Using Non-Clinical Data to Assess Immunogenicity Risk:

Are we there yet?

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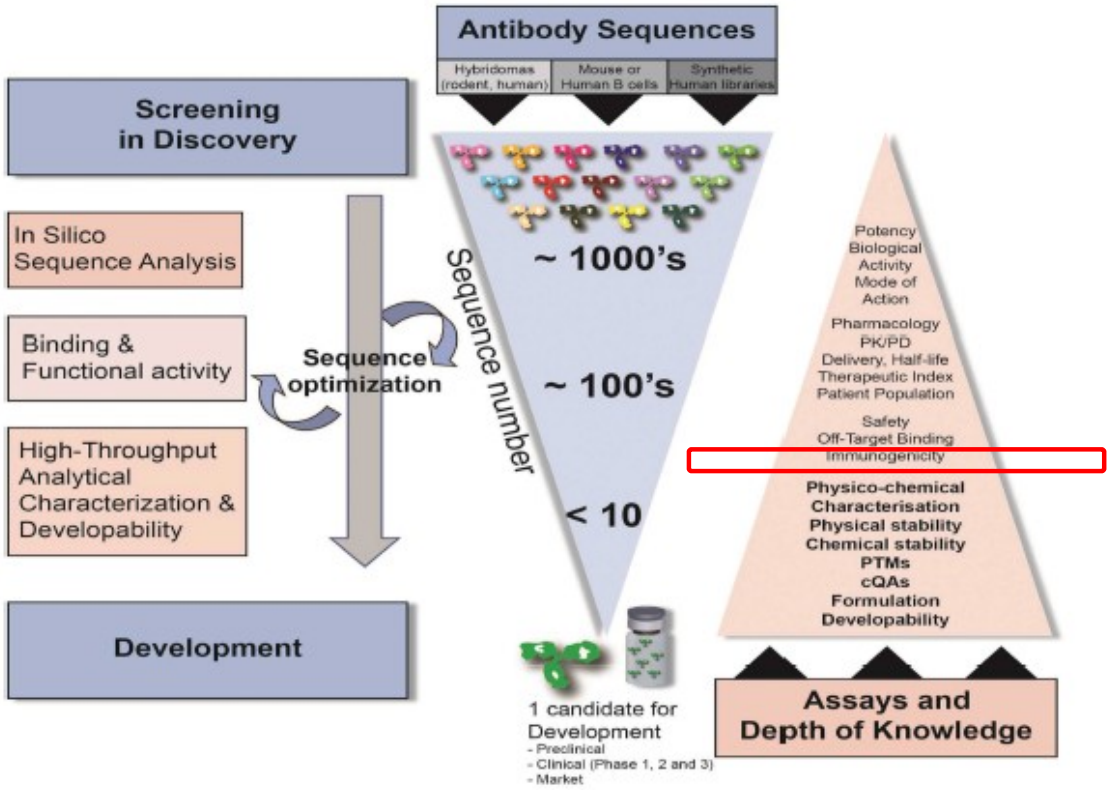
FDA Workshop on Non-clinical Immunogenicity Assessment of Generic Peptides
26 Jan 2021

Immunogenicity is one of many considerations in a Therapeutic Developability Risk Assessment



Bailly et al 2020

Many Factors may Impact the Immunogenicity of Protein Therapeutics

Drug Product Related Factors

- Amino acid sequence
- Post translational modifications
- Higher Order Structure
- Product related variants:
  - Aggregates, Sequence Variants…

  Process related impurities:
  - Host Cell Proteins, Host Cell DNA, Endotoxins
- Container/closure related impurities
- Formulation and storage conditions
- Mechanism of action

Clinical Factors

- Immunologic status and competence of patient
- Prior sensitization to protein therapeutics
- Route, dose, frequency and duration of administration
- Patient genetics
- Age and gender
- Underlying disease
- Concomitant medications
- Pre-existing antibodies
- Tolerance

Protein therapeutics are often extensively engineered:
immunogenicity risk assessment strategies may help mitigate risk of unwanted immune responses.

