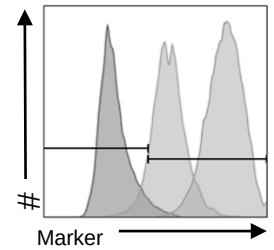


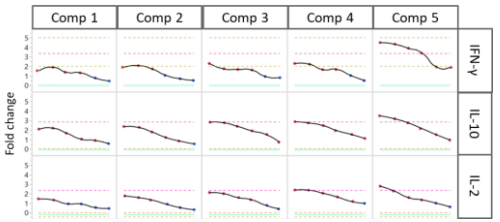
Primary cells of NHP, mice, hamsters,...
Cell lines

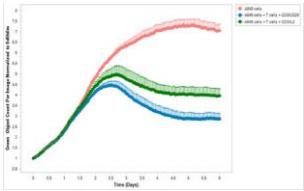
Activation/Proliferation Immune Cells

Cytokine Production

Killing

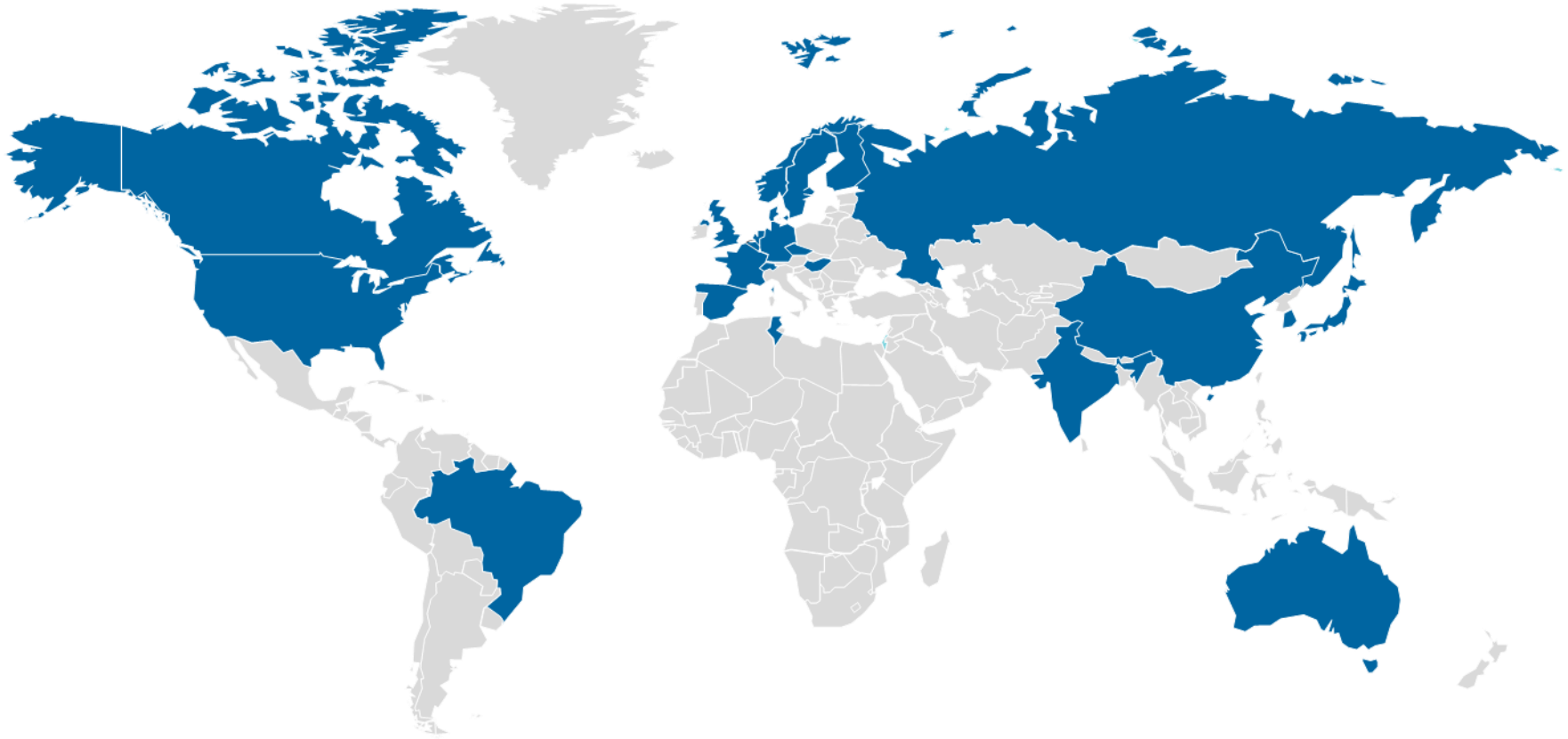






CUSTOMER | IMXP' BIOBANK | READ-OUTS | DELIVERABLES

Global Footprint



Clients across 25+ countries including Large Biopharma, Small to Medium or Startups & Virtual Biotech

Team

- 30 team members:
- Seasoned immunology experts with R&D focus
- Specialized dedicated biostatistician expert

We think with you

Facilities

Currently

400 m^2 (4,300 ft^2) laboratories/offices
incl. BSL2+
(access to) BSL3

End 2023

800 m^2 dedicated laboratories
400 m^2 offices
70 m^2 cryostorage

Cell isolation, banking and culture facilities (BE biobank license)

Technologies

- BD Fortessa
5 lasers
up to 20 parameters

Macs Quant®10
3 lasers
up to 10 parameters

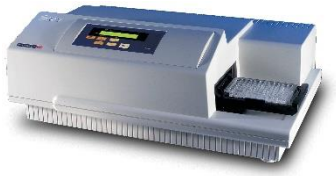
BD FACSSymphony™ A1
4 lasers
up to 16 parameters

BD FACSMelody™ Cell Sorter
3 lasers
up to 11 parameters

Luminex

ELISpot/FluoroSpot

Glomax Explorer



Spectramax

IncuCyte SX5

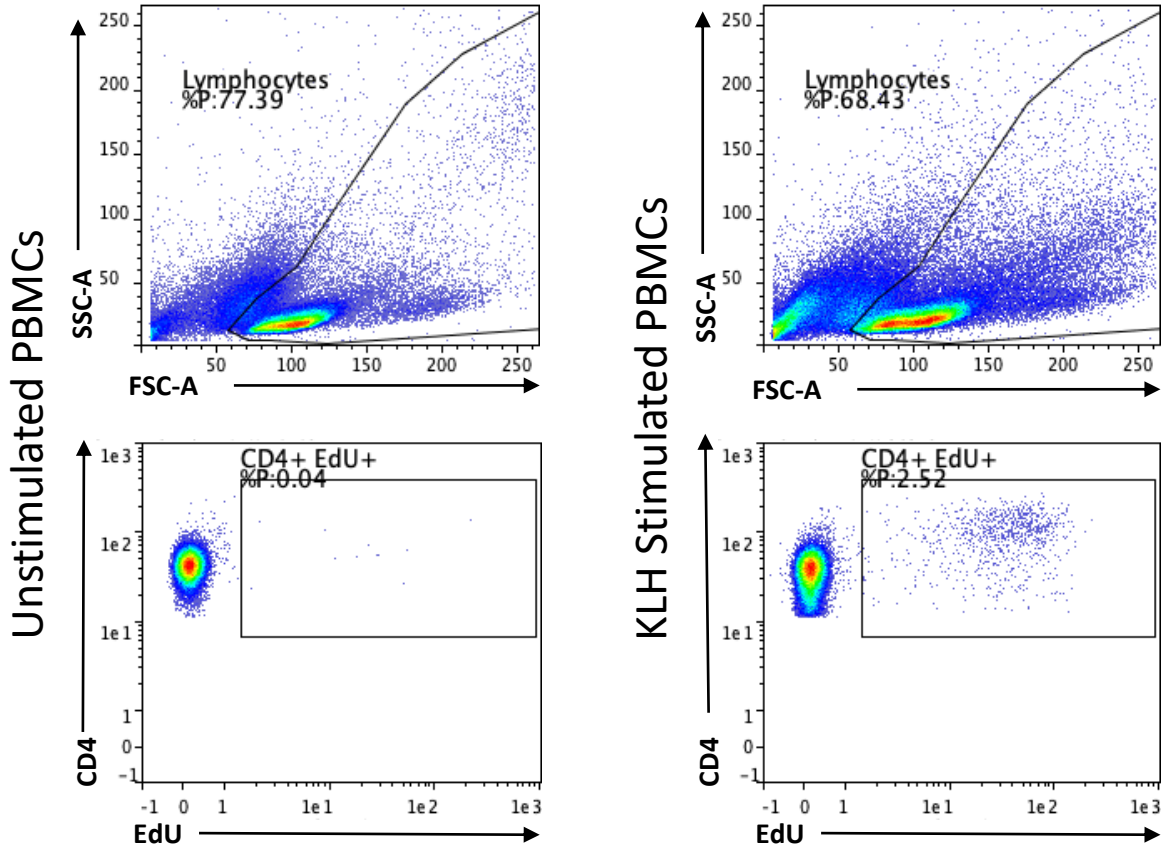
Figures adopted from BD, Miltenyi Biotec, Luminex Corporation, Mabtech, Promega, Molecular Devices and Satorius

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)
  - Plus 1000 healthy donor samples (4-digit HLA typed)

Functionality Assessment

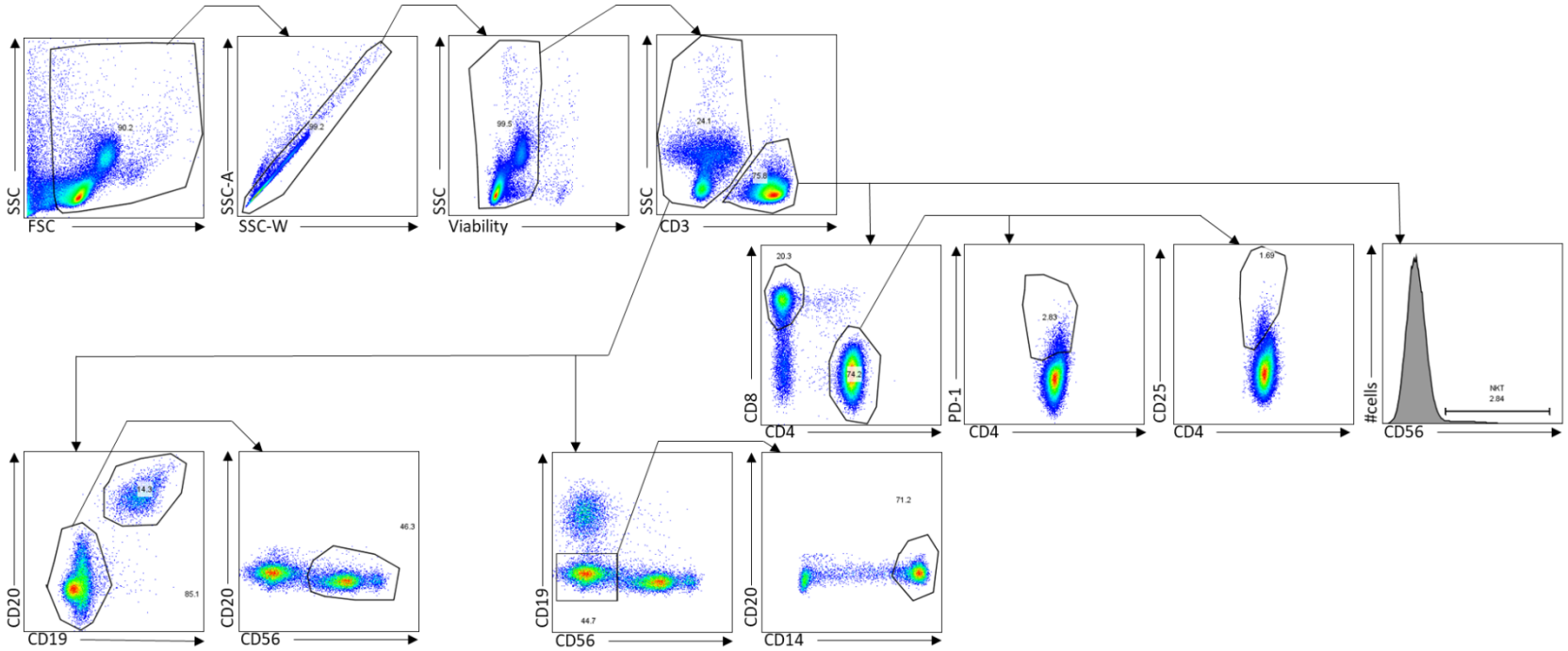
- Assessment of proliferative response towards polyclonal stimulation (anti-CD3 antibody)
- Assessment of proliferative response towards naïve antigen Keyhole Limpet Hemocyanin (KLH)



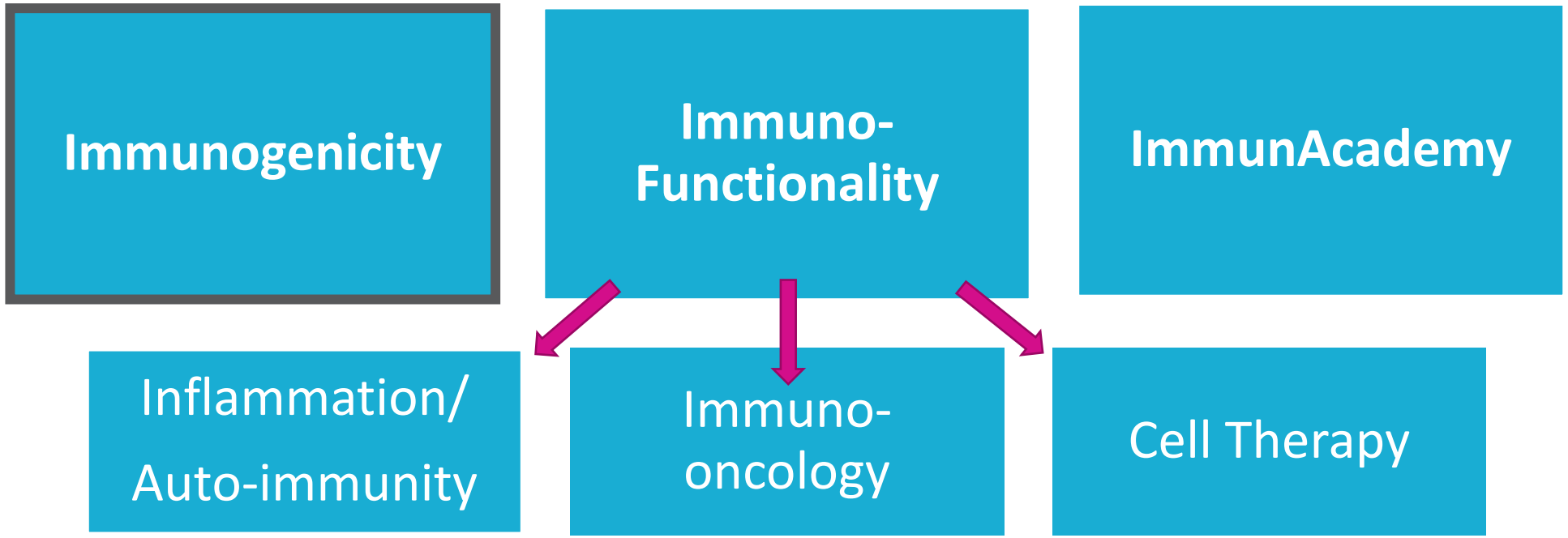
Subpopulation Analysis

- Classic Surface Marker staining:
  - 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

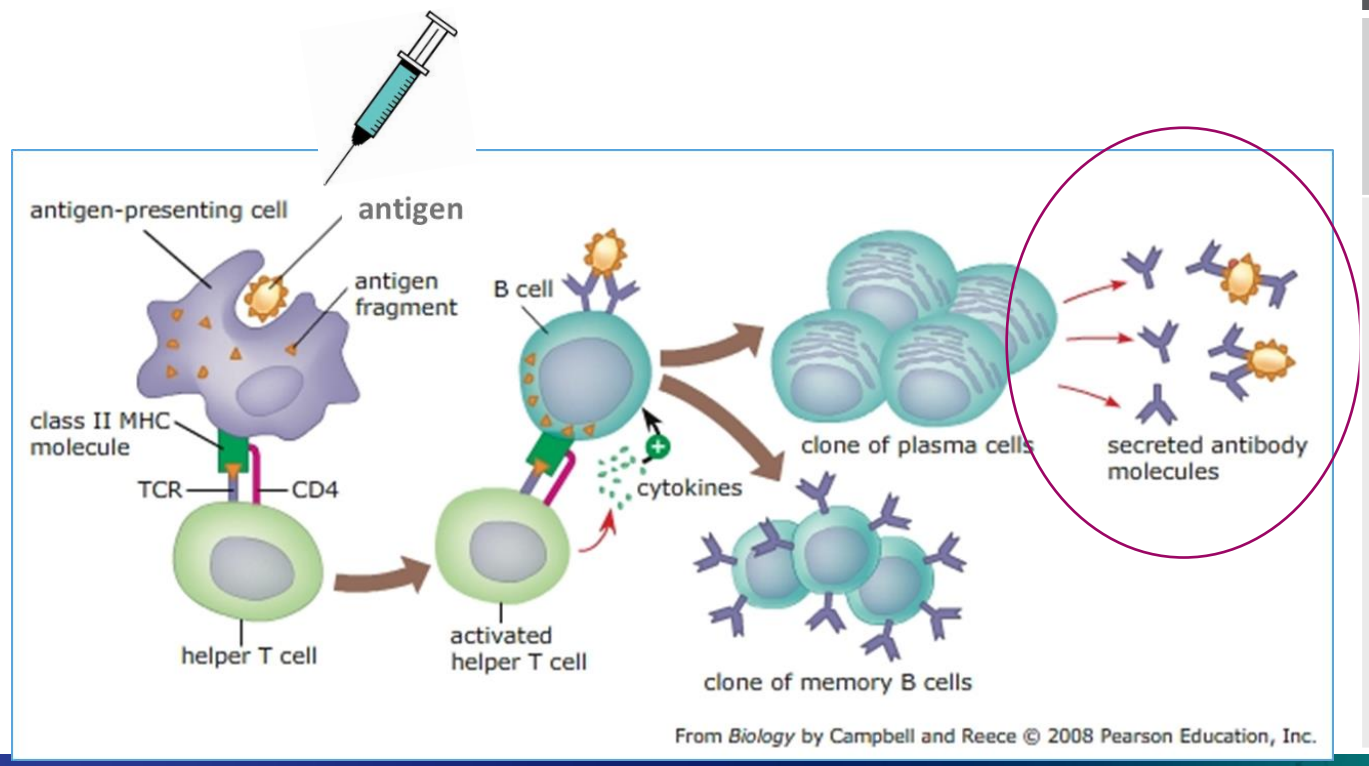


ImmunXperts' Services



Immunogenicity

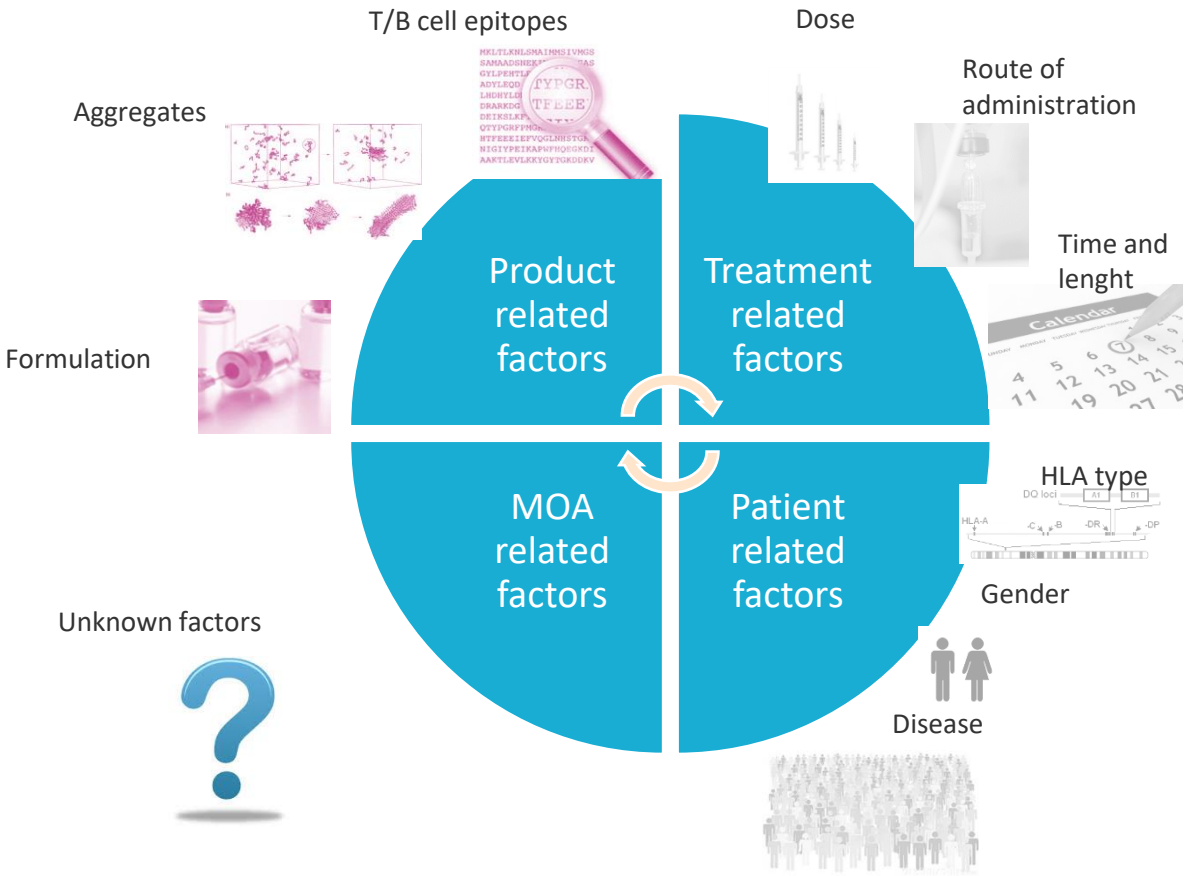
"The ability of a particular substance, such as an antigen or epitope, to induce an immune response"



WANTED	UNWANTED	
Vaccines	**Therapeutic proteins**	**CGT 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 effects

Unwanted Immunogenicity

Factors impacting Immunogenicity



Consequences of Unwanted Immunogenicity

J Young Pharm. 2010 Jul-Sep; 2(3): 332–336.
doi: 10.4103/0975-1483.66810

PMCID: PMC2964774
PMID: 21042496

TGN1412: From Discovery to Disaster

H Attarwala

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Abstract

Go to: ▸

After a drug is confirmed as safe and efficacious in preclinical studies, it is tested in healthy human volunteers for first in man trials. In 2006, a phase I clinical study was conducted for a CD28 superagonist antibody TGN1412 in six human volunteers. After very first infusion of a dose 500 times smaller than that found safe in animal studies, all six human volunteers faced life-threatening conditions involving multiorgan failure for which they were moved to intensive care unit. After this particular incident, a lot was changed over how first in man trials are approved by regulatory authorities and the way clinical trials are conducted. This review primarily deals with preclinical studies conducted by TeGenero, results of which encouraged them to test the antibody on human subjects, reasons why this drug failed in human trial and aftermath of this drug trial. In addition, another drug—Fialuridine which failed in phase 2 clinical trial leading to death of five human subjects is briefly reviewed.

Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2964774/

Consequences of Unwanted Immunogenicity

Bayer drops hemophilia candidate BAY 86-6150 on safety concerns

06-05-2013 COMMENTS (0)

BAY 86-6150 | Bayer | Pharmaceutical | Research

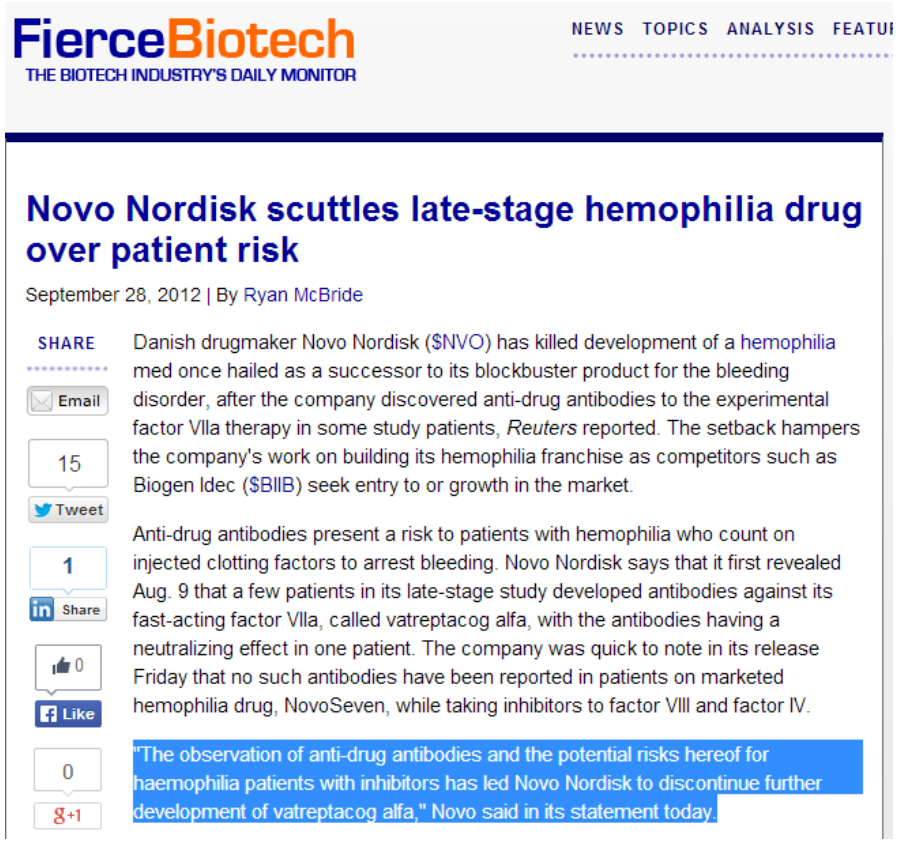
German drug major Bayer (BAYN: DE) said on Friday (May 3) that it has discontinued a Phase II/III trial evaluating the efficacy and safety of BAY 86-6150 in people with hemophilia A and hemophilia B with inhibitors has been discontinued.

The company said that the hope that BAY 86-6150 might help patients with inhibitors to achieve better control of their disease could not be fulfilled due to the detection of a neutralizing antibody in the trial.

"Patient safety is our primary concern when designing clinical trials and evaluating BAY 86-6150," said Kemal Malik, a member of the Bayer HealthCare executive committee and head of global development, adding: "Due to safety concerns, we are discontinuing the BAY 86-6150 trial as a precautionary measure."

