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Human PBMC-based assays for the immunogenicity risk assessment of therapeutic peptides

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Non-clinical Immunogenicity Assessment of Generic Peptide Products: Development, Validation, and Sampling | Tuesday 26 January 2021

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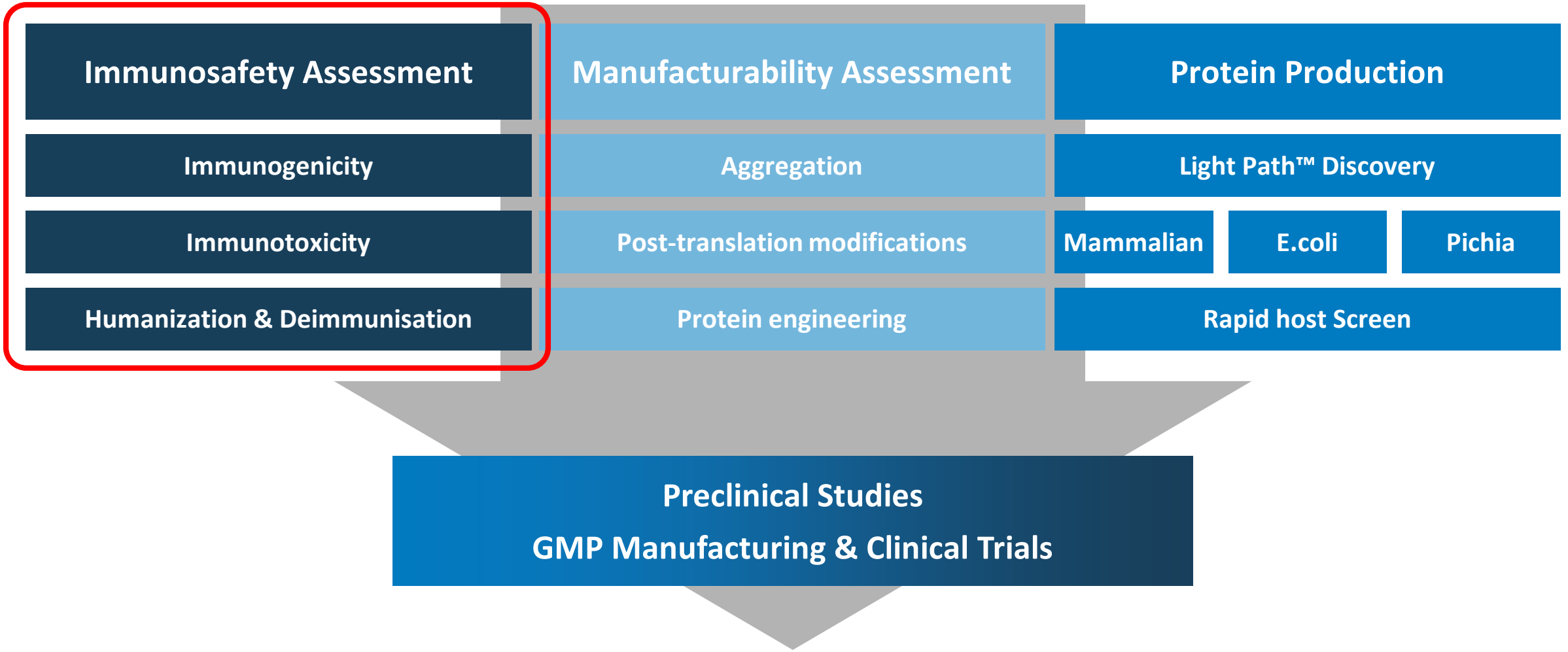
Developability Assessment

Toolbox



Immunogenicity of Biopharmaceuticals

Potential causes

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Product and process-related factors

The Product
Host Cell Protein
Aggregates
Formulation excipients
Product and process-related impurities

For peptide-based products these can include: insertions, deletions, substitutions, truncations, PTMs, conformation changes etc.

