Safety of proteins – lessons from non-clinical assessment and in-silico prediction of immunogenicity of therapeutic proteins

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Disclaimer

The presented views are my personal views
and do not necessarily represent the view
of the Paul-Ehrlich-Institut or
the European Medicines Agency.

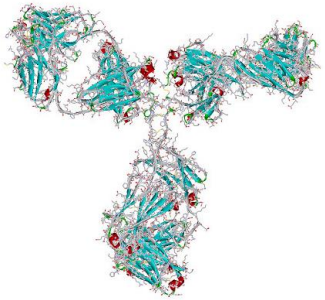
I have no involvement with or financial interest
in any of the organisations mentioned.

Immunogenicity

propensity of a protein to trigger an immune response

beneficial
(vaccine)

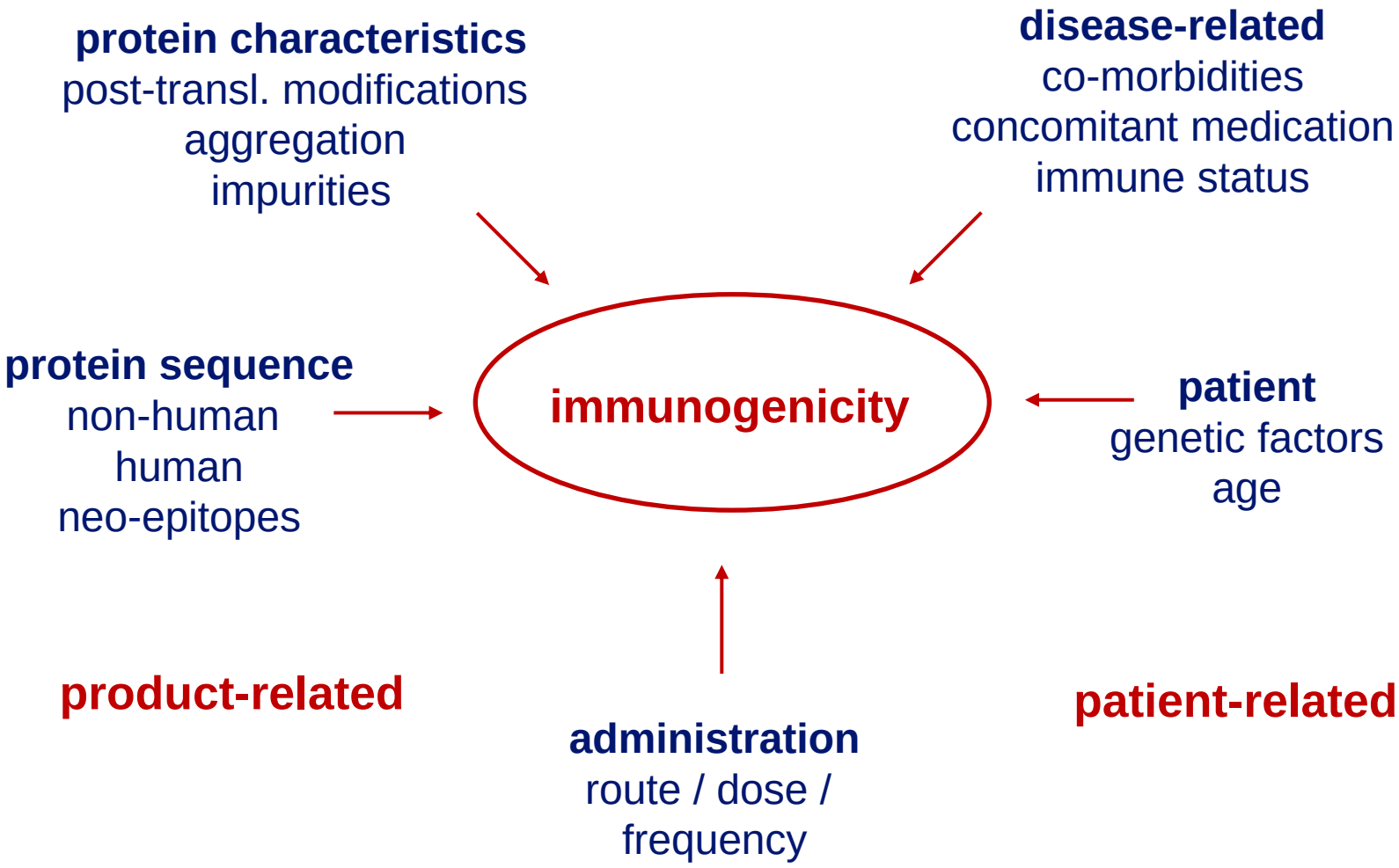
immune protection
against infectious
disease



