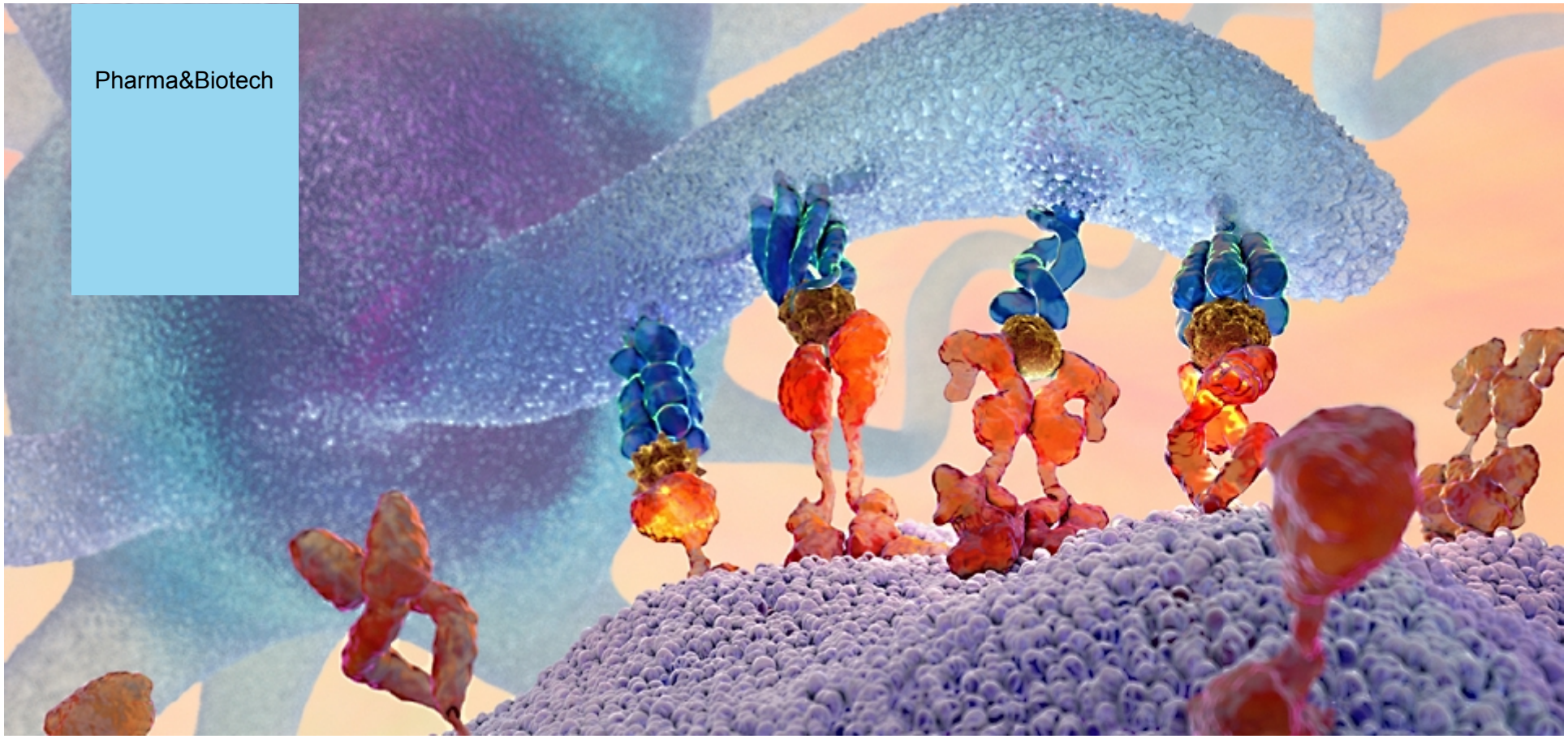


Immunogenicity Prediction

Where are we?