The Protein
Species of origin
T and B cell epitopes
Post-translational modifications

Immunogenicity

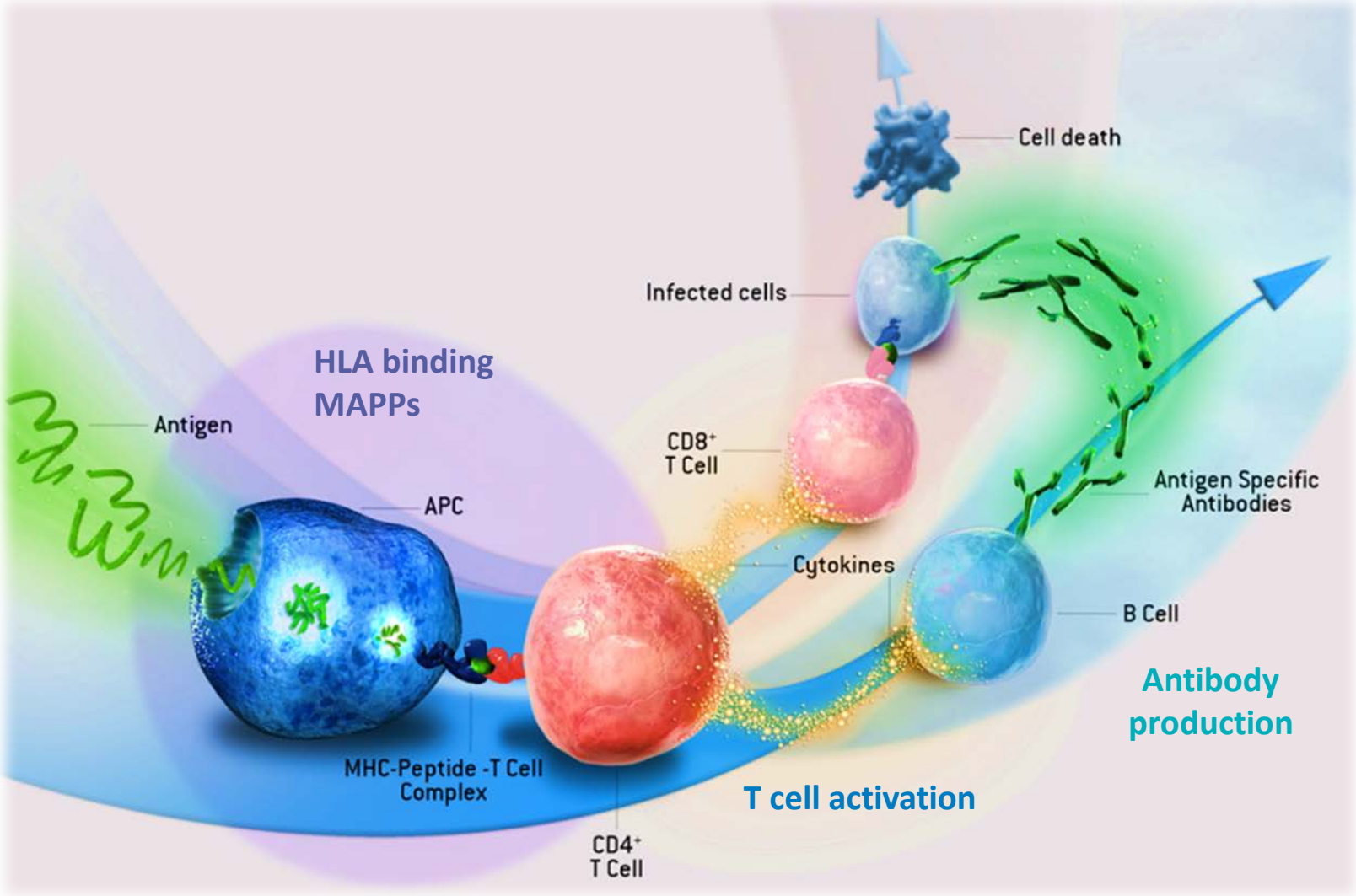
The Patient
HLA genotype
Immune status

The Treatment
Route of administration, dose, regimen
Concomitant medication

Clinical factors

Adaptive immune response

Overview



Immunosafety assessment of biotherapeutics

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Human PBMC bank

- Large highly characterized human primary cell bank (0.2-12 billion PBMC/donor)
- PBMC often provide a better correlation with patient response than animal models; ideal for comparator studies
- Continuous access to healthy donors with the ability to source patients when required
- Serum samples collected from all donors
- High resolution HLA typing and QC for all PBMC preparations (recovery, viability, naïve/memory T cell functionality)

Lonza's immunology platform

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Assessment of the human immune response *in vitro*

Innate immune response

- Whole PBMC/DC activation – impact of product and process-related impurities

Adaptive immune response

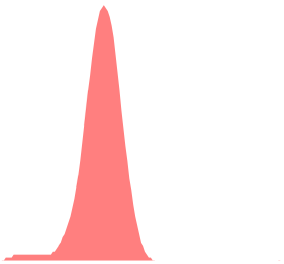
- Naïve T cell activation/pre-existing immunity

Immunotoxicity risk

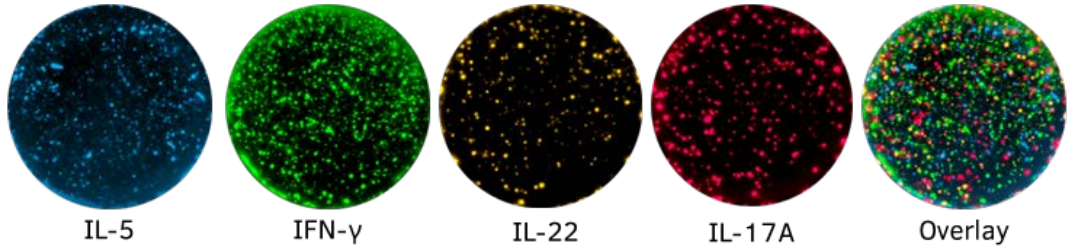
- Impact of mode of action or off-target binding