unwanted
(therapeutic or food)

immune-related adverse reactions
hypersensitivity / anaphylaxis

Factors influencing immunogenicity

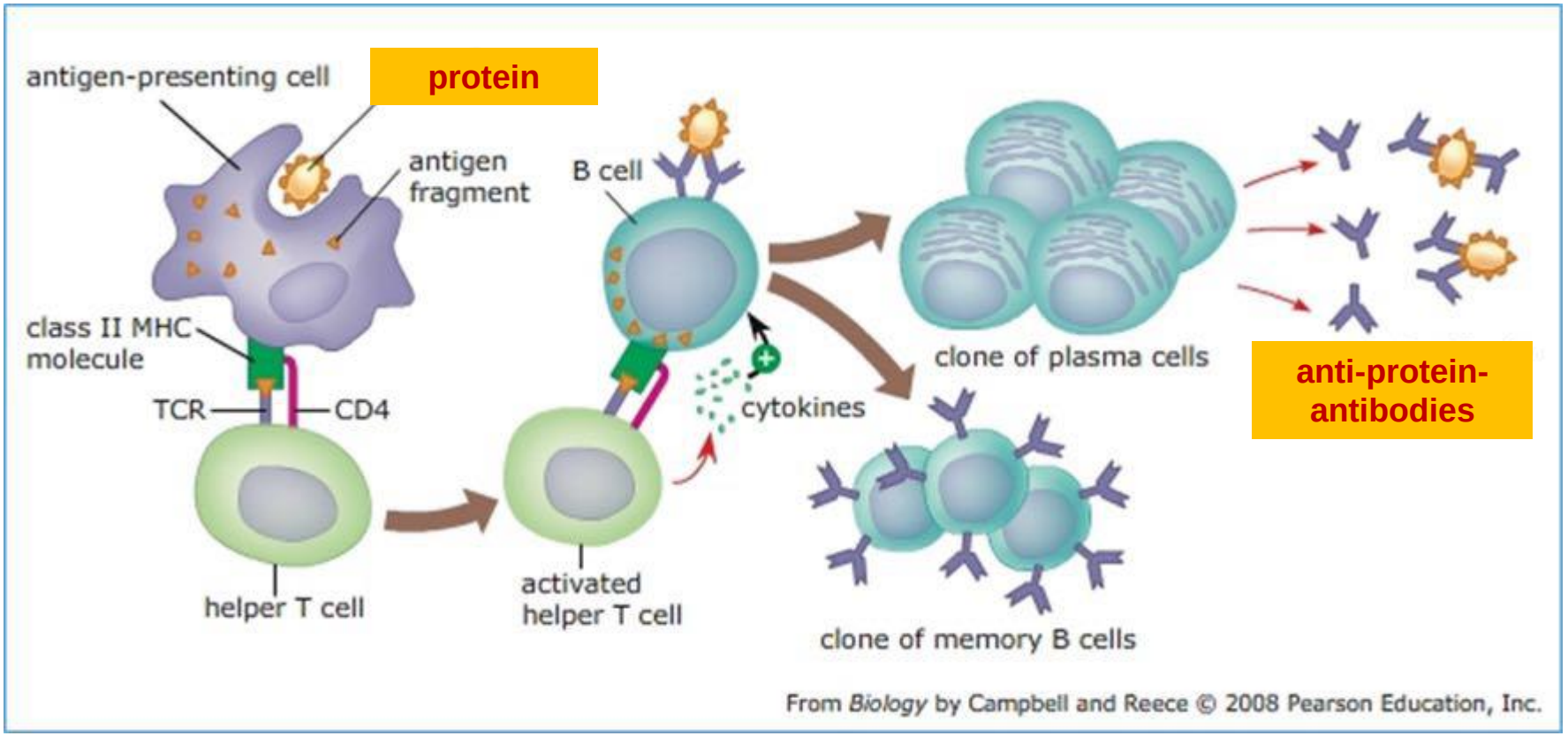


Immunogenicity risk assessment

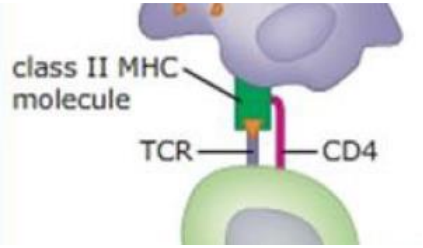
Multi-step approach

- in silico analyses
- in vitro / ex vivo assays
- combination with clinical data
- protein de-immunisation (if feasible)

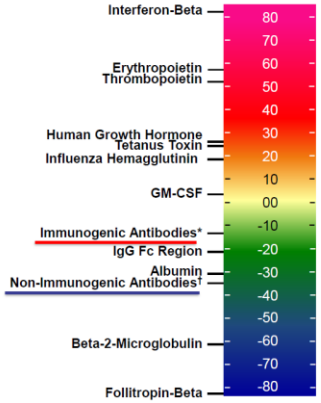
T cell-dependent B cell immune response



In silico screening