Source: https://www.thepharmaletter.com/

Consequences of Unwanted Immunogenicity



FierceBiotech
THE BIOTECH INDUSTRY'S DAILY MONITOR

NEWS TOPICS ANALYSIS FEATU

Novo Nordisk scuttles late-stage hemophilia drug over patient risk

September 28, 2012 | By Ryan McBride

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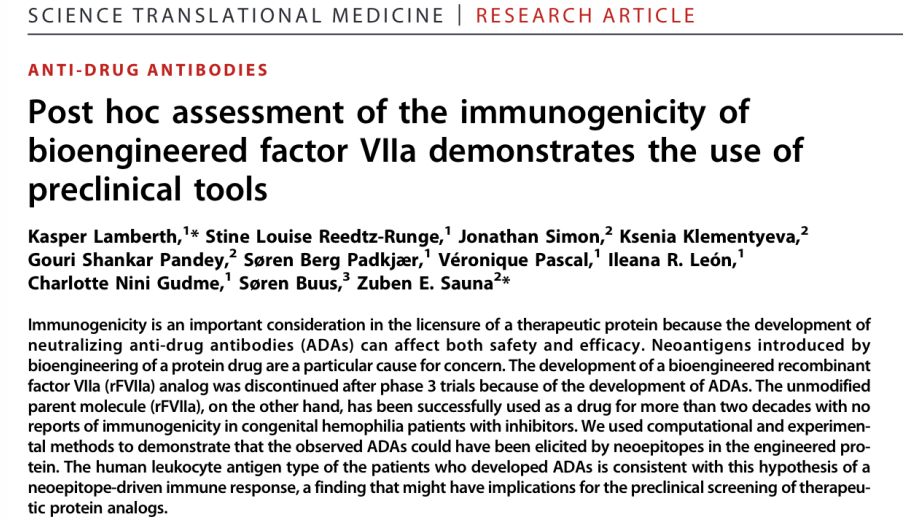
Danish drugmaker Novo Nordisk ($NVO) has killed development of a hemophilia med once hailed as a successor to its blockbuster product for the bleeding disorder, after the company discovered anti-drug antibodies to the experimental factor VIIa therapy in some study patients, *Reuters* reported. The setback hampers the company's work on building its hemophilia franchise as competitors such as Biogen Idec ($BIIB) seek entry to or growth in the market.

Anti-drug antibodies present a risk to patients with hemophilia who count on injected clotting factors to arrest bleeding. Novo Nordisk says that it first revealed Aug. 9 that a few patients in its late-stage study developed antibodies against its fast-acting factor VIIa, called vatreptacog alfa, with the antibodies having a neutralizing effect in one patient. The company was quick to note in its release Friday that no such antibodies have been reported in patients on marketed hemophilia drug, NovoSeven, while taking inhibitors to factor VIII and factor IV.

"The observation of anti-drug antibodies and the potential risks hereof for haemophilia patients with inhibitors has led Novo Nordisk to discontinue further development of vatreptacog alfa," Novo said in its statement today.

Source: www.fiercebiotech.com



SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

ANTI-DRUG ANTIBODIES

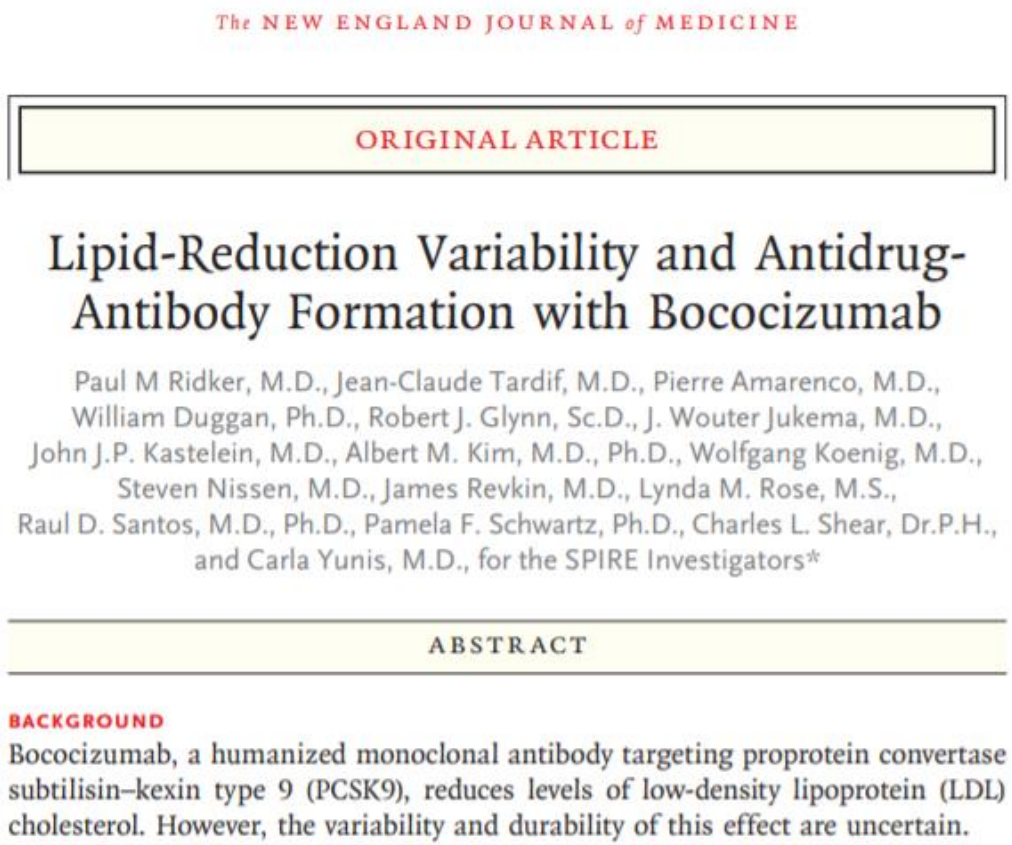
Post hoc assessment of the immunogenicity of bioengineered factor VIIa demonstrates the use of preclinical tools

Kasper Lamberth,[1]* Stine Louise Reedtz-Runge,[1] Jonathan Simon,[2] Ksenia Klementyeva,[2] Gouri Shankar Pandey,[2] Søren Berg Padkjær,[1] Véronique Pascal,[1] Ileana R. León,[1] Charlotte Nini Gudme,[1] Søren Buus,[3] Zuben E. Sauna[2]*

Immunogenicity is an important consideration in the licensure of a therapeutic protein because the development of neutralizing anti-drug antibodies (ADAs) can affect both safety and efficacy. Neoantigens introduced by bioengineering of a protein drug are a particular cause for concern. The development of a bioengineered recombinant factor VIIa (rFVIIa) analog was discontinued after phase 3 trials because of the development of ADAs. The unmodified parent molecule (rFVIIa), on the other hand, has been successfully used as a drug for more than two decades with no reports of immunogenicity in congenital hemophilia patients with inhibitors. We used computational and experimental methods to demonstrate that the observed ADAs could have been elicited by neoepitopes in the engineered protein. The human leukocyte antigen type of the patients who developed ADAs is consistent with this hypothesis of a neoepitope-driven immune response, a finding that might have implications for the preclinical screening of therapeutic protein analogs.

Transl Med. 2017 Jan 11;9(372)

Unwanted Immunogenicity: One Hurdle of the Drug Development Cycle



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Lipid-Reduction Variability and Antidrug-Antibody Formation with Bococizumab

Paul M Ridker, M.D., Jean-Claude Tardif, M.D., Pierre Amarenco, M.D., William Duggan, Ph.D., Robert J. Glynn, Sc.D., J. Wouter Jukema, M.D., John J.P. Kastelein, M.D., Albert M. Kim, M.D., Ph.D., Wolfgang Koenig, M.D., Steven Nissen, M.D., James Revkin, M.D., Lynda M. Rose, M.S., Raul D. Santos, M.D., Ph.D., Pamela F. Schwartz, Ph.D., Charles L. Shear, Dr.P.H., and Carla Yunis, M.D., for the SPIRE Investigators*

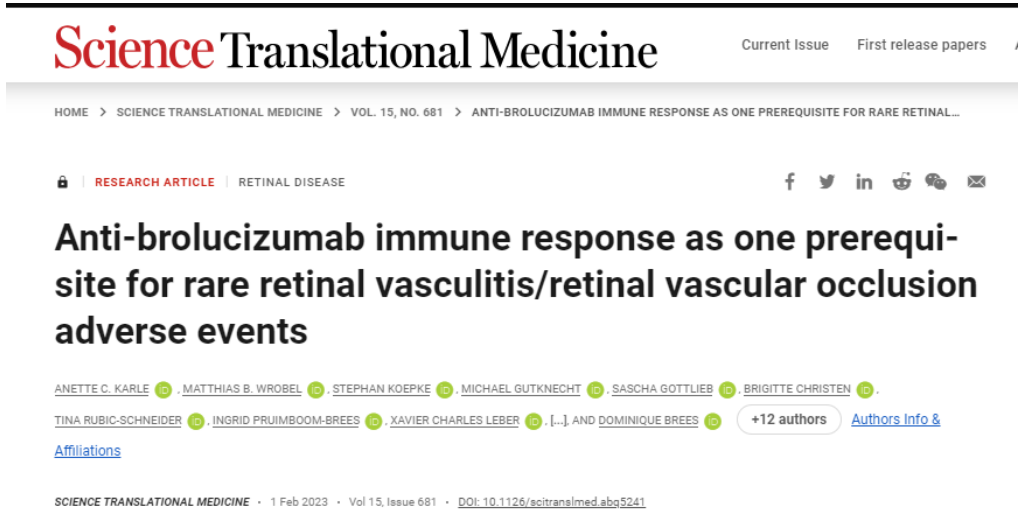
ABSTRACT

BACKGROUND

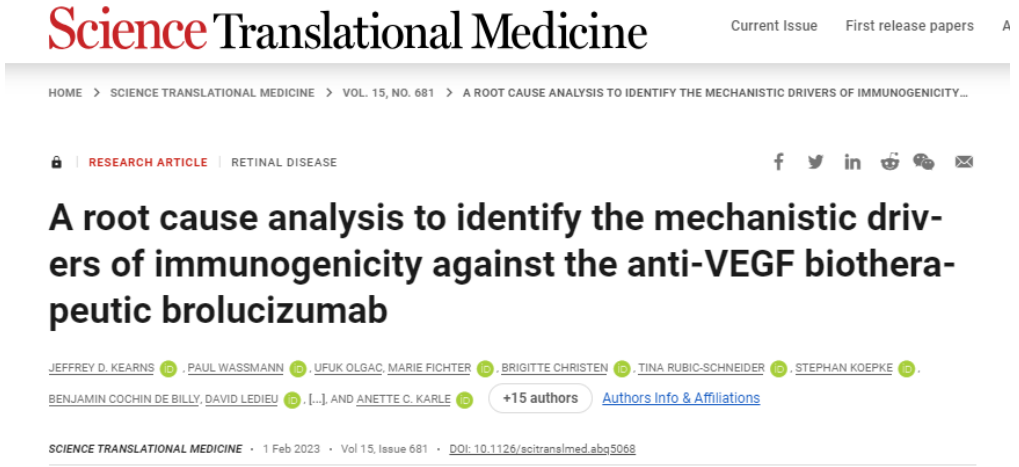
Bococizumab, a humanized monoclonal antibody targeting proprotein convertase subtilisin–kexin type 9 (PCSK9), reduces levels of low-density lipoprotein (LDL) cholesterol. However, the variability and durability of this effect are uncertain.

Source: N Engl J Med 2017; 376:1517-1526

Unwanted Immunogenicity: One Hurdle of the Drug Development Cycle

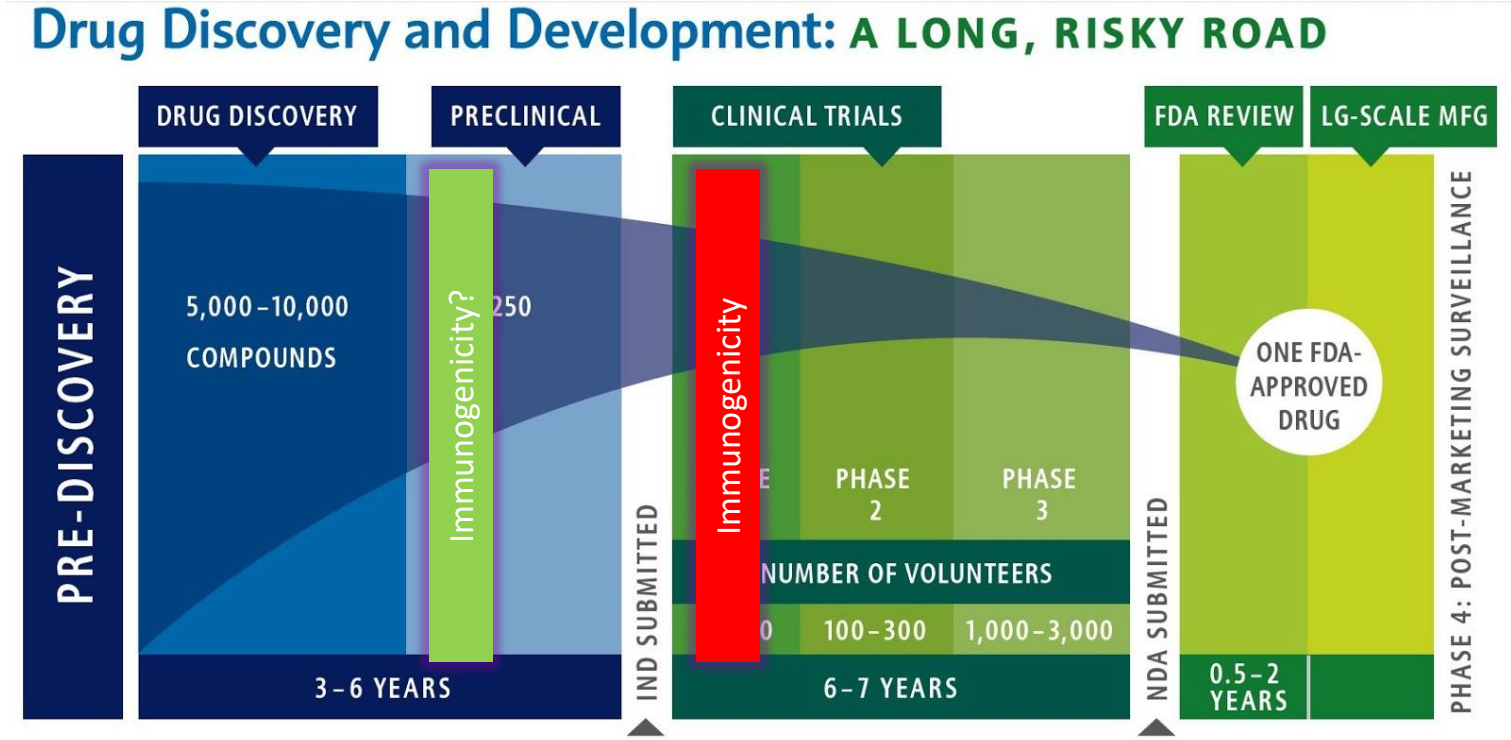


Source :
https://www.science.org/doi/10.1126/scitranslmed.abq5241



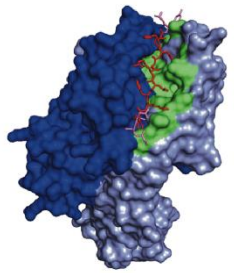
Source :
https://www.science.org/doi/10.1126/scitranslmed.abq5068

Consequences of Unwanted Immunogenicity

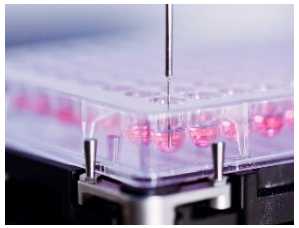


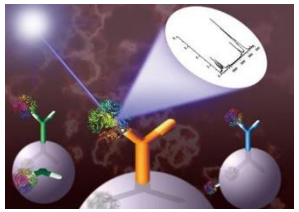
Adapted from Medicines in Development Leukemia & Lymphoma 2013

Early Immunogenicity Assessment Tools

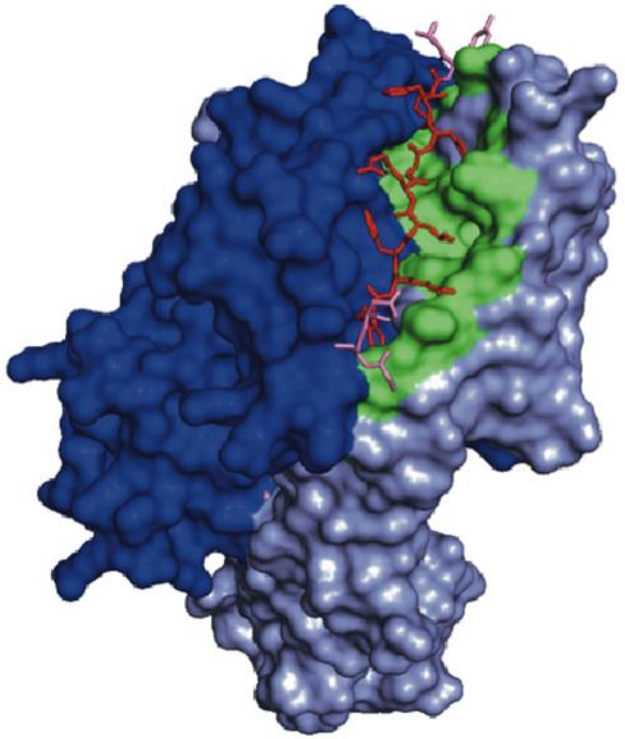


In silico Assays	1. Computer based assessment of T cell epitopes De-immunization
In vitro early/Innate Assays	2. Monocyte Activation Assay 3. DC Activation/Maturation Assay 4. Reporter cell line Assay 5. Cytokine Release Assay
In vitro T cell Assays	6. CD8-depleted PBMC Assay 7. DC-T cell Assay 8. Peptide Assay
MAPPS Assay	9. Identification of processed and presented epitopes
ADA Assay	10. Pre-existing ADA analysis in serum



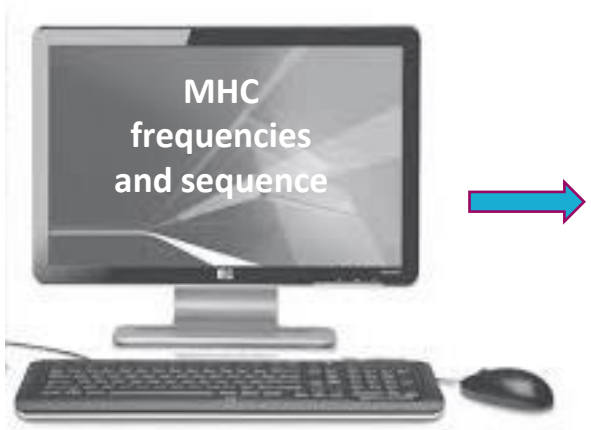


In Silico Assays



- Net MHC II pan
- Net MHC Class I and II

1. Computer-based Assessment of T cell Epitopes



Identify potential epitopes

Test sequence → Test population → Algorithm → Assess immunogenicity potential/risk

1. Computer-based Assessment of T cell Epitopes

In silico T cell epitope prediction:

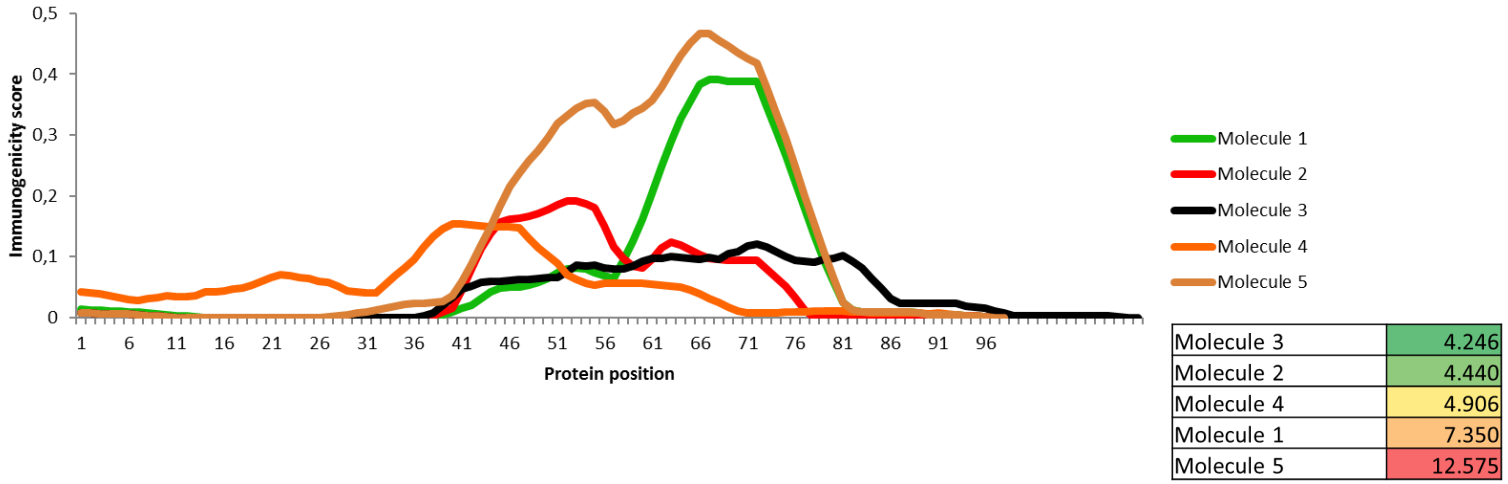
- Class I/II epitope prediction: NetMHC(II)pan
- Immunogenicity assessment and ranking of immunogenic potential
- De-immunization

Benefits:

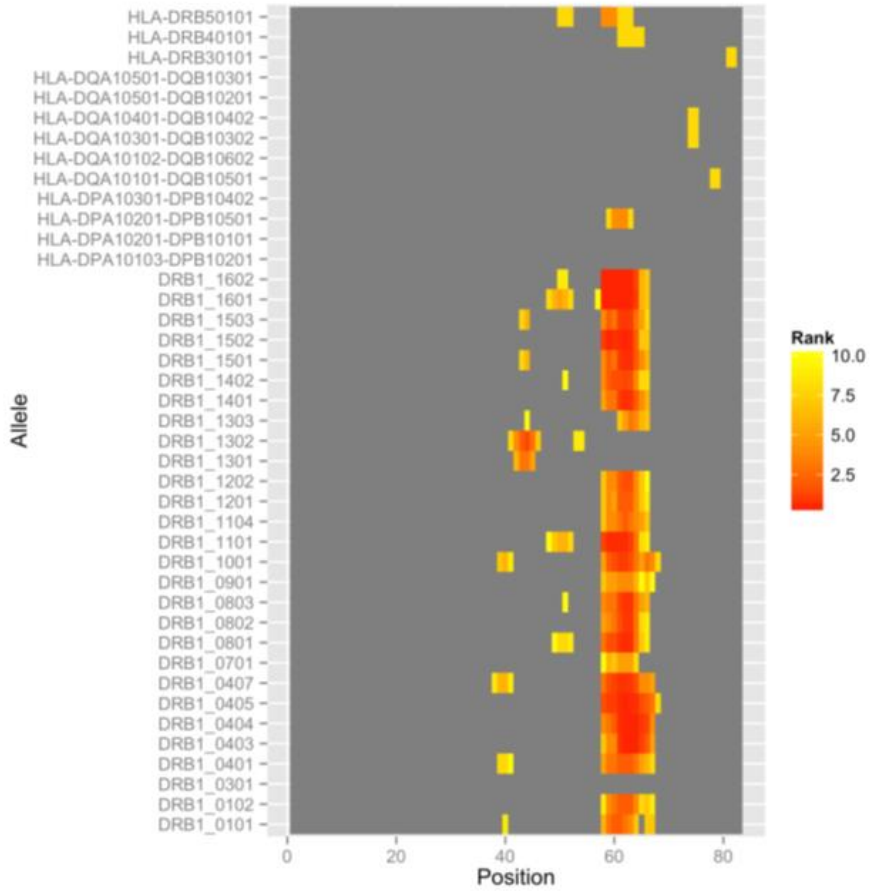
- State of the art, well documented and bench marked tools
- Transparency: several publications available
- Pan-specific, availability HLA's
- Continuous optimization at the Technical University of Denmark, Prof. Morten Nielsen

1. Computer-based Assessment of T cell Epitopes

1) Epitope Mapping
2) Population Risk Score Calculation
3) Amino Acid Mutation
4) Evaluation

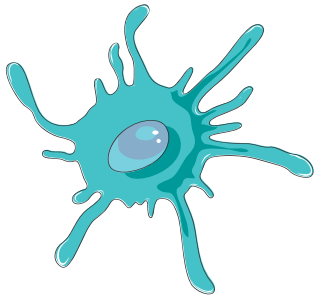


Molecule 3	4.246
Molecule 2	4.440
Molecule 4	4.906
Molecule 1	7.350
Molecule 5	12.575

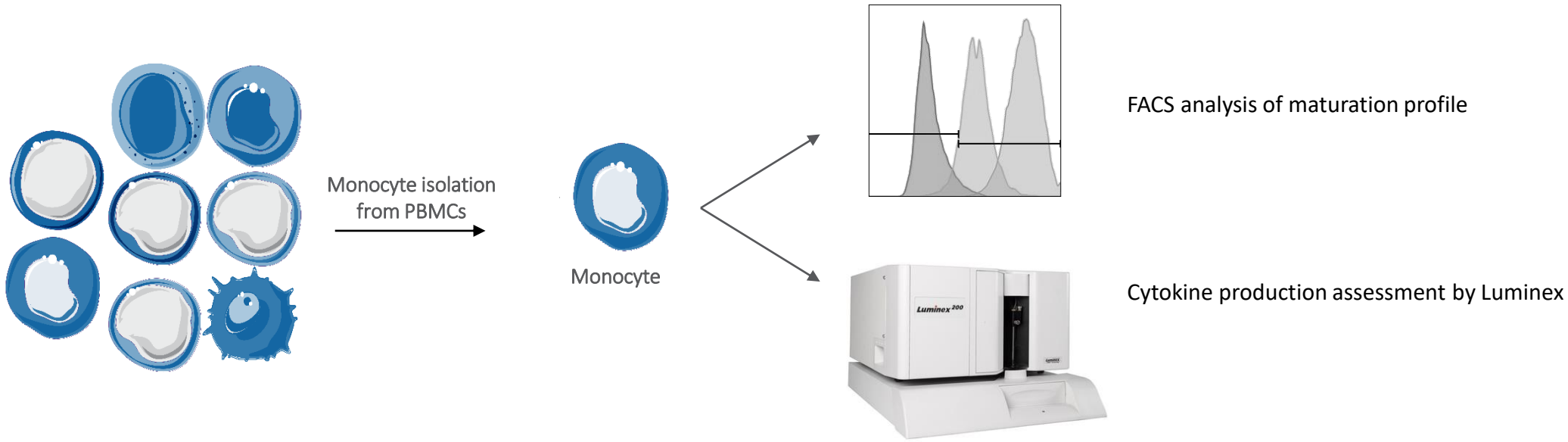


In vitro Tools: Early/Innate Assays

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

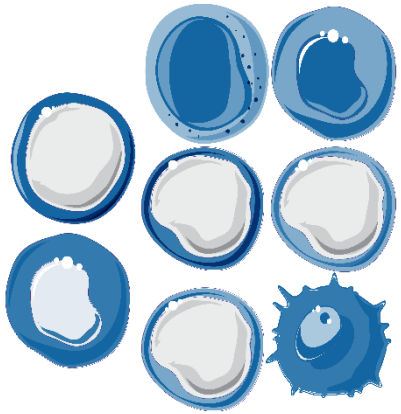


2. Monocyte Activation Assay



- PBMCs or isolation of monocytes via CD14 negative or positive selection
- *In vitro* activation with LPS or another agent
- ROs:
  - Measurement of cytokines/chemokines in the supernatant (Elisa/Luminex/HTRF)
  - Evaluation of activation/maturation markers (Flow cytometry)