Luminex®

FACS



FluoroSpot

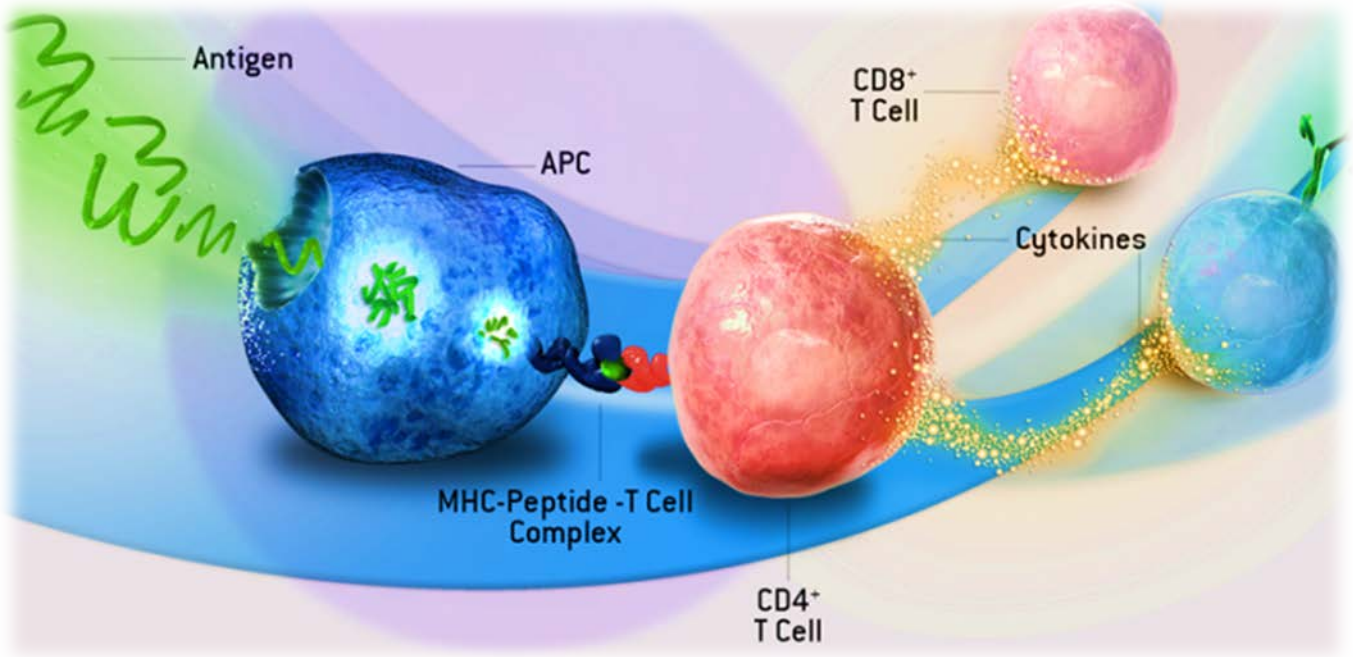


Adaptive immune response risk

T cell assays

- Whole proteins/peptides can be used to assess the risk of raising a T cell response
  - Lead selection
  - Assess the impact of engineering
  - **Generic/follow-on products**

- T cell assay formats:
  - PBMC proliferation assay
  - DC:CD4 proliferation assay
  - DC:CD4 re-stimulation assay