European Immunogenicity Platform, 24th February 2016

Lonza

Immunogenicity of Biopharmaceuticals

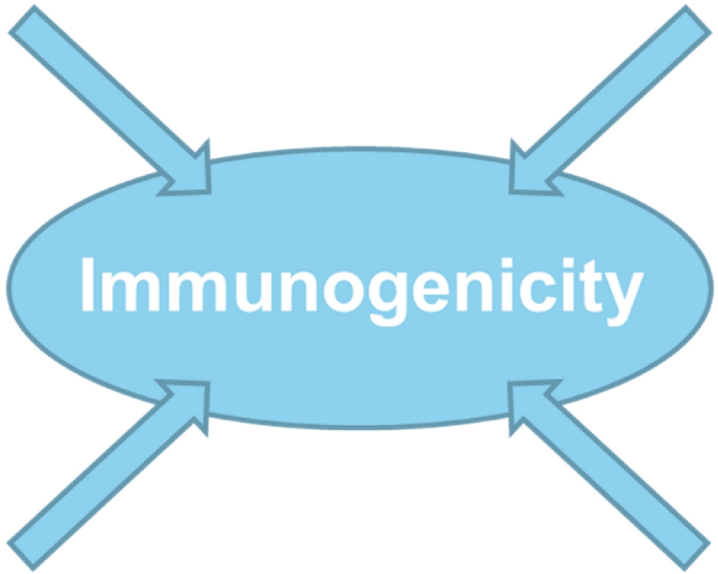
Potential causes

The Protein Sequence & Structure

Source/Species
T & B cell epitopes
Post-translational modifications

The Product

Expression system
Production contaminants
Aggregates
Formulation Excipients



The Clinical Agent

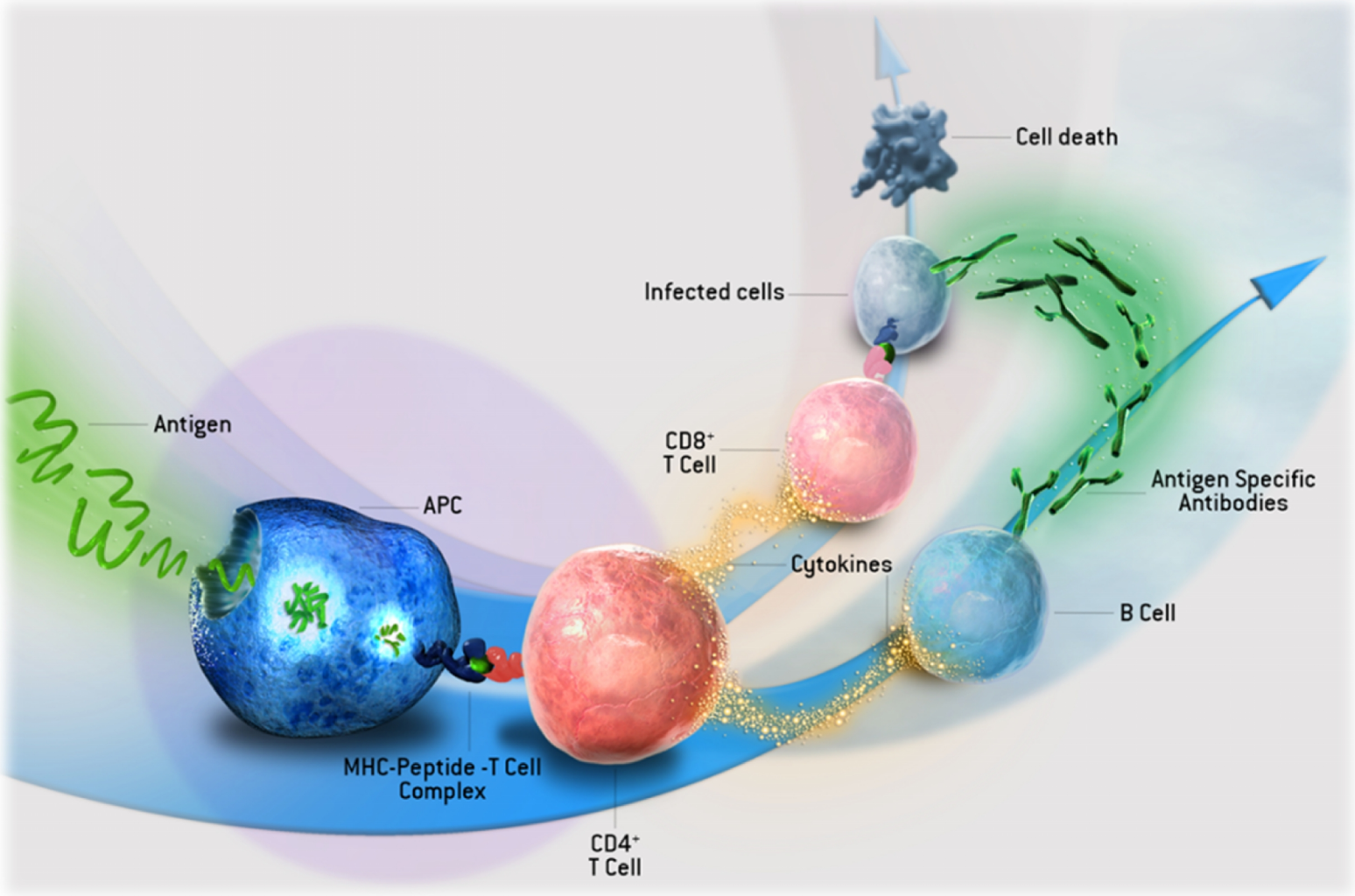
Route of application
Dose
Treatment regimen

