Non-clinical Immunogenicity Assessment of Generic Peptide Products: Development, Validation, and Sampling

T cell immunogenicity assays: time for standardization and harmonization

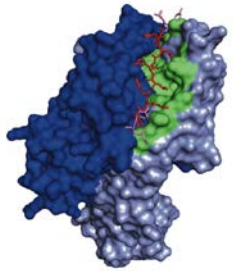
SOFIE PATTIJN, CTO – FOUNDER IMMUNXPERTS, A NEXELIS GROUP COMPANY

JANUARY 26, 2021

ImmunXperts

ImmunXperts
a Nexelis group company

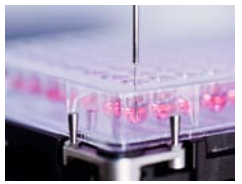
Tools for early immunogenicity assessment



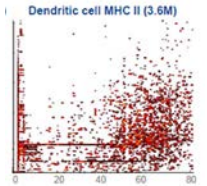
In silico
- Computer based assessment of T cell epitopes
- De-immunization

In vitro early/Innate assays
- DC activation assays
- Cytokine release assays

In vitro T cell assays
- (CD8-depleted) PBMC assays
- DC-T cell assays

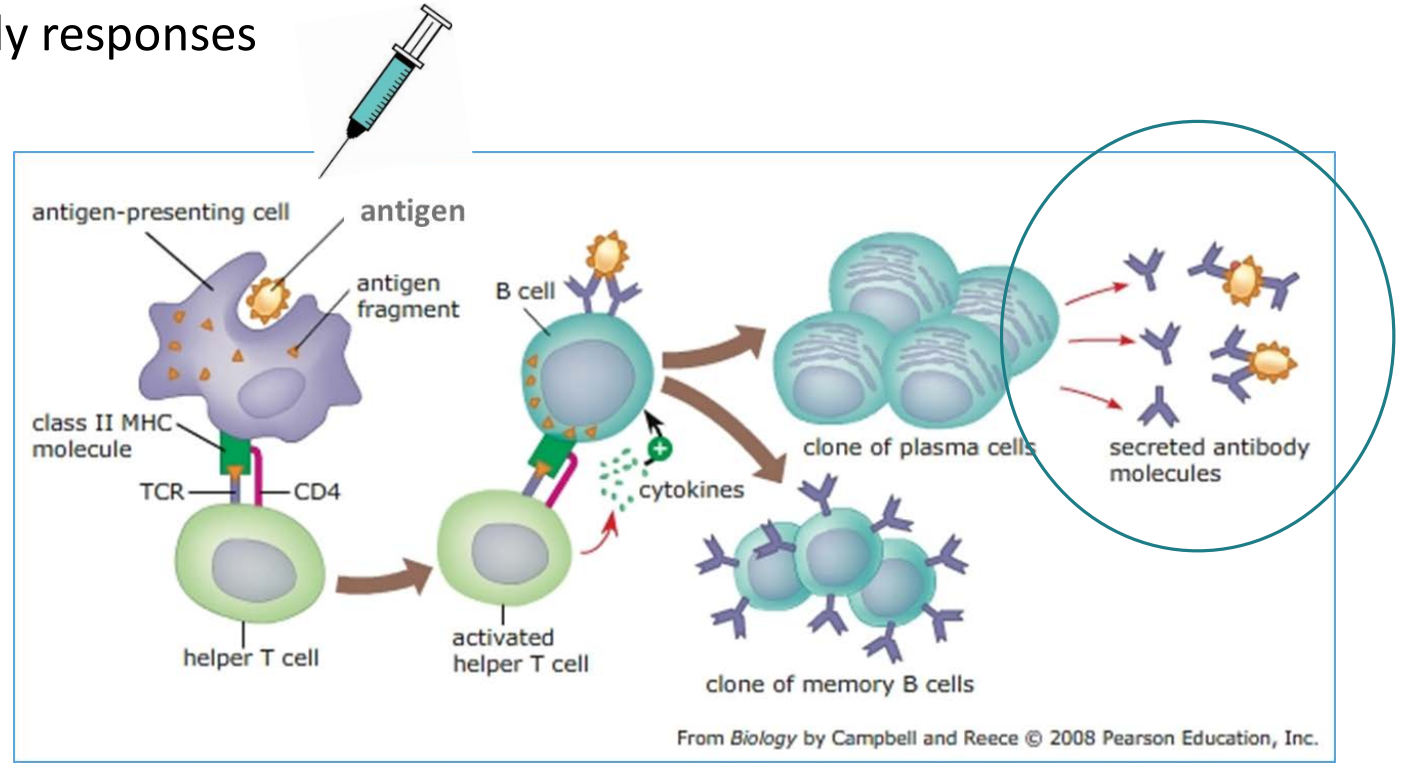


MAPPS assay
- Identification processed and presented epitopes

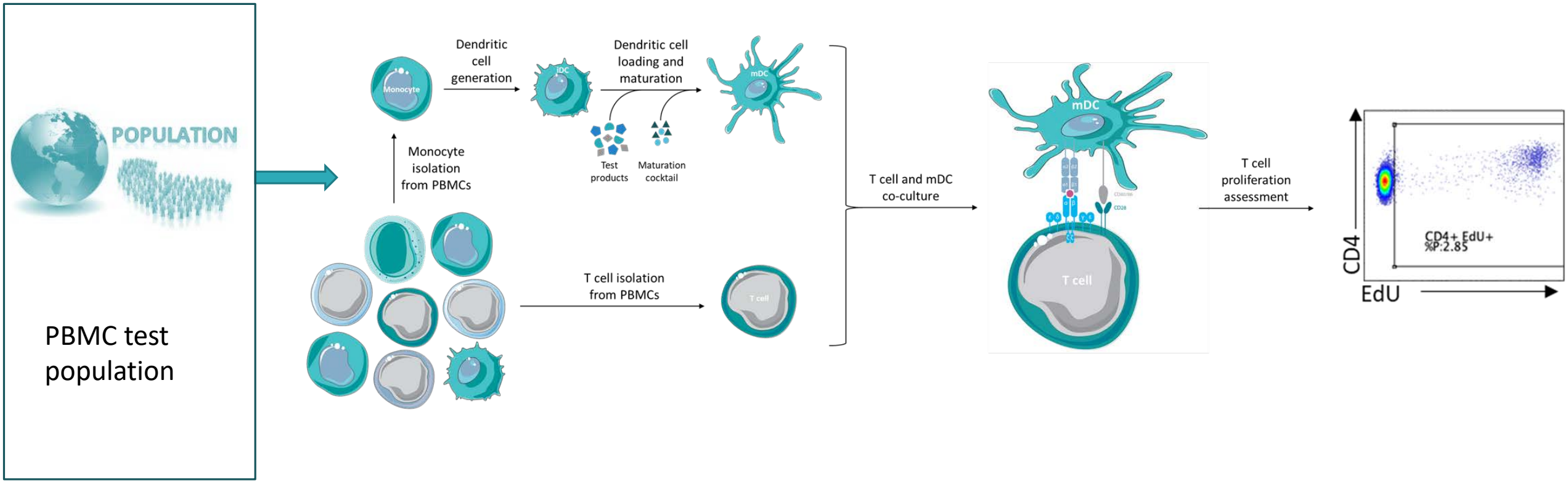


In vitro tools: T cell proliferation assays

T cell activation/proliferation assays using human PBMC can be used as a **surrogate marker** for antibody responses

