

***In Silico & In Vitro* Tools for Preclinical Immunogenicity Risk Assessment**

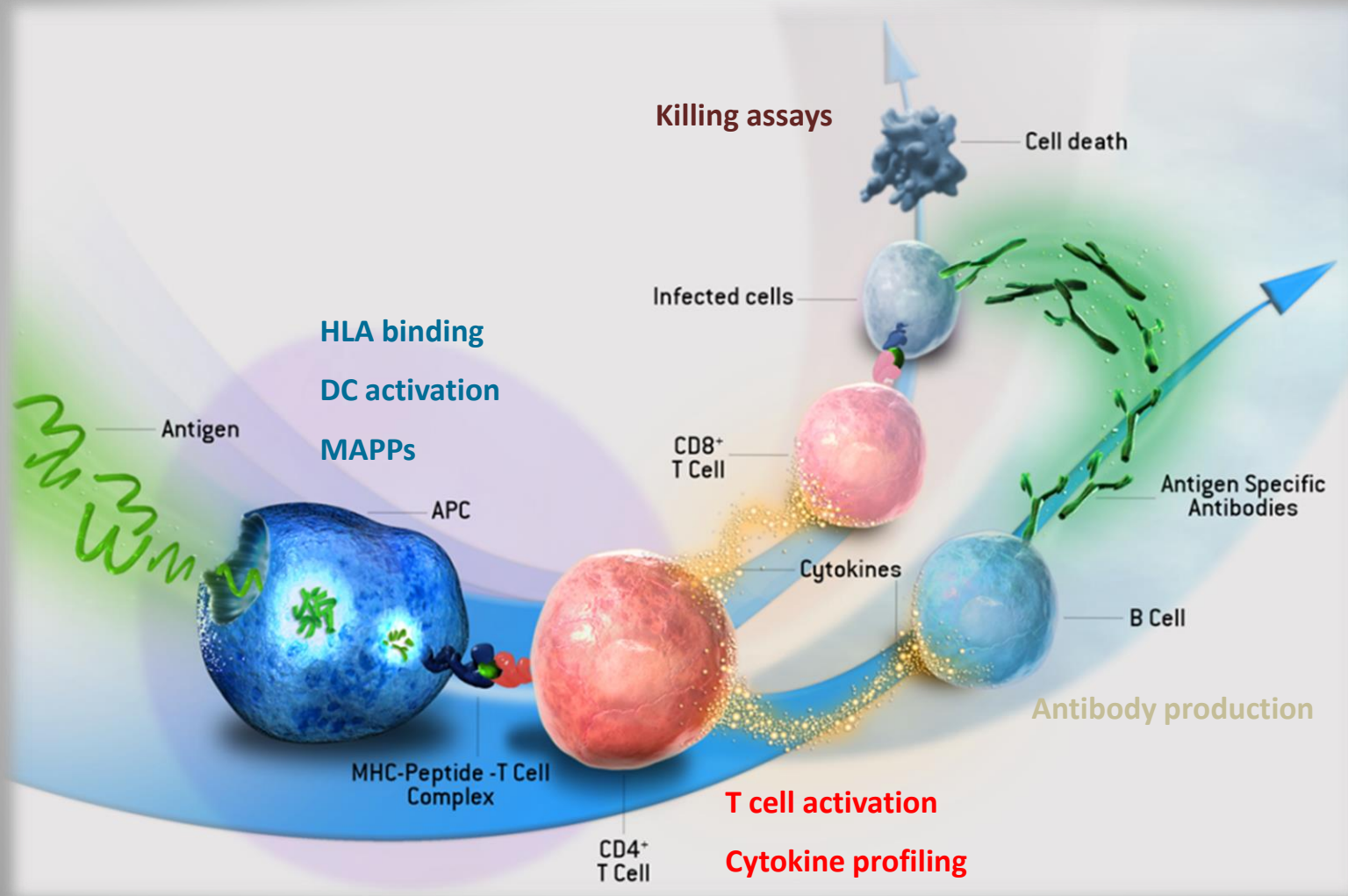
Noel Smith

EIP Symposium

25th February 2019



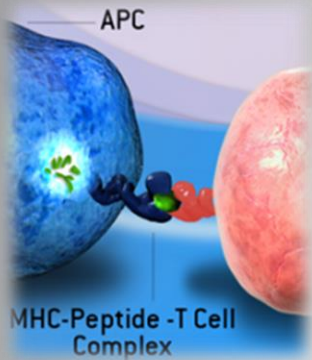
The Immune Response



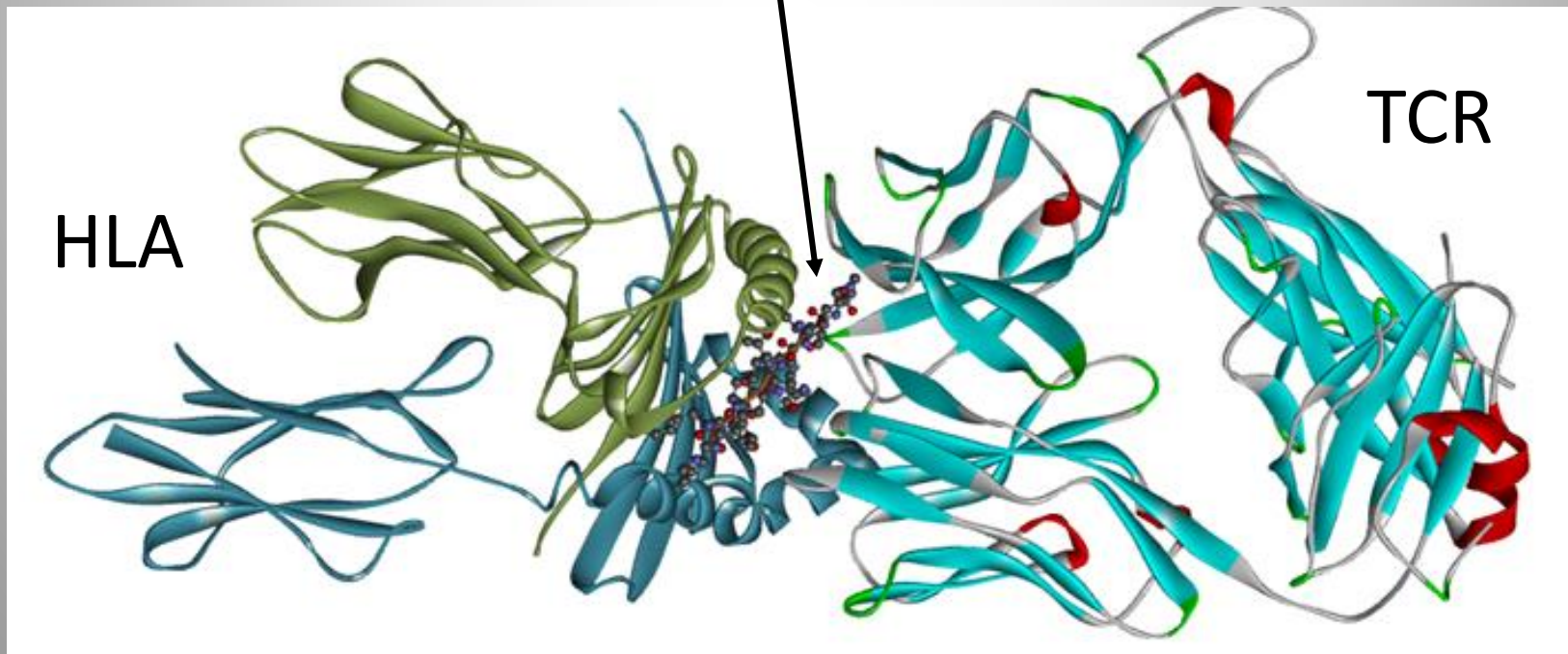
Overview

- **HLA binding**
 - *In silico*
 - *In vitro*
- **Primary cell assays**
 - Processing assays (MAPPs)
 - T cell activation assays
 - Whole PBMC assays
 - DC:T cell assays
 - Memory B cell assays
 - Pre-existing antibodies
 - Innate immune response assays
 - Whole PBMC assays
 - DC assays

