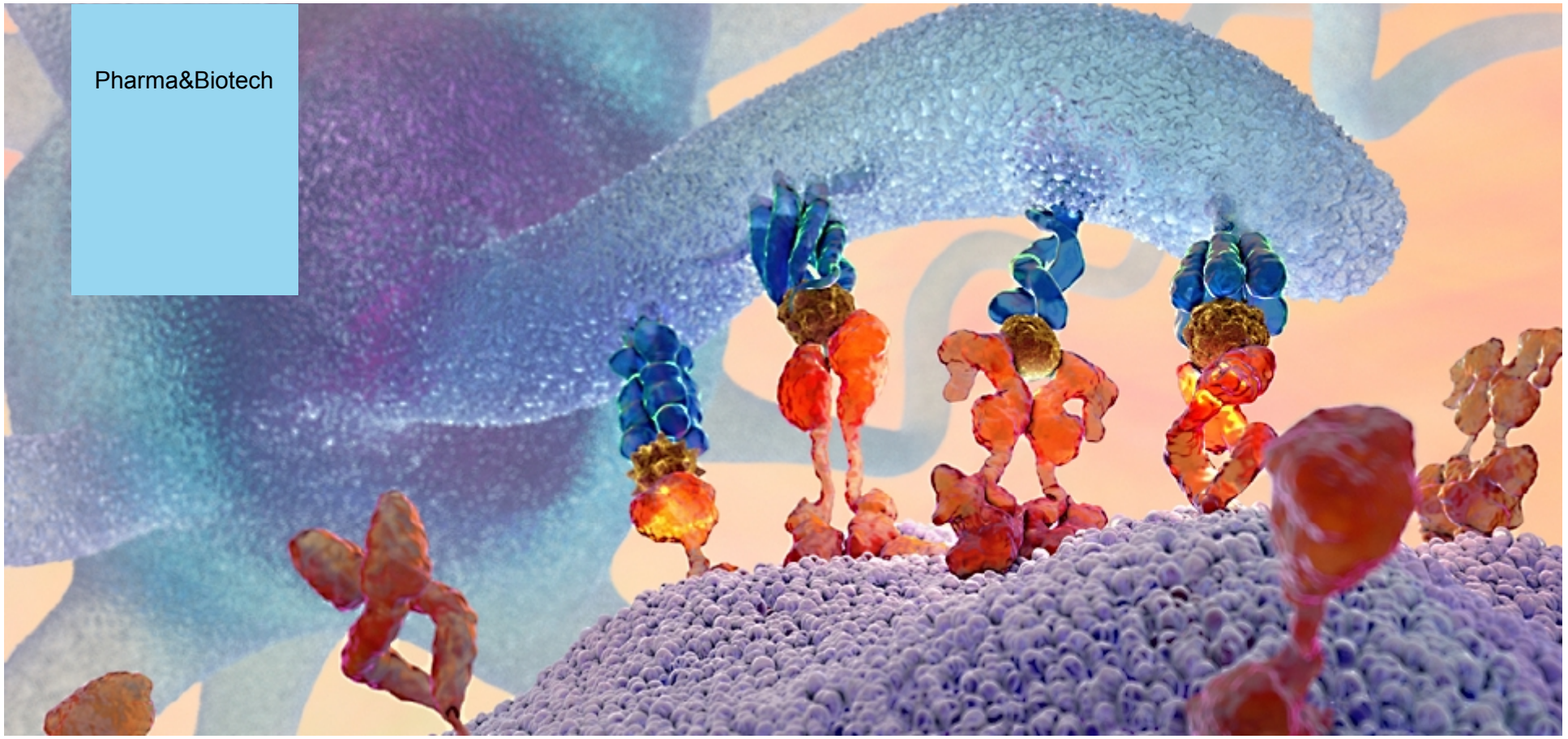
Pharma&Biotech



# Immunogenicity Prediction

## Introduction & Discussion

European Immunogenicity Platform, 22nd Feb 2016

Lonza

# Immunogenicity of Biopharmaceuticals