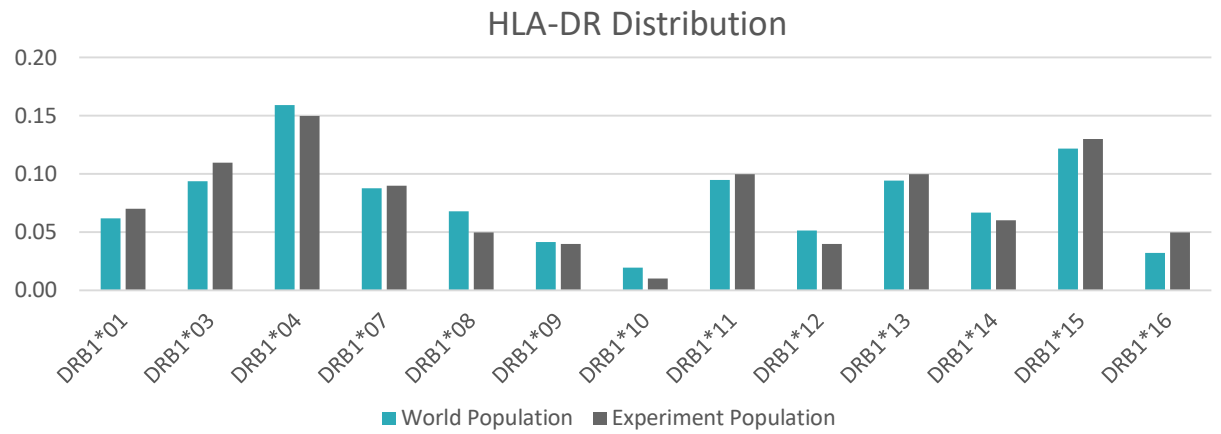


In vitro tools: DC-T cell assays: procedure



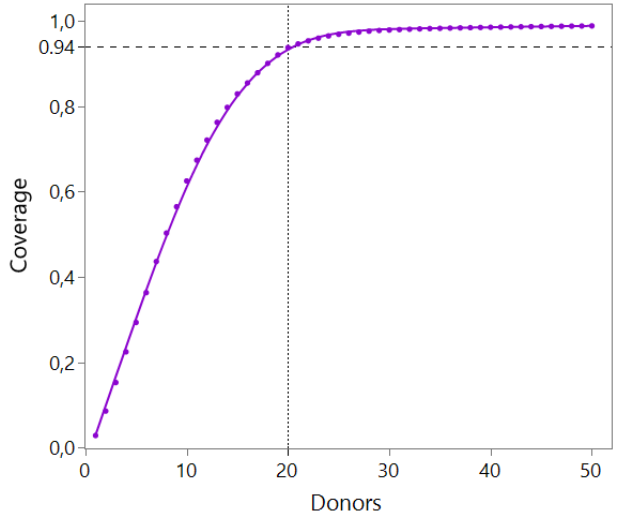
Test population: Donor selection

The number of donors should be sufficient to represent the **HLA distribution** of the target population and **allow the detection of a positive and negative response**