Factors that may Impact the Immunogenicity of ANDA Peptide Therapeutics

Drug Product Related Factors

- Amino acid sequence and physicochemical properties
- Aggregation propensity/aggregate types
- Peptide related impurities
- Other impurities or contaminants
- Container/closure related impurities
- Formulation and storage conditions
- Mechanism of action

Clinical Factors

- Immunologic status and competence of patient
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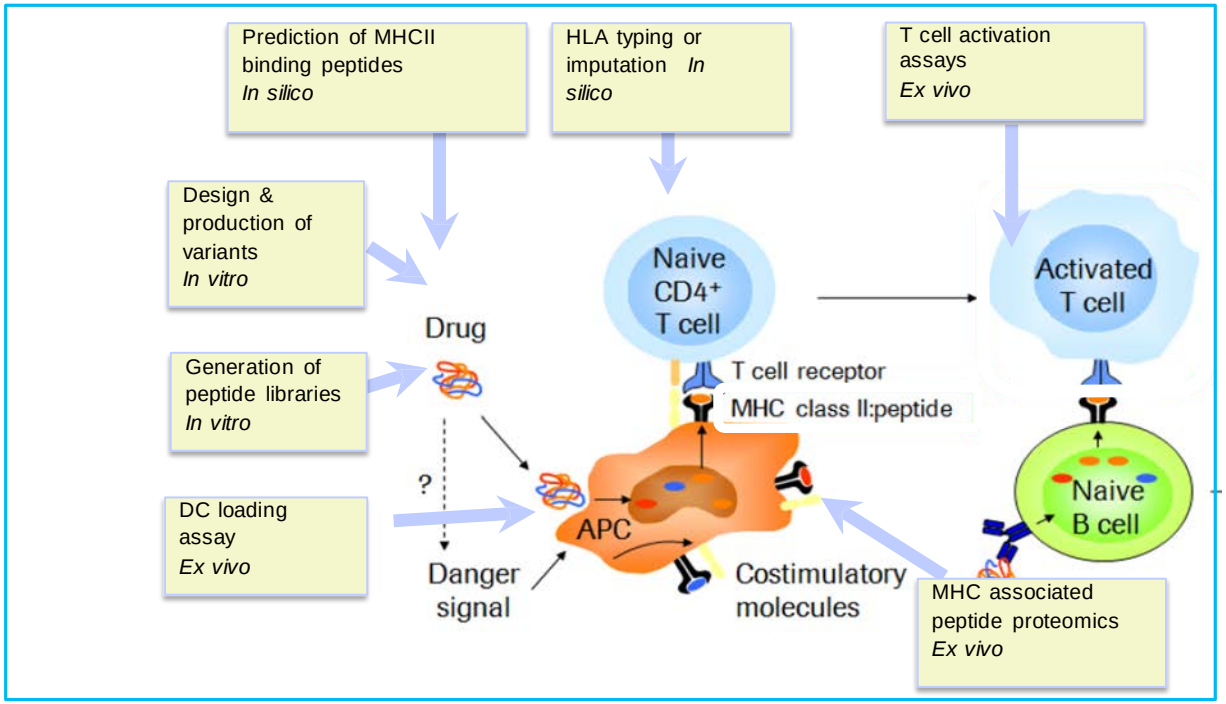
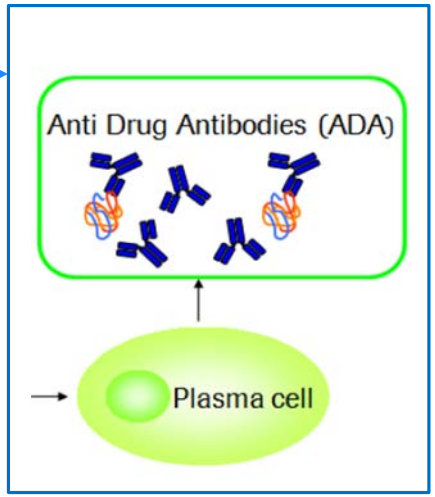
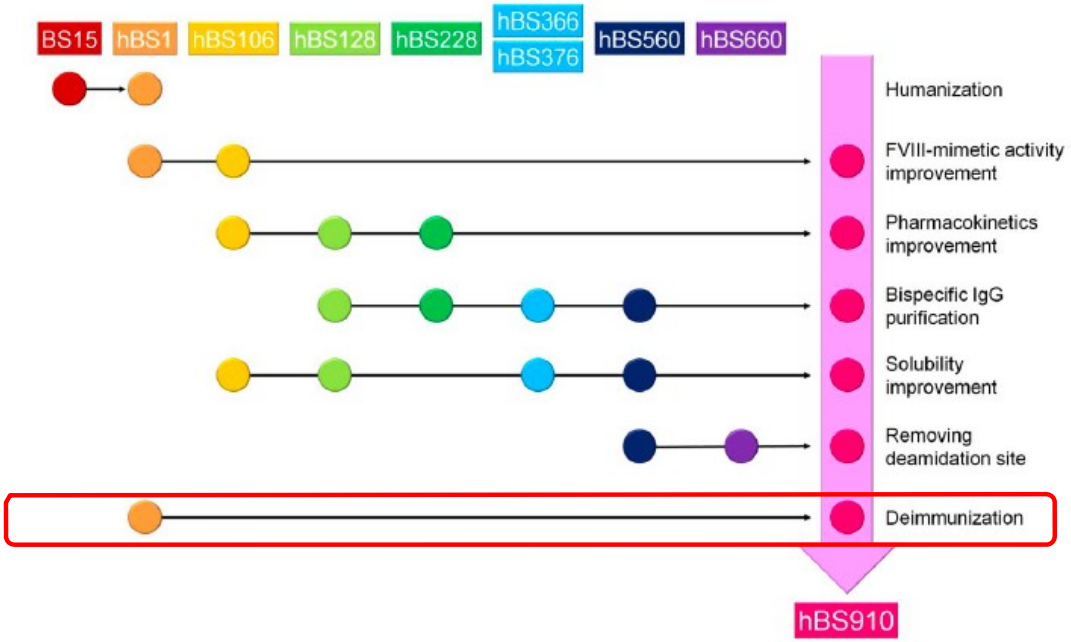
Assessment of Immunogenicity Risk for Protein Therapeutics