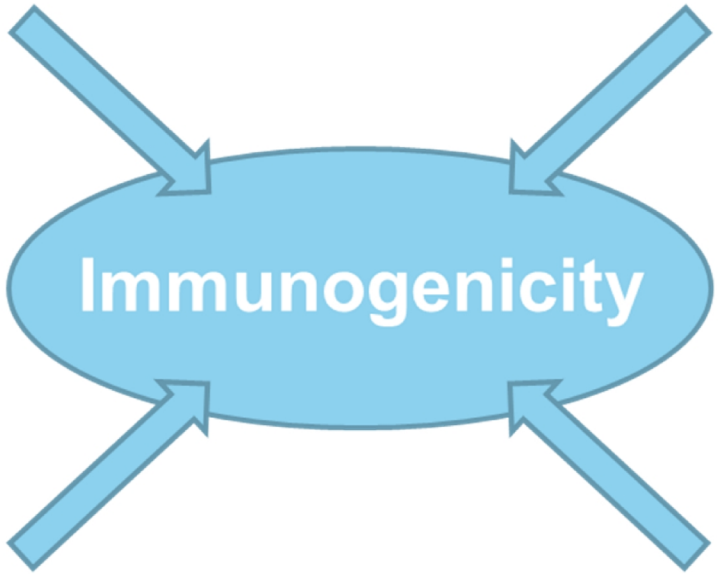
## Potential causes

### 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

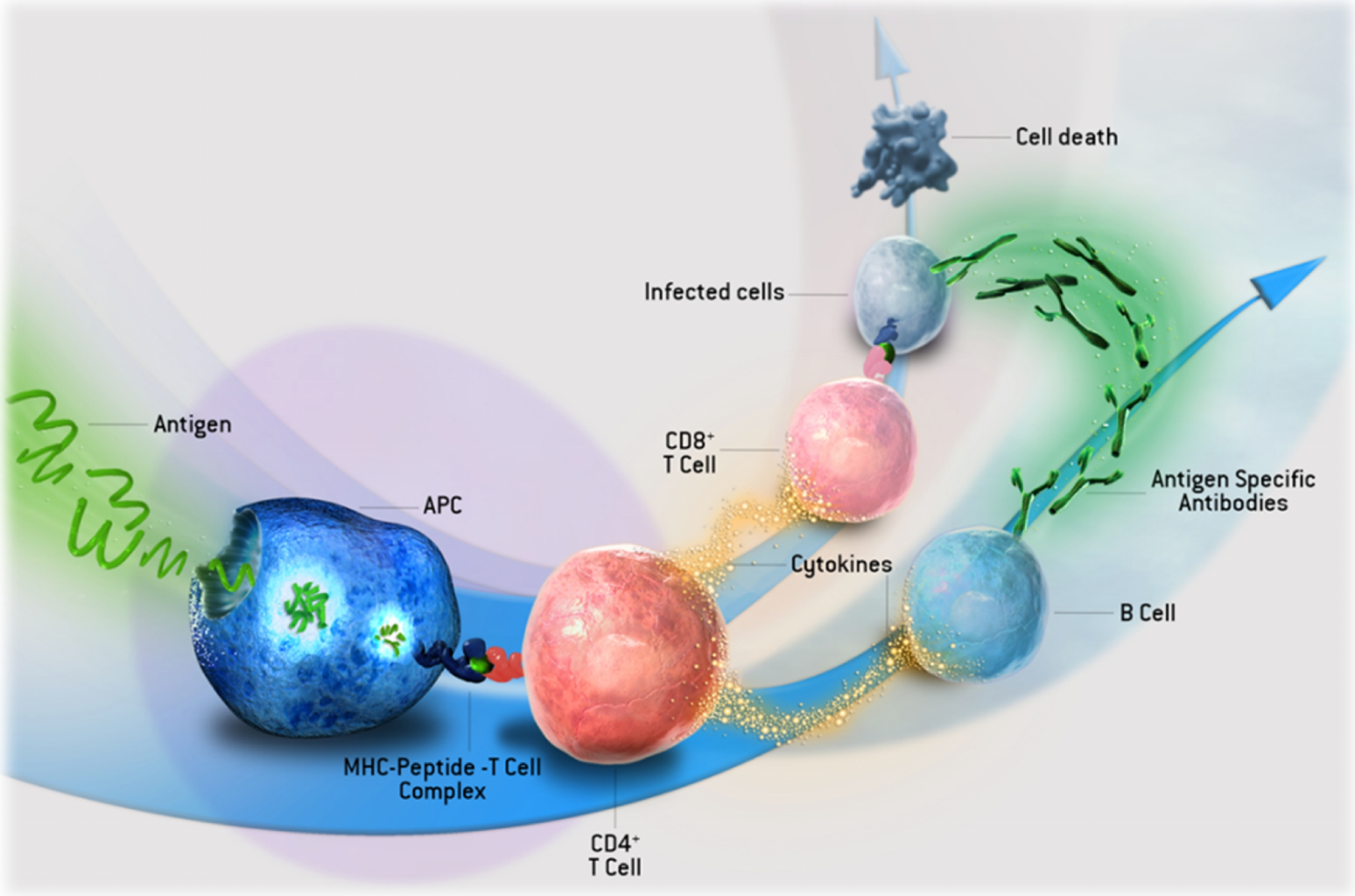
Route of application
Dose
Treatment regimen