- **in silico** assessment of T cell epitopes based on protein sequence
  - predict binding of peptides to MHC II
  - identify epitopes recognised by effector T cells vs. regulatory T cells *(De Groot et al., Blood 2018)*
  - comparison of predictions with epitope data bases (e.g. *www.iedb.org*)



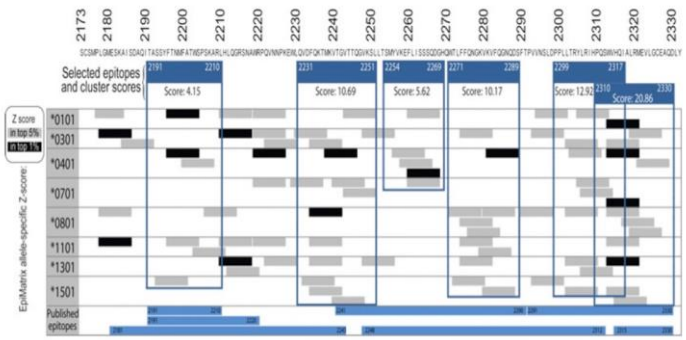
(epivax.com)

- **immunogenicity score / ranking**

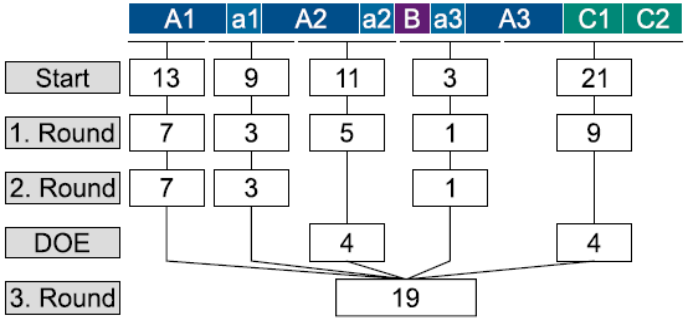
- **de-immunisation**: modify protein sequence to remove T cell epitopes