3. DC Activation/Maturation Assay



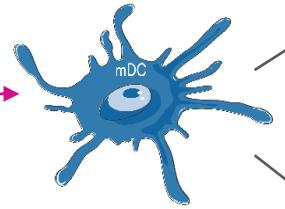
Monocyte isolation from PBMCs

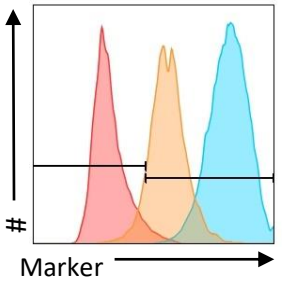
Monocyte

DC generation

+

Test Products





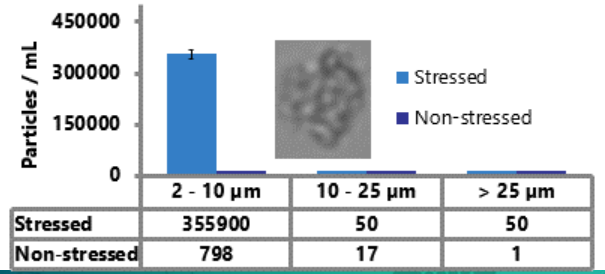
FACS analysis of the maturation profile

Cytokine production assessment by Luminex

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

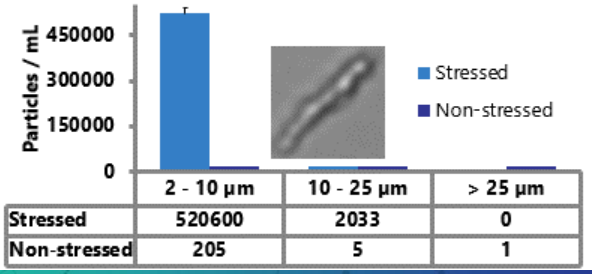
- RO:
  - Measurement of cytokines/chemokines in the supernatant (Elisa/Luminex/HTRF)
  - Evaluation of maturation markers (Flow cytometry)

Nivolumab



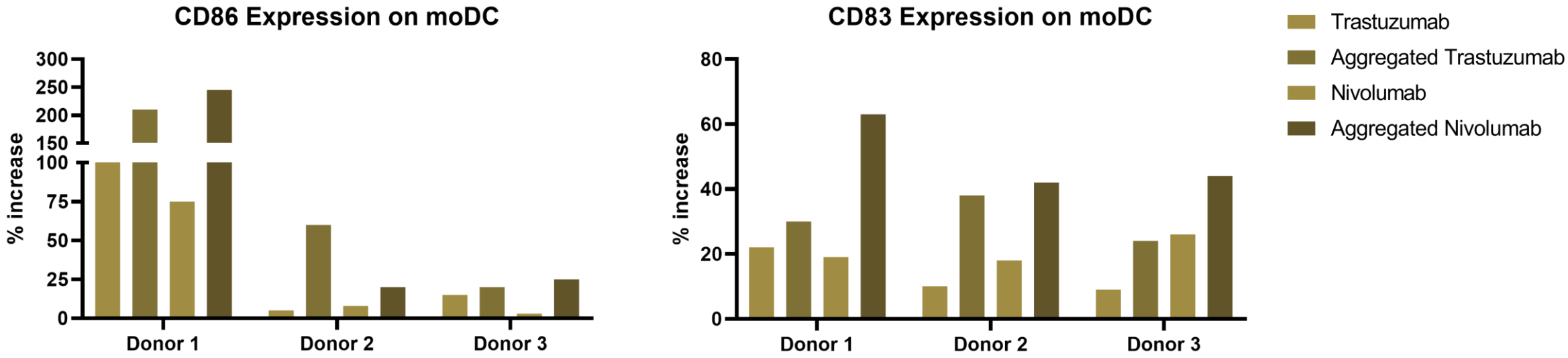
	2 - 10 µm	10 - 25 µm	> 25 µm
Stressed	355900	50	50
Non-stressed	798	17	1

Trastuzumab



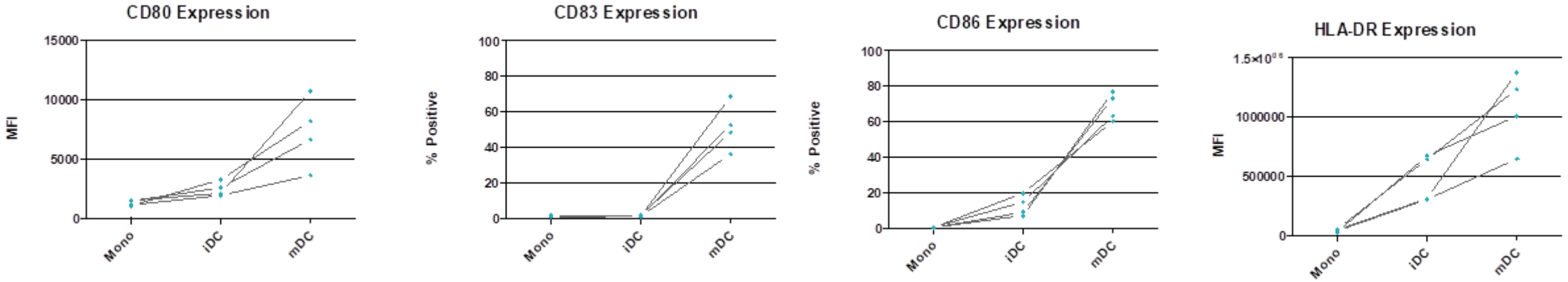
	2 - 10 µm	10 - 25 µm	> 25 µm
Stressed	520600	2033	0
Non-stressed	205	5	1

3. DC Activation/Maturation Assay



Increased expression of CD86 and CD83 upon incubation with aggregated therapeutics

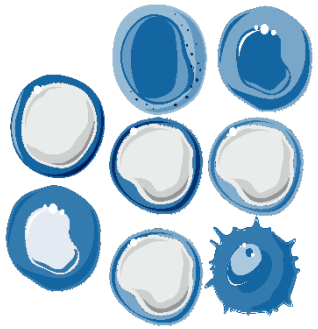
3. DC Activation/Maturation Assay



Upregulation of maturation/activation markers upon stimulation with test compounds

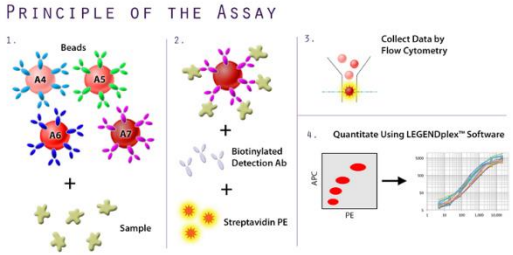
4. Cytokine Release Assay

PBMCs



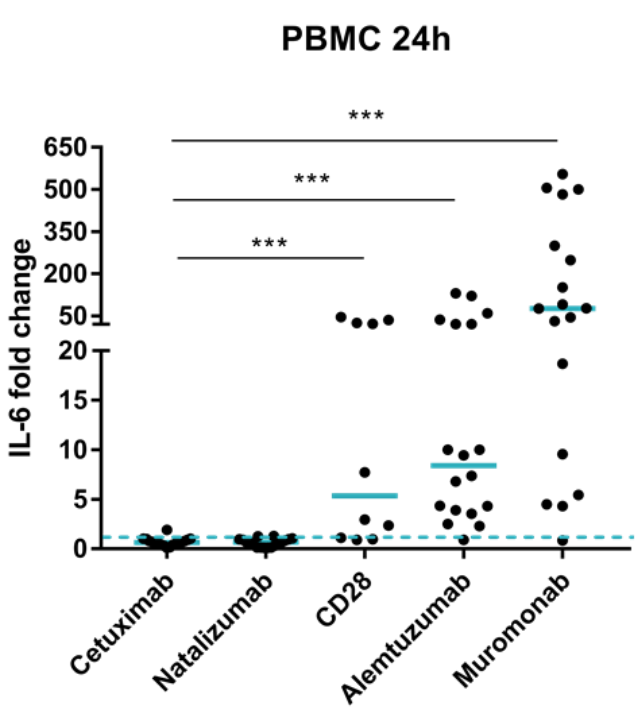
Whole blood

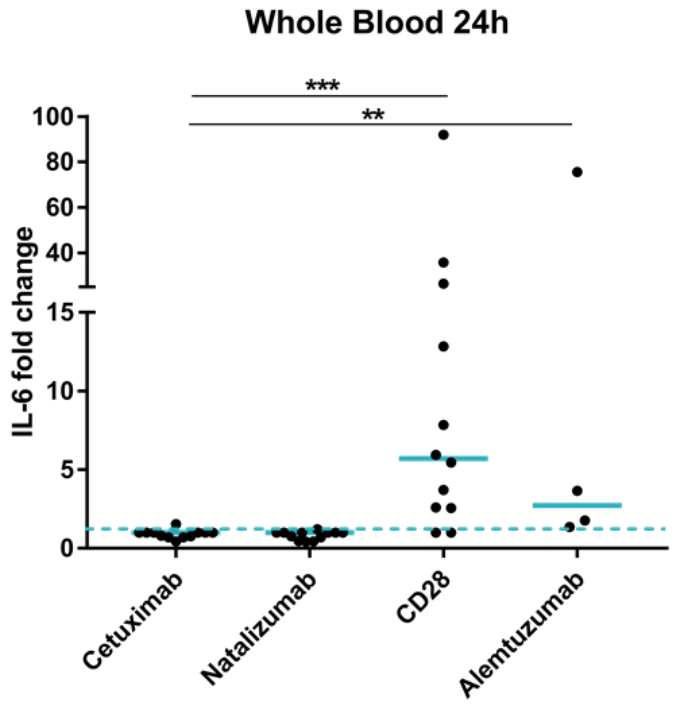
PBMCs or whole blood incubation with test products → Supernatant or plasma harvesting → Cytokine secretion analysis via Multiplex



- 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

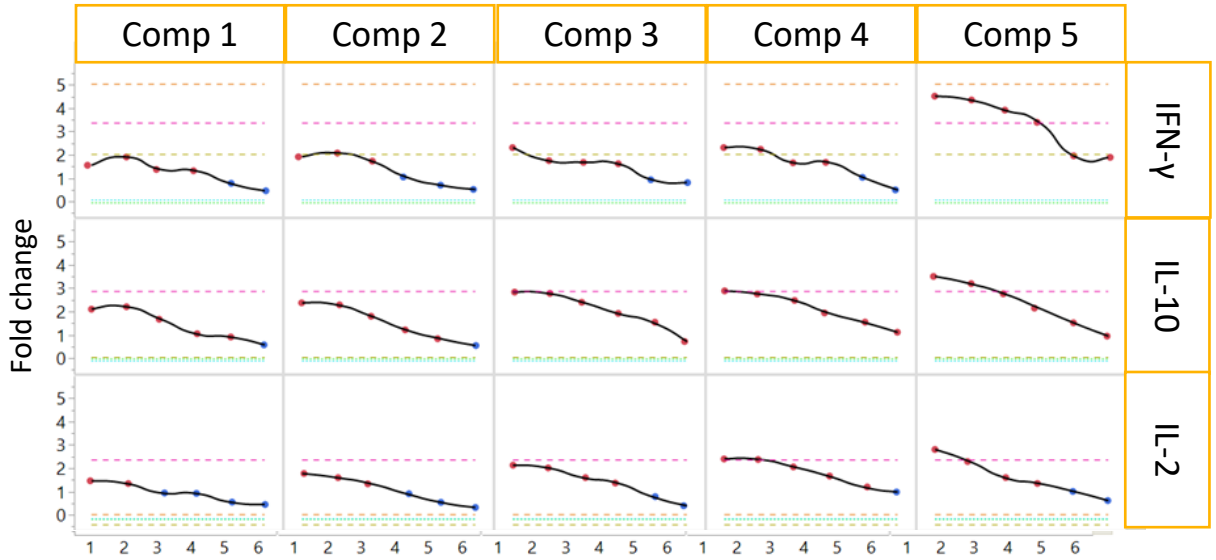
4. Cytokine Release Assay

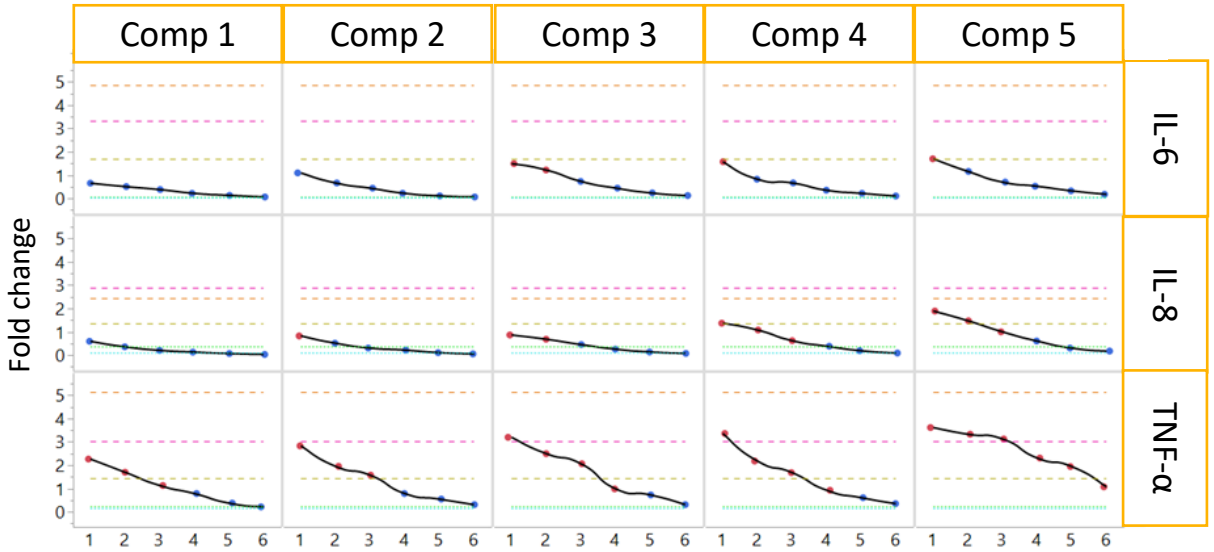


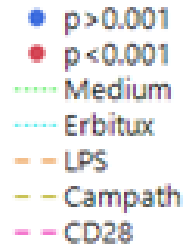


Increased secretion of IL-6 upon incubation with CD28 super-agonist and test antibodies

4. Cytokine Release Assay

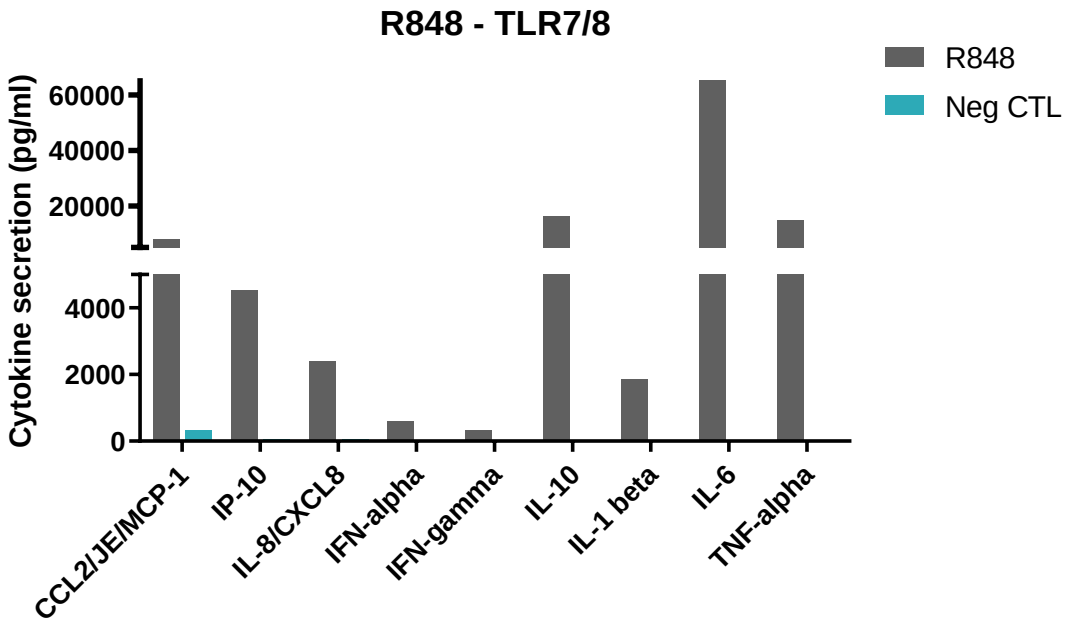


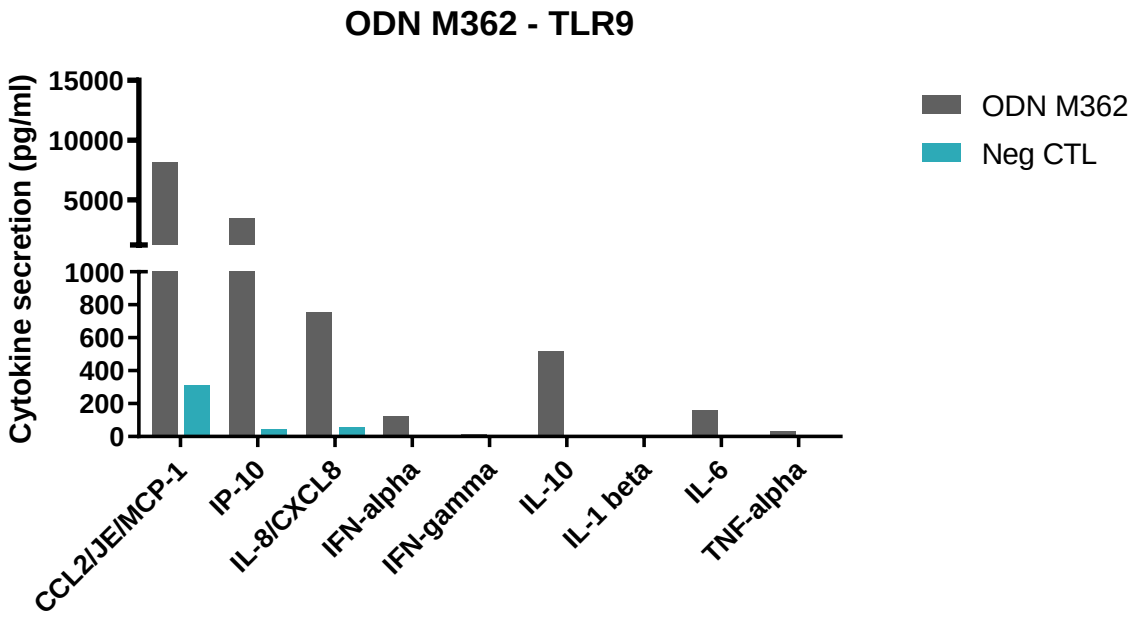




Stimulation of whole blood/PBMCs with antigens/cytokines, evaluation of test compounds

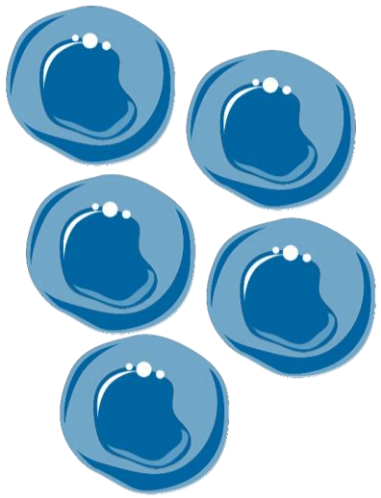
4. Cytokine Release Assay





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

5. Reporter Cell line Assay



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

Test product addition

Luciferase reporter

Luminescence analysis

SEAP reporter

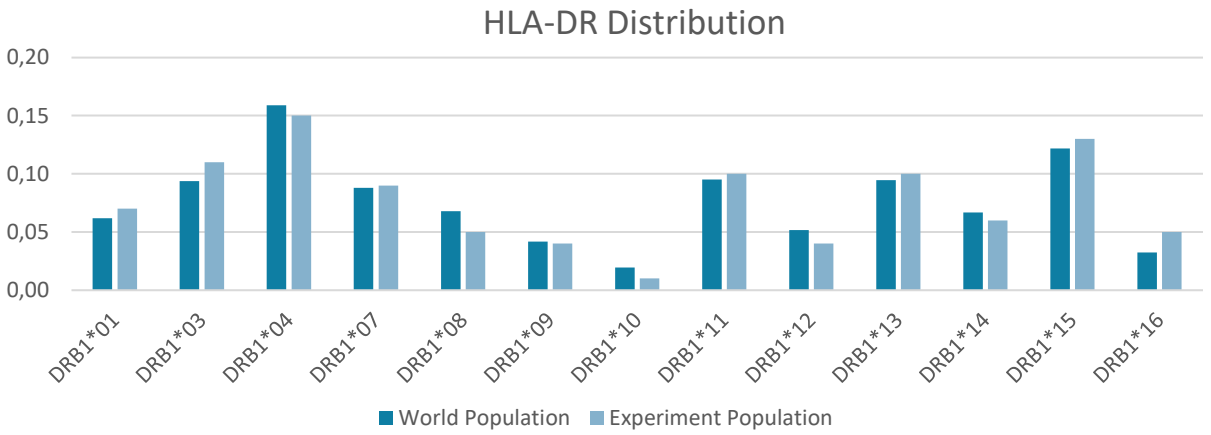
Absorbance analysis

- 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

Importance of Donor Selection



PBMCs = critical reagent

Need for high quality and functionality

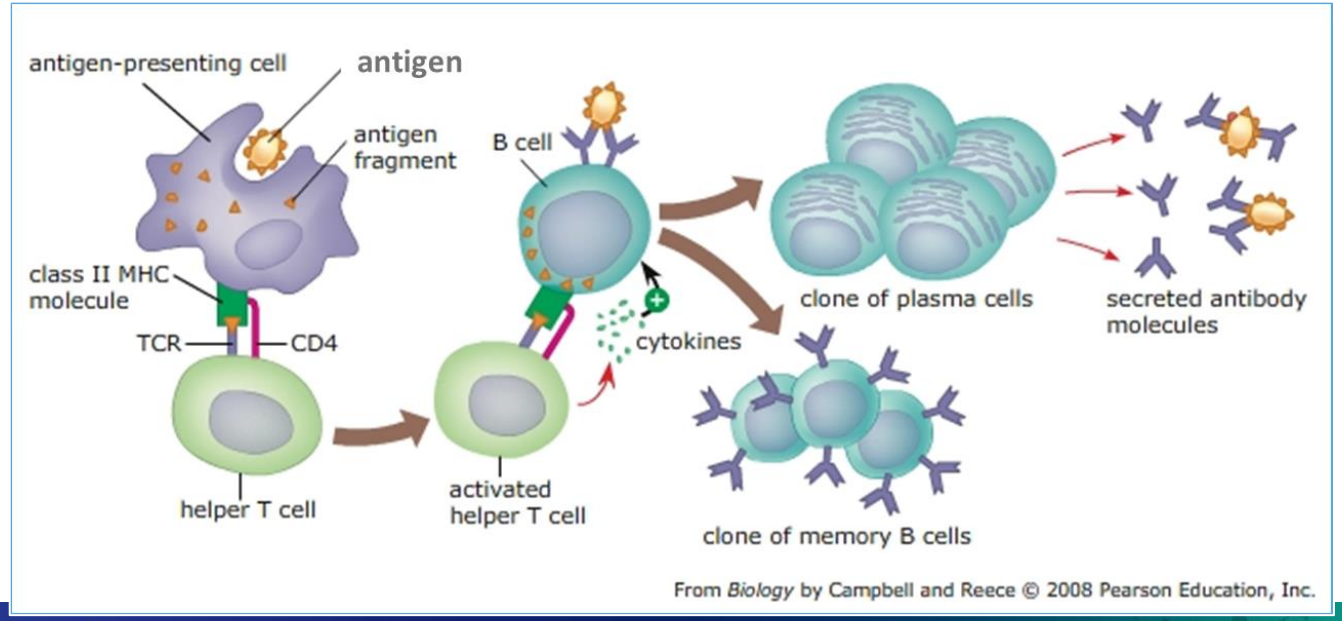
4-digit HLA-typed donor samples

Plus 1000 cell preparations

Immunogenicity

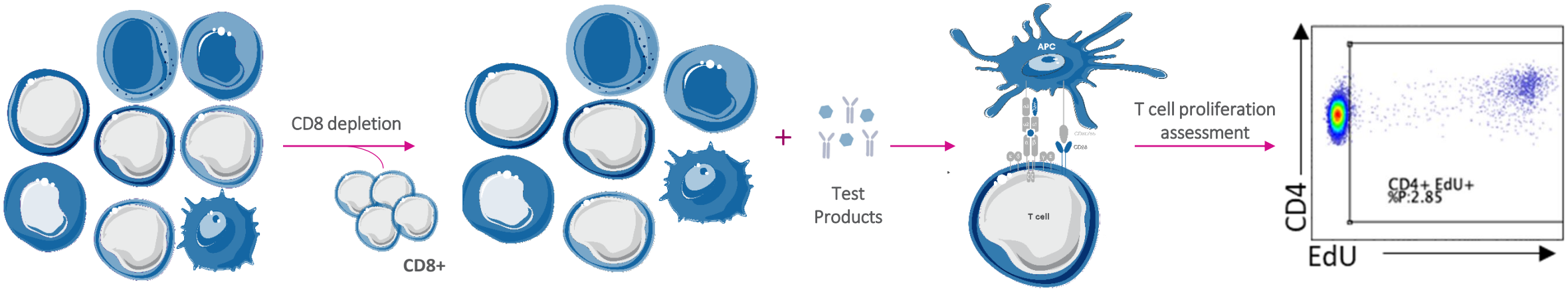
"The ability of a particular substance, such as an antigen or epitope, to induce an immune response"

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).