- NOTE: CD8+ T cell assays also available in similar formats

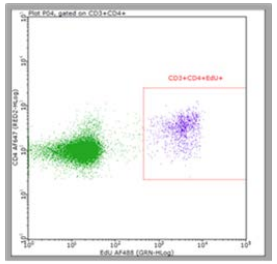
T Cell activation assays

1) PBMC proliferation assay

Assay Readout

Flow Cytometry Analysis

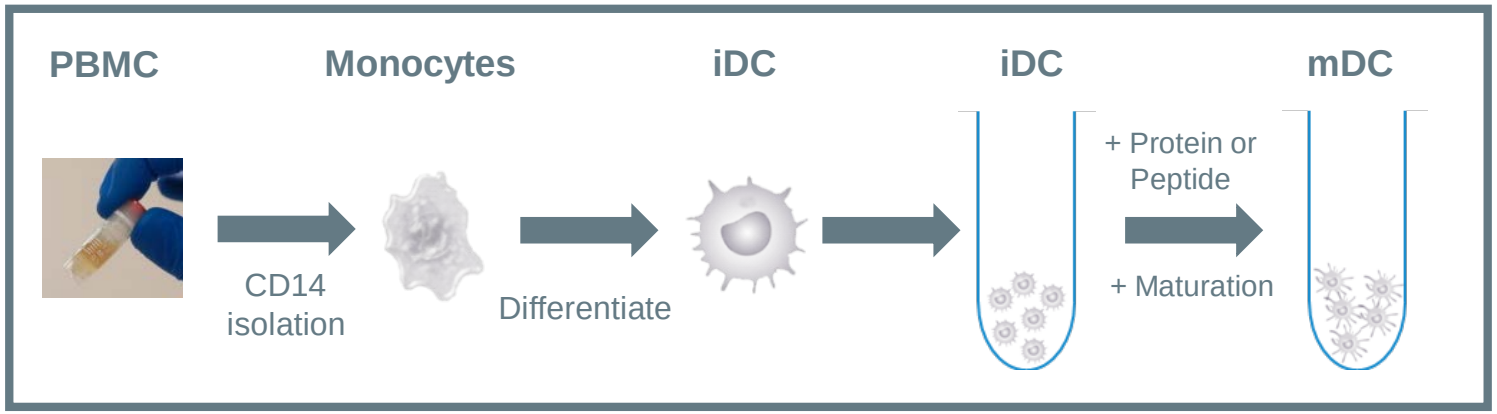
- CD3+CD4+ T cell proliferation

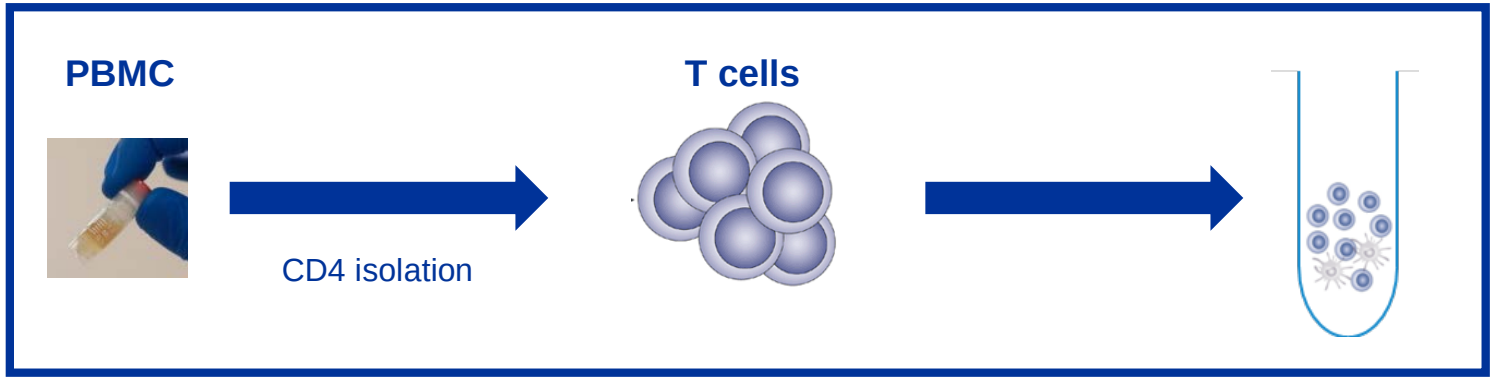


T Cell activation assays

2) DC:CD4 proliferation assay

DC Generation & loading

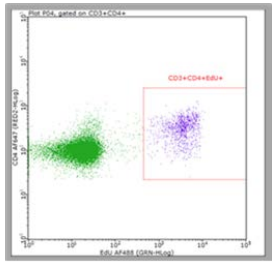




CD4⁺ T cell isolation & co-culture

Flow Cytometry Analysis

- CD3+CD4+ T cell proliferation

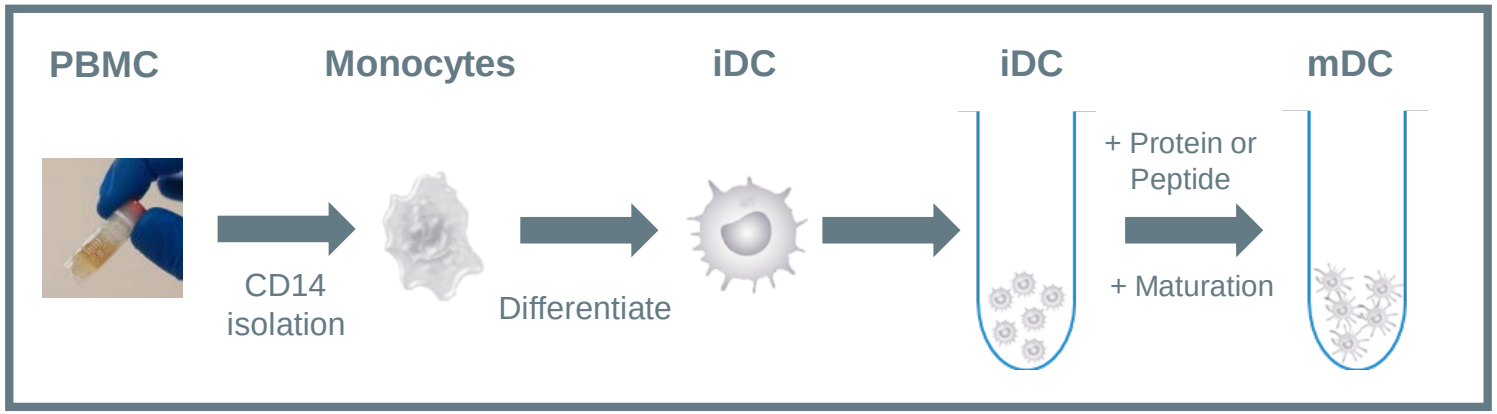


Assay Readout

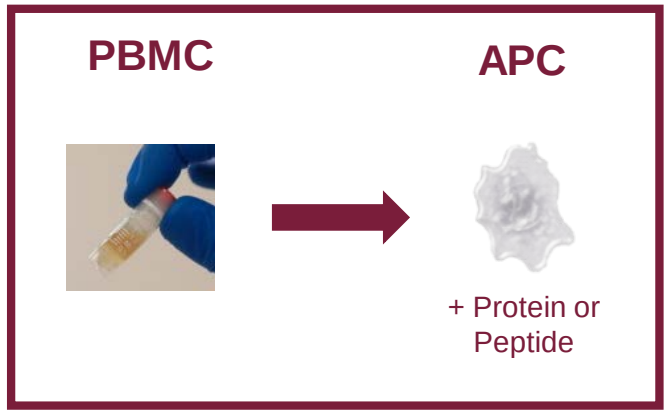
T Cell activation assays

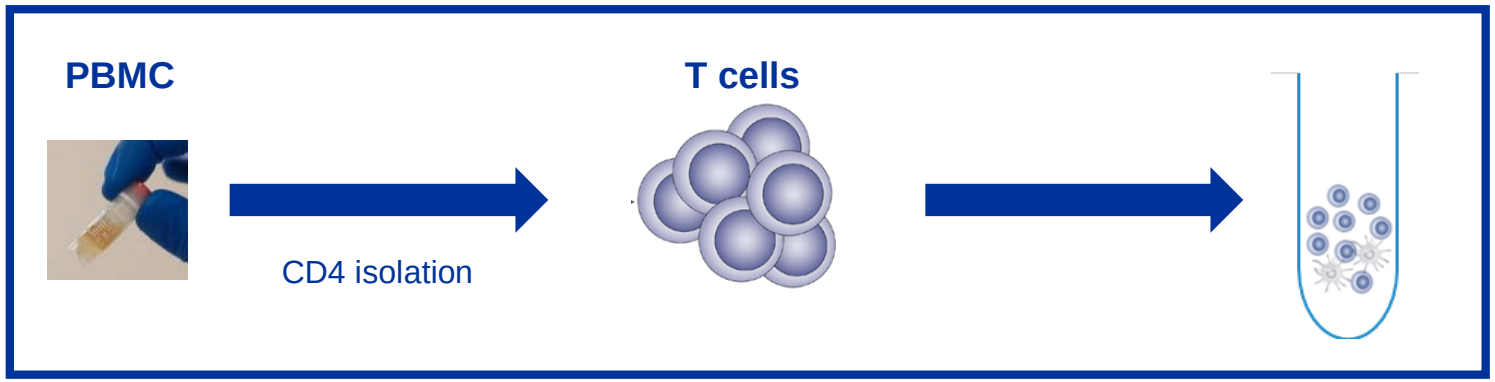
3) DC:CD4 re-stimulation assay

DC Generation & loading



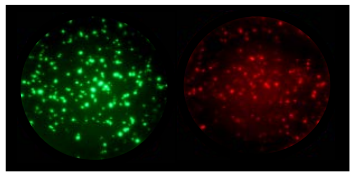
Re-stimulation





FluoroSpot analysis

- IFNγ and IL-5 secreting cells