Case study - de-immunised coagulation factor VIII

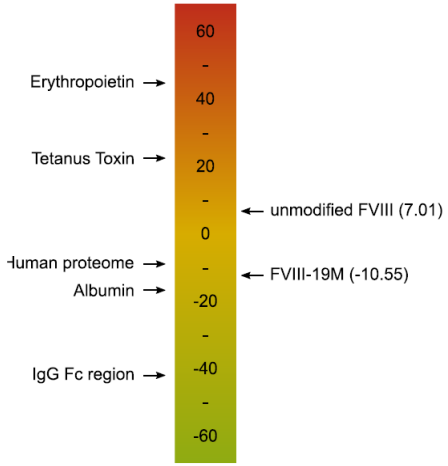
Predicted HLA II epitope map of FVIII C2 domain



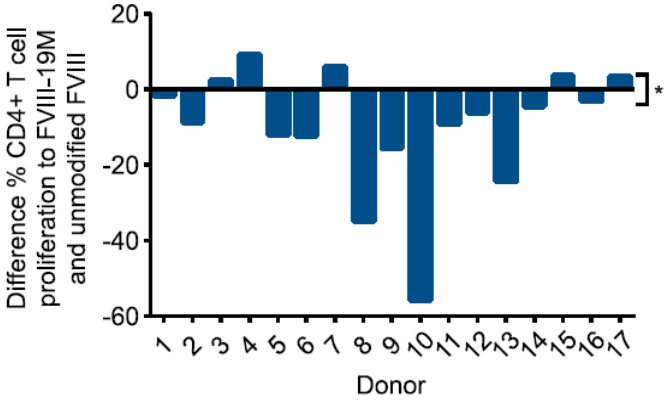
Incorporation of aa substitutions in FVIII sequence



Immunogenicity scale



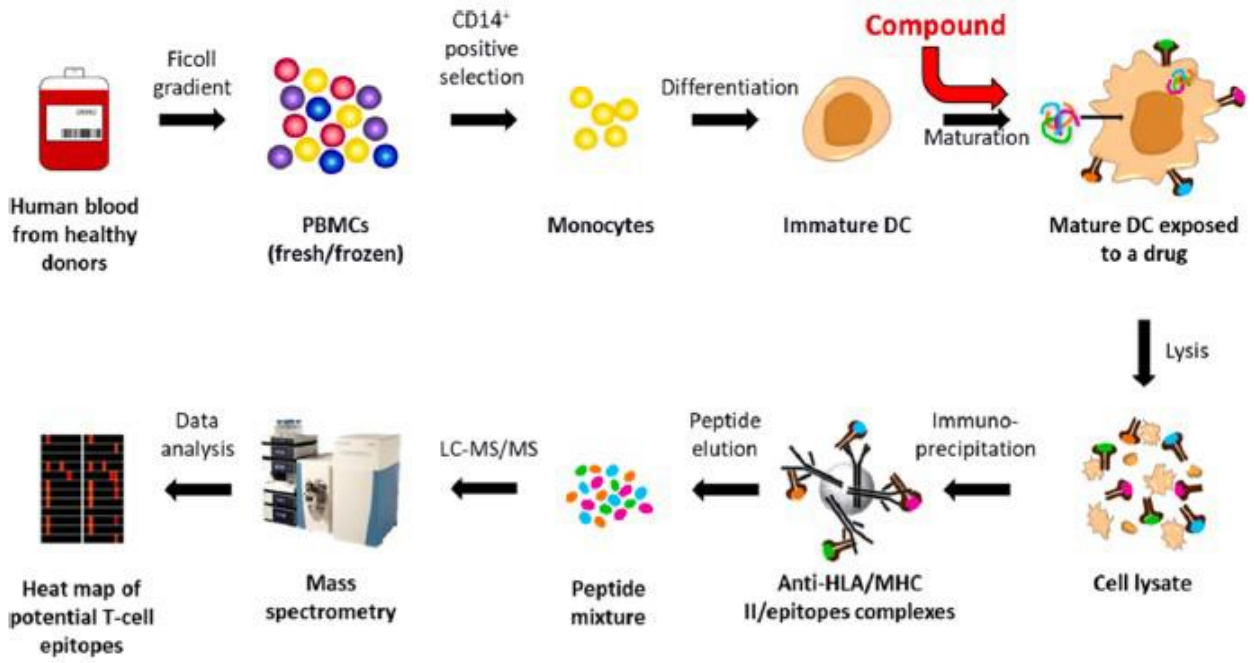
In vitro T cell response to modified FVIII