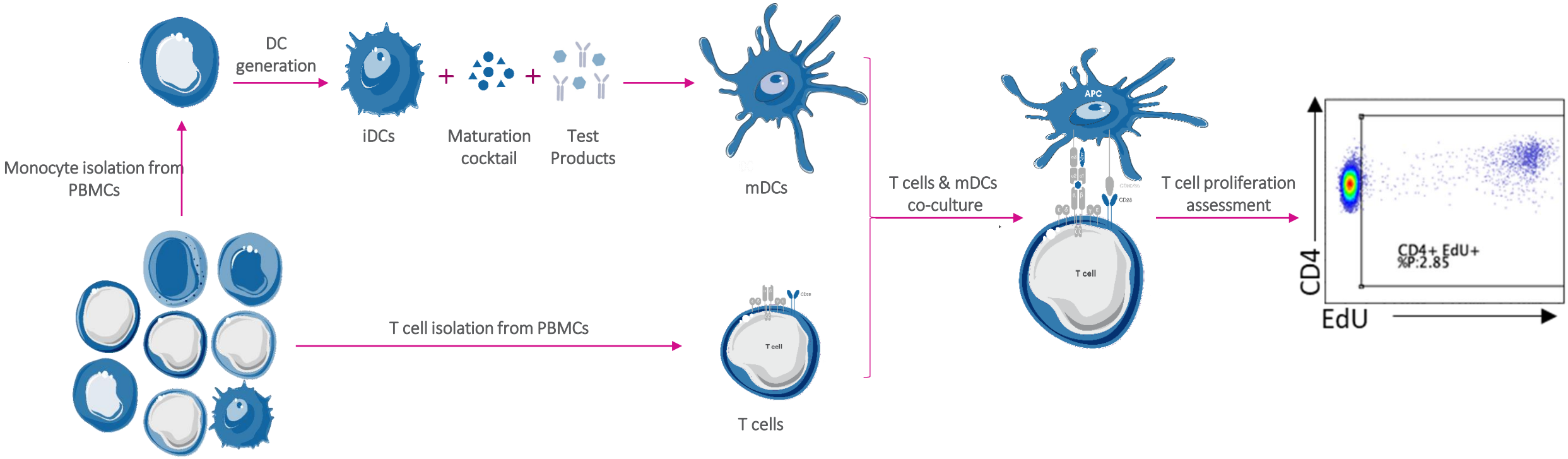
UNWANTED	
Therapeutic proteins	**CGT Products**
Production of antidrug- antibodies (ADAs), possibly neutralising the therapeutic effects of the treatment and, in rare cases, inducing adverse effects.	Cellular and humoral responses Anti HLA antibodies Immune rejections Potential safety effects

6. CD8-depleted PBMC Assay



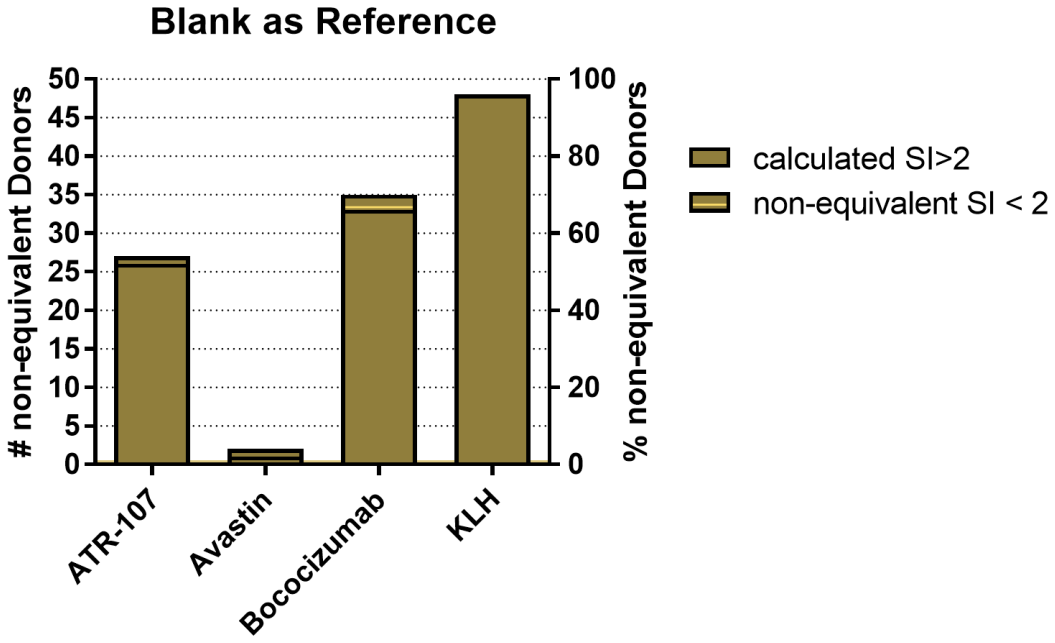
- T cell activation and proliferation assays to assess and compare the immunogenicity potential of test molecules
- Format depends on the nature and function of the test products:
  - The CD8-depleted PBMC format is used for test products with non-immuno-modulatory functions

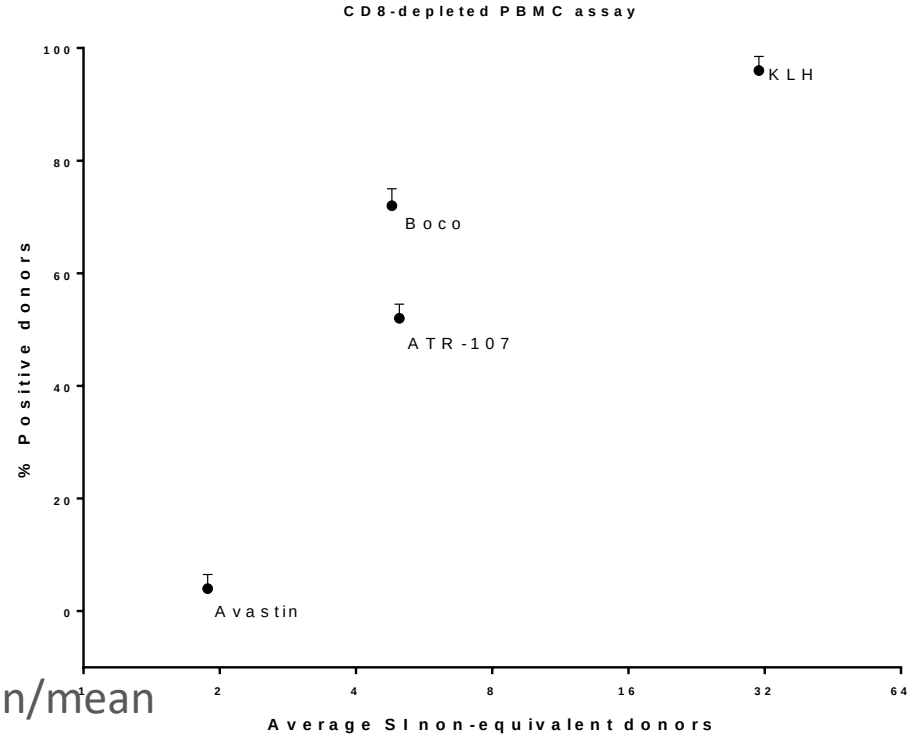
7. DC-T Cell Assay



- 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

In vitro tools – T Cell Assays: Outcome

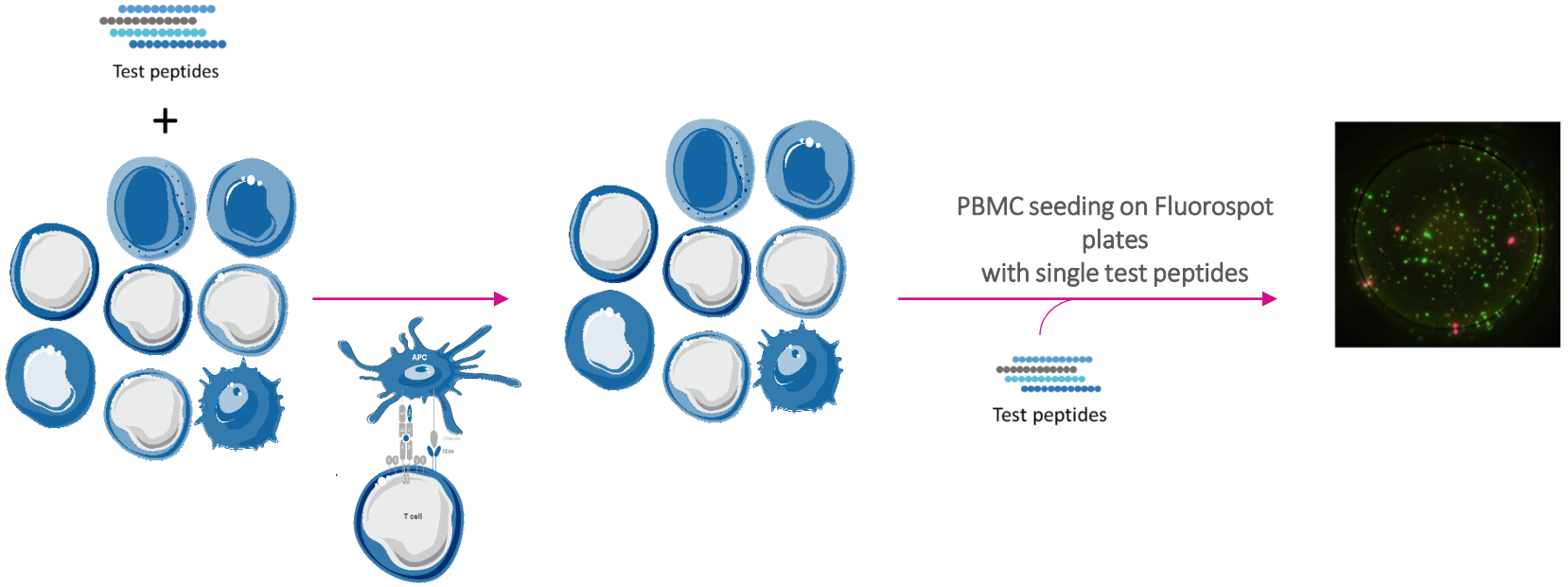




- Per donor, a stimulation index (mean response in test condition/mean response in blank condition) is calculated.
- All reactions with a calculated SI > 2 are considered positive.

8. Peptide Assay

Sample PBMCs from test population



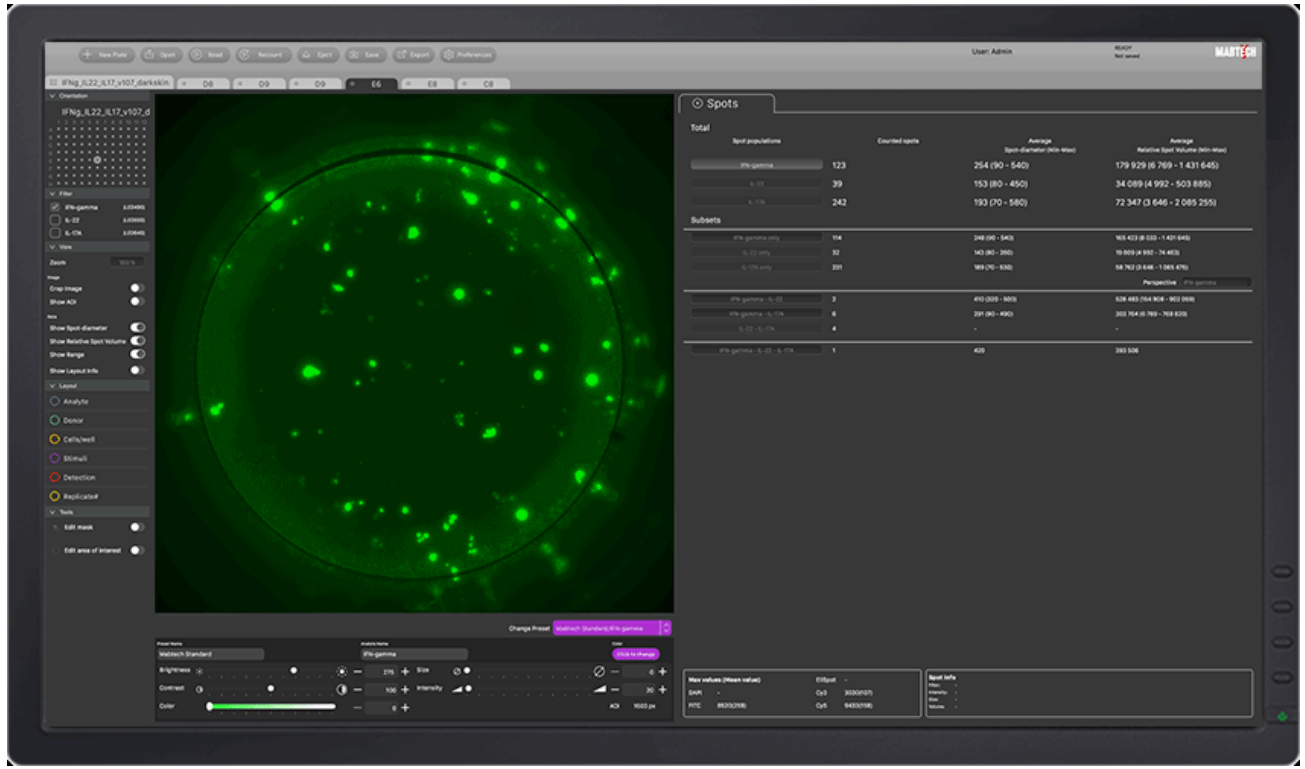
- PBMC priming with test peptides
- PBMC re-stimulation with test peptides (pool vs. individual)
- RO:
  - Measurement of cytokines/chemokines (EliSpot/FluoroSpot)

8. Peptide Assay

- Example data peptide screening (Infliximab peptides)

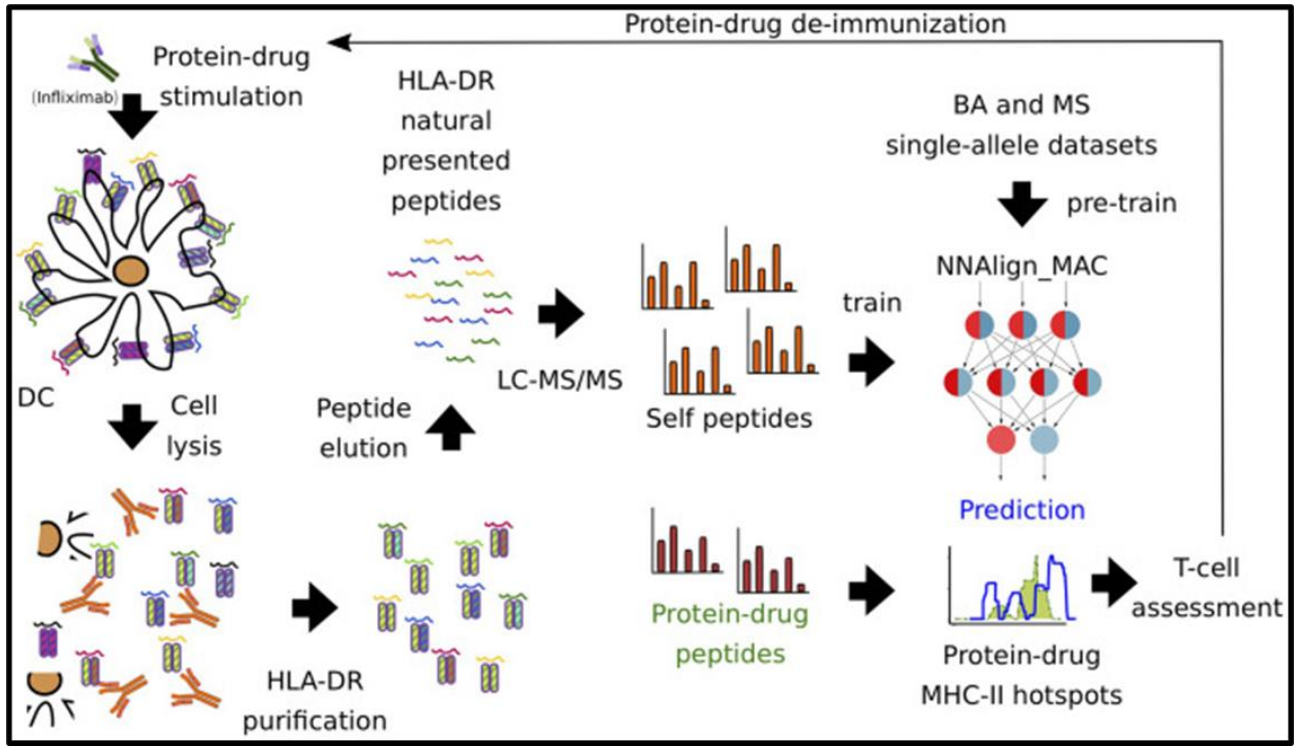
MABTECH
Capture | Detect | Discover

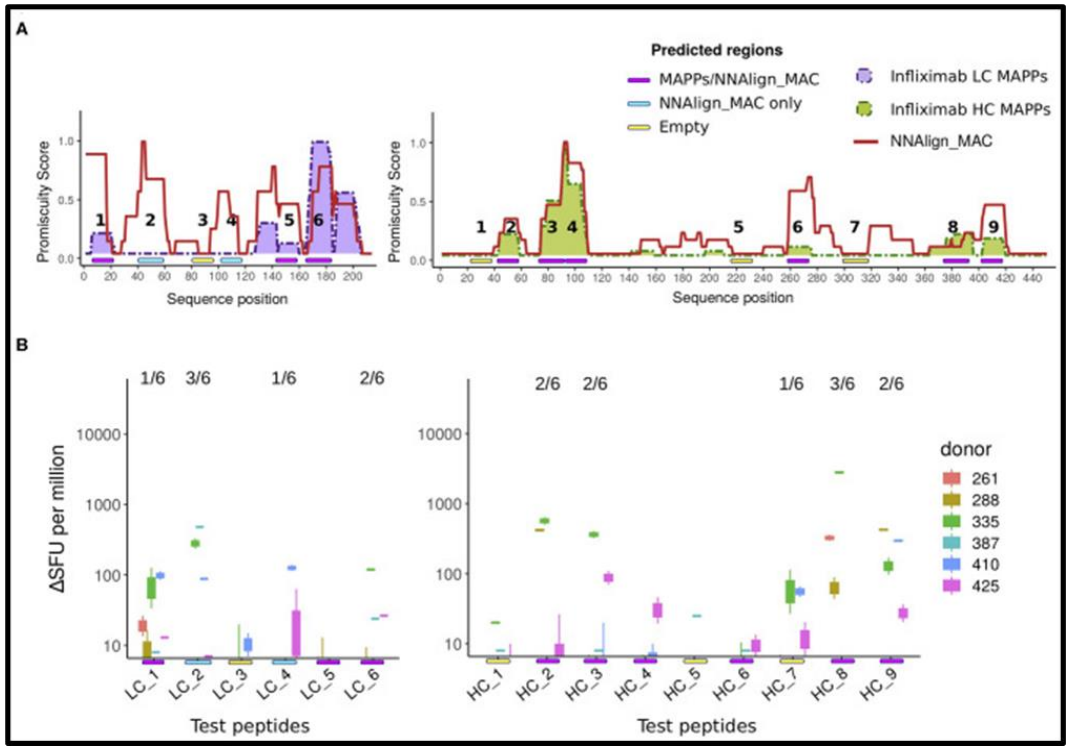
IRIS™



8. Peptide Assay

- Example data peptide screening (Infliximab peptides)

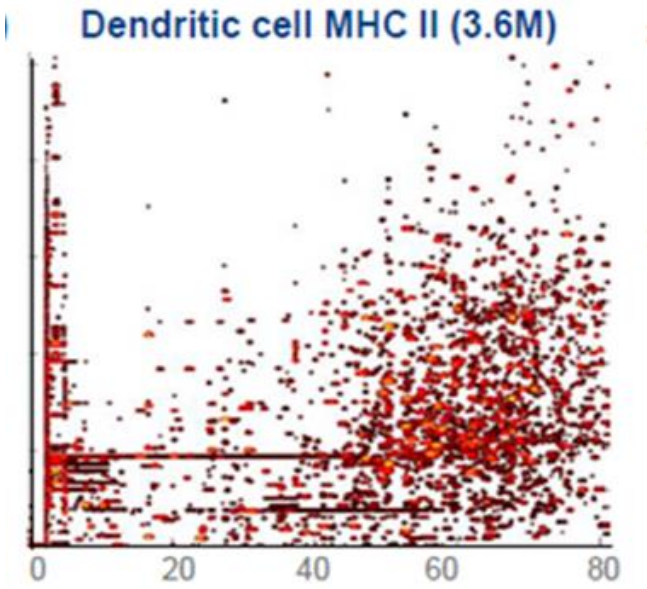




Immunopeptidomic Data Integration to Artificial Neural Networks Enhances Protein-Drug Immunogenicity Prediction
Front Immunol. 2020 Jun 23;11:1304. doi: 10.3389/fimmu.2020.01304. eCollection 2020.

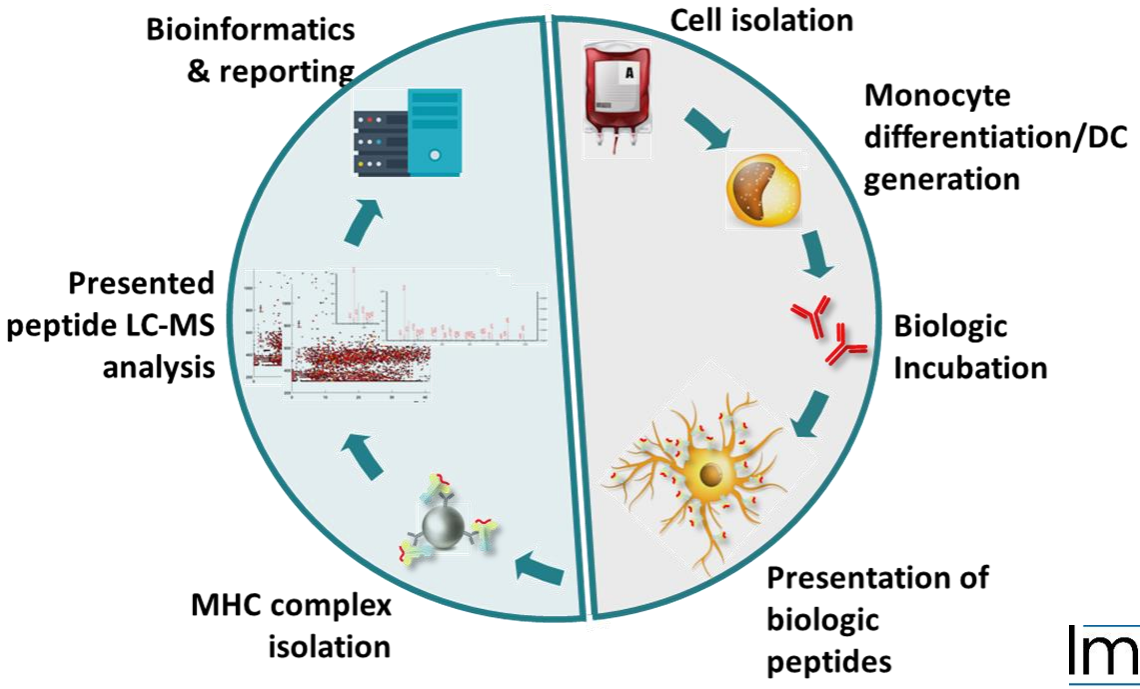
In vitro MAPPs Assay

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



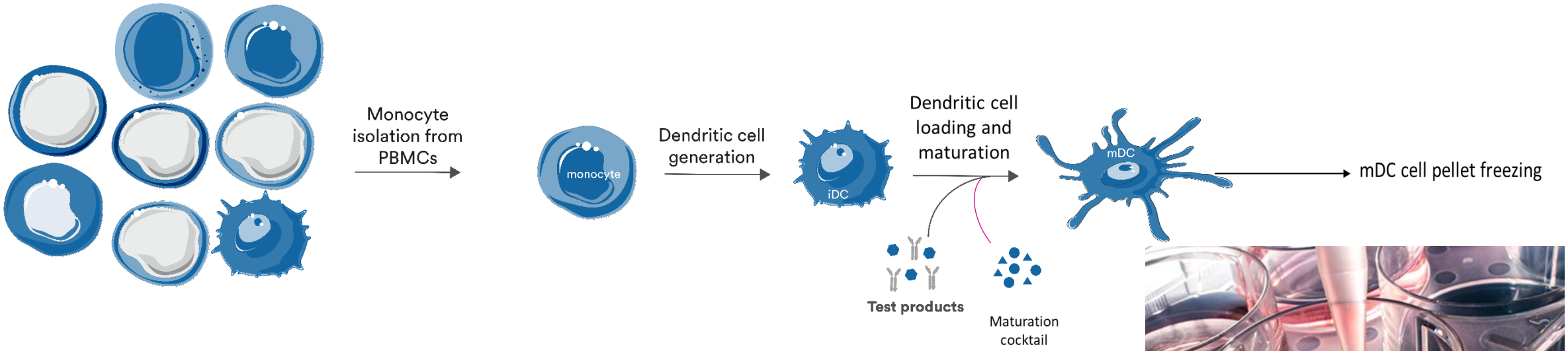
9. MAPPS Assay

PARTNER

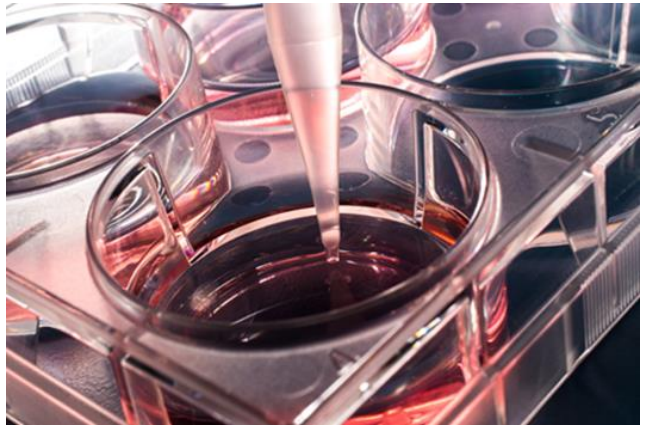


ImmunXperts
a Q² Solutions Company

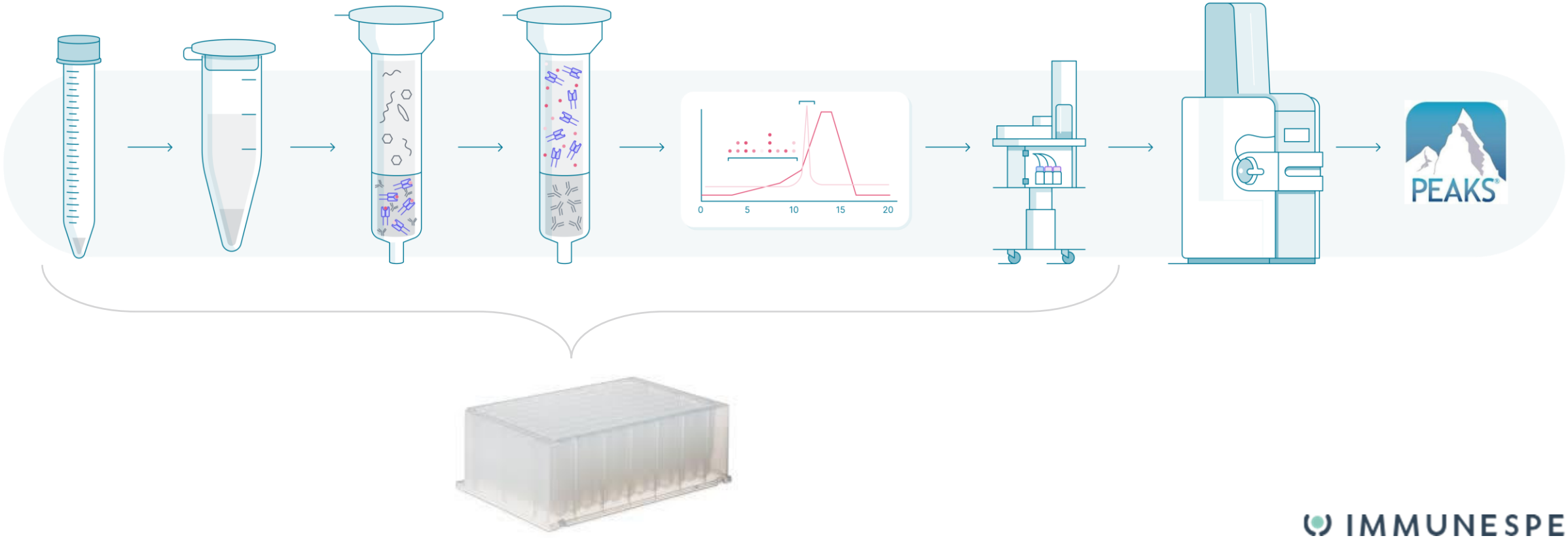
9. MAPPS Assay – IMXP



- Generation of immature dendritic cells
- Incubation with test products and maturation agent
- mDC cell pellet freezing