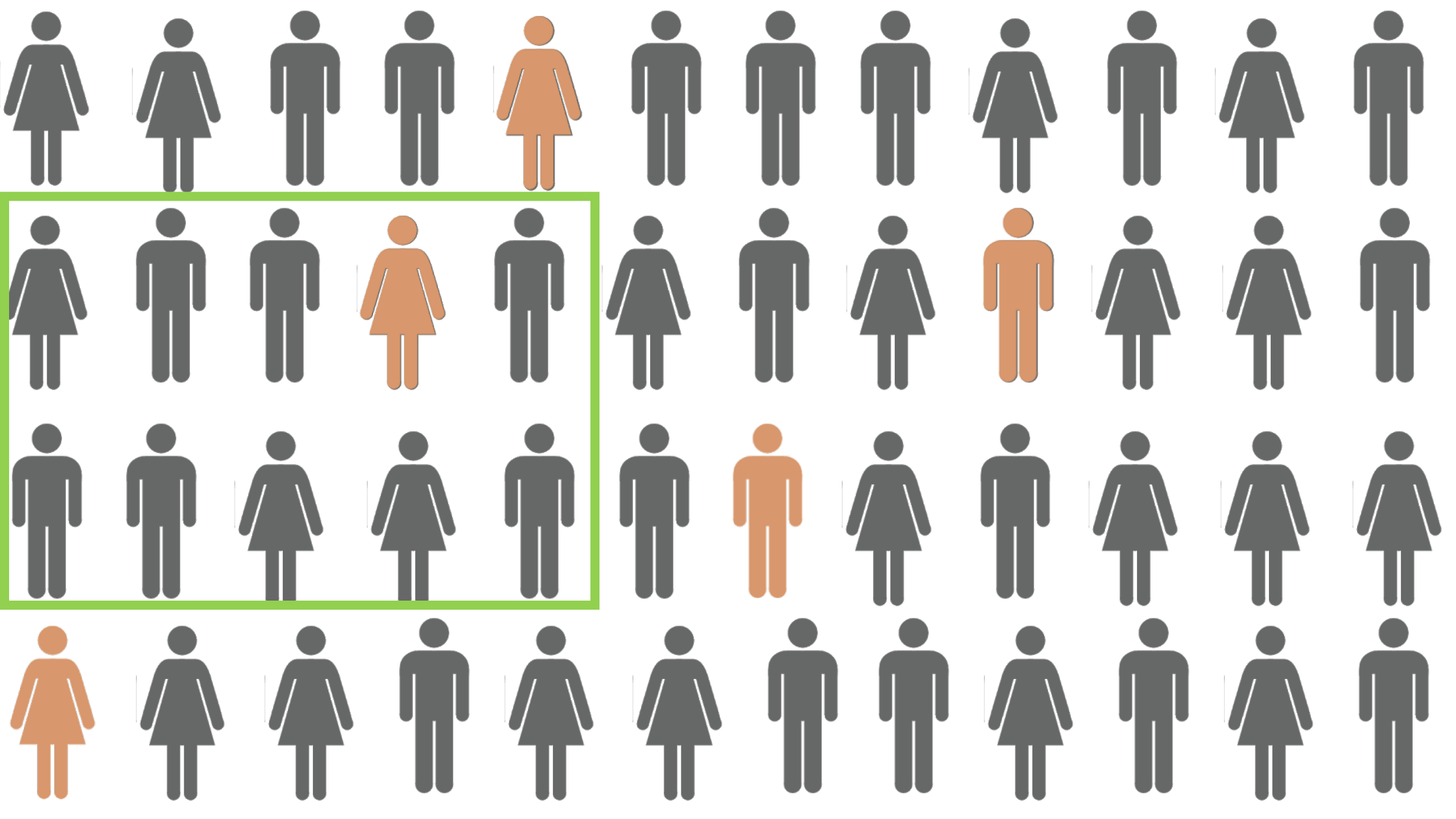
Donor selection: HLA distribution

The coverage of world population in function of number of donors.



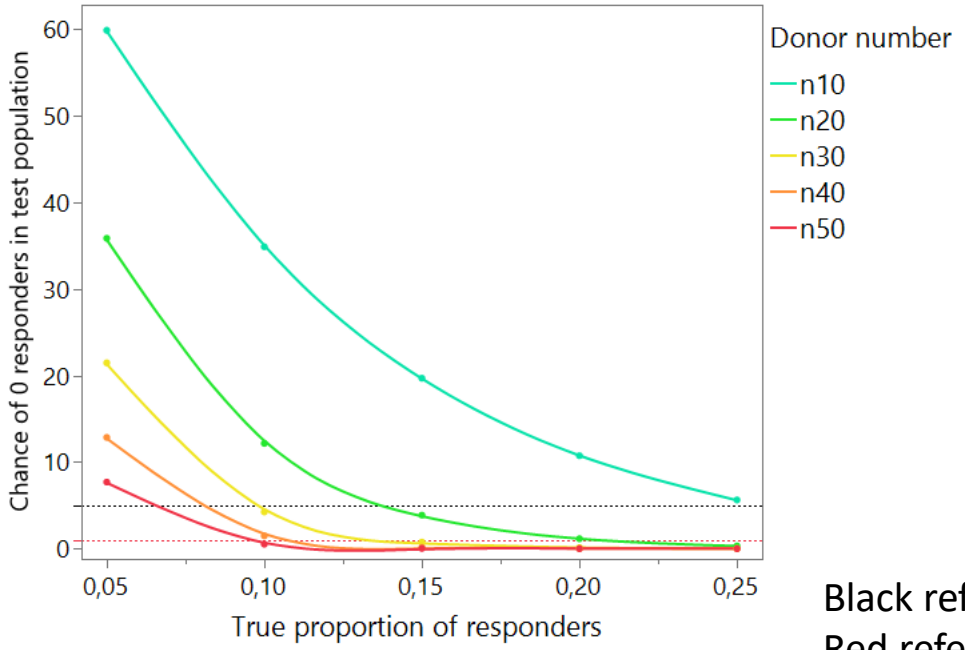
Donor selection: rationale number of donors

- The number of donors to include in the screening depends on the proportion of positive subjects in the population that would be of concern
- Depending on the proportion of positive subjects in the population, there is a chance that a random/representative sample of the population (test donors) would not include any reactive donors and thus the compound would be classified as negative:
  - ✓ For a fixed number of donors, the chance of including only negative donors increases if the proportion of positive subjects in the population decreases
  - ✓ For a fixed proportion of positive subjects in the population, the chance of not including any positive donor decreases if the number of donors increases
  - ⇒ If it is acceptable to consider a compound negative if less than 25% of subjects in population are negative you need less donors than when you can only consider a compound negative if less than 10% of subjects in the population are negative
- Chance that a random/representative sample of the population (test donors) would not include any reactive donor (cfr. next slide) = basis for the calculation of number of donors



Donor selection: rationale number of donors

- Graph showing the chance of not including any positive donors (completely missing immunogenicity) in function of sample size (color code) and probability of subjects responding in the population (x-axis) based on the binomial distribution.



With 10 donors, you have 20% chance of not including any reactive donor in test population even if 15% of the population would show a response.

With 40-50 donors you have high sensitivity to detect positive responses if only <10% of population would show a response.

Black reference line: 5%
Red reference line: 1%

Donor selection: rationale number of donors

- Calculations on previous slide are minimal number of donors required

  - ➢ It only takes into account sampling variability in selecting donors that are true responders but assumes 100% power of the assay to classify true responders as positive

    - ✓ Power of the assay to classify positive donors as positive depends on the variation specific for the assay and number of replicates per donor

Test population: PBMC quality

Quality of the primary cells:

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

Test population: preserving PBMC quality

ImmunXperts
a Nexelis group company

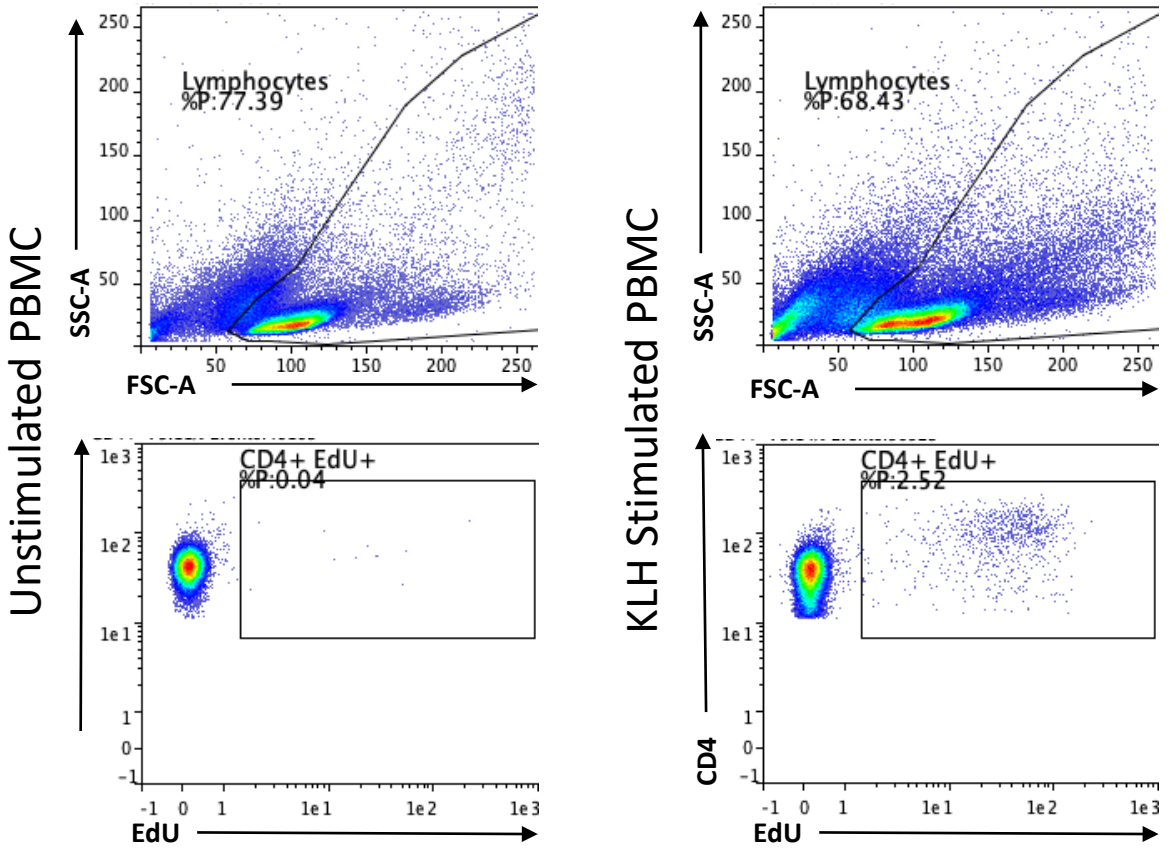
Preserving Quality of the primary cells:

- Standardized procedures for sampling, shipping, isolation, cryopreservation, thawing, handling, …
- Start isolation of PBMC within 8 hours after blood draw
- Alternatively, use CPT™ tubes for blood sampling
- Perform an extended quality control on all cell preparations

Cryopreserved PBMC: functionality assessement

- Assessment of proliferative response towards polyclonal stimulation (anti-CD3 antibody)

- Assessment of proliferative response towards **naïve antigen Keyhole Limpet Hemocyanin (KLH)**



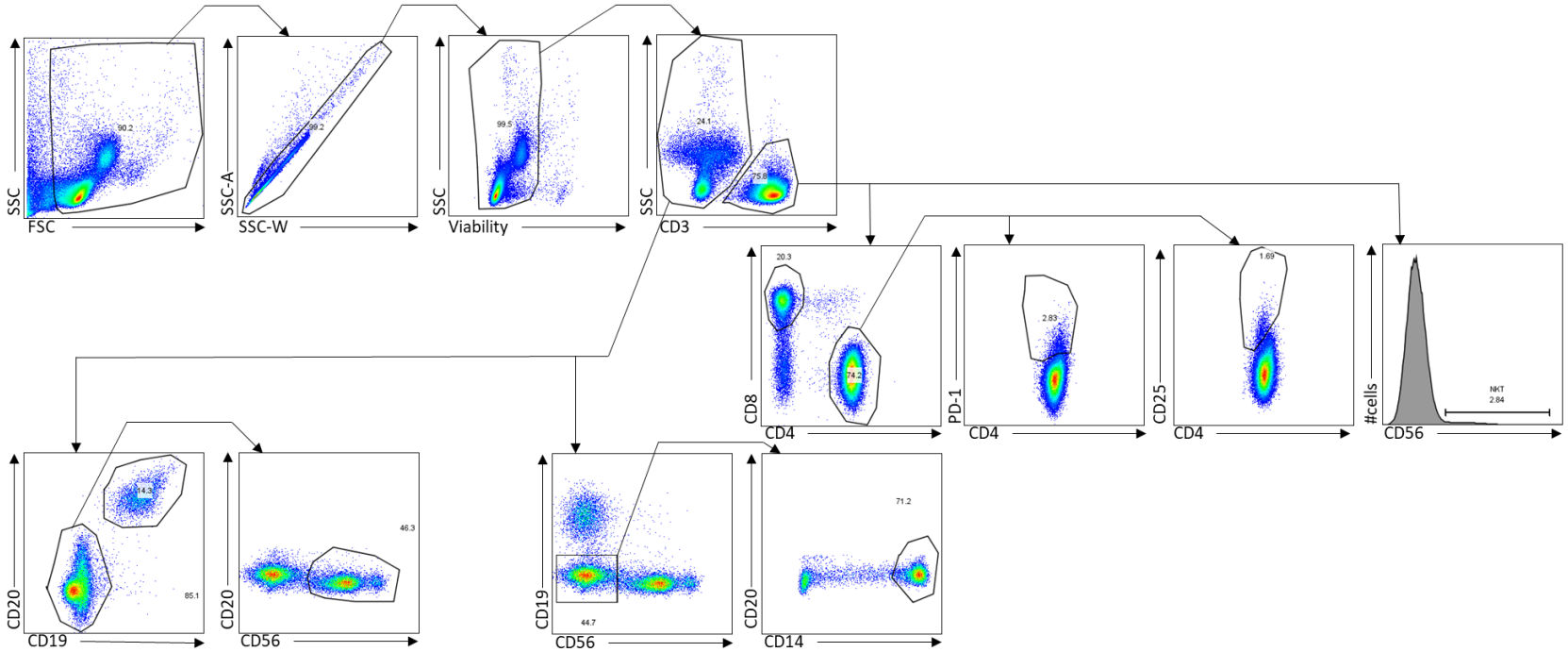
Cryopreserved PBMC: subpopulation analysis

Classic Surface marker staining for:

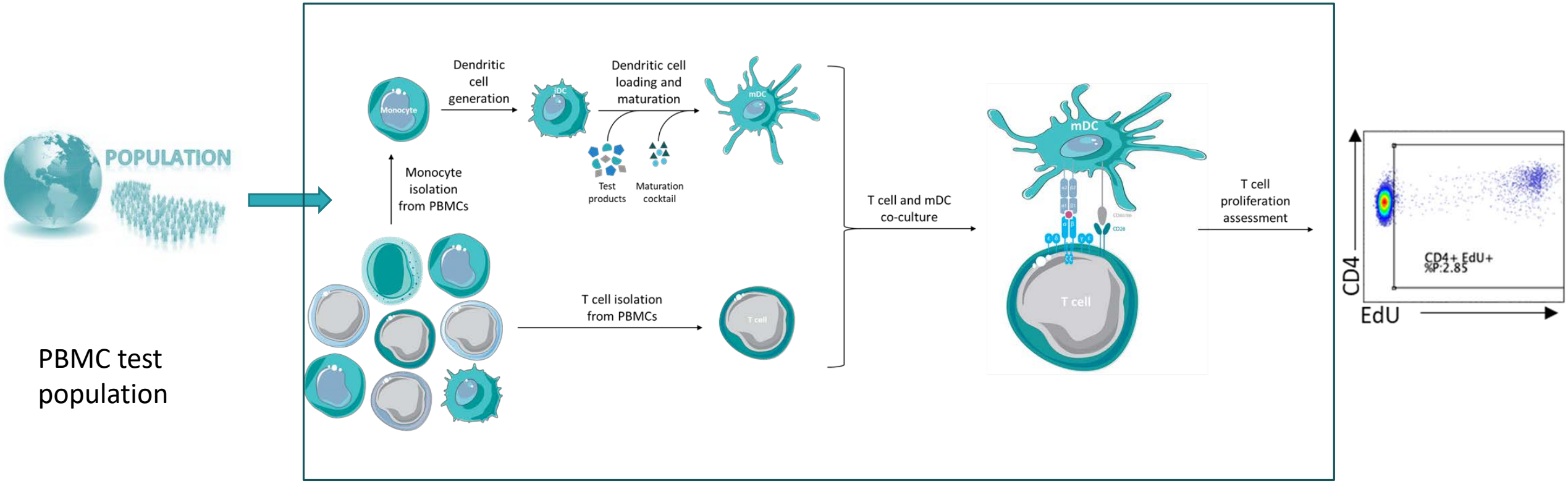
- CD14: Monocytes
- CD3: T cells
- CD4: Helper T cells
- CD8: Cytotoxic T cells

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



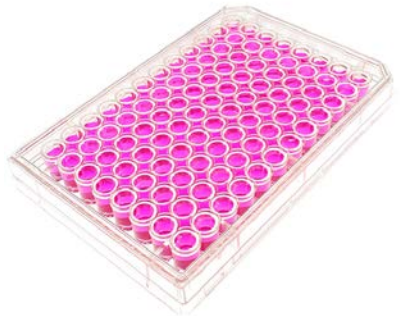
In vitro tools: DC-T cell assays: procedure



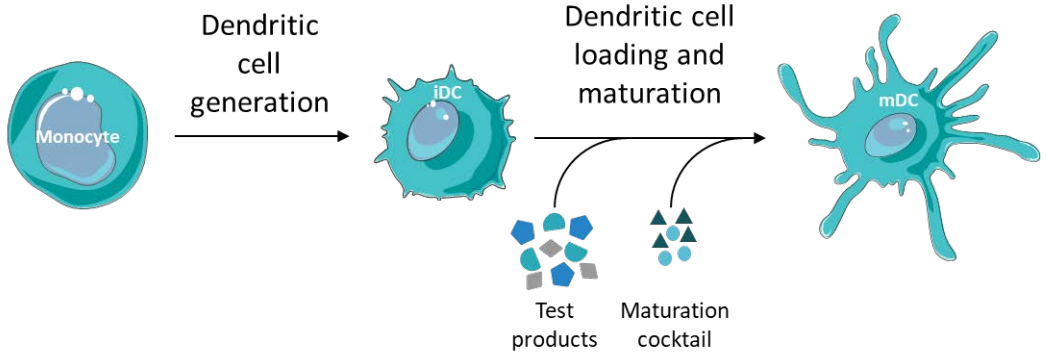
PBMC test population

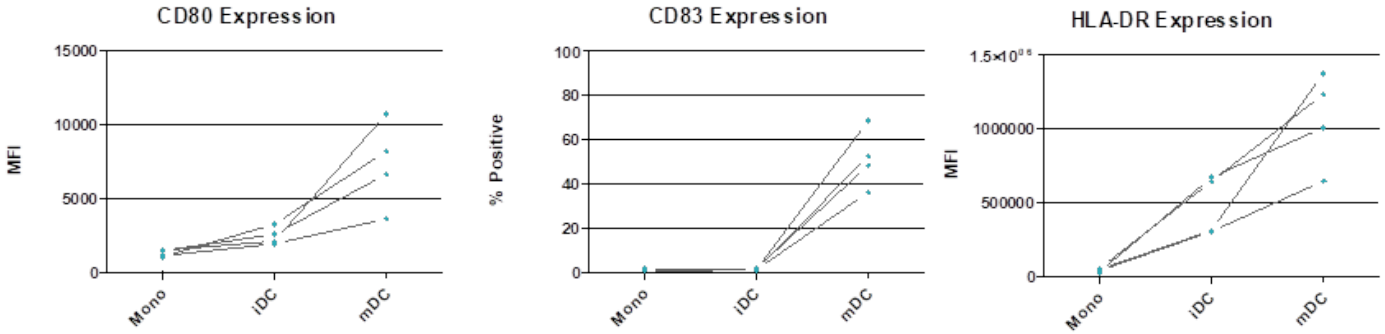
Assay optimization and standardization

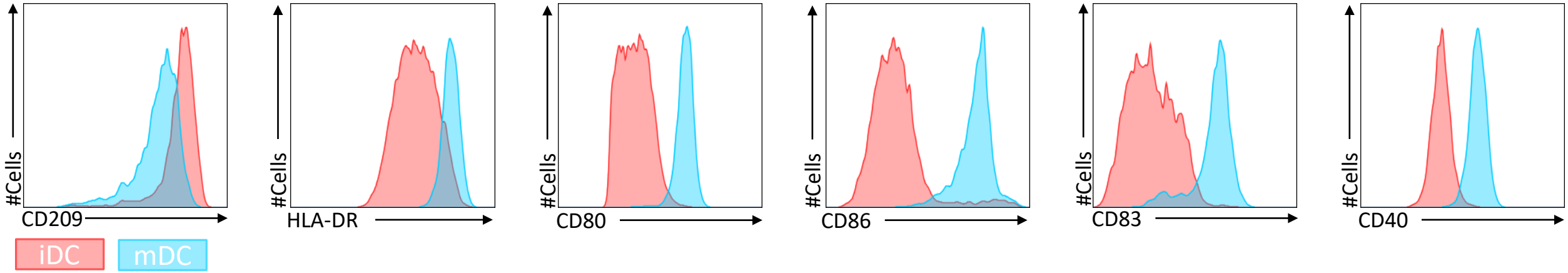
- Optimized procedures for generation of dendritic cells, use of premium grade cytokines
- Quality control of dendritic cells at each stage (monocytes, iDC and mDC)
- Optimized procedures for T cell isolation and in vitro culture (format, cell number, culture media, number of replicates …)
- Selection of positive and negative controls and benchmark controls