Moise et al., 2012, Clin. Immunol.; Winterling et al., 2021, J. Thrombosis Hemostasis

In vitro analysis of immunogenicity

MAPPs assay: MHC-associated peptide proteomics for identification of processed and presented epitopes



Steiner et al., 2020. J. Proteom Res.

In vitro analysis of immunogenicity

Early / innate assays

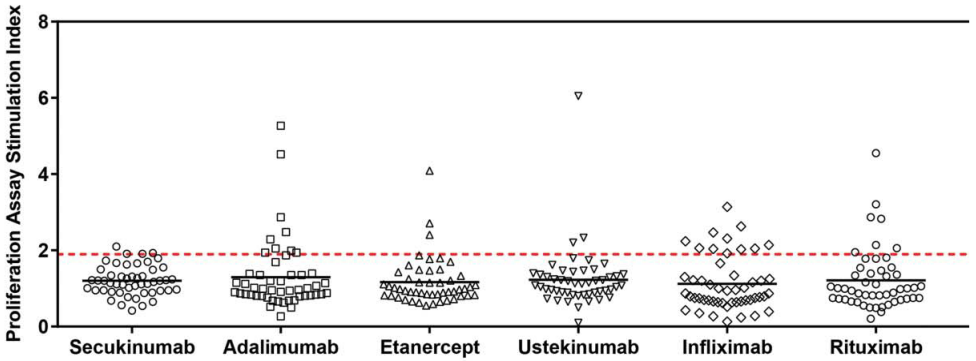
ability to activate antigen-presenting cells

- DC activation / maturation assay
  (can detect protein aggregation)
- cytokine release assay

T cell assays

assessment of T cell responses towards protein / epitope

- (CD8-depleted) PBMC
- DC-T cell assay
- peptide assay



Karle et al., 2016, mAbs

In vitro assays - considerations

Benchmarking of in vitro assays

- use of **reference molecules** with known immunogenic potential (low, high)

- if feasible use **PBMC from same donor** for different assays e.g. T cell assay and MAPPs

- assess relationship of **in vitro results and clinical data** (once available)

Caveats

- results for one protein with different assays may not agree
- results from in vitro assays may not agree with clinical data