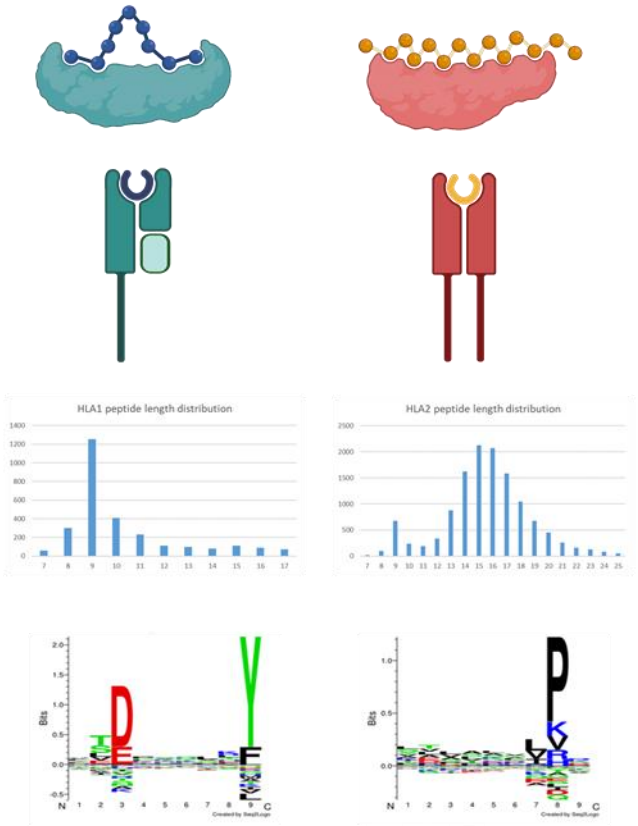


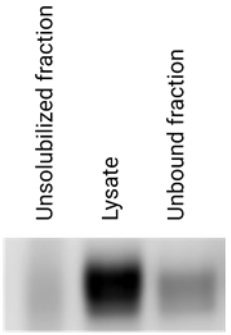
MAPPs Assay: Isolation and identification of Presented Peptides



IMMUNESPEC

MAPPs Assay: Identification of Presented Peptides



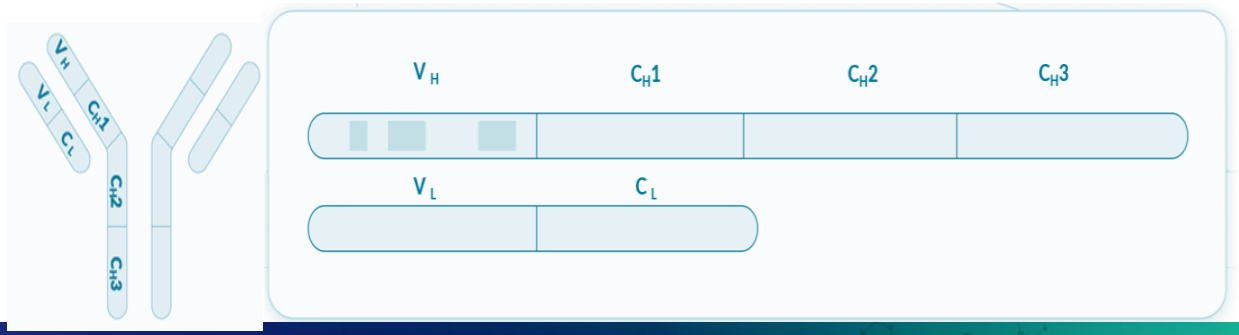


- Peptide size distribution
- Major peptide motifs
- Western Blot
- List of identified immune peptides:
  - Peptide sequence
  - Peak areas
  - Parent protein ID
- For test product:
  - Heat map of identified peptides
  - Distribution of identified peptides

MAPPs Assay: case study ATR-107

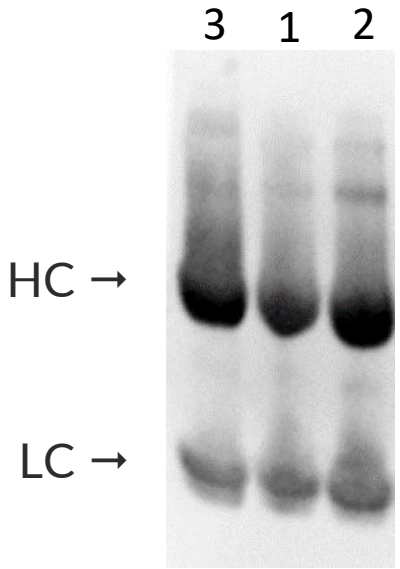
Evaluation of 3 different batches of ATR-107 at 3 different concentrations (control Bet V1), 3 donors

Name	Batch	Stock concentration	Test concentration
ATR-107	Batch 1	1 mg/ml	50, 25 and 5 µg/ml
ATR-107	Batch 2	1 mg/ml	50, 25 and 5 µg/ml
ATR-107	Batch 3	5 mg/ml	25 and 5 µg/ml



MAPPs Assay: case study: properties ATR-107

- Western Blot



AB: Goat pAB α-human IgG/IgM/IgA-HRP

- Protein concentration stock solution ATR-107

↺ 20 000g

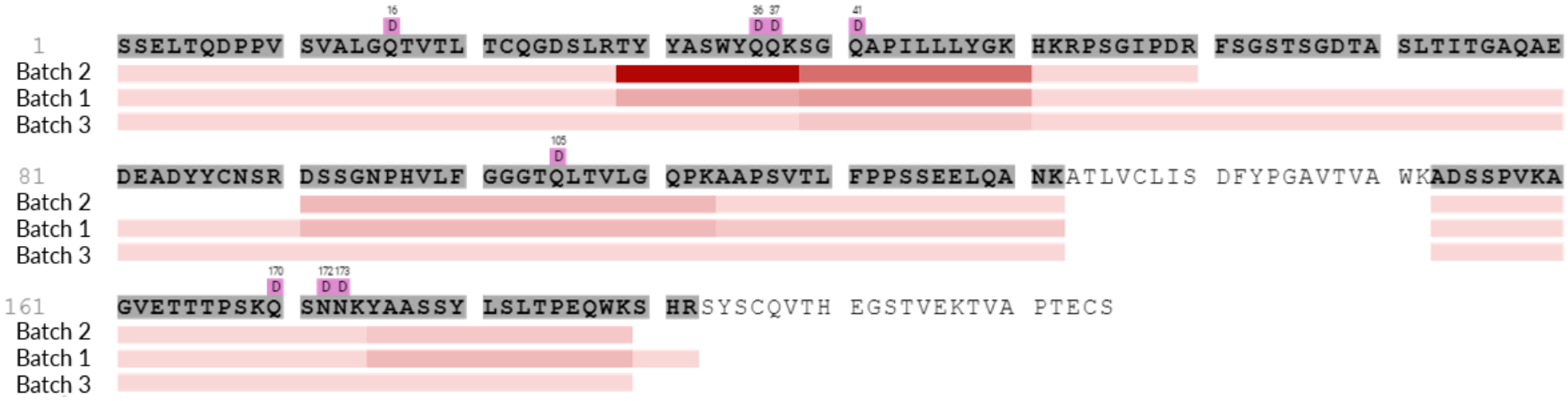
Batch 2	6.08 mg/mL	→	5.95 mg/mL
Batch 1	1.02 mg/mL	→	1.00 mg/mL
Batch 3	1.12 mg/mL	→	1.12 mg/mL

MAPPs Assay: case study: properties ATR-107

ATR-107 – Heavy Chain: Heat Map - Tryptic digest (50 ng ATR-107 – TimsTOF – SCP)

IMMUNESPEC

MAPPs Assay: case study: properties ATR-107



ATR-107 – Light Chain: Heat Map - Tryptic digest (50 ng ATR-107 – TimsTOF – SCP)

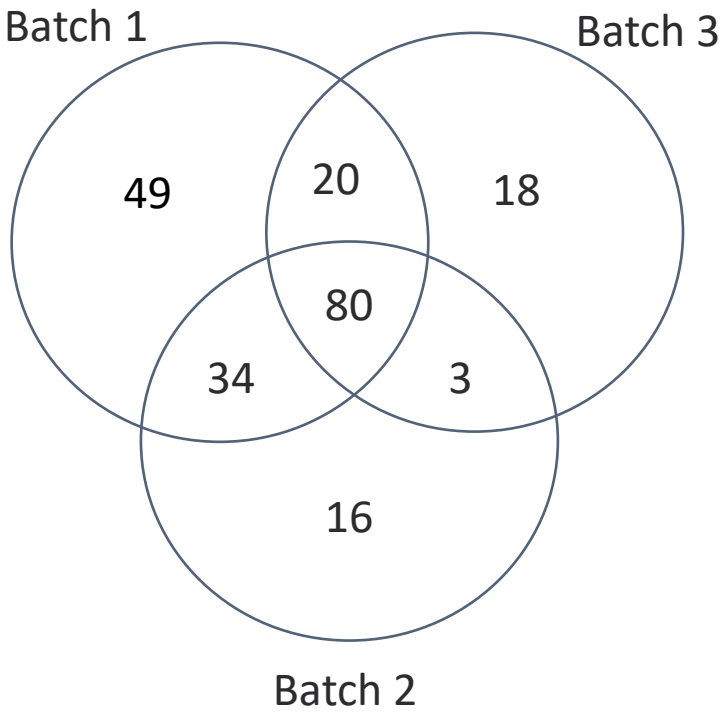
MAPPs Assay: case study: properties ATR-107

Total # protein groups identified in samples different batches:

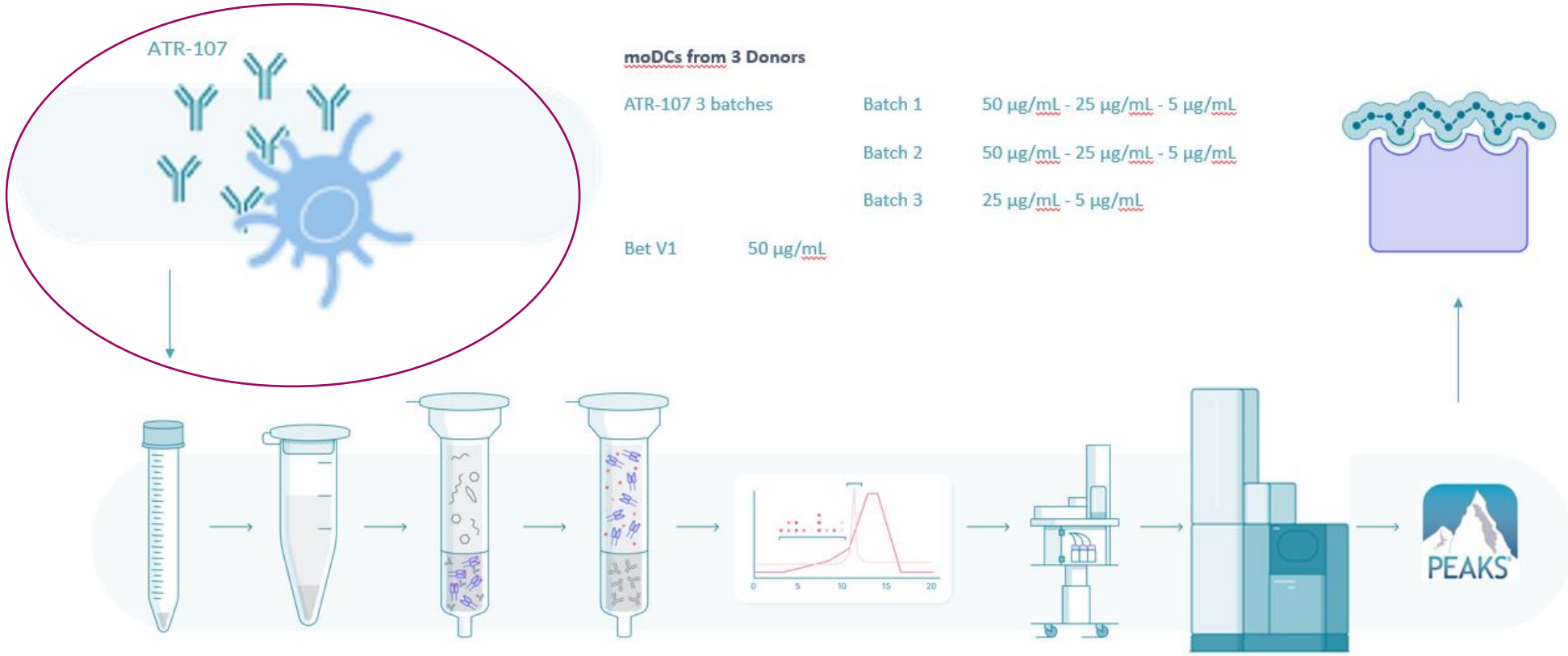
ATR-107 Batch 1 #184

ATR-107 Batch 2 #141

ATR-107 Batch 3 #121

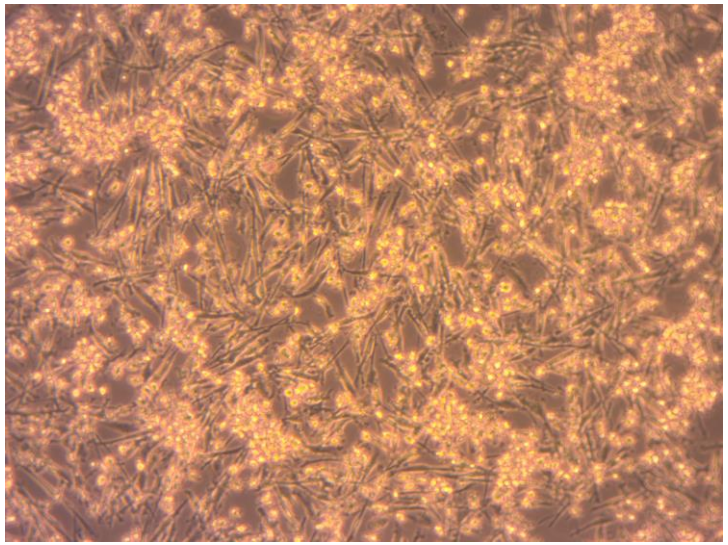


MAPPs Assay: case study: overview

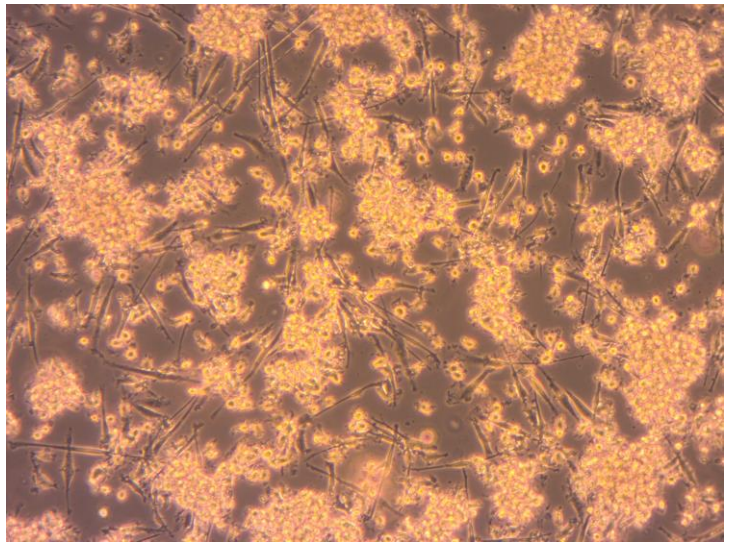


IMMUNESPEC

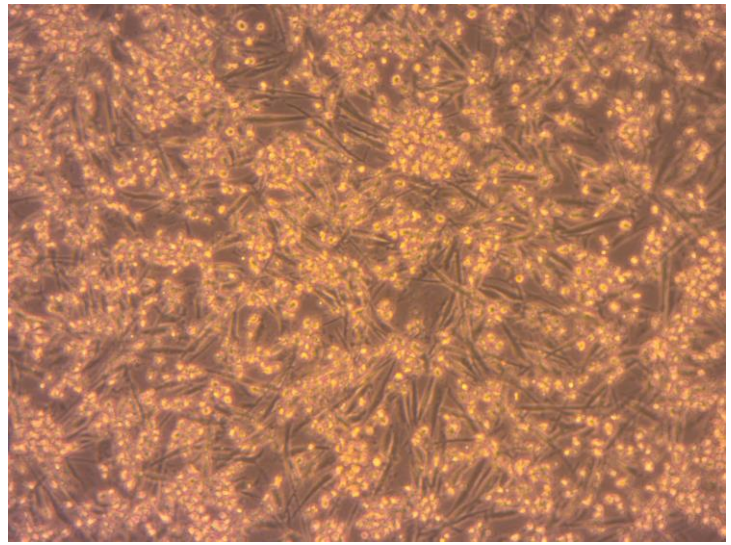
MAPPs Assay: case study ATR-107: mDC



mDC Donor 1

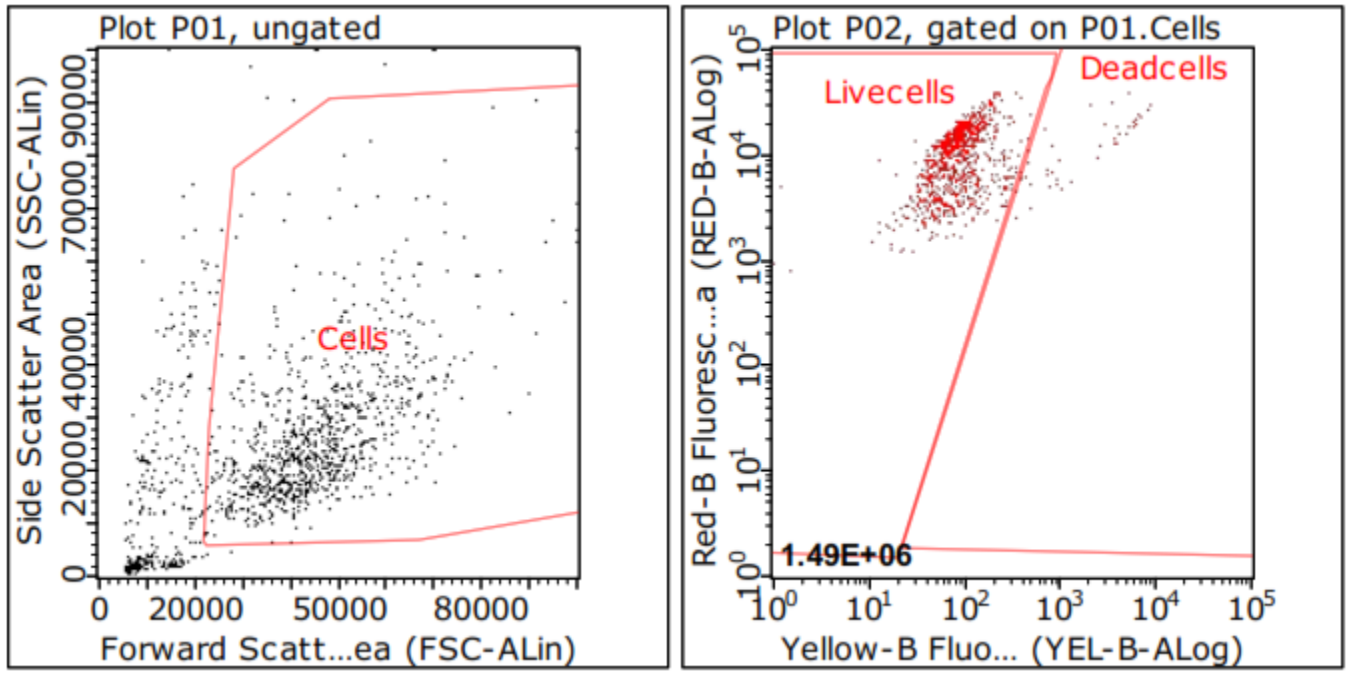


mDC Donor 2



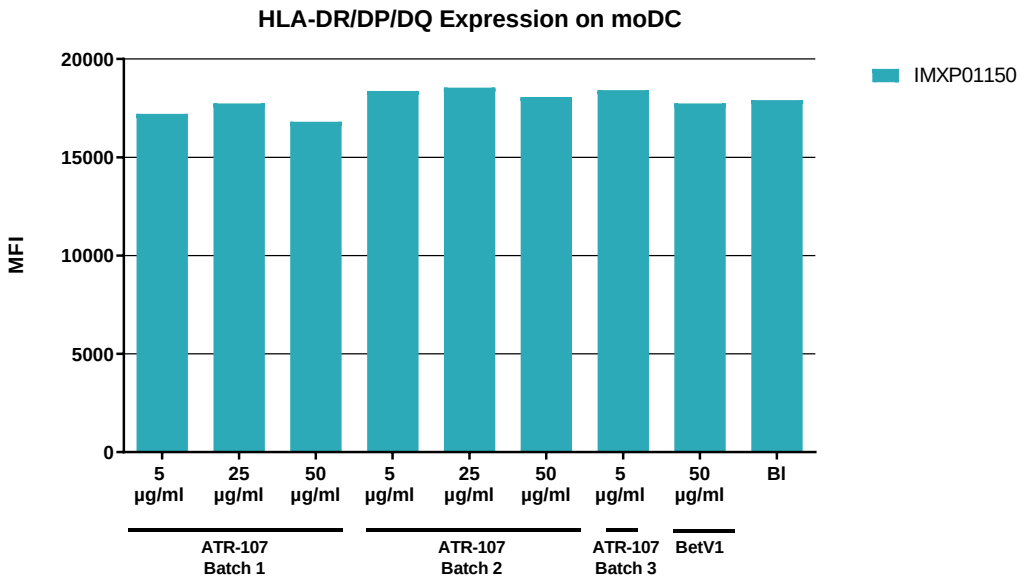
mDC Donor 3

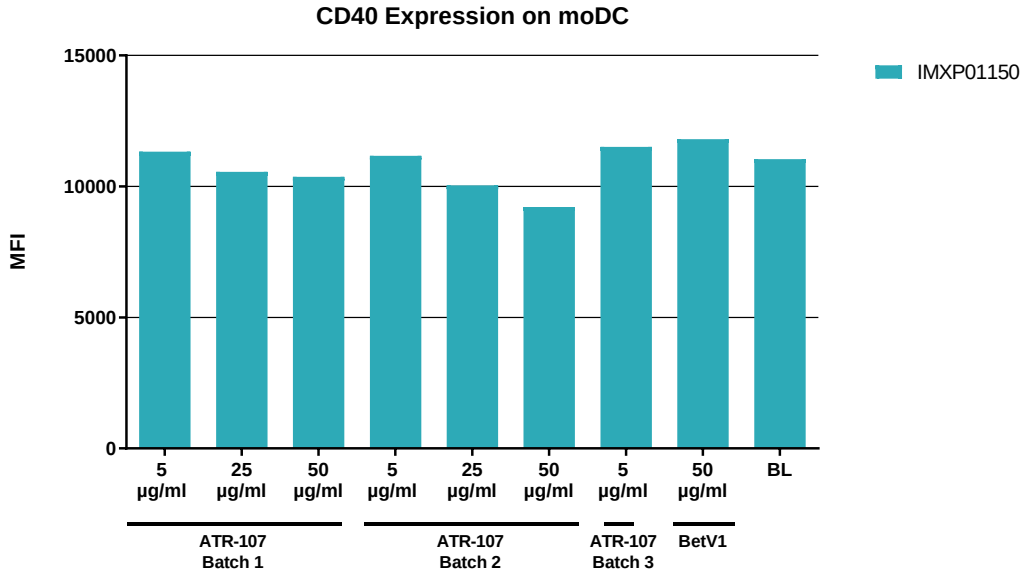
MAPPs Assay: case study ATR-107: mDC QC and counts