Quality control DC at each step of the DC-T assay

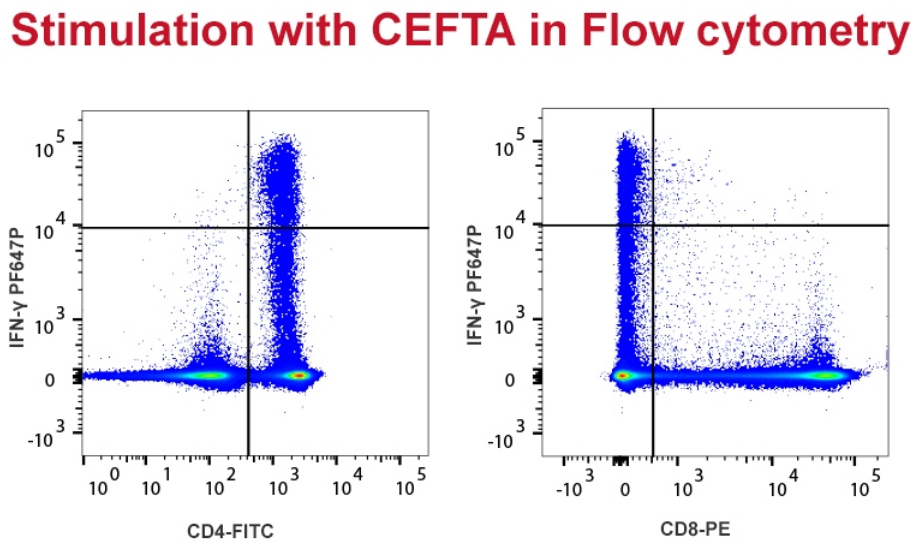






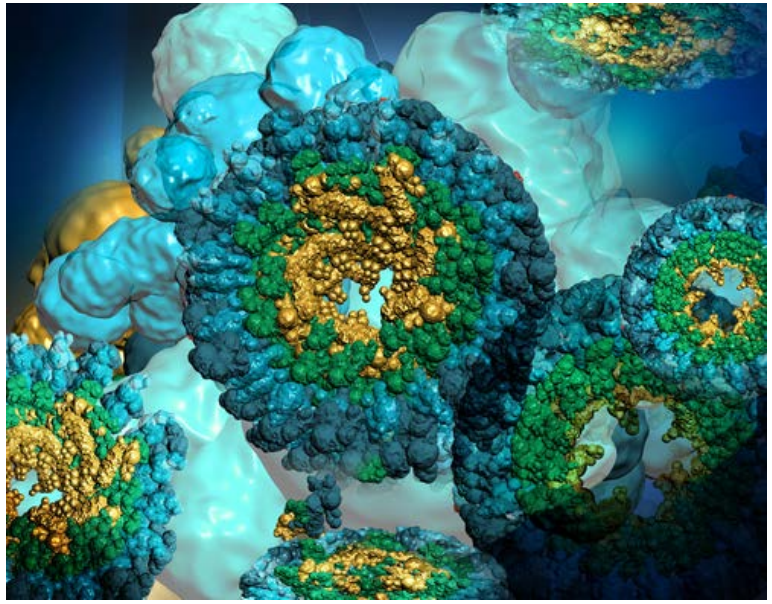
Selection of positive and negative controls

- Positive control(s): peptide mix of recall peptides: **CEFTA** Peptide Mix Mabtech and DMSO control or a strong immunogenic antigen like CMV or KLH



Selection of **positive** and **negative** controls

- Positive control(s): peptide mix of recall peptides: CEFTA Peptide Mix Mabtech and DMSO control or a strong immunogenic antigen like CMV or **KLH**



- The outcome with different brands/types of KLH can be varying a lot
- (Some) toxicity observed with certain lots/brands
- Always perform a lot specific titration
- Stability KLH, storage temperature
- Endotoxin levels

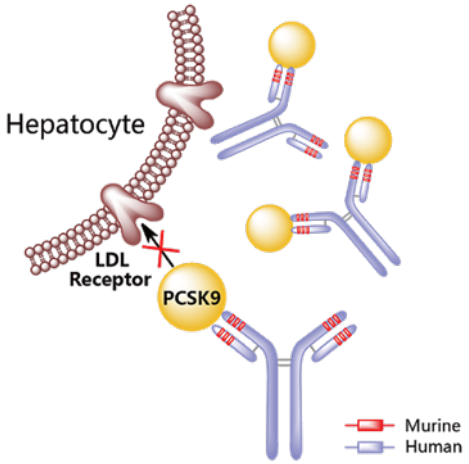
Selection of **benchmark/reference peptide** controls

- Benchmark/reference peptides : PADRE (universal T cell epitope sequence AKFVAAWTLKAAA) or internal positive and negative peptide mix
- Internal **reference positive 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.
- Internal **reference negative 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 **non binders** were identified.