The Patient

Immune status
HLA allotype
Medical history
Pre-existing antibodies

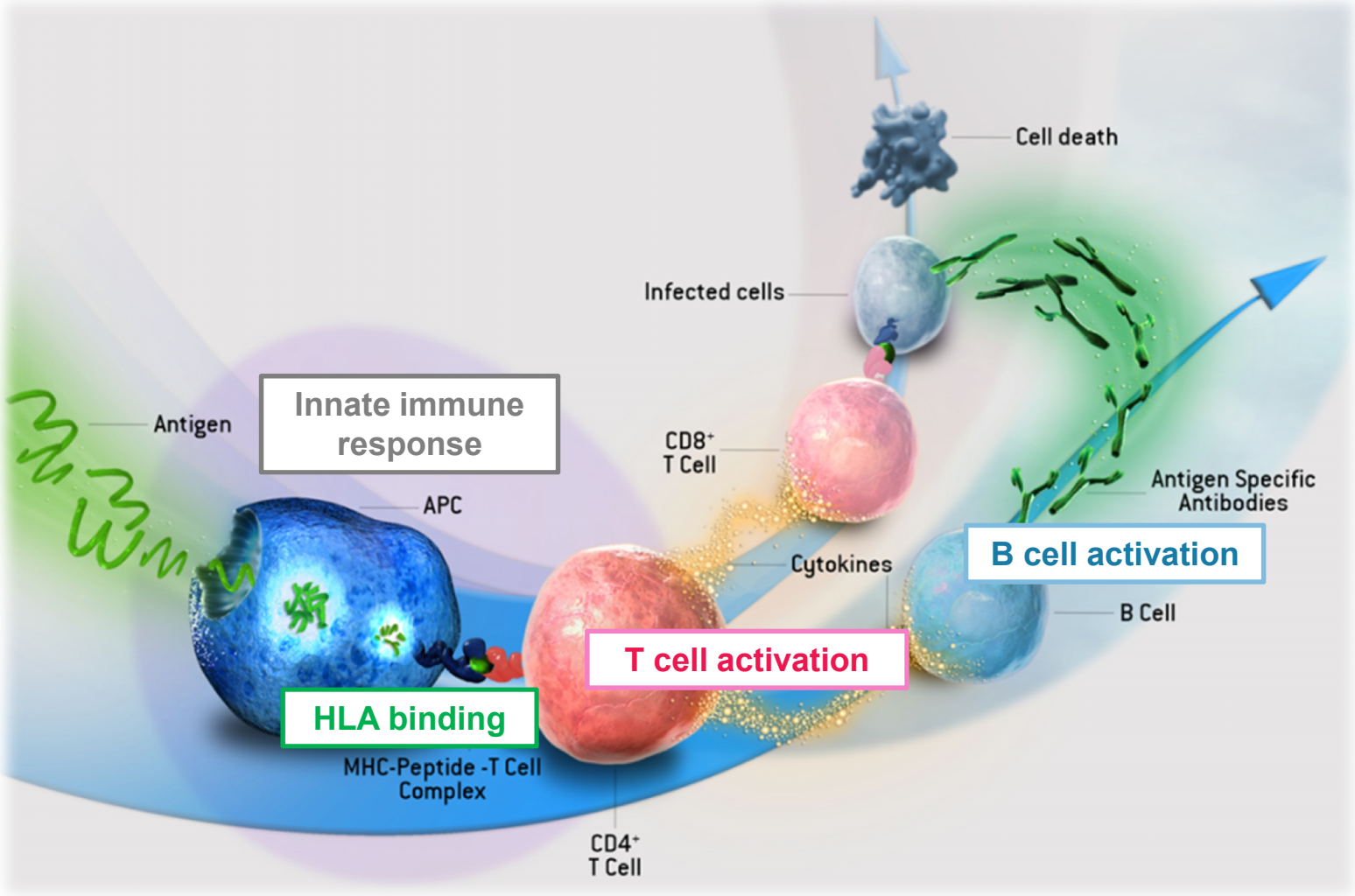
Pre-clinical Immunogenicity Prediction

Overview



Pre-clinical Immunogenicity Prediction

Overview



Immunogenicity Prediction

Pre-clinical Screening Tools

- Mentioned in the regulatory guidelines but are not currently a requirement
- *In silico* tools
  - Computer-based tools
  - Uses the protein sequence/structure
- *In vitro* tools
  - Use human immune cells to assess the immune response to a therapeutic protein
- Humanized mouse models
  - Mice with parts of their immune system replaced with human cells
- Artificial Lymph node
  - Human *in vitro* system to mimic a lymph node