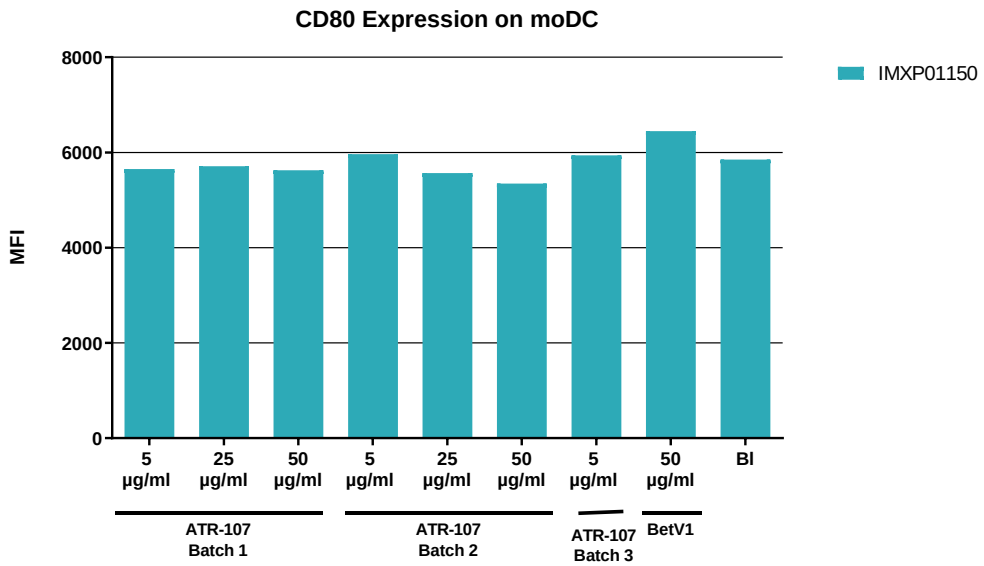
Average viability mDC: 95%

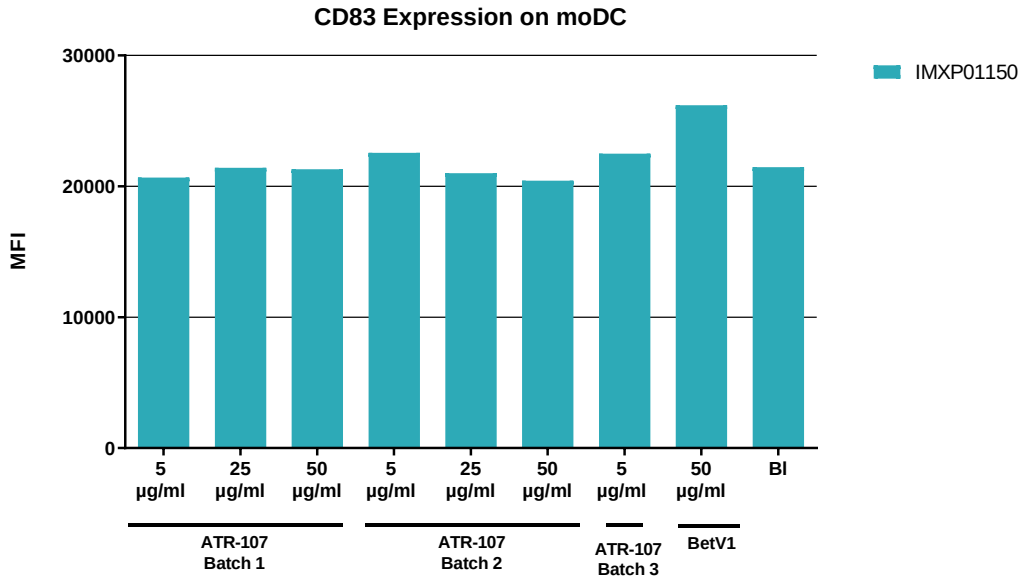
MAPPs Assay: case study ATR-107: mDC membrane marker analysis



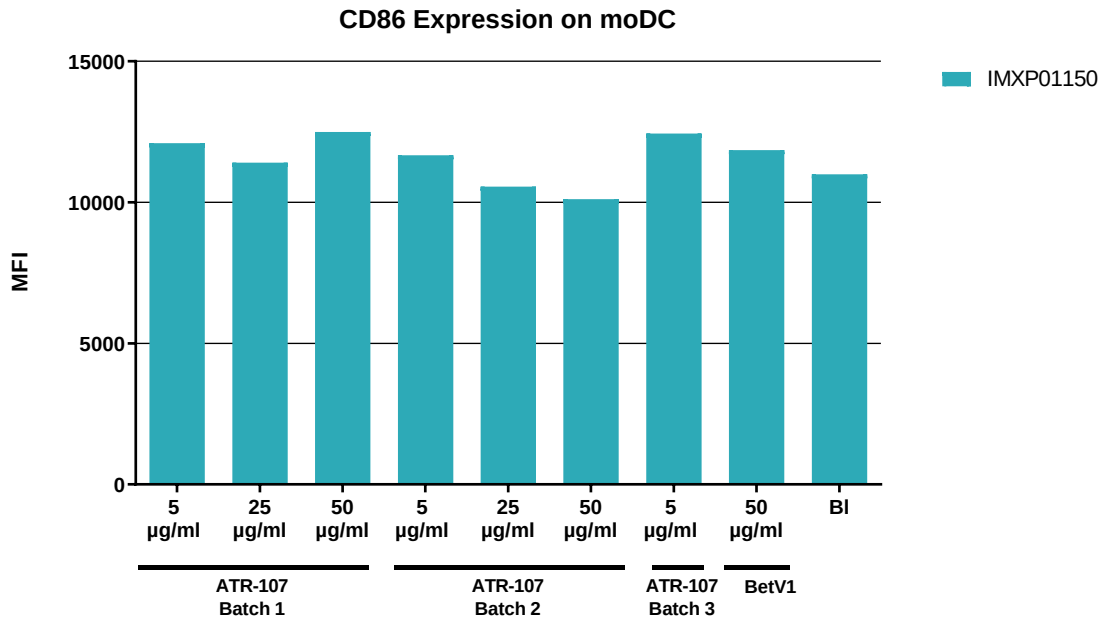


MAPPs Assay: case study ATR-107: mDC membrane marker analysis

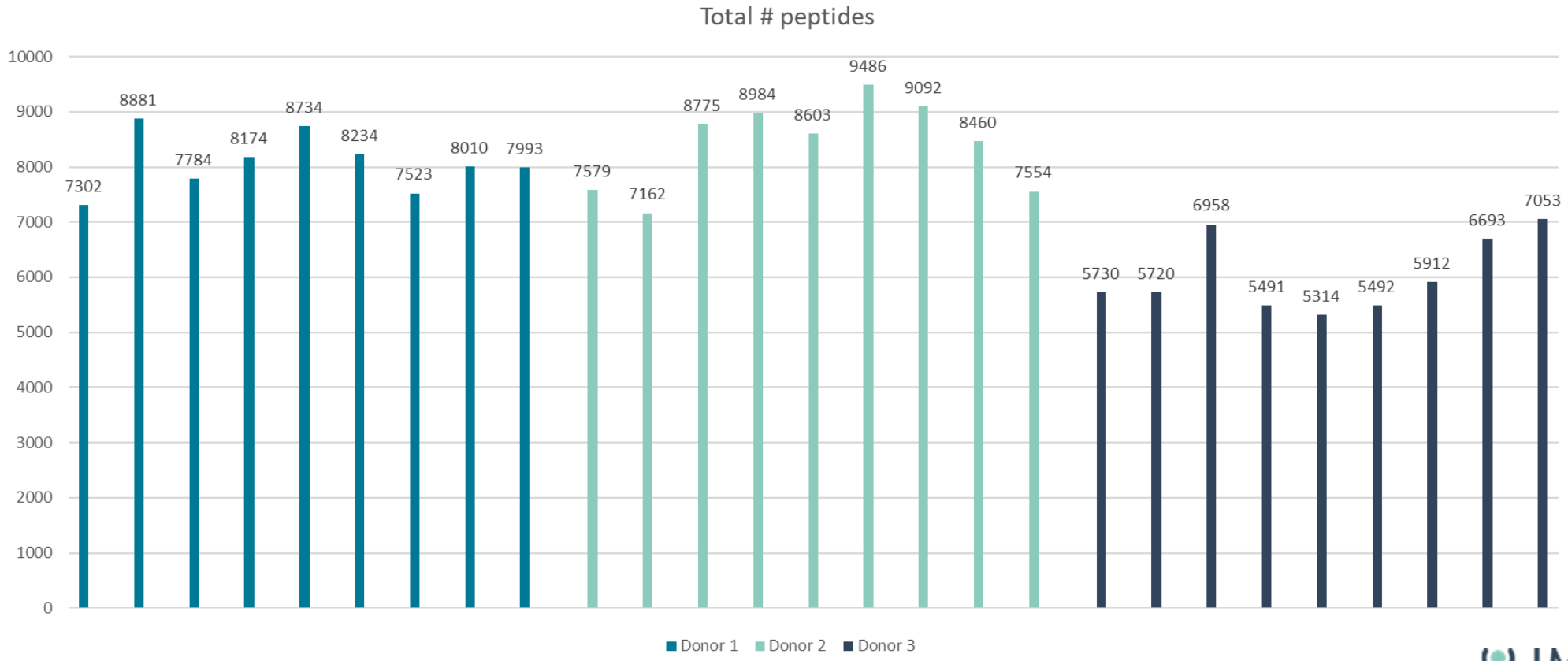




MAPPs Assay: case study ATR-107: mDC membrane marker analysis



MAPPs Assay: case study ATR-107: total #nr of identifications



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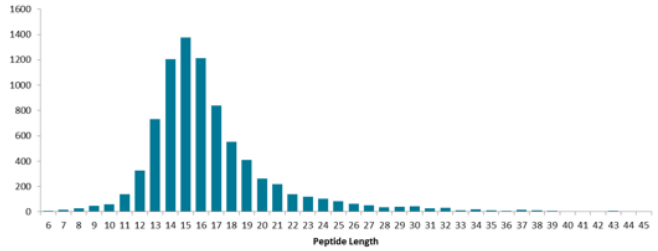
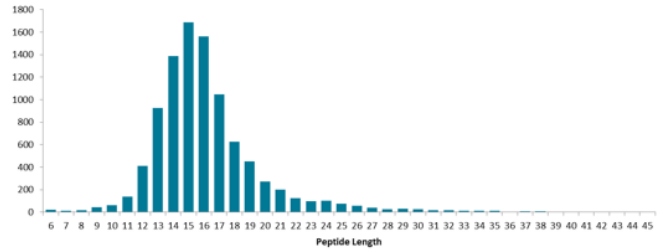
MAPPs Assay: case study ATR-107: QC Size distribution

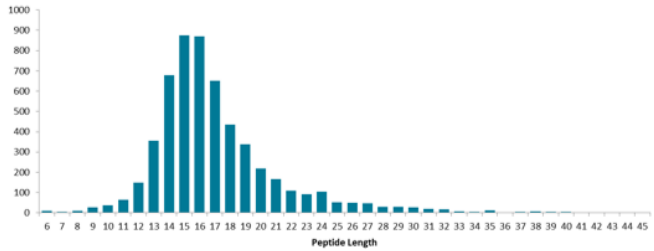
Donor 1
ATR 107 Batch 2 25 µg



Donor 2
ATR 107 Batch 2 25 µg



Donor 3
ATR 107 Batch 2 25 µg



	# Peptides total
D1 - B2	8234
D2 - B2	9486
D3 - B2	5492

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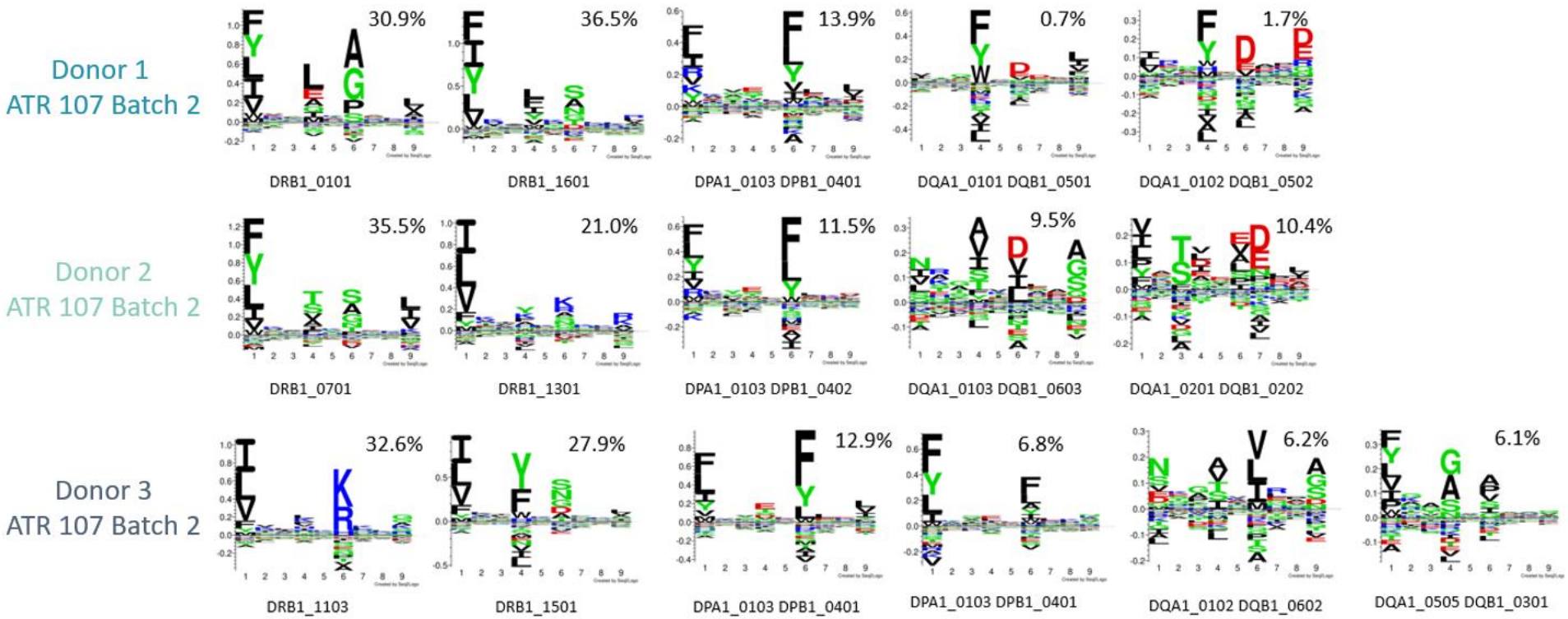
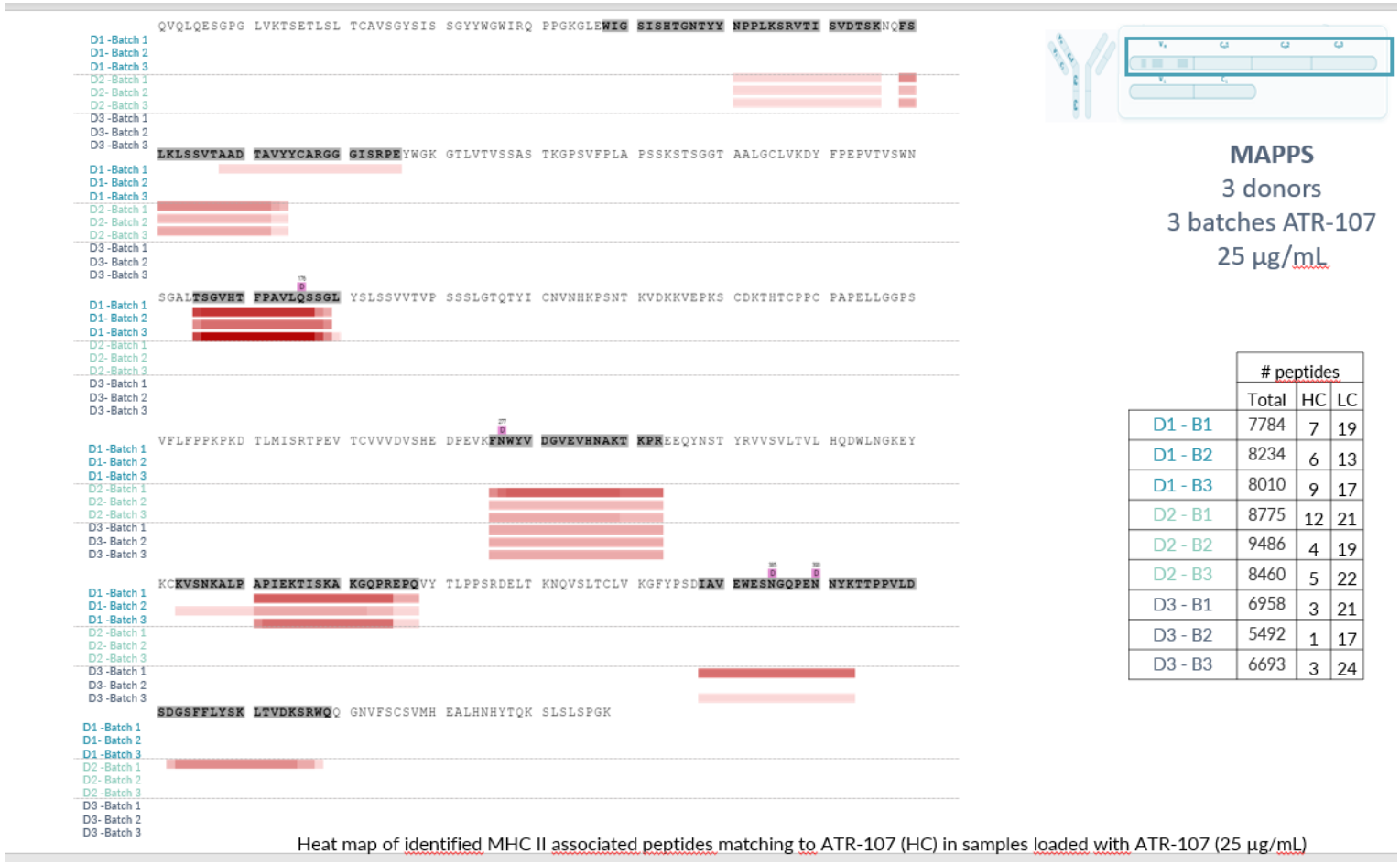
MAPPs Assay: case study ATR-107: QC – MHC Motif Decon Tool



MHC Motif Decon tool – Morten Nielsen
Kaabinejadian et al, 2022

MAPPs Assay: case study ATR-107: #nr of specific identifications per batch/donor (25 µg/ml) HC



MAPPS
3 donors
3 batches ATR-107
25 µg/mL

	# peptides		
	Total	HC	LC
D1 - B1	7784	7	19
D1 - B2	8234	6	13
D1 - B3	8010	9	17
D2 - B1	8775	12	21
D2 - B2	9486	4	19
D2 - B3	8460	5	22
D3 - B1	6958	3	21
D3 - B2	5492	1	17
D3 - B3	6693	3	24

Heat map of identified MHC II associated peptides matching to ATR-107 (HC) in samples loaded with ATR-107 (25 µg/mL)

IMMUNESPEC

MAPPs Assay: case study ATR-107: #nr of specific identifications per batch/donor (25 µg/ml) LC

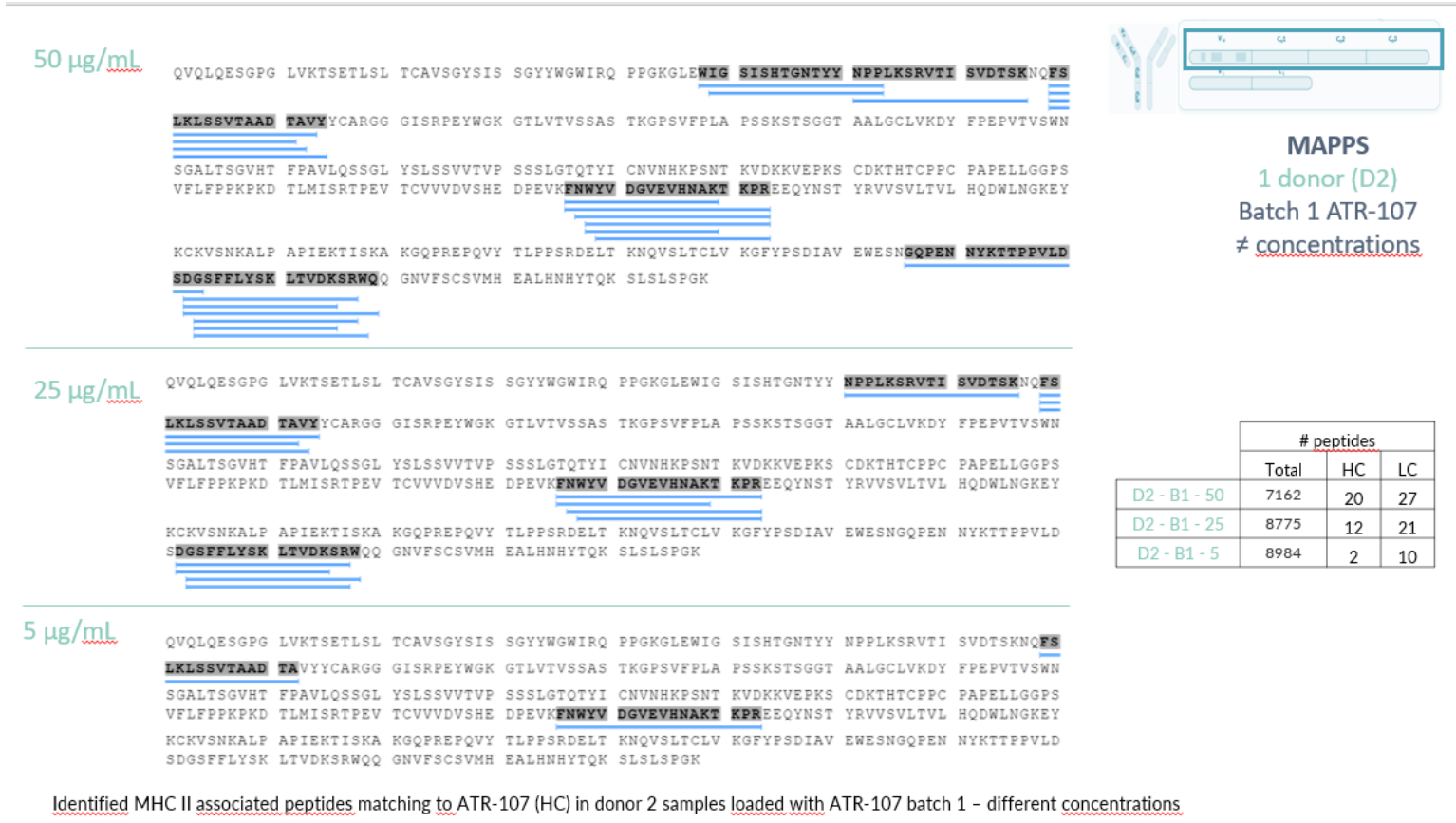
MAPPS
3 donors
3 batches ATR-107
25 µg/mL

	# peptides		
	Total	HC	LC
D1 - B1	7784	7	19
D1 - B2	8234	6	13
D1 - B3	8010	9	17
D2 - B1	8775	12	21
D2 - B2	9486	4	19
D2 - B3	8460	5	22
D3 - B1	6958	3	21
D3 - B2	5492	1	17
D3 - B3	6693	3	24

Heat map of identified MHC II associated peptides matching to ATR-107 (LC) in samples loaded with ATR-107 (25 µg/mL)

IMMUNESPEC

MAPPs Assay: case study ATR-107: #nr of specific identifications per concentration HC



	# peptides		
	Total	HC	LC
D2 - B1 - 50	7162	20	27
D2 - B1 - 25	8775	12	21
D2 - B1 - 5	8984	2	10

Identified MHC II associated peptides matching to ATR-107 (HC) in donor 2 samples loaded with ATR-107 batch 1 – different concentrations

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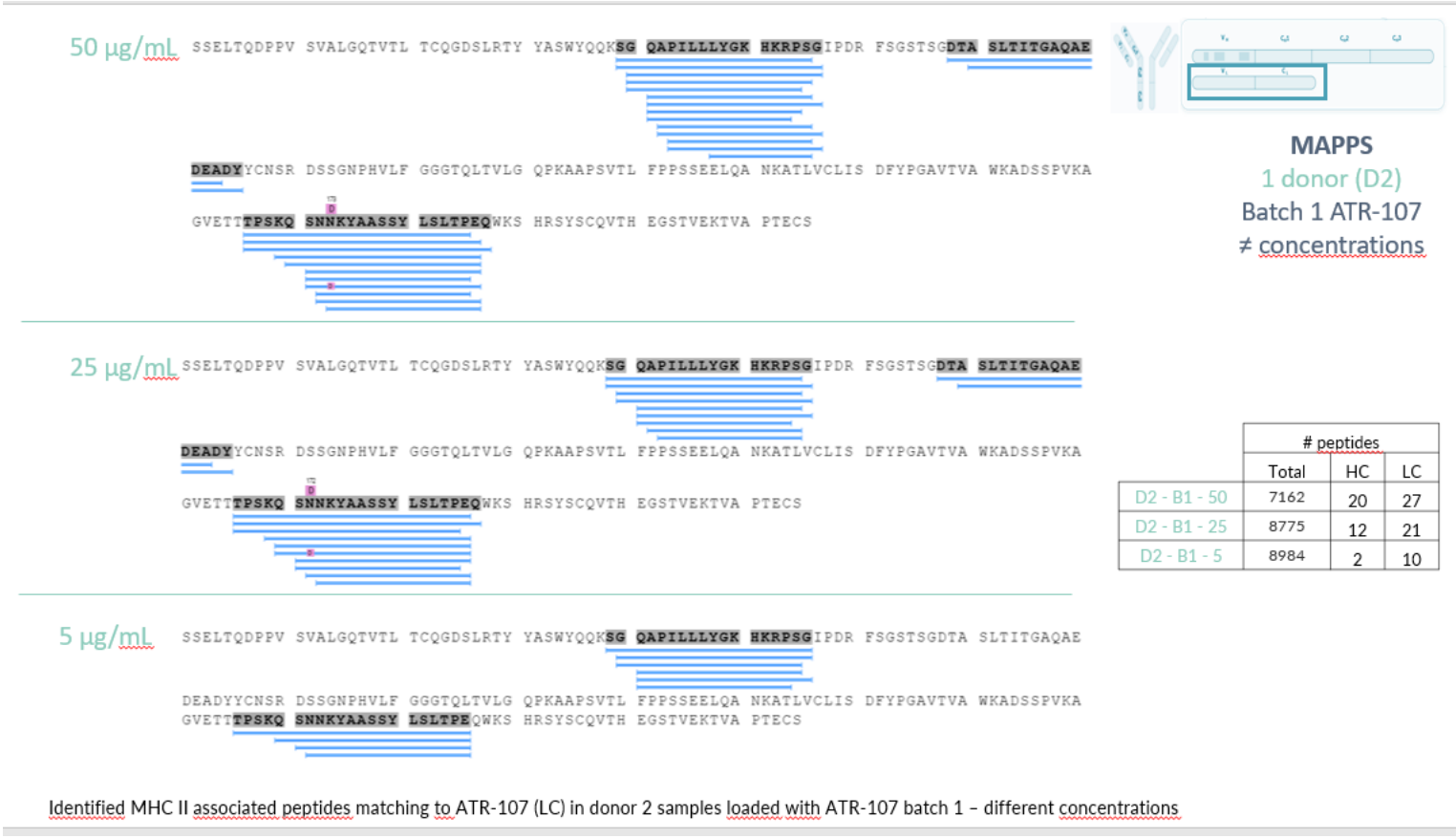
MAPPs Assay: case study ATR-107: #nr of specific identifications per concentration LC