In silico work performed by Prof. Morten Nielsen – DTU Denmark

Selection of benchmark/reference **antigen/antibody** controls

- 2 reference monoclonals, Bococizumab and ATR-107

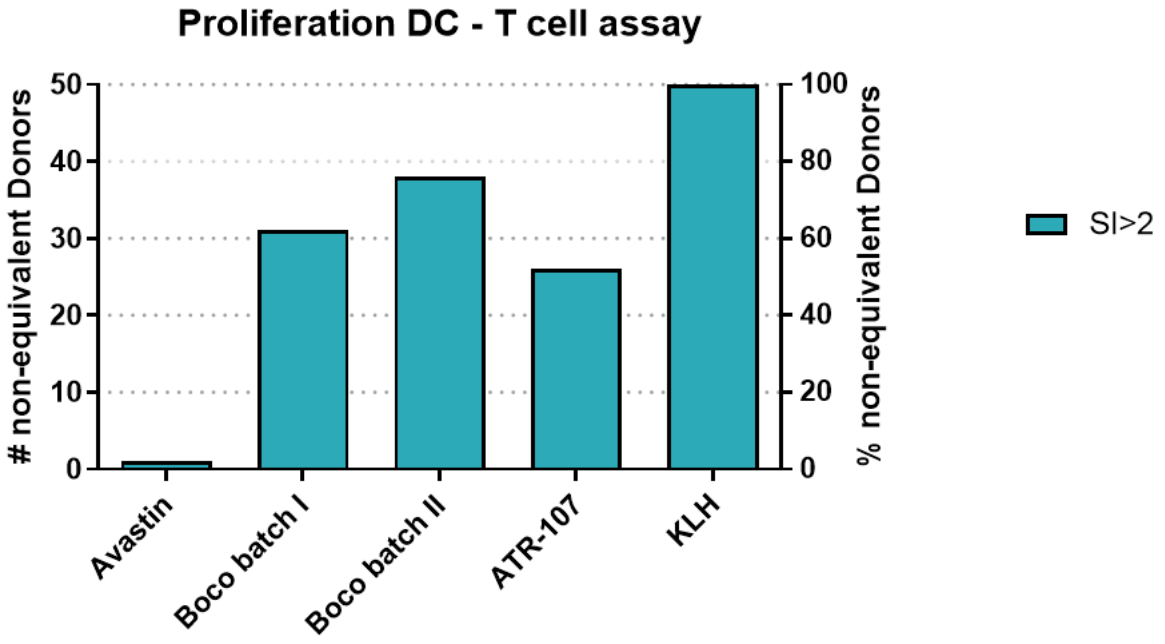


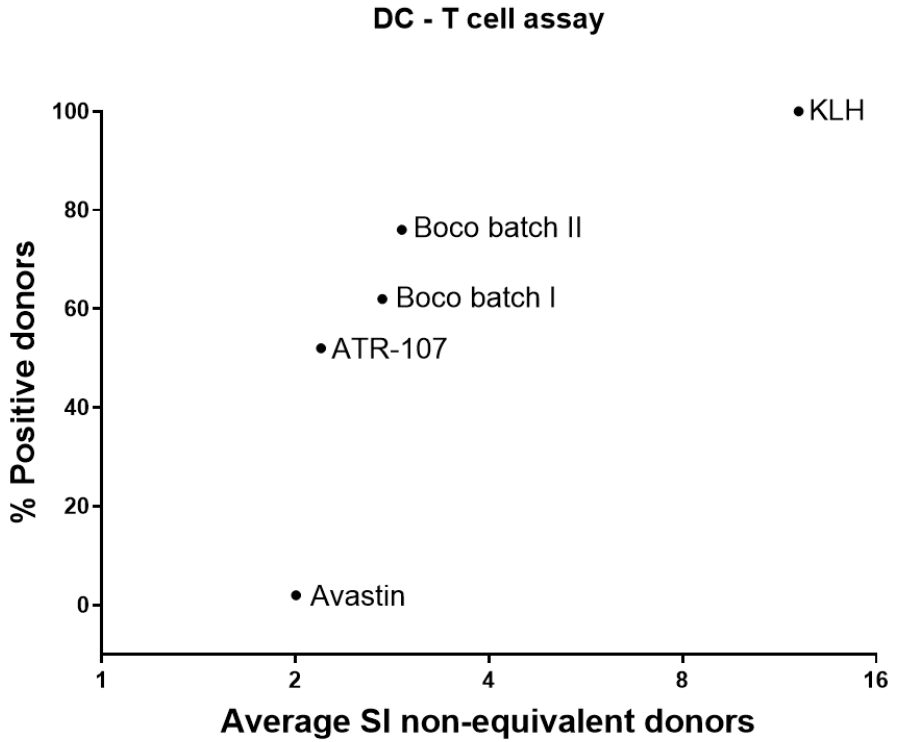
Bococizumab

ATR107

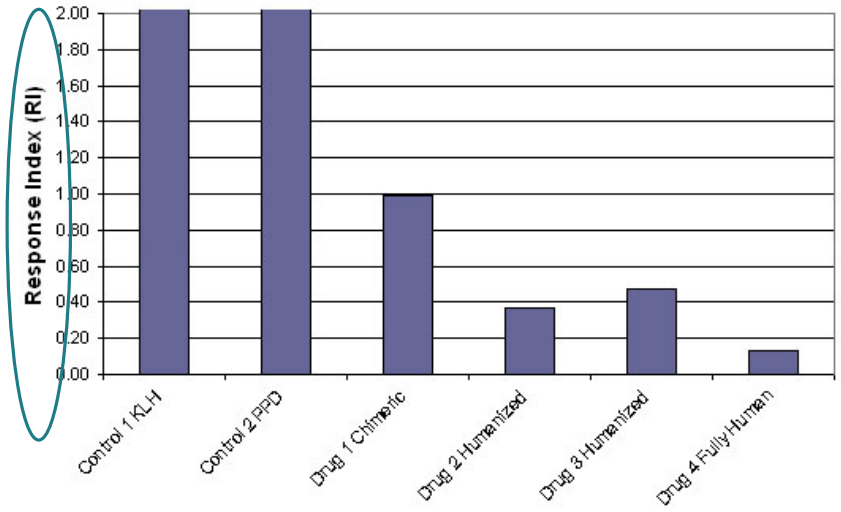
Anti-IL21 Receptor Monoclonal Antibody (ATR-107): Safety, Pharmacokinetics, and Pharmacodynamic Evaluation in Healthy Volunteers: A Phase I, First-in-Human Study
DOI: 10.1002/jcph.158