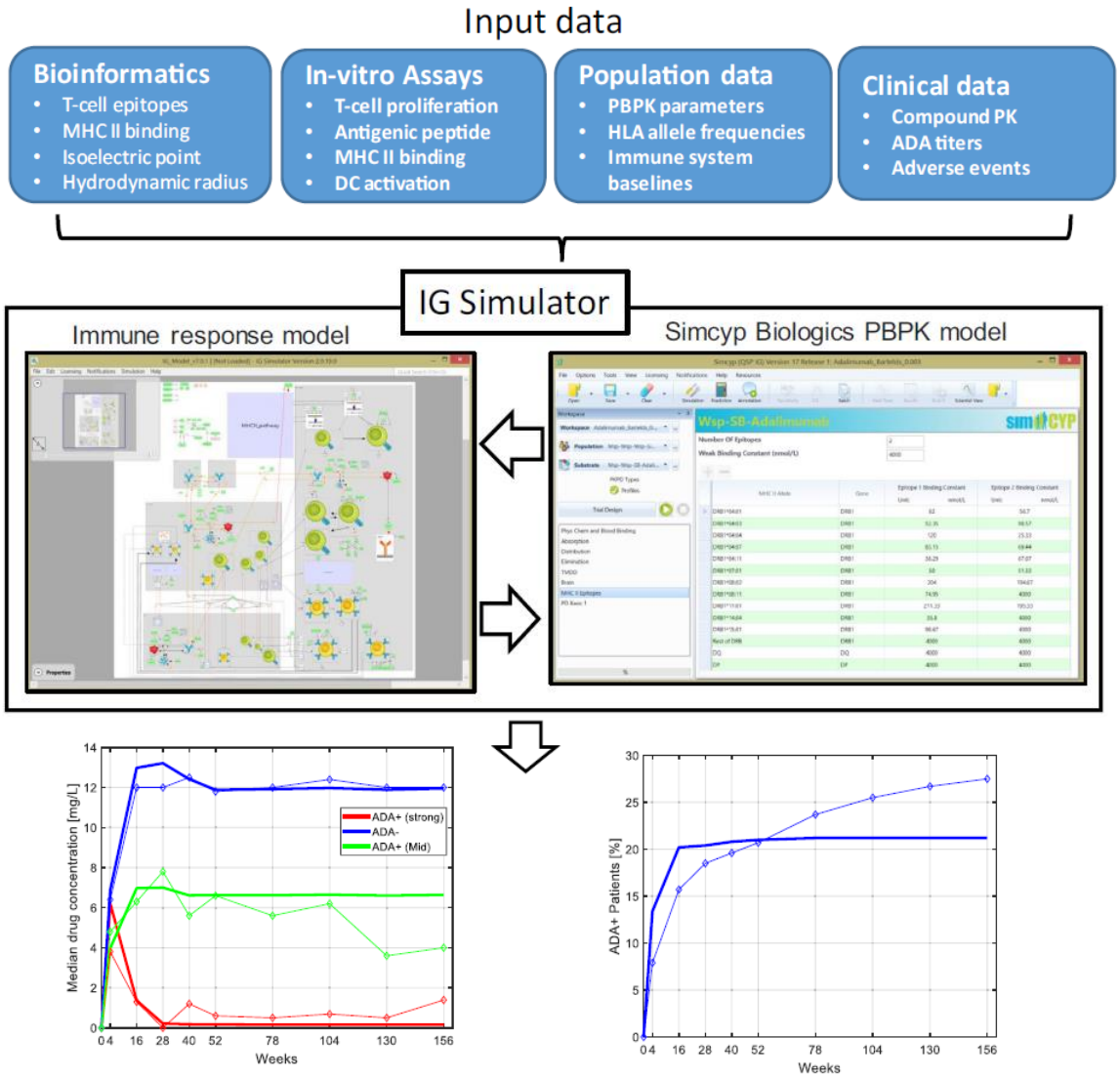
QSP approch to predict immunogenicity

- Quantitative Systems Pharmacology
  - combination of computational modelling and experimental data
  - integration of in vitro assay data and in silico model data with clinical data

- Mathematical model of immunogenicity of therapeutic proteins (theoretical model and application)
  (Chen et al., 2014, CPT Pharmacometrics Syst. Pharmacol.)

- Certara / Pharma consortium (established in 2017)
  development of Immunogenicity Simulator
  - prediction of ADA incidence
  - prediction of impact on PK and on PD

IG Simulator



Clinical data: Bartelds et al., 2011; simulations: Kierzek et al., 2019, CPT; Shaknovich et al., 2020, Clin. Transl. Sci.

QSP approach - considerations

- baseline assumption:
  T cell epitopes play pivotal role in B cell-driven ADA response

- multiple in silico / in vitro methods can be used
  - each method must be carefully implemented
  - characterisation of assay performance parameters
  - use of benchmarking molecules

- integrate data from several orthogonal methods

- limitation:
  knowledge of patient-related factors influencing the development of ADA

Prediction of food immunogenicity ?

Likely feasible, but would require modification of the models

- known food allergens are proteins
  in food: mixtures of proteins / substances
  → which protein exactly is the antigen / allergen ?

- oral uptake and digestion (protein cleavage and modification)
  → which peptide is available to the immune system ?

- specific characteristics of mucosal immune system, IgE responses

- scientific challenge: knowledge of processes inducing the immunogenic / allergenic response

- regulatory challenge: model validation for use in risk assessment

Thanks for your attention.