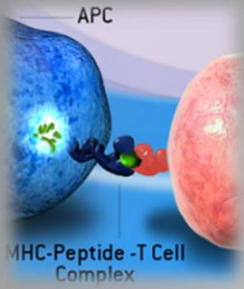
HLA Binding and TCR activation



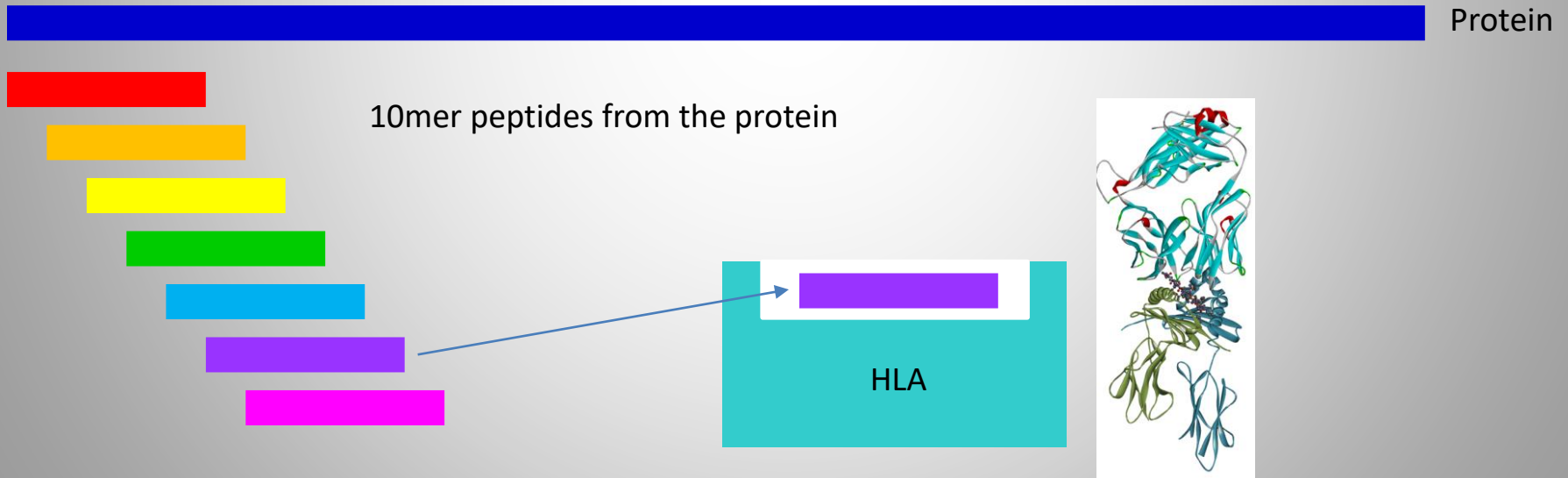
Peptide

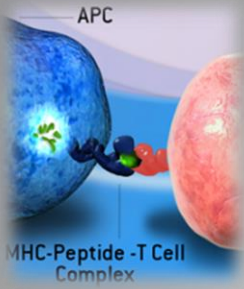


In Silico HLA Binding



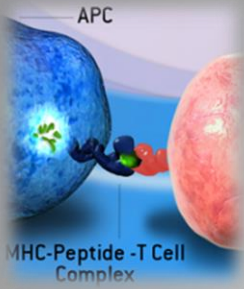
- Algorithms built on available HLA:peptide binding data
- Algorithms can take into account:
 - Binding affinity
 - HLA promiscuity
 - HLA frequency





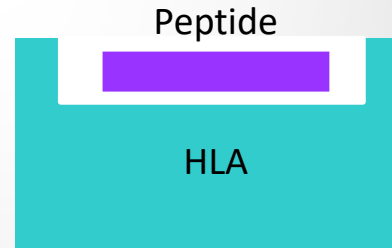
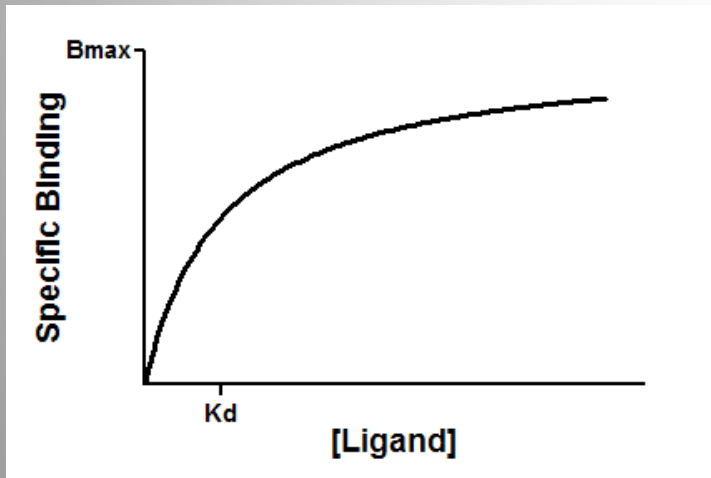
In Silico HLA Binding

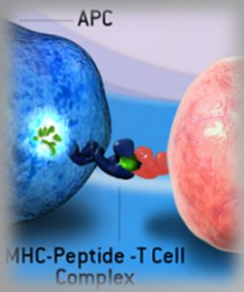
- High throughput, low cost
- Wide HLA coverage
- Filters for human proteins
- False positives
 - Overestimation of peptides (no processing)
 - No information on TCR activation
- No assessment of the influence of product MoA on uptake and processing
- No assessment of the impact of PTMs



In Vitro HLA Binding Assays

- Recombinant HLA-DR, DP, DQ (or Class I)
- Synthetic peptides
- Binding affinity, stability
- Wide range of HLA possible





In Vitro HLA Binding Assays

- Need to select peptides
- No information on TCR activation
- Medium cost/throughput
- Actual HLA:peptide interaction
- Can potentially assess the influence of PTMs