### The Patient

Immune status
HLA allotype
Medical history
Pre-existing antibodies

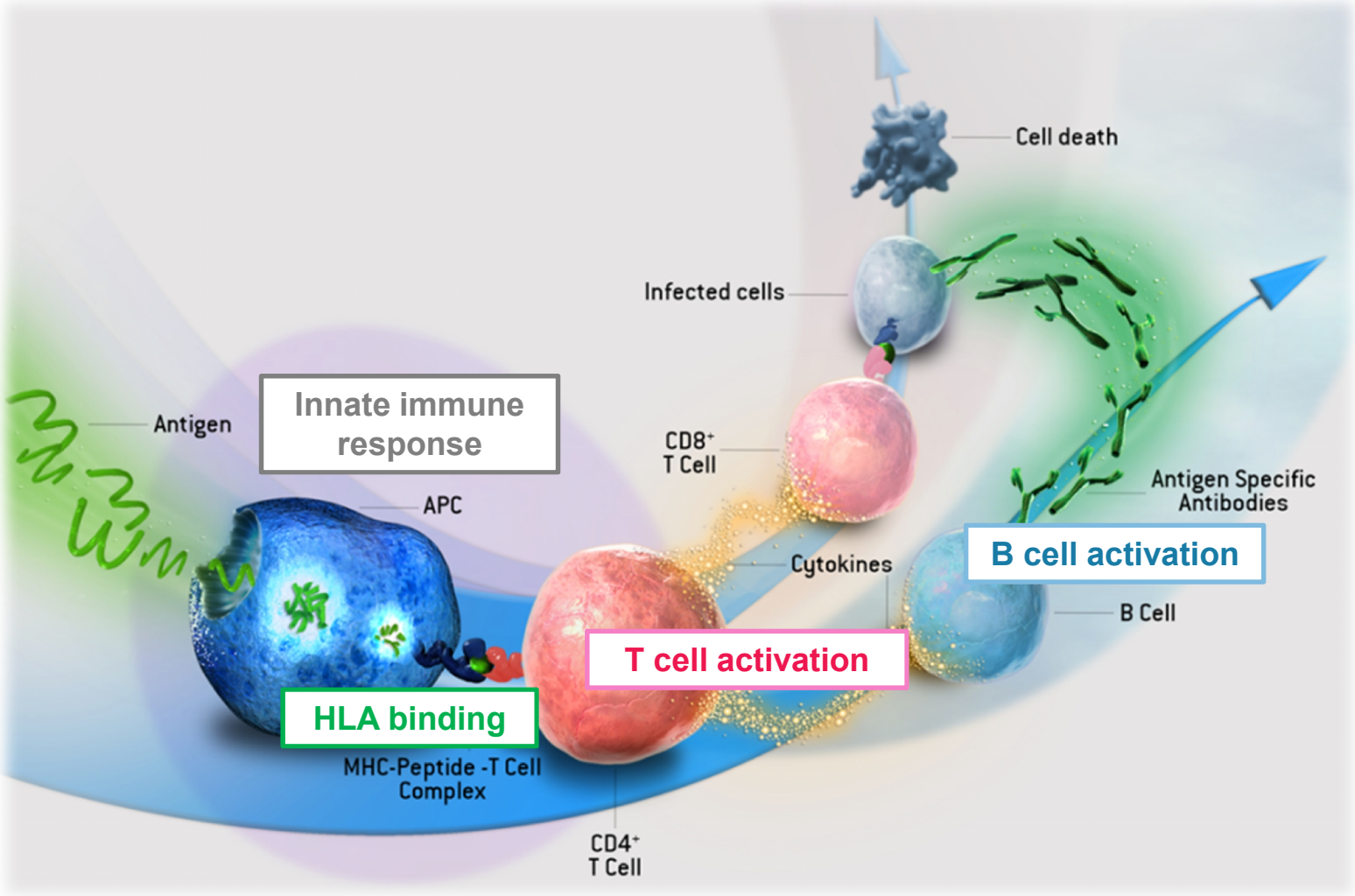
# Pre-clinical Immunogenicity Prediction