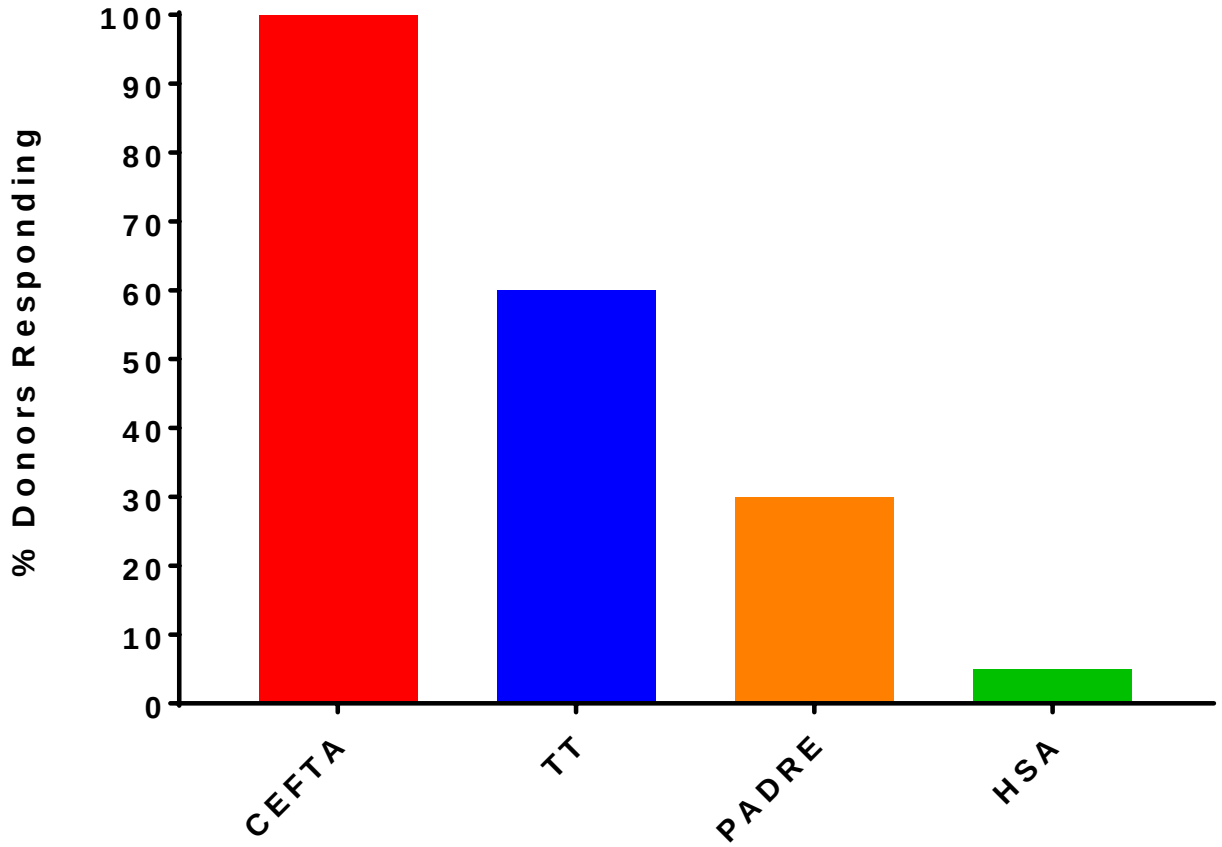
CD4⁺ T cell isolation & co-culture

Assay readout

Benchmarking of T cell activation assays

Peptide responses in the DC:CD4 re-stimulation assay

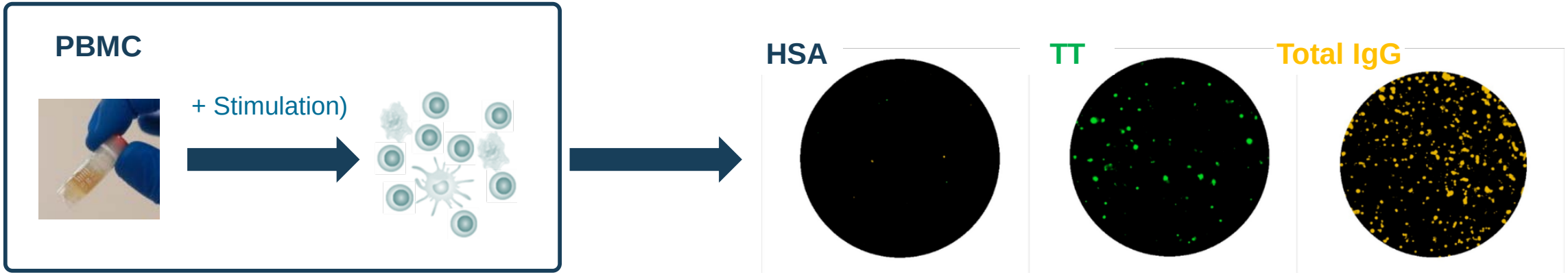
- DC:CD4 re-stimulation assay with IFNγ & IL-5 FluoroSpot readout
- Graph shows % of donors with a significant T cell response (IFNγ and/or IL-5)
- Healthy donor response to different products
- **Very strong response to CEFTA**
- **Strong response to individual TT peptides**
- **Response to naïve PADRE peptide**
- **Low response to HSA peptides**



What about B cells?

Assessment of pre-existing antibodies

- Can conformational changes in the peptide be bound by pre-existing antibodies?
- Polyclonal stimulation of memory B cells in PBMC from naïve healthy donors
- Memory B cells differentiate into plasma cells and secrete antibody