Primary Cell Assays

- Source and quality of immune cells is very important

Blood
sampling

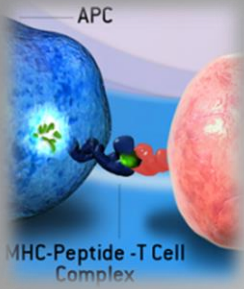
PBMC
isolation

Counting
& vialing

Controlled
rate
freezing

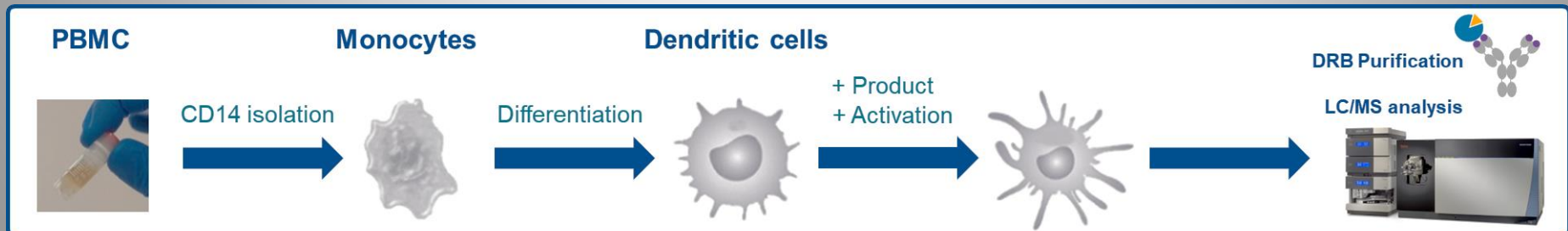
Storage &
sample
management

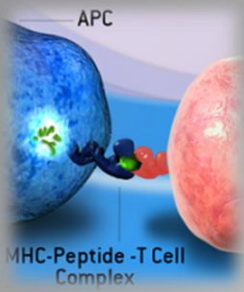
- High cell quality is essential to having sensitive, robust assays
- Informed consent and ethics
- Better correlation with clinical response than animal studies
- Healthy donors and/or patient samples?
- High resolution HLA typing and QC



In Vitro Processing Assays

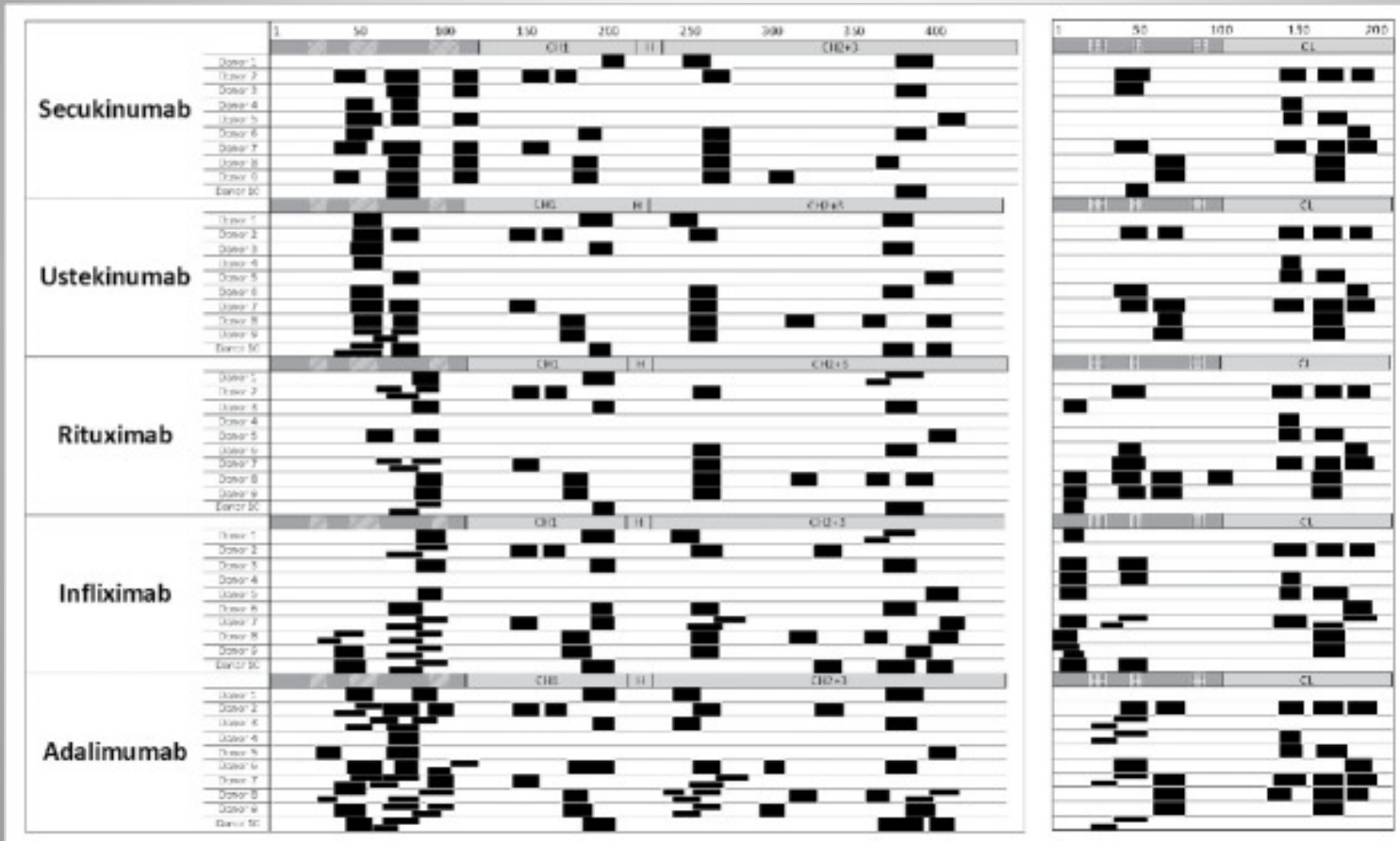
- MAPPs – MHC-Associated Peptide Proteomics
- Dendritic cells (DC) used to naturally process whole proteins and present short peptides via HLA
- Generate DC from PBMC *in vitro*
- Load DC with test protein and activate with e.g. LPS
- Lyse DC and purify HLA:peptide complexes
- Elute peptides from HLA and purify
- ID peptide sequences by MS and map back to protein sequence

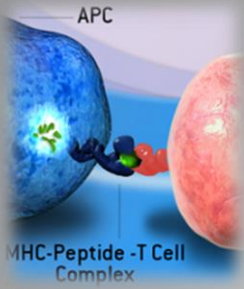




In Vitro Processing Assays

- Example data for monoclonal antibodies



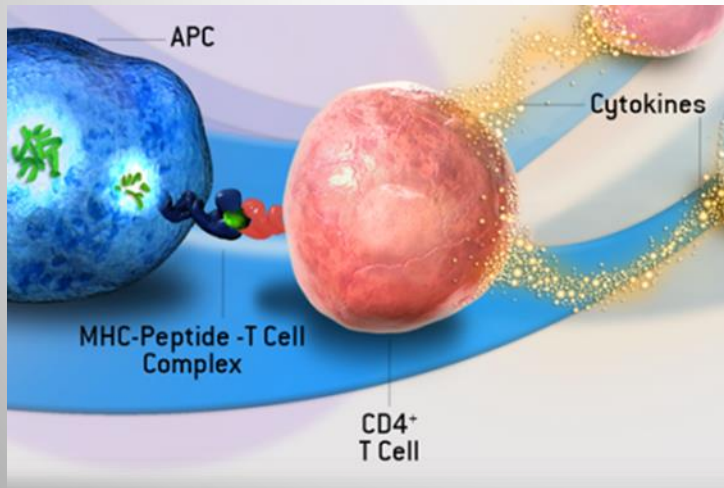


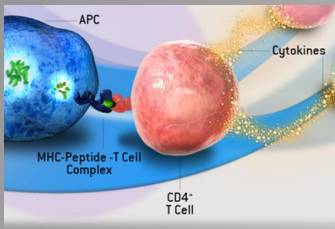
In Vitro Processing Assays

- MAPPs – MHC Associated Peptide Proteomics
- Accurate identification of naturally processed and presented peptides
- Can assess the impact of aggregation, PTMs, formulation, contaminants etc.
- Competition of peptides for HLA
- Expensive
- Large numbers of cells required
- Technically challenging
- Are *in vitro* moDC representative?
- No information on the interaction with the TCR

T Cell Assays

- Directly measure T cell activation
- Proliferation, cell surface markers, cytokine release





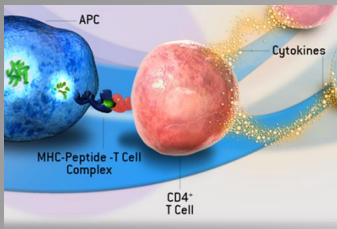
T Cell Assays

- Whole PBMC assays



- # or % activated T cells
- Impact of product on multiple cell types
- Medium throughput
- Medium cost
- Few DC present to drive a naïve response
- Often not suitable for immune modulators

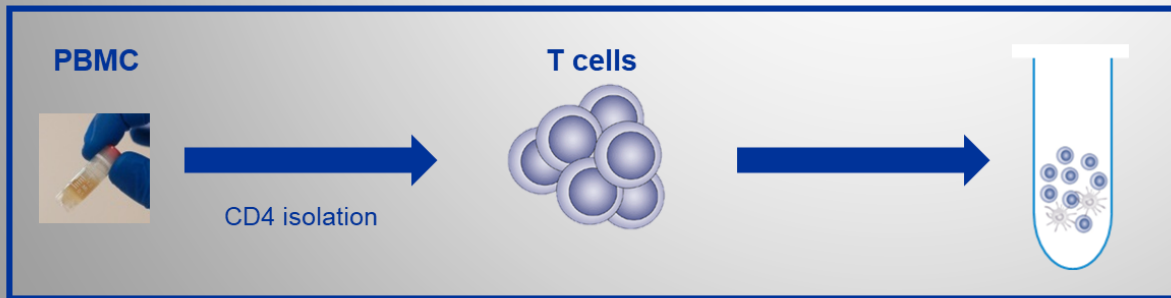
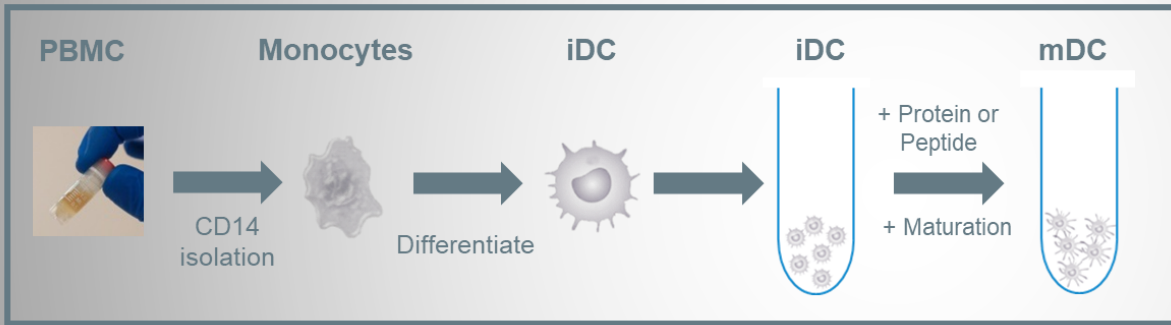
- Proliferation
 - FACS
 - Thymidine
- Cytokines
 - ELISpot
 - FluoroSpot
 - ICS (FACS)
 - Luminex[®]



T Cell Assays

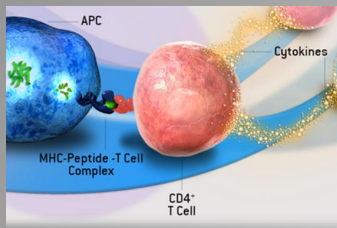
- DC:T cell assays

DC Generation & loading



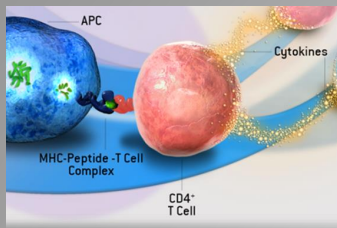
CD4⁺ T cell isolation & co-culture

- Proliferation
 - FACS
 - Thymidine
- Cytokines
 - ELISpot
 - FluoroSpot
 - ICS (FACS)
 - Luminex[®]

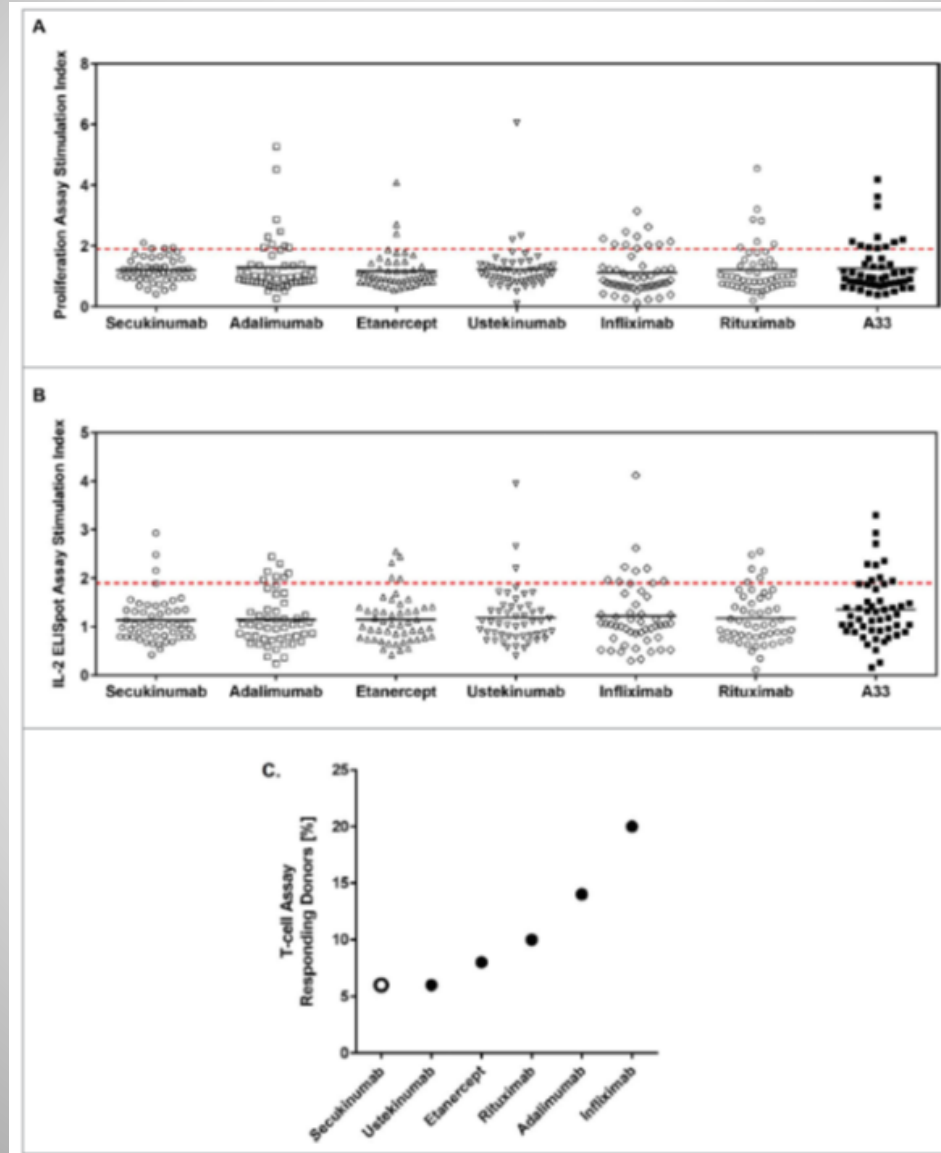


T Cell Assays

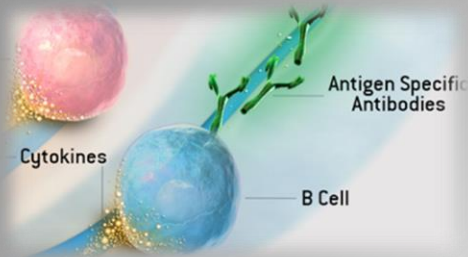
- DC:T cell assays
- # or % activated T cells
- Focus on interaction of DC and CD4+ T cells
- High sensitivity for naïve CD4+ T cell response
- Suitable for immune modulators
- Lower throughput
- Labour intensive
- Technically challenging
- Higher cost



T Cell Assays

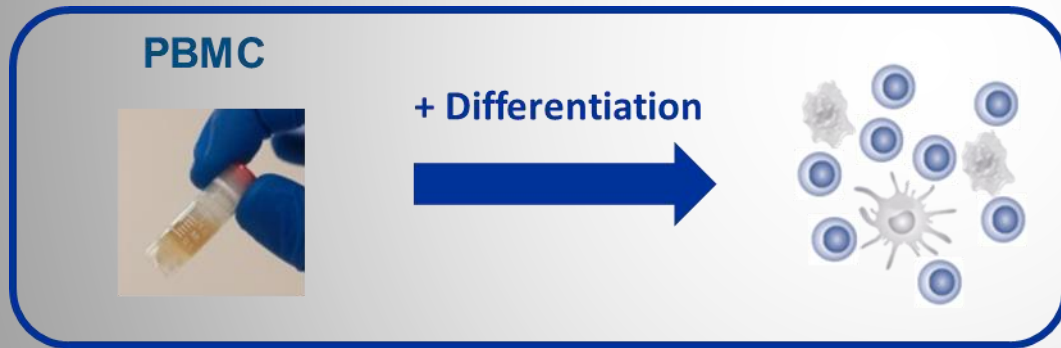


Karle *et al.* MAbs.
2016 Apr; 8(3):
536–550



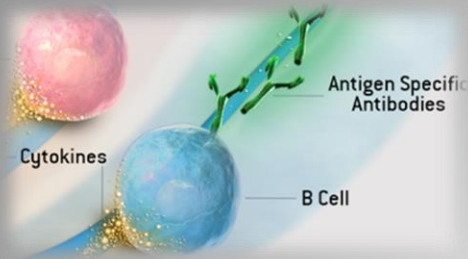
B Cell Assays

- Polyclonal stimulation of memory B cells induces differentiation into plasma cells
- Detect what antibody individual B cells are secreting



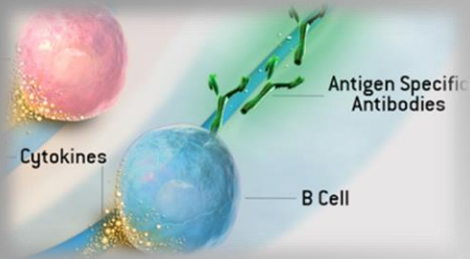
- ELISpot/FluoroSpot
 - IgA, IgE, IgG, IgM
 - Total
 - Protein-specific

- Detection of pre-existing antibodies
 - Protein component
 - PEGylation
- Detection of ADA-secreting B cells during treatment



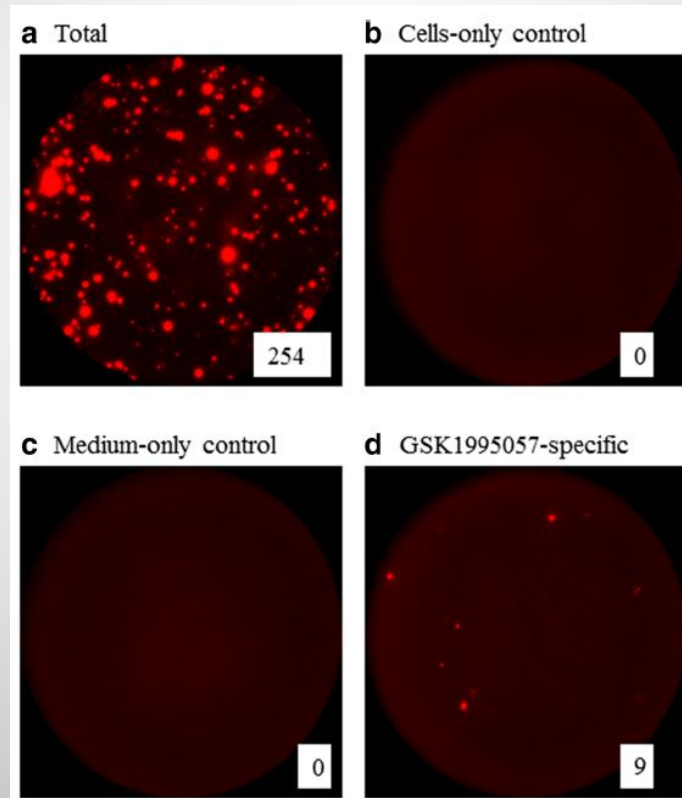
B Cell Assays

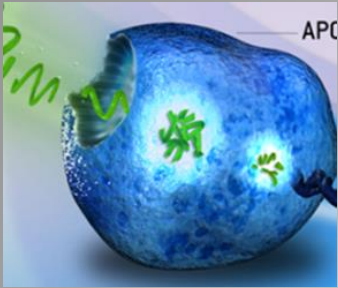
- No specific ADA assay development required
- No free drug present in the assay
- Identification of individual B cells
- Only a portion of the memory B cells accessible
- Clinical validation?



B Cell Assays

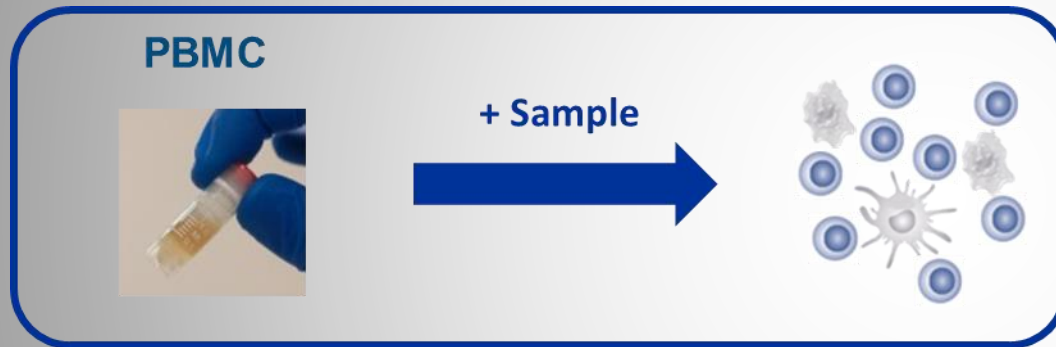
- Liao *et al.* AAPS Journal 2018, 20 (51)





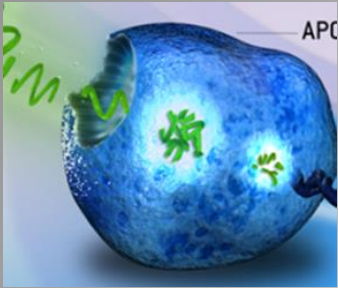
Innate Assays

- Whole PBMC assays



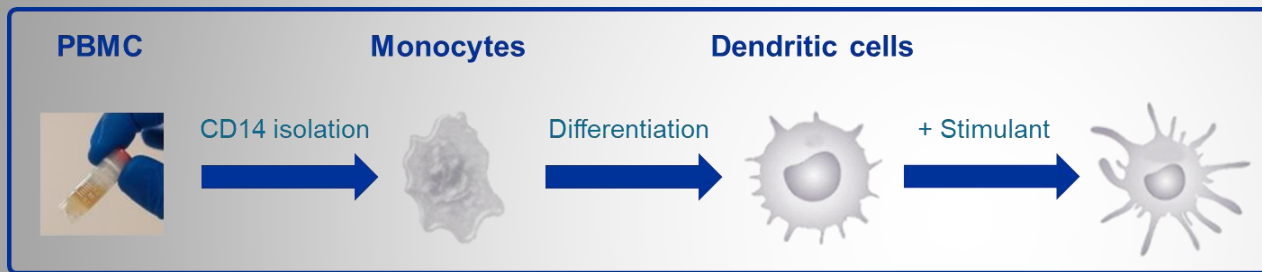
- Cytokines/chemokines
 - ELISpot
 - FluoroSpot
 - ELISA
 - ICS (FACS)
 - Luminex[®]
 - Gene expression

- Fully human system
- Detects responses to known inducers of cytokine storms
- Sensitive for MoA-related activation (TGN1412) & impurities (IIRMI)
- Relatively low cost and throughput
- Low numbers of innate cells (DC) impacting sensitivity?



Innate Assays

- DC activation assays



- Cytokines/chemokines
 - ELISpot
 - FluoroSpot
 - ELISA
 - ICS (FACS)
 - Luminex®
 - Gene expression

- Technically challenging
- Lower throughput and higher cost than PBMC-based assays
- No assessment of the influence on other cells
- Specialised innate immune cells
- Increased sensitivity to impurities (IIRMI)

Discussion

- *In silico* and *in vitro* tools are now widely used during discovery/development
- Not currently a regulatory requirement but this data is often looked upon favorably with regulators as part of the preclinical immunogenicity risk assessment
- Recent FDA workshop
- Common uses:
 - Lead selection
 - Humanisation/deimmunisation
 - Process changes (process-related impurities)
 - Biosimilars/Biobetters/next generation products
 - Clinical samples?
- Selection of tools should be product/project-specific

Questions?