## Overview



# Pre-clinical Immunogenicity Prediction

## Overview



# Immunogenicity Prediction Tools

- *In silico* tools
  - Computer-based tools
  - Uses the protein sequence/structure
- *In vitro* tools
  - Use human primary 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

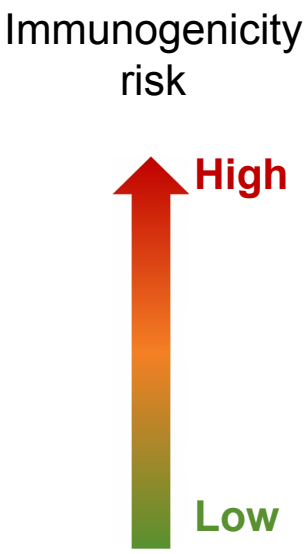
# *In Silico* Tools

- Identification of T cell and/or B cell epitopes in a protein sequence
  - T cell epitopes: HLA binding linear peptides presented to T cells
  - B cell epitopes: linear/conformational epitopes bound by the BCR
- Built and trained on human data
- Wide HLA coverage (A, B, DR, DP, DQ)
- Whole protein risk assessment and/or identification of individual epitopes for deimmunization
- High throughput & low cost
- Overpredictive (false positives)
- Uses only the  linear aa sequence

# *In Silico* Tools
## Therapeutic Antibody 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 |



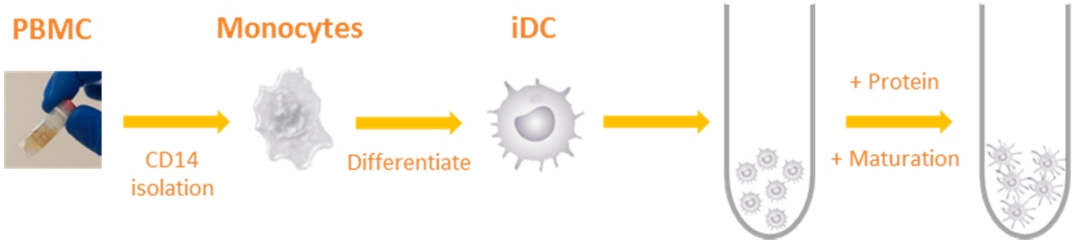
# *In Vitro* Tools

- Human primary immune cells (PBMC)
- Activation of innate immune response
- Naturally processed HLA binding peptides
- T cell activation
- B cell activation
- Human primary cells (healthy donors or patient samples)
- Wide HLA coverage
- Takes into account formulation, aggregates, contaminants etc.
- Large banks of primary human cells required
- More costly & time consuming compared to *in silico* tools
- *In vitro* B cell responses difficult