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In Silico Screening Tools

- Manufacturability assessment
  - Aggregation & PTMs
- T cell and B cell epitope prediction
  - Predicts the likelihood of regions of the protein binding to HLA or BCR
  - Algorithms built on published *in vitro* data
- Rapid, high throughput, cost effective
- Wide HLA coverage
- Used to aid lead selection or to identify hot spots for engineering
- Overpredictive
  - Aggregation & PTMs influenced by other factors
  - Does not take into account the processing and presentation of the protein
  - HLA binding does not mean TCR activation

In Silico Screening Tools
Whole protein screening

- Ranking of protein leads
- Cumulative score of all the potential T cell epitopes within a protein sequence

Type	DRB Score	Epitope count			
		DRB1 strong	DRB1 medium	DRB3/4/5 strong	DRB3/4/5 medium
Chimeric	1940	15	38	5	24
Humanized A	1530	14	28	2	26
Humanized B	1040	7	25	3	14
Human C	890	6	17	0	12
Human D	680	5	20	2	14
Human E	280	0	15	0	10

In Vitro Tools

- Human primary immune cells (PBMC) stimulated *in vitro* with the test protein (or peptides/peptide pools)
- Select your HLA coverage
- Takes into account formulation, aggregates, contaminants etc.
- Can be used to aid lead selection or to identify individual epitopes for deimmunization
- More costly & time consuming compared to *in silico* tools
- Naïve B cell responses *in vitro* difficult

In Vitro Tools

- Activation of innate immune response
  - DC activation (cell surface markers, cytokine profiles)
- *In vitro* HLA binding
- Naturally processed HLA binding peptides
  - MHC-Associated Peptide Proteomics (MAPPs)
- T cell activation
  - DC:T cell assays
- B cell activation
  - Activation of memory B cell responses
- Cytokine release assays
  - Not traditional immunogenicity but also very important

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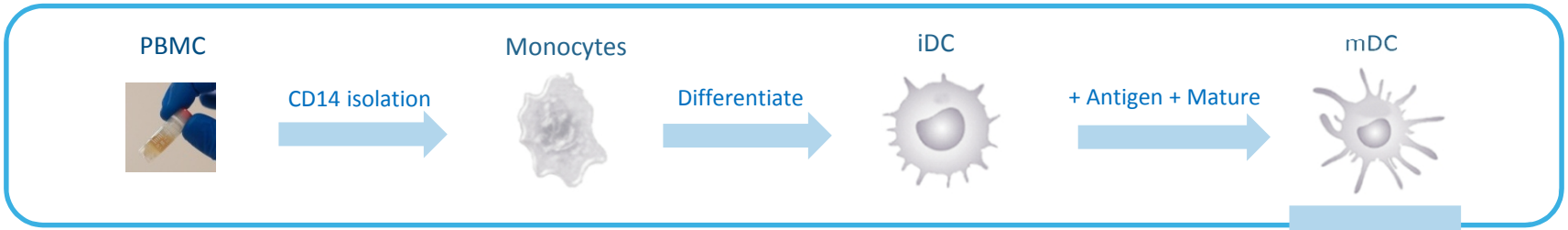
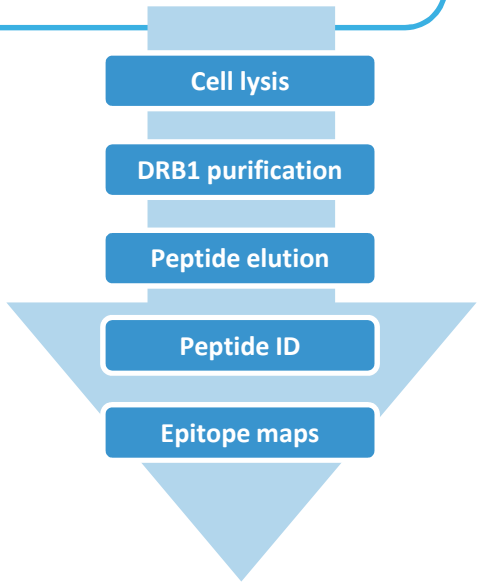
MHC-Associated Peptide Proteomics (MAPPs)