Stability/performance bench mark controls

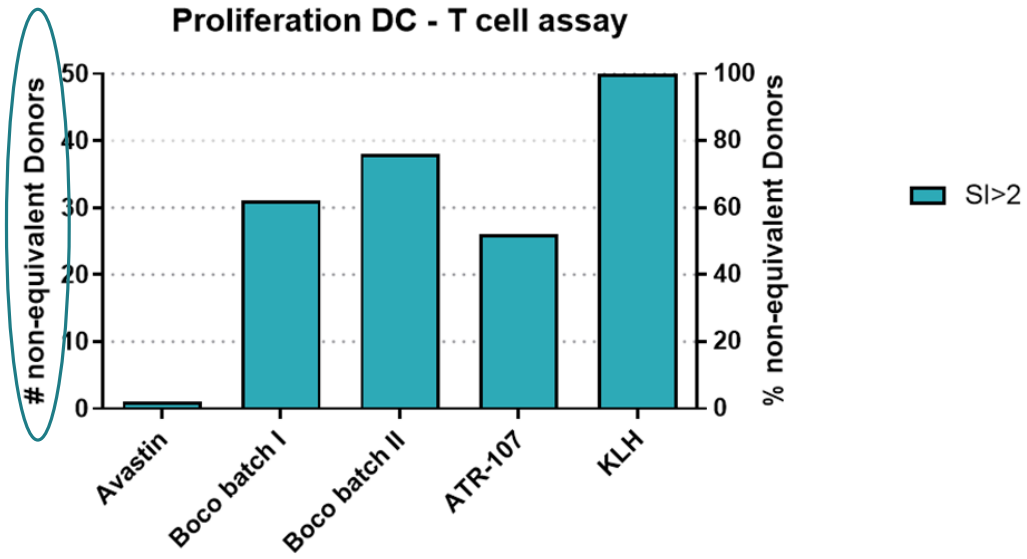




Definition of positive responses



© Proimmune

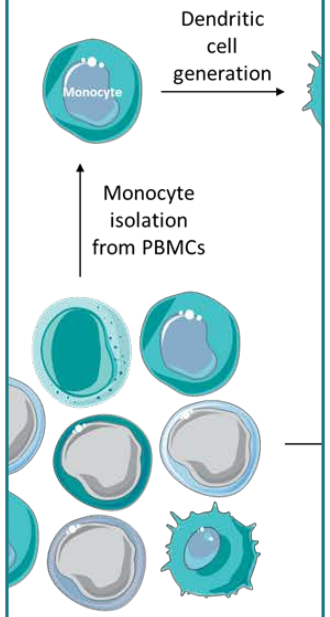


© ImmunXperts

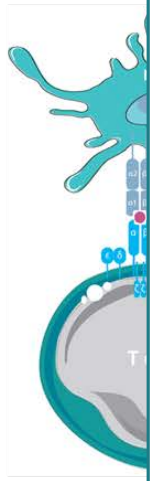
Harmonisation/standardisation DC-T cell assays at every level

ImmunXperts
a Nexelis group company

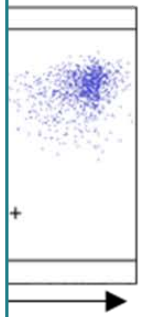
Number of donors
Source of donors
Fresh vs cryopreserved



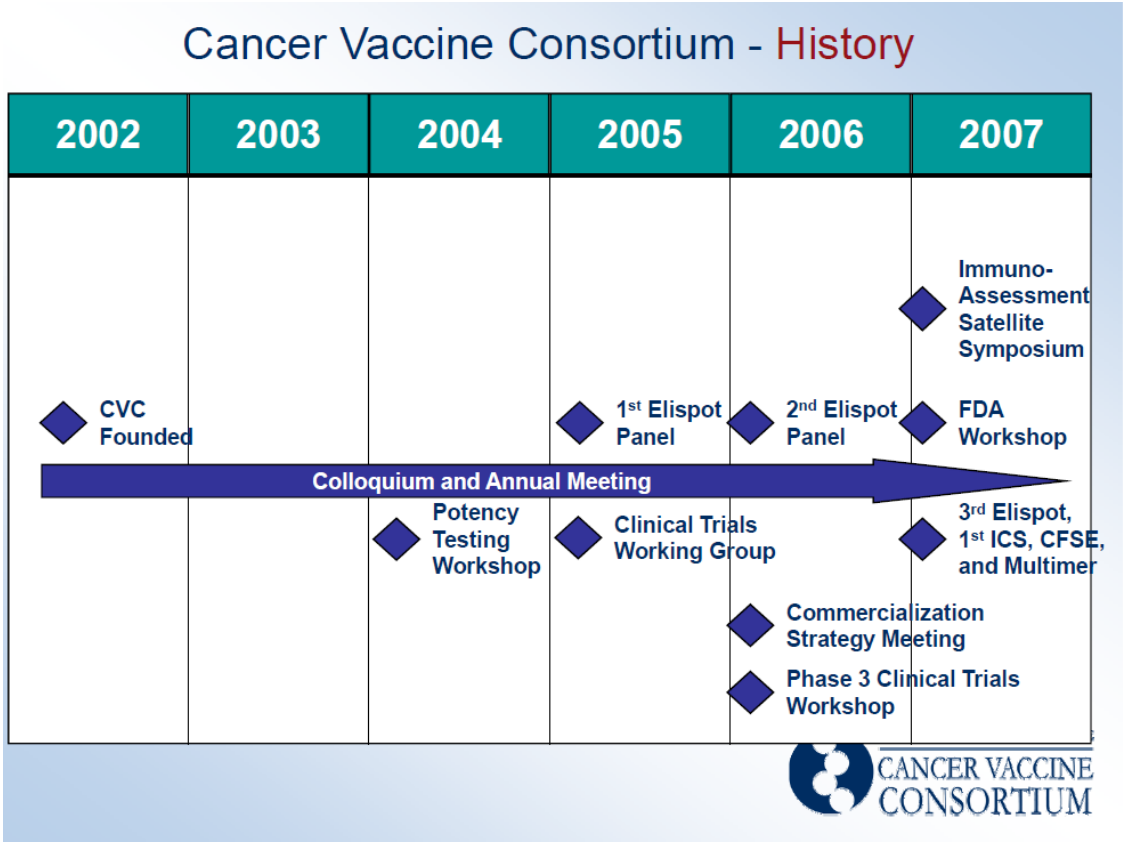
DC generation
CD4 isolation
Culture medium
Plate format
Number of replicates
Incubation time
Reference molecules



Read out platform
Flow cytometry/Elispot
Analysis method (number of events, gating strategy, statistics, …)



Harmonisation/standardization initiatives



2015

Guidelines for the automated evaluation of Elispot assays

Authors:
Sylvia Janetzki [6], Leah Price [7], Helene Schroeder [1], Cedrik M Britten [3,4] ... Axel Hoos [5]

show more details

Harmonisation/standardization initiatives

ImmunXperts
a Nexelis group company

Cytokine: X
Volume 2, Issue 4, December 2020, 100042

Development of the first reference antibody panel for qualification and validation of cytokine release assay platforms – Report of an international collaborative study

Sandrine Vessillier [a], Madeline Fort [b], Lynn O'Donnell [c], Heather Hinton [d], Kimberly Nadwodny [e], Joseph Piccotti [f], Peter Rigsby [a], Karin Staflin [g], Richard Stebbings [h], Divya Mekala [i], Aarron Willingham [j], Babette Wolf [k], participants of the study [a, b, c, d, e, f, g, h, i, j, k]

Show more

NIBSC
Confidence in Biological Medicines

Thank you

ImmunXperts SA
Rue Auguste Piccard 48
6041 Gosselies
Belgium

www.immunxperts.com

info@immunxperts.com