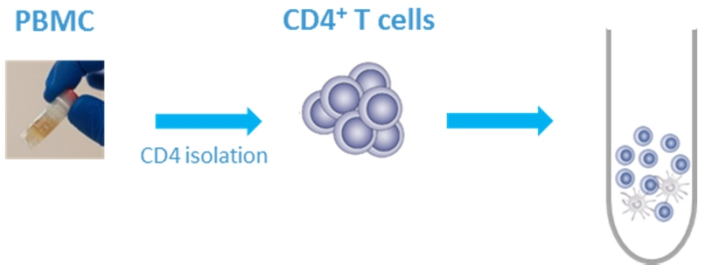
# *In Vitro* T Cell Assay Platforms

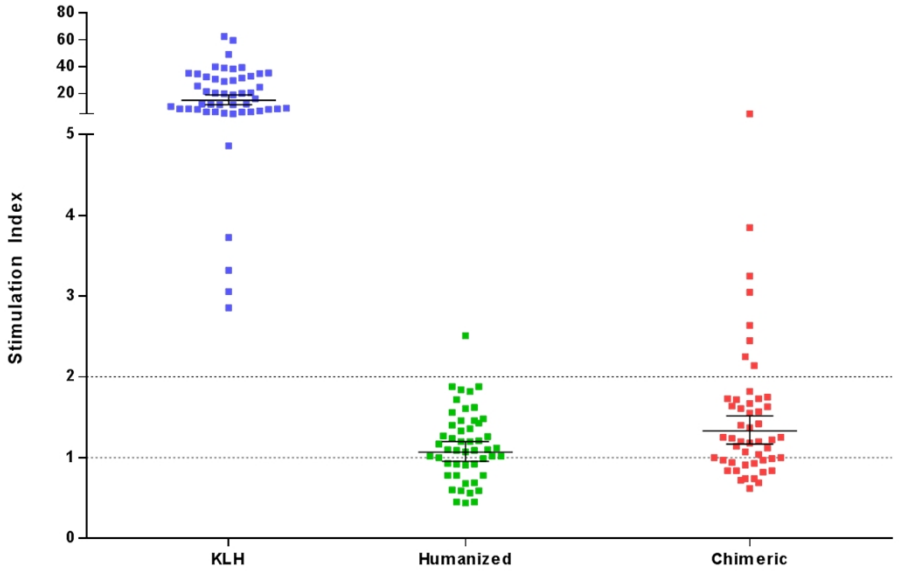
## Whole Protein-induced T cell responses

**DC Generation & loading**




**CD4⁺ T cell isolation & co-culture**



# Humanized mouse models

- Mouse models with human immune systems
- *In vivo* system – all components of the immune system
- Route of administration/dose assessment
- Costly & time consuming
- Difficult to get wide genetic coverage

# Artificial Lymph node

- Cell systems in vitro to mimic a human lymph node
- Closer to a human immune system than PBMC-based assays
- T and B cell responses?
- Costly & time consuming
- Difficult to get wide genetic coverage

# Common Questions

1. Are there any clinical case studies that ‘validate’ the predictive tools?
2. Predictivity of IS/IV tools compared to pre-clinical rodent/NHP models?
3. Correlation between IS and IV tools?
4. Number of donors to use in an IV study?
5. When to use these tools?
6. Prediction vs risk assessment?
7. Can these tools detect aggregation/stability issues?
8. Biosimilar testing?