- Analysis of total and antigen-specific ASC (antibody secreting cells) by B cell FluoroSpot
- Assay can detect the presence of cross-reactive antibodies binding to impurities



Non-clinical immunogenicity assessment of generic peptide products

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Assessment of the T cell response: practical considerations

- Choice of assay format is important (DC:CD4-based assays typically improve sensitivity)
- Multiple RLD, DS and DP batches recommended
  - Batches should represent fresh lots along with lots at the end of shelf life to represent maximum impurity content
  - DP can be compared to the RLD
  - DS can be compared to the impurities
- Individual impurities (>0.1%) should also be included where possible
  - Can these be purified from the product (stressed samples)?
  - Synthetic peptides with insertions/deletions/substitutions/truncations and/or PTMs?
- Relevant controls should also be included (e.g. HLA Class II peptide pools and individual peptides)
- Number of donors? For T cell assays 30-50 donors recommended to ensure broad HLA coverage (DR, DQ, DP)
- *In silico* data can also be used to inform the *in vitro* study design

Non-clinical immunogenicity assessment of generic peptide products

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Assessment of the T cell response: example project

- INNATE: PBMC activation assay (12-plex Luminex® readout)
  - 10 donors
  - Untreated and LPS controls
  - RLD (3 lots) and DP (3 lots)
  - DS (3 lots) and individual impurities
- ADAPTIVE: DC:CD4 re-stimulation assay (IFNγ/IL-5 FluoroSpot readout)
  - 30 donors (selected primarily on HLA-DRB1 alleles)
  - Untreated and CEFTA controls
  - RLD (3 lots) and DP (3 lots)
  - DS (3 lots) and individual impurities
- OPTIONAL: Memory B cell assays to assess the risk of pre-existing antibodies

Non-clinical immunogenicity assessment of generic peptide products

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Assessment of the T cell response: data analysis and interpretation

- Data analysis typically includes the frequency and magnitude of the response over the donor population
  - Positive donor response threshold?
    - SI > 2 fairly standard in the field
  - What difference in response frequency should be considered 'different'?
  - Is a lower response than the RLD acceptable?
  - Equivalence testing?
    - Use the RLD batches as a reference
  - Should the assay be optimized for each individual product?
  - Product test concentration?
    - *In vivo* relevance or optimal for the assay?

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Thank you for your attention

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