Multi-Dimensional Optimization Process w/ Immunogenicity Risk Mitigation

Hemlibra anti-FIXa/FX bi-specific IgG for Hemophilia A



Sampei et al 2013, Uchida et al 2015

Several orthogonal methods are typically used to mitigate immunogenic risk

Hemlibra anti-FIXa/FX bi-specific IgG for Hemophilia A:

- Prospective data from in silico & T cell activation assays generated during lead optimization suggested that Hemlibra would have low immunogenic potential.
- Retrospective data from MAPPS also suggested that Hemlibra would have low immunogenic potential.
- Clinical immunogenicity data from registrational studies showed low immunogenic potential: 4% treated patients in HAVEN studies developed ADAs

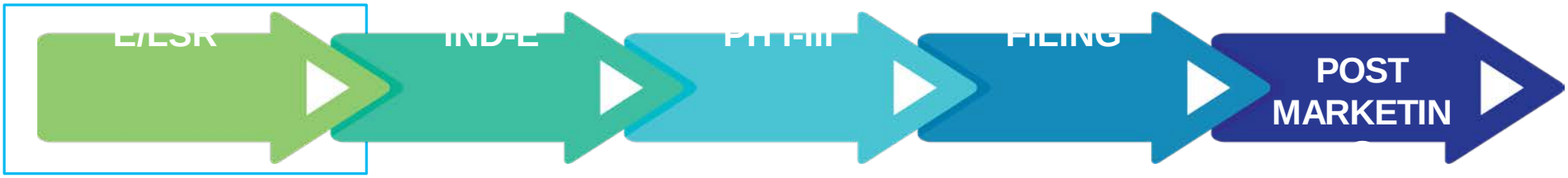
Sampei et al 2013, Uchida et al 2015, Hemlibra USPI