MAPPS
1 donor (D2)
Batch 1 ATR-107
≠ concentrations

	# peptides		
	Total	HC	LC
D2 - B1 - 50	7162	20	27
D2 - B1 - 25	8775	12	21
D2 - B1 - 5	8984	2	10

Identified MHC II associated peptides matching to ATR-107 (LC) in donor 2 samples loaded with ATR-107 batch 1 – different concentrations

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MAPPs Assay: ATR-107 case study conclusions

ATR-107 input material:

- Different ‘contaminants/HCPs’ in different batches
- Concentrations not always accurate
- Large batches and bridging required (reference panel)

DC/MAPPS data:

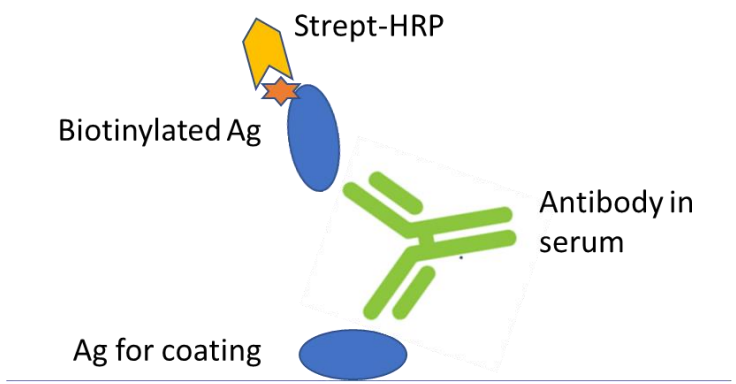
- No large differences observed between batches in activation markers
- Almost no difference in specific peptides between the 3 batches
- Dose dependent number of identifications per cluster and at lower dose, some hits missing
- For ATR-107, recommended to use higher dose for loading

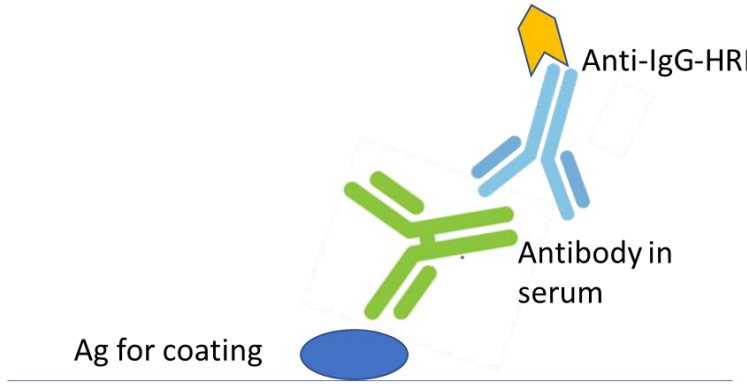
In vitro ADA Assay

10. Analysis of pre-existing anti-drug antibodies in serum

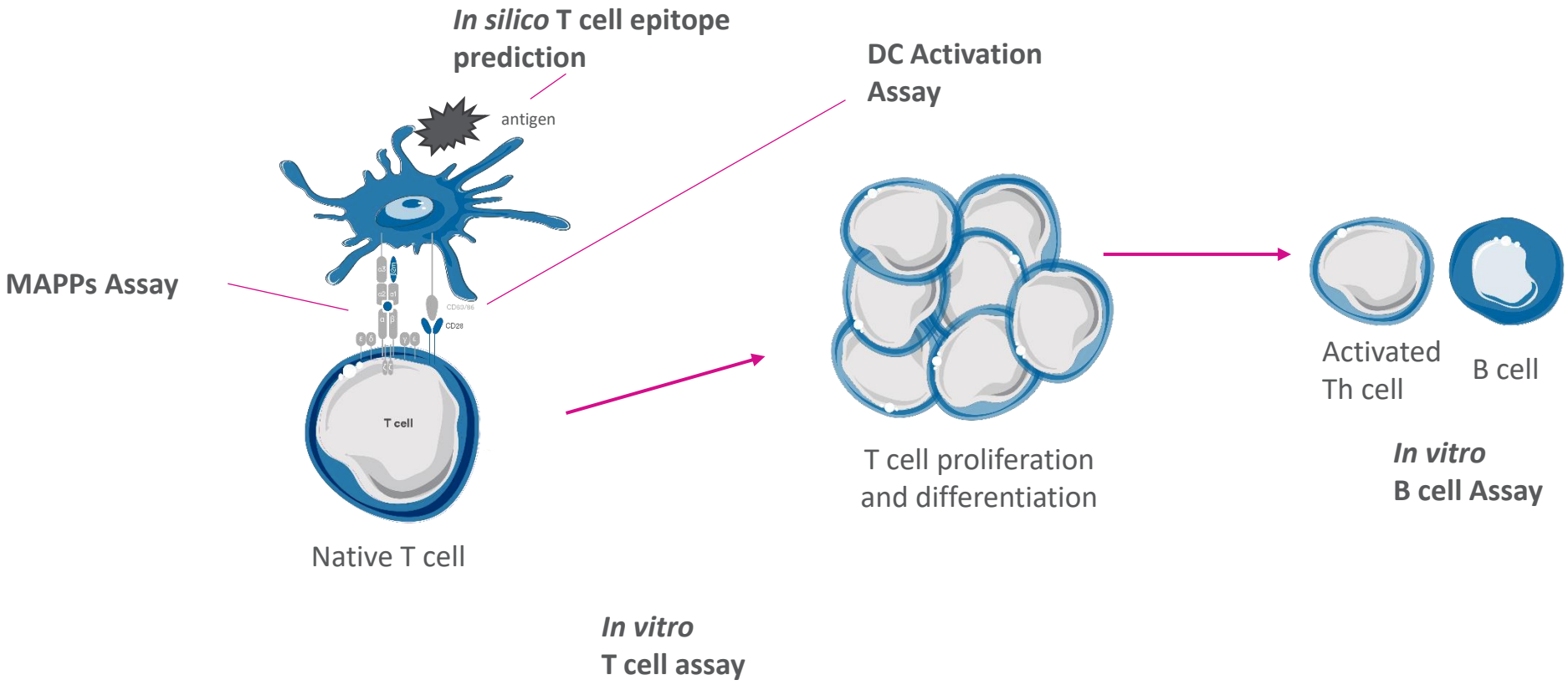
10. Pre-existing ADA Analysis in Serum

Serum from 50 healthy donors
2 possible assay formats
Set-up screening and confirmation cut-points using training set
Analysis pre-existing ADAs in serum

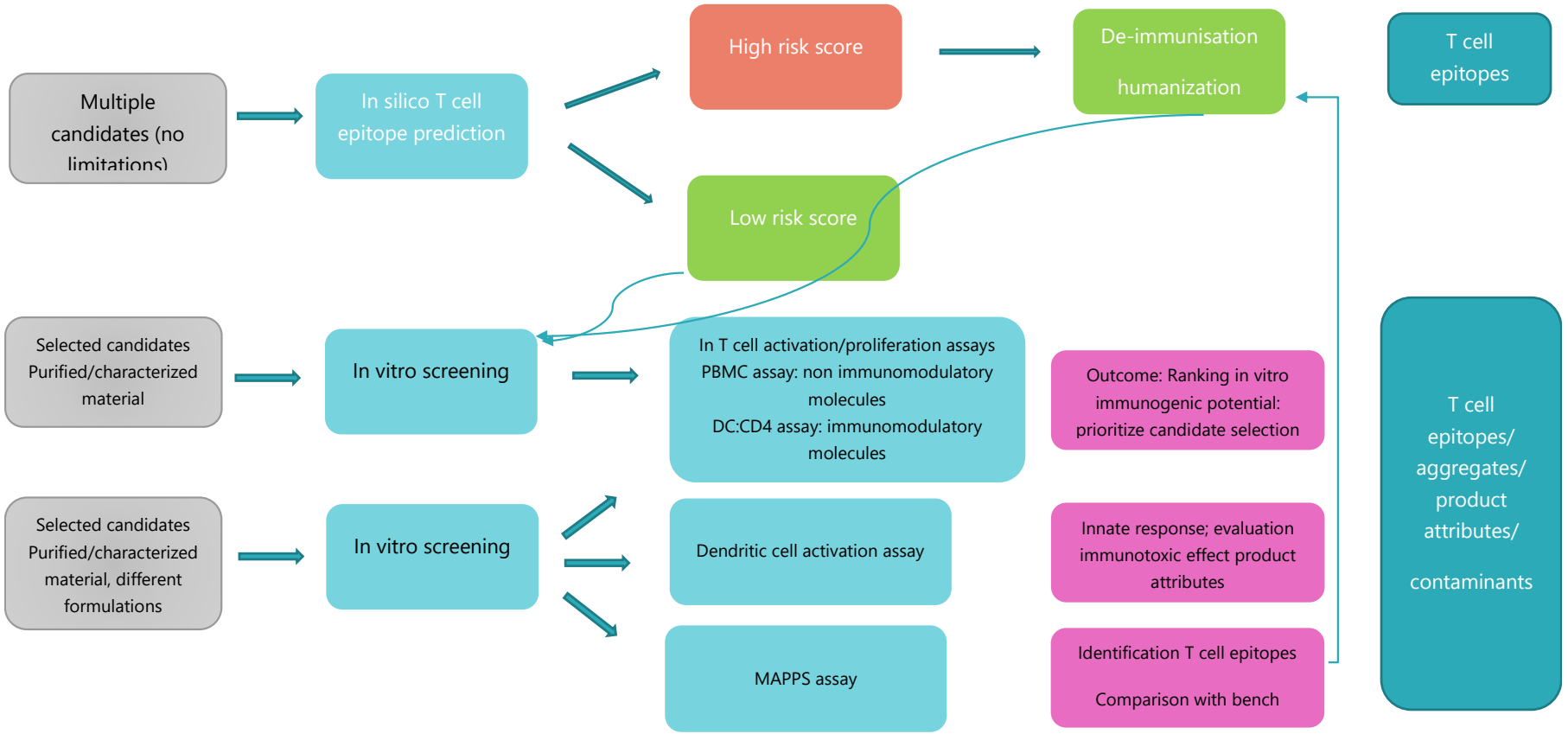




Early Immunogenicity Assessment Tools



Early Immunogenicity Risk Mitigation/Prediction

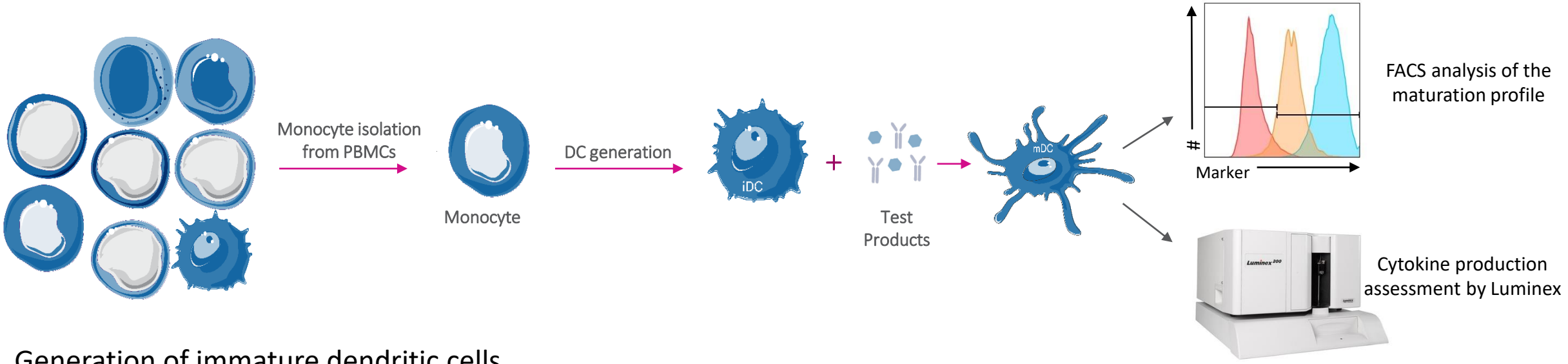


Benefits Early Immunogenicity Risk Mitigation/Prediction

- **Assessment/predictive tools have several benefits in the development and design of less-immunogenic drugs and can be used at an early stage to**:
  - Improve the safety profile by testing and re-engineering (de-immunization and humanization) or adapted formulations
  - Select the candidates with the lowest immunogenic potential
  - Evaluate the immune responses in different or specific test populations
  - Add an additional quality tag to the pipeline candidates
  - Learn and understand immunological mechanisms of the test products
  - Compare the immunogenic potential of originator and biosimilar candidate

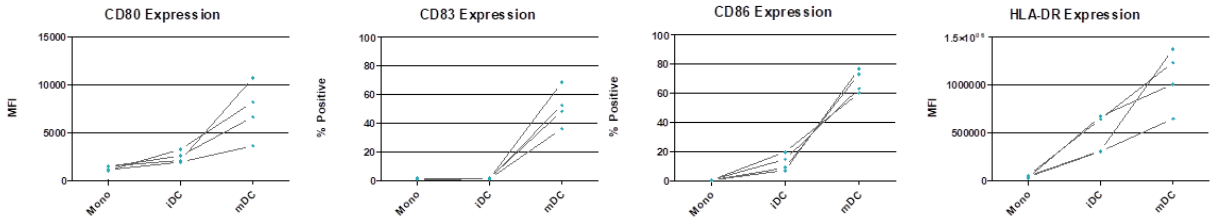
Wanted Immunogenicity

DC Activation/Maturation Assay

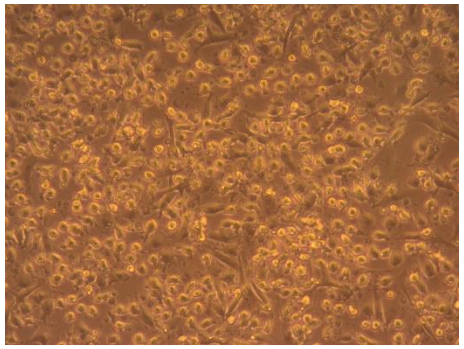


- Generation of immature dendritic cells
- Incubation with vaccine products or adjuvants

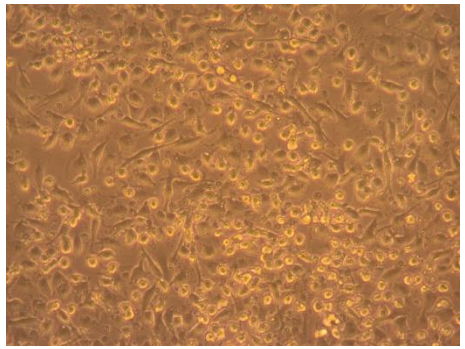
- ROs:
  - Measurement of cytokines/chemokines in supernatant (Elisa/Luminex/HTRF)
  - Evaluation of maturation markers (Flow cytometry)



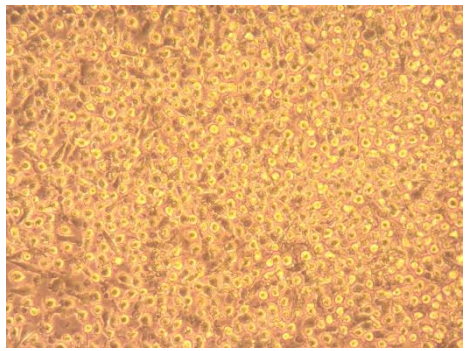
DC Activation/Maturation Assay - Results



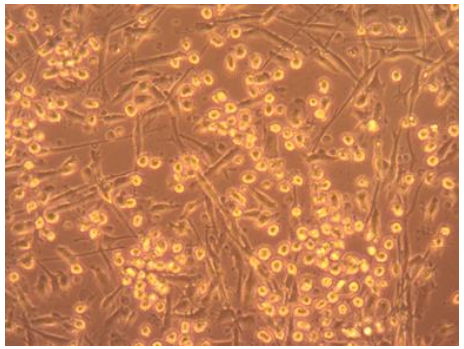
D1 20X iDCs



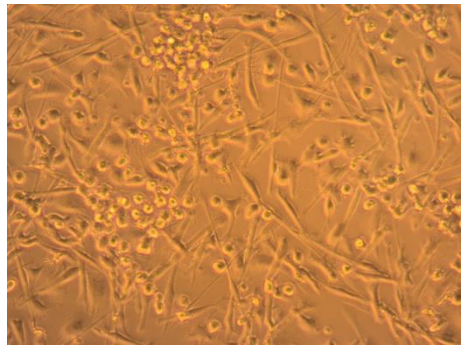
D2 20X iDCs



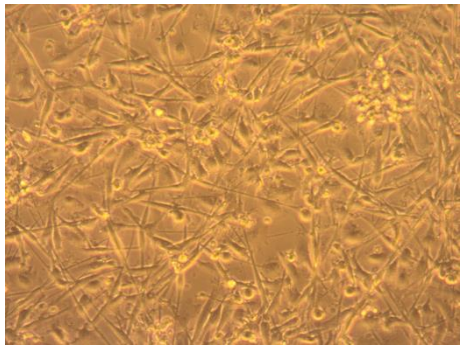
D3 20X iDCs



D1 20X mDCs



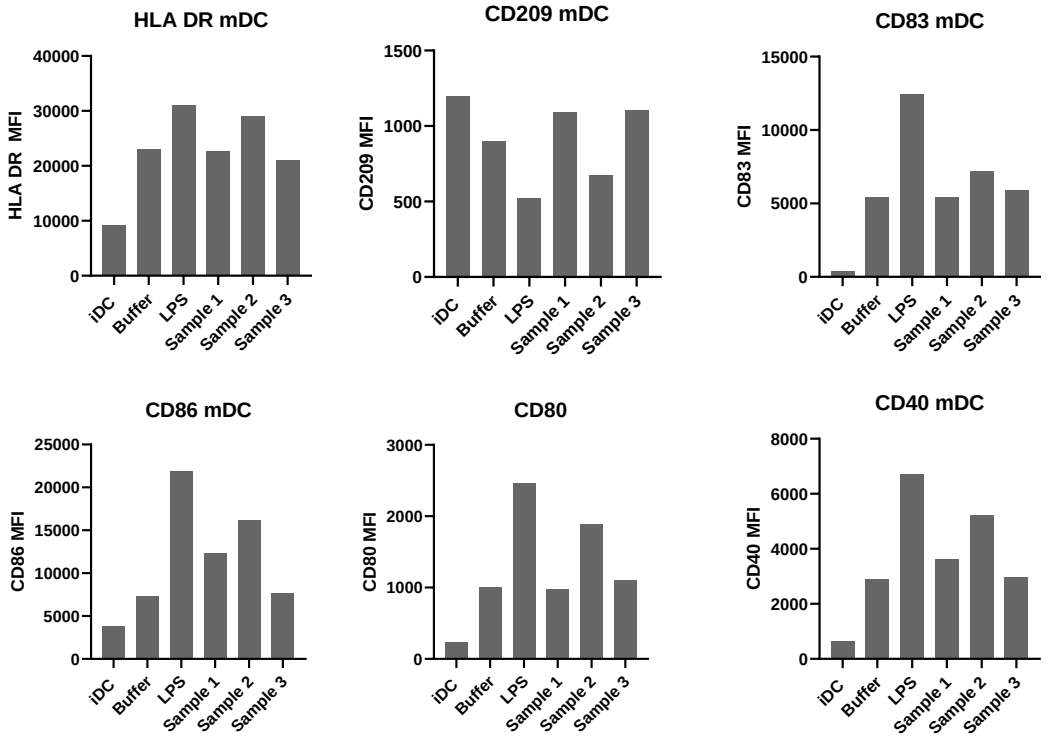
D2 20X mDCs



D3 20X mDCs

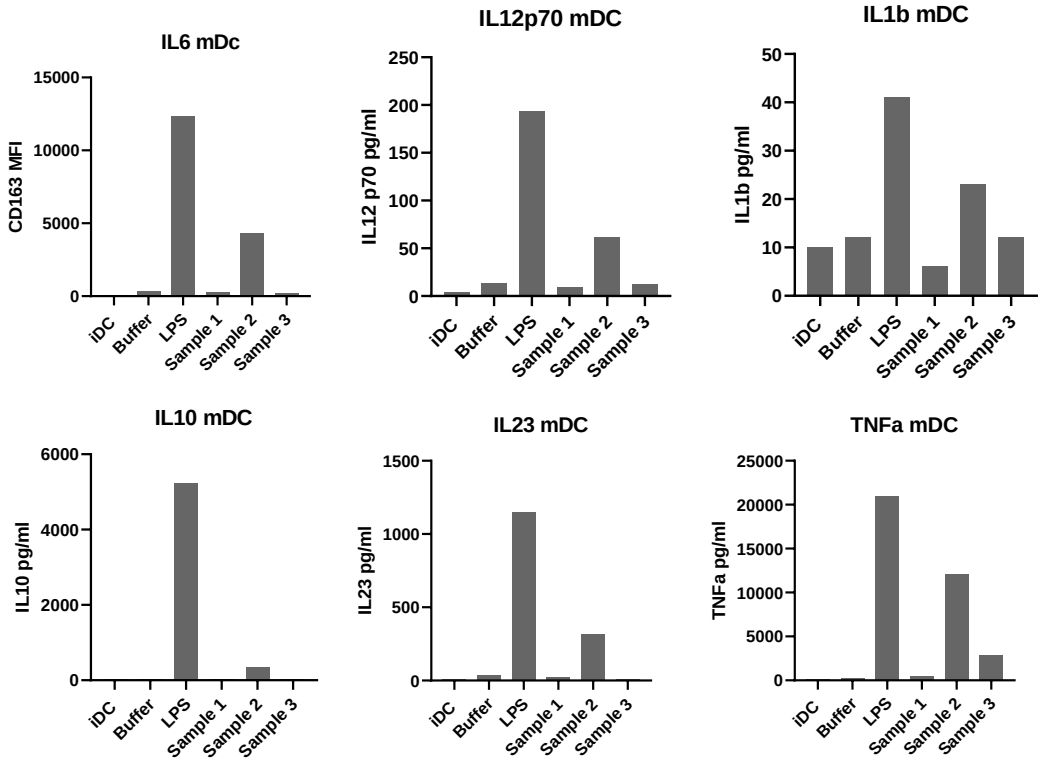
DC Activation/Maturation Assay - Results

Flow cytometry

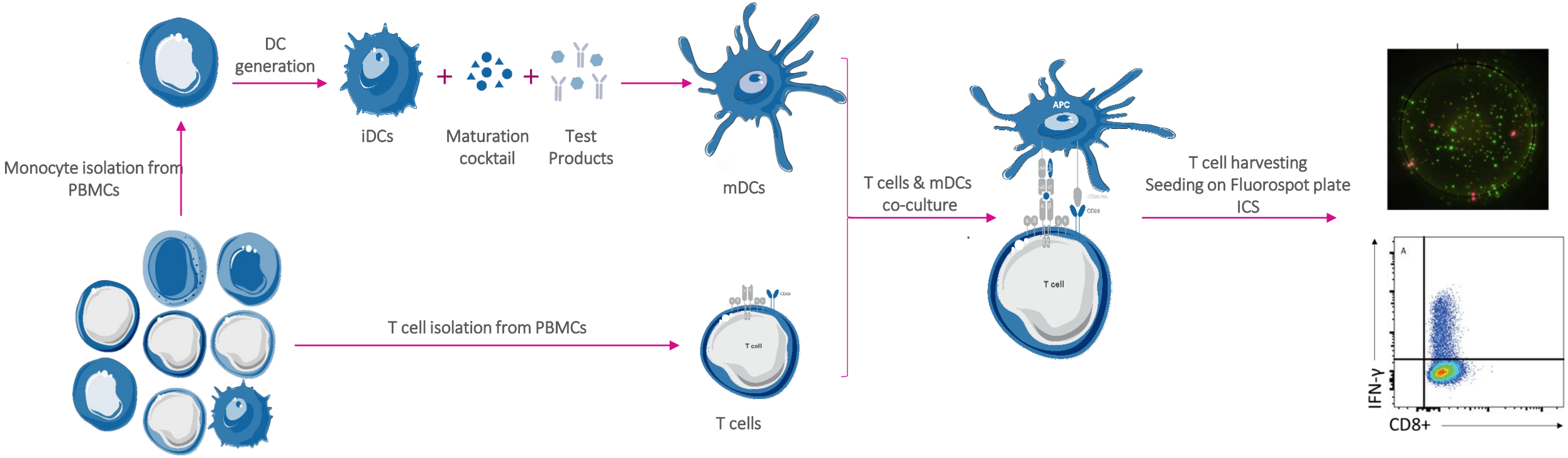


DC Activation/Maturation Assay - Results

Luminex

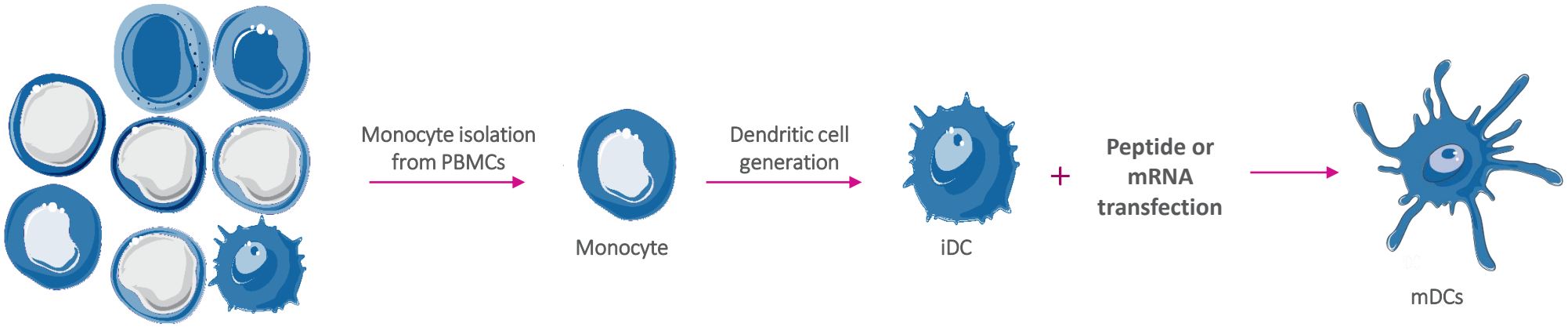


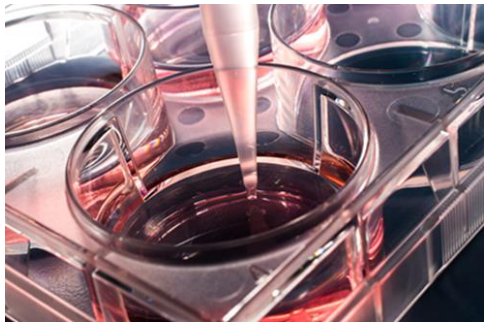
DC-T Cell Assay



- Upon co-incubation with autologous T-cells, the potential to induce a vaccine specific immune response is evaluated via multicolor Fluorospot or intracellular cytokine evaluation via flow cytometry