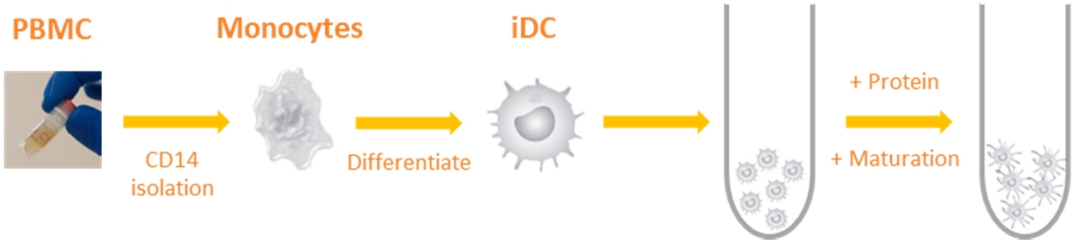
- Accurate identification of potential T cell epitopes
- Takes into account protein uptake, cleavage and processing within the dendritic cells
- Identifies naturally processed HLA binding peptides from therapeutic proteins
- Can select HLA-DRB donors that represent major allotypes concentrate on specific HLA-DRB allotypes of interest
- Assay process
  - PBMC preparation
  - DC generation
  - anti-DR antibody production
  - DRB1 purification
  - MS analysis



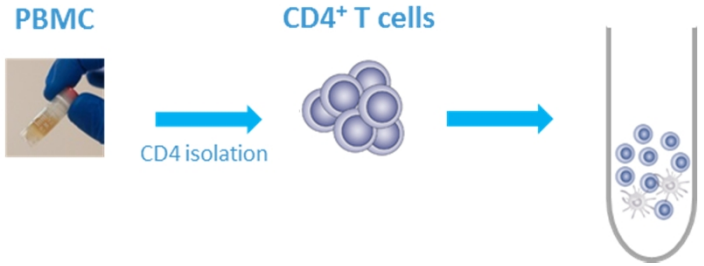
In Vitro T Cell Assay Platforms

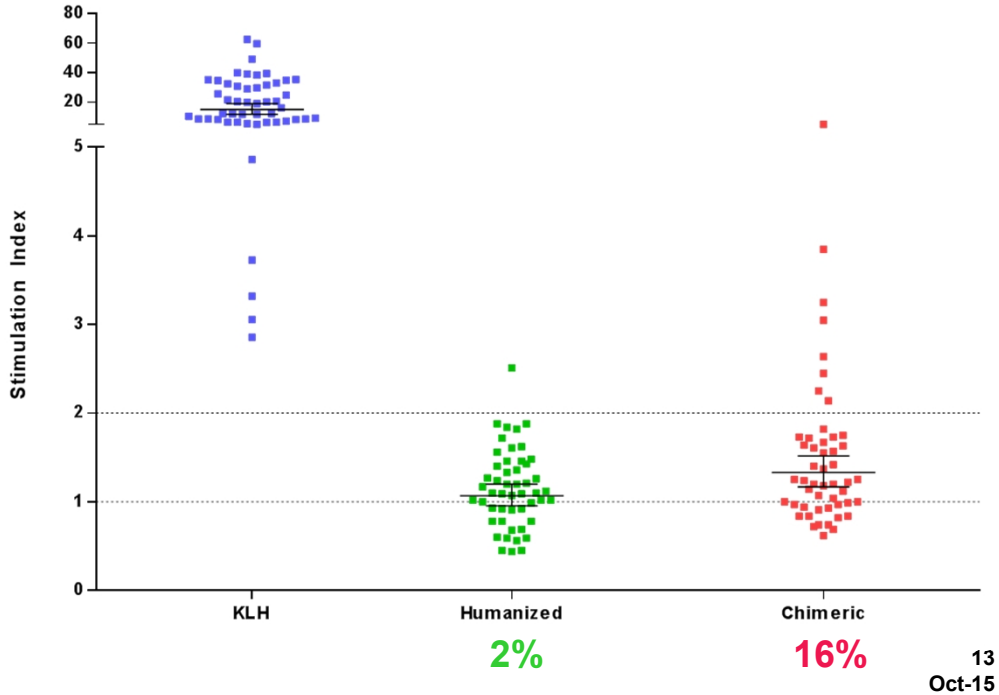
DC:T cell assay

DC Generation & loading



CD4⁺ T cell isolation & co-culture





Immunogenicity Prediction

Where are we?

- Technology has moved on in the past few years with lots of different platforms available to assess different stages of the immune response
- Now being more widely used during lead selection
- Currently focused on T cell responses
- May become more important for immunomodulators?
- Assay standardization?