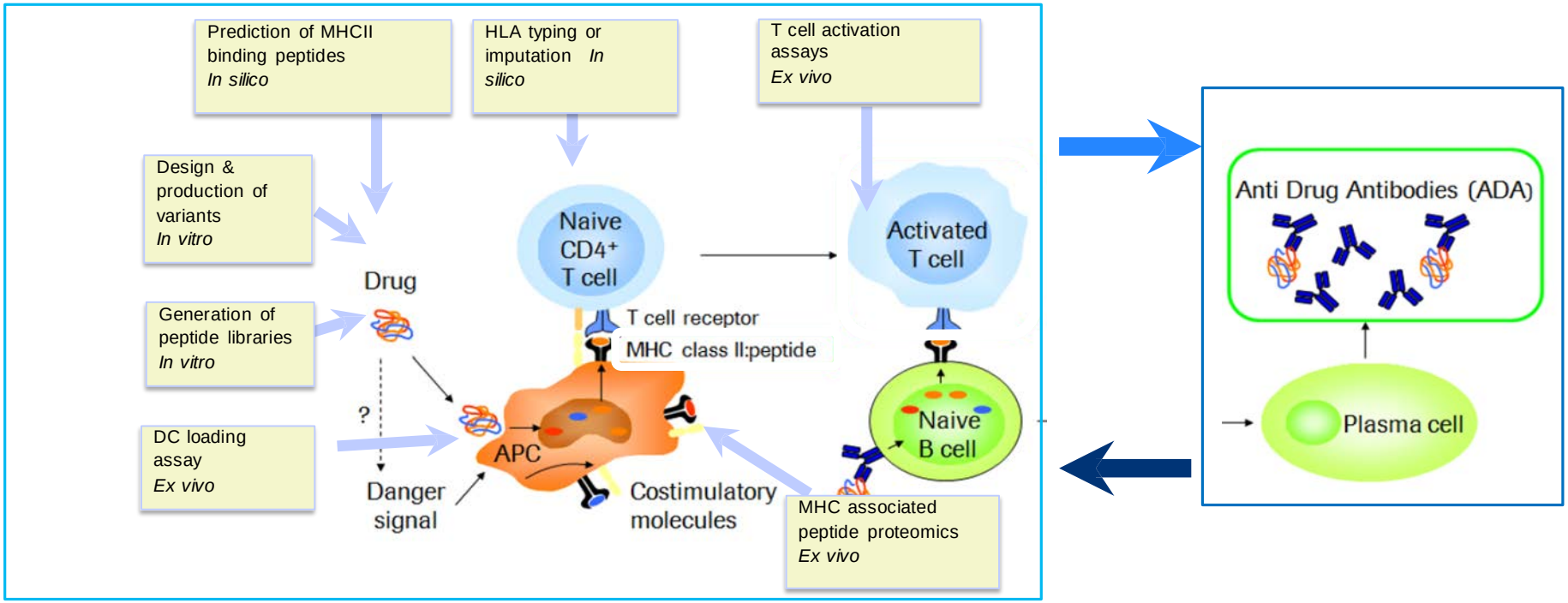
Several orthogonal methods are typically used to mitigate immunogenic risk

How is utility of methods assessed?

- Benchmarking data are generated during method optimization using sets of molecules with known low and high immunogenic potential.
- Decision thresholds for methods are established based on data from benchmarking molecules.
- Important to re-assess relationship between methods & clinical data as soon as clinical data emerge.

Assessment of Immunogenicity Risk for Protein Therapeutics





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How is utility of methods assessed?

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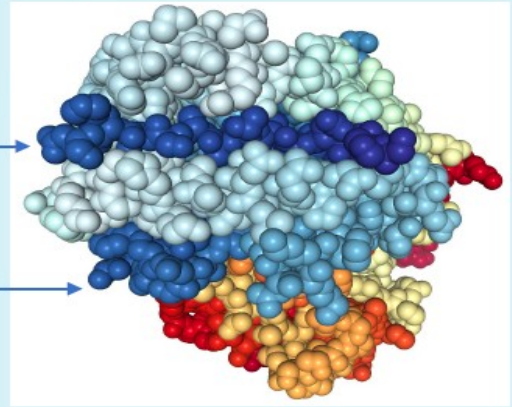
- Decision thresholds for methods are established based on data from benchmarking molecules.
- Important to re-assess relationship between methods & clinical data as soon as clinical data emerge.
- CAVEAT - results for same molecules from different methods may not agree
- CAVEAT - results from methods may not agree with clinical outcomes

CAVEAT –*in silico* data do not always mesh w clinical results.

Bioinformatics



IG is mostly tackled preclinically:

- Bioinformatics prediction of peptides that bind strongly to major histocompatibility (MHC) II receptors;
- Protein engineering to avoid strong binding.

FROM MOLECULE TO PATIENT

Genentech
A Member of the Roche Group

(Kapil Gadkar & Jennifer Rohrs)

Antibody Drug	# Binding peptides*	# MHC II alleles	% ADA+ Patients
Bococizumab (Pfizer)	2	12	68% (Ridker, 2017)
Alirocumab (Regeneron)	1	1	5.1% (Roth, 2017)
Evolocumab (Amgen)	0	0	0.1% (Henry, 2016)
GNE anti-PCSK9 (Genentech)	2	8	4% (GENE data*)

**Based on Phase II clinical study with ~200 subjects*

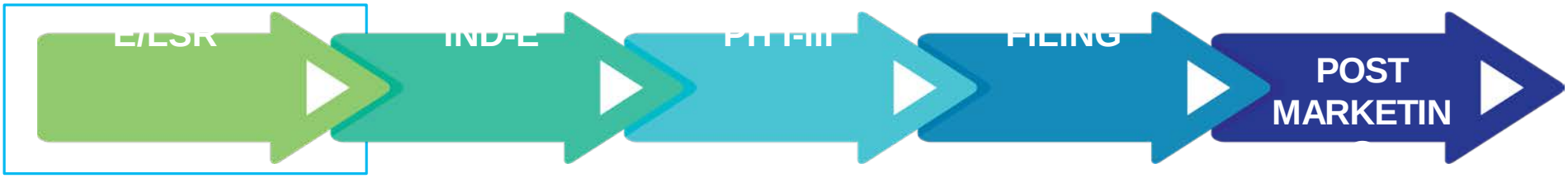
Limited power of bioinformatics approach to predict clinical outcome indicates that other factors than MHC II binding are important (e.g. co-therapy, disease, age).

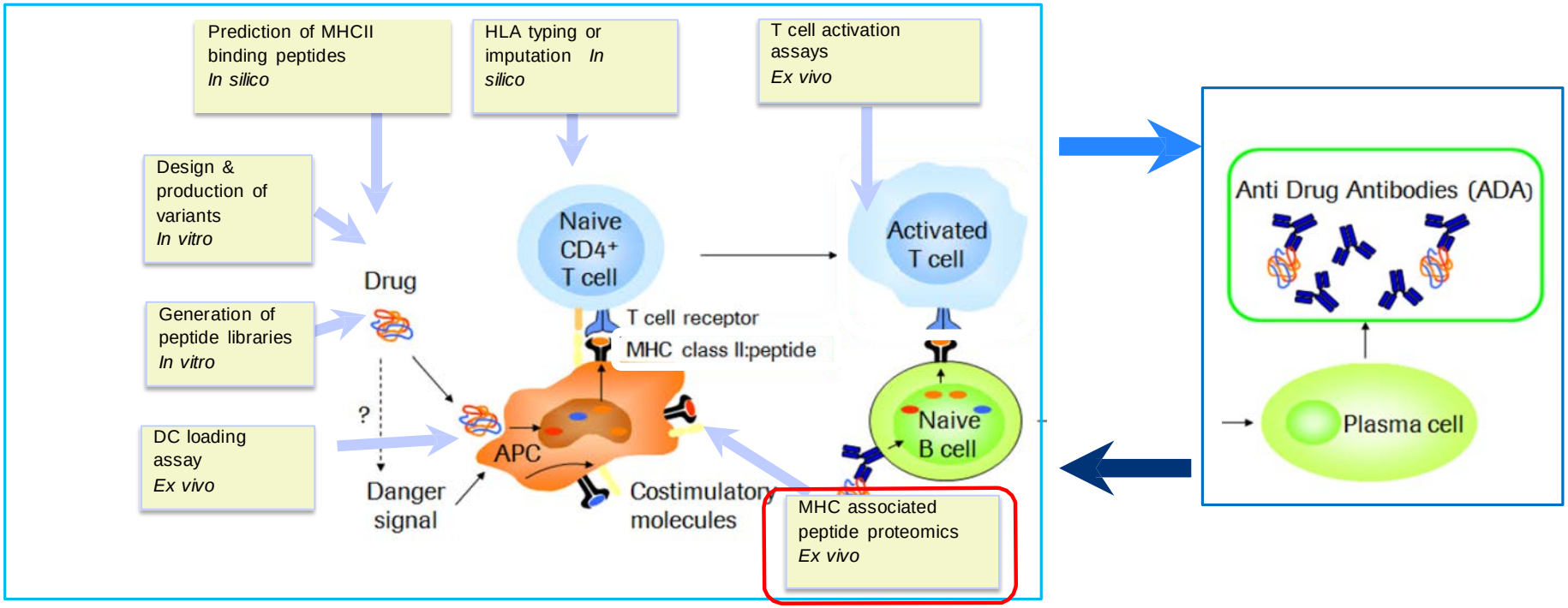
CAVEAT - *in vitro* data do not always mesh w clinical results.

ABIRISK T cell assay comparison