Priming with Peptide-loaded or mRNA-transfected Dendritic Cells

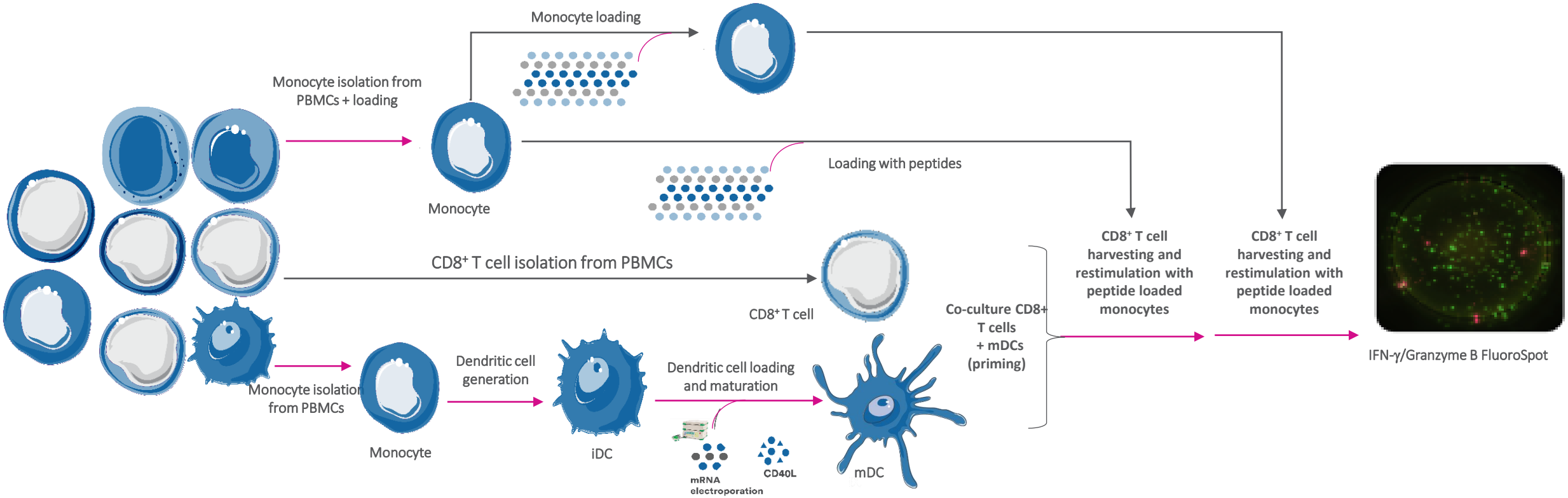




Gene Therapy – Potency/Immunogenicity

- *In vitro* evaluation of DNA, mRNA constructs (electroporation and lipid-based transfection dendritic cells)
  - Biorad Gene Pulser, Trans IT, Lipofectamine, custom agents

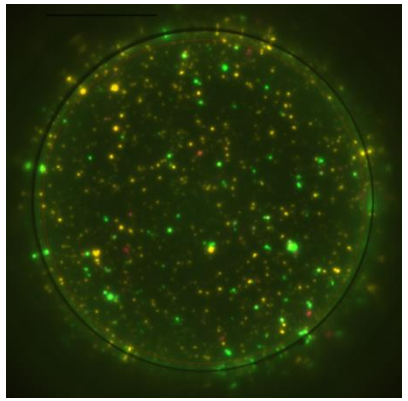
Gene Therapy – Potency/Immunogenicity



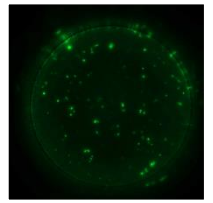
Dendritic cell transfection/electroporation (priming) +/- further *in vitro* enrichment (mRNA or peptides)

RO: Evaluation of the response using FluoroSpot or ICS

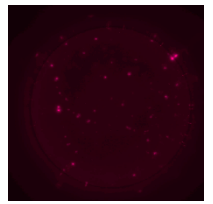
DC-T Cell Assay: Fluorospot Read-out



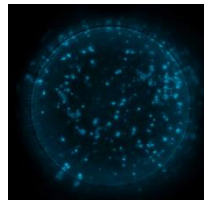
	Elispot	Fluorospot
Polyfunctionality	Single analyte	Up to 4 analytes
Sensitivity	++	+++
Cost	+	+(+)
Input material (cells/antigen)	=	=



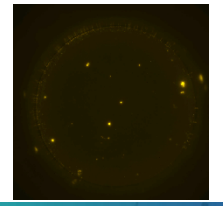
IFN-γ



IL-17A



IL-22



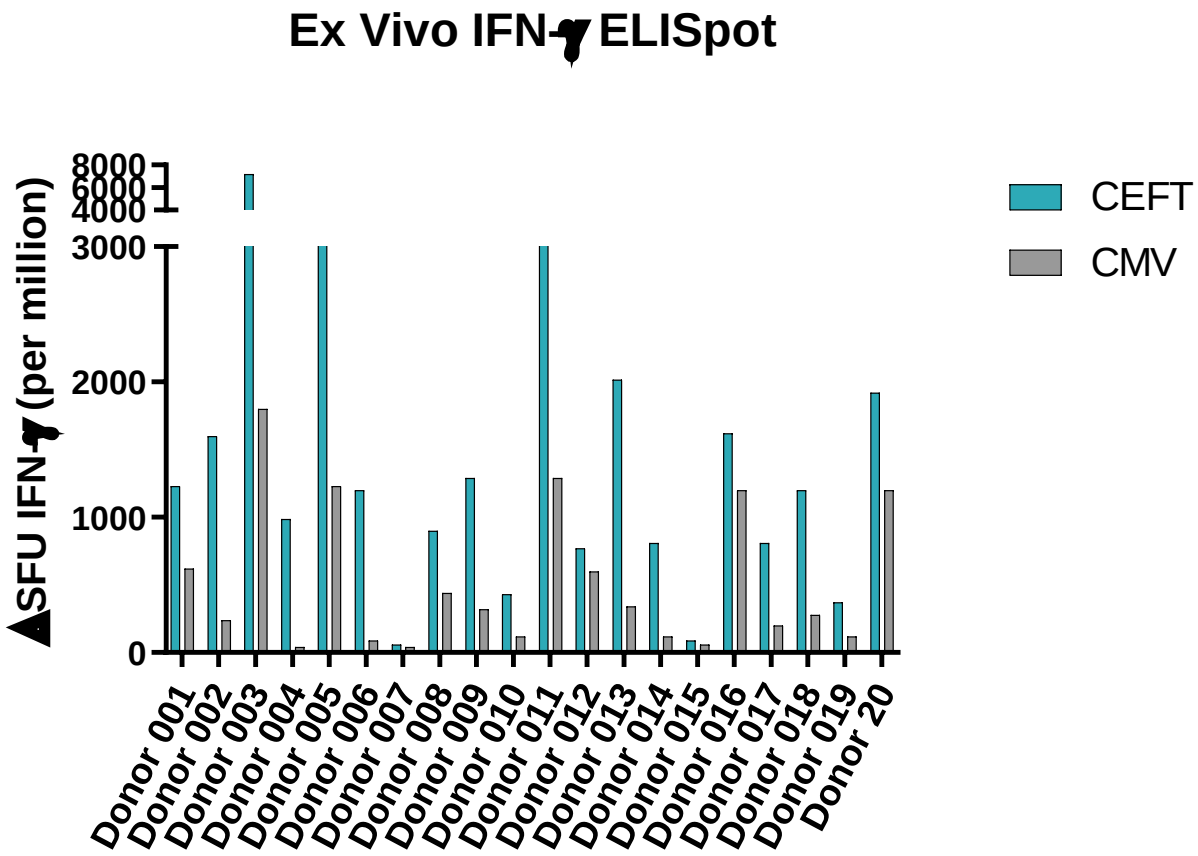
IL-5

DC-T Cell Assay: Fluorospot Read-out

Development:

- Pre-screening of healthy donor PBMCs with **peptide mixes**
- Optimization of **cell concentration**
- Optimization of **peptide concentration, evaluation interference**
- Optimization of **incubation time**
- Evaluation of **sample quality** (clinical samples)
- Identification **positive** and **negative** control samples

DC-T cell Assay: Fluorospot Assay Controls



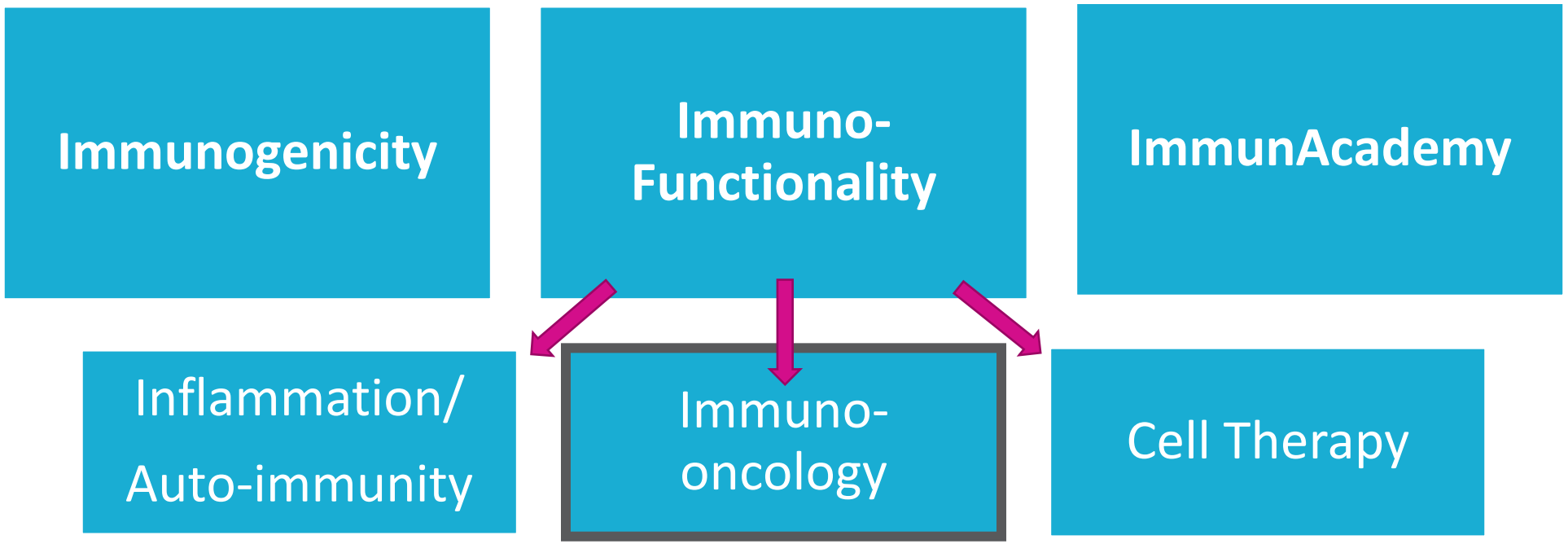
Positive Controls:

- Polyclonal stimuli: PHA, Con A, SEB, anti CD3/CD28
- Antigen-specific controls: CEF(T)(A) peptide pool, CMV lysate or peptide pool, EBV or COVID peptide pools
- Internal peptide mix: Binding of a set of 250,000 random natural non-human 15mer peptides was evaluated for 43 HLA-II alleles with NetMHCIIpan-4.0 and strong binders were identified. Out of these, **5 peptides** with the highest degree of promiscuity were selected. (In silico work performed by Prof. Morten Nielsen – DTU Denmark)

Negative Controls:

- Medium control
- DMSO control

ImmunXperts' Services



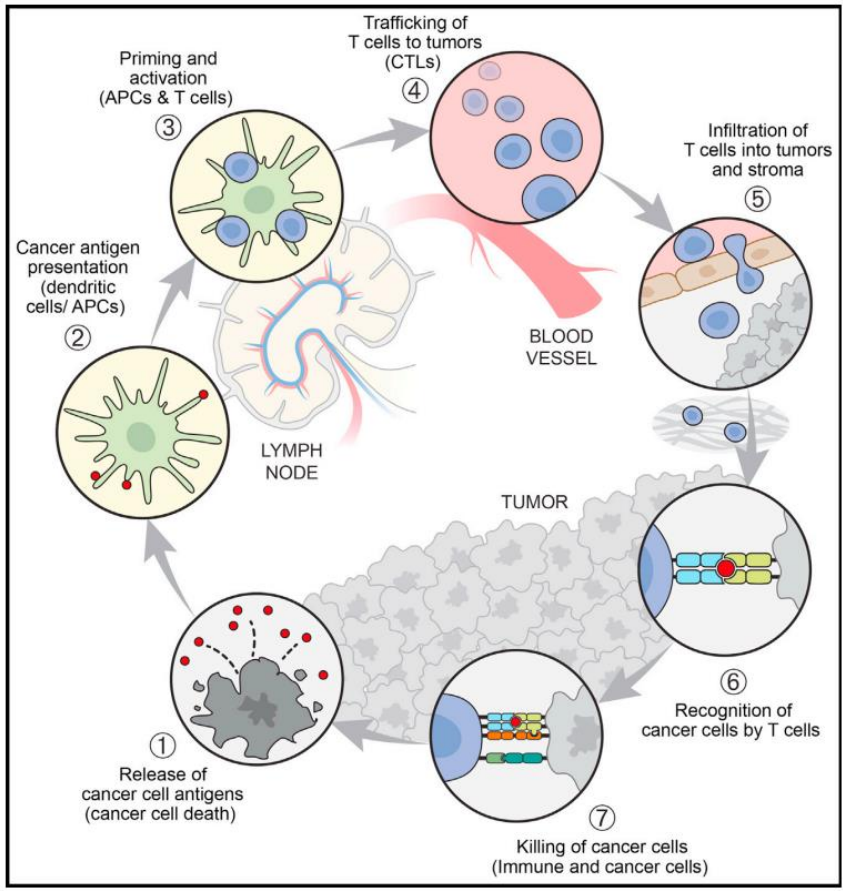
Tools to Accelerate **Immuno-Oncology** Therapy Development

Established assays

Staggered approach for new assays

→ **Fine tuning before addition of compounds**

Stage 1 → **Go/No go** → **Stage 2** → **Go/No go** → **Stage 3** →



Adapted from Mellman, I. et al., (2023)

Tools to Accelerate **Immuno-Oncology** Therapy Development

***In vitro* screening**

Functional screenings

Cellular disease models

Drug mechanism of action

Myeloid cell Assays

- Macrophage Polarization Assay
- Macrophage Suppression Assay
- Antibody-dependent Cellular Phagocytosis (ADCP) Assay

NK cell Assays

- NK Activation Assay
- Antibody-dependent Cellular Cytotoxicity (ADCC) Assay
- NK Proliferation Assay

T cell Assays

- Mixed Lymphocyte Reaction (MLR) Assay
- Antigen CMV/SEB (re-)Activation Assay
- Treg Suppressive CD3/CD28 Activation Assay
- Treg Suppressive MLR Assay
- T cell Exhaustion Assay
- Pan T cell killing Assay

Neutrophil Assays

- Neutrophil activation assay
- Neutrophil killing assay

Cynomolgus Assays

- Mixed Lymphocyte Reaction (MLR) Assay
- Macrophage Assays

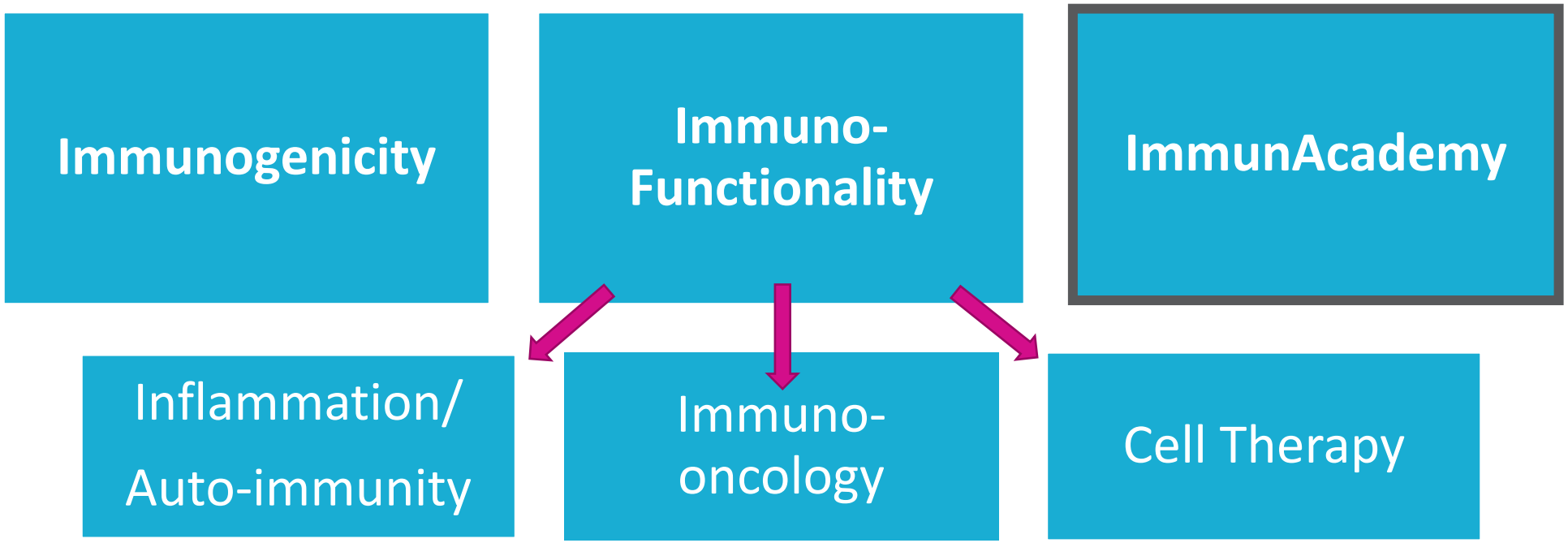
Mouse Assays

- Mixed Lymphocyte Reaction (MLR) Assay

3D spheroid culture model

- PBMC coculture killing assay

ImmunXperts' Services



ImmunXperts' goal is to work hand-in-hand with its Customers to create rich partnerships

- In this context, ImmunAcademy is ImmunXperts' offer of:
  - In house theoretical courses on immunology and hands-on lab training
  - Assay transfer
  - Training PBMC Isolation and Cryopreservation
  - On-site technical support for Customers interested in setting up their own immunology lab
  - Advise on the analysis and interpretation of immunology data
  - Coaching of Customers’ staff to implement an immunogenicity assessment and risk mitigation strategy

PBMC Isolation & Cryopreservation Site Training

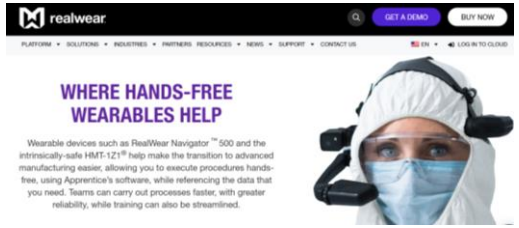
- Training Preparation

- Creation of a site questionnaire.
- Creation of a PowerPoint slide deck for virtual training based on the Customer's preferred isolation protocol.
- Standardized communication with sites (e-mail templates).
- Discussion and agreement with Customer on procedural details (isolation protocol, training blood volume, number of donors, number of operators, acceptance criteria, etc.).
- Bi-weekly status updates, including preparation and follow up.

PBMC Isolation & Cryopreservation Site Training

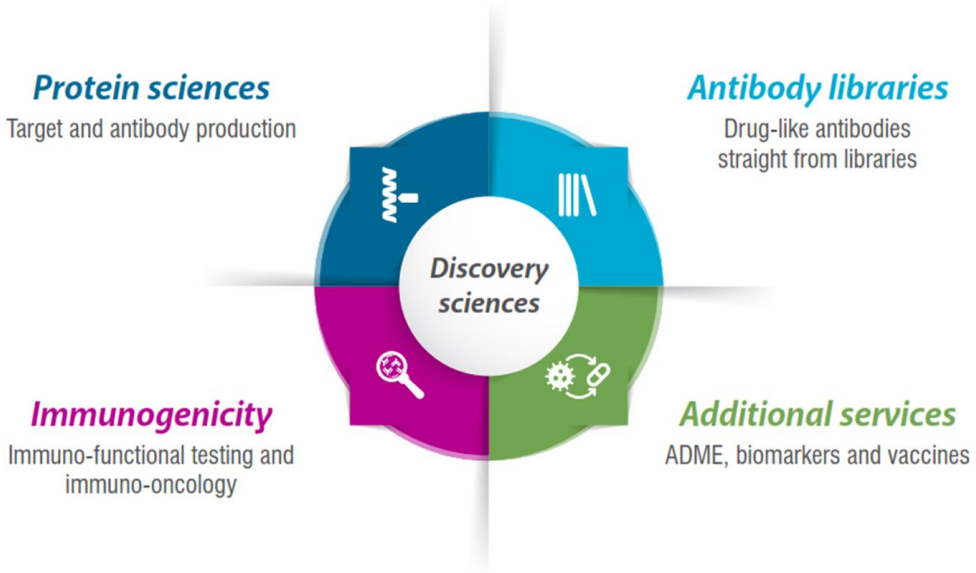
- <u>Site Training</u>

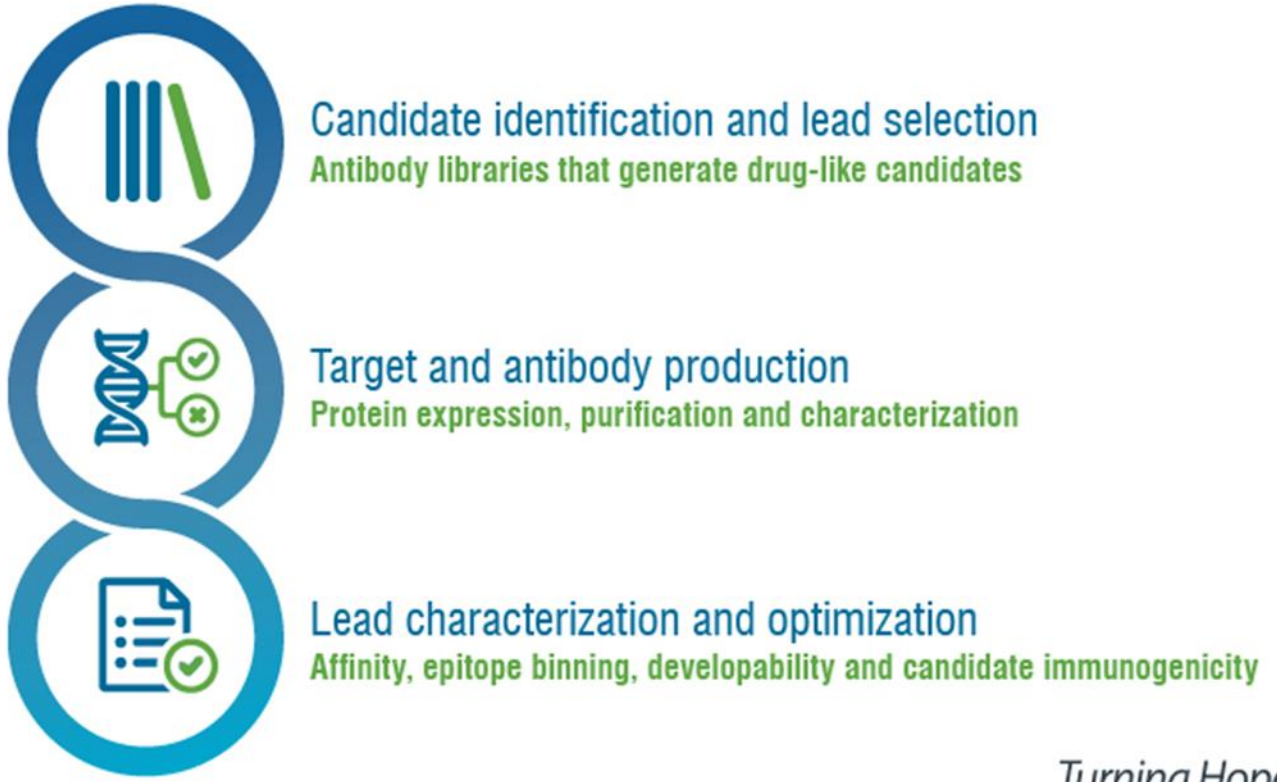
- Sending and review of the questionnaire; to assess the study site's general experience and infrastructure/organization.
- Call to review the completed questionnaire; to ensure full understanding of the situation and identification of any gaps or special considerations for on-site training.
- Virtual training by IMXP on the procedure, including data collection requirements *(in presence of Customer)*.
- Hands-on training at the different sites including demonstration of critical steps by the IMXP' trainer and observation of the procedure performed by the technicians of the study site.
  Additional/alternative training via Realwear Smart Glasses:



- Evaluation of PBMC quality by dry runs:
  Evaluation of viability and functionality for the selected read-out parameters (Fluorospot/ELISpot or ICS) using CEFTA peptide mix/other recall antigens and a polyclonal stimulation, versus IMXP's healthy donor PBMCs

Overview Discovery Sciences





Turning Hope Into Help™

Acknowledgments

- Sofie Pattyn
- Jana Schockaert
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- Ursula Fels
- Ellen Boelen
- Mareva Cervellin
- Elise Pepermans
- Morten Nielsen

Elise Pepermans

CEO & CO-FOUNDER

elise@immunespec.com

IMMUNESPEC

Morten Nielsen

CEO EIR Sciences

eir@eirsciences.com

EIR SCIENCES

Questions

Your partner in Immunology projects
"We think with You"

ImmunXperts SA, a Q^2 Solutions Company
Rue Auguste Piccard 48
6041 Gosselies
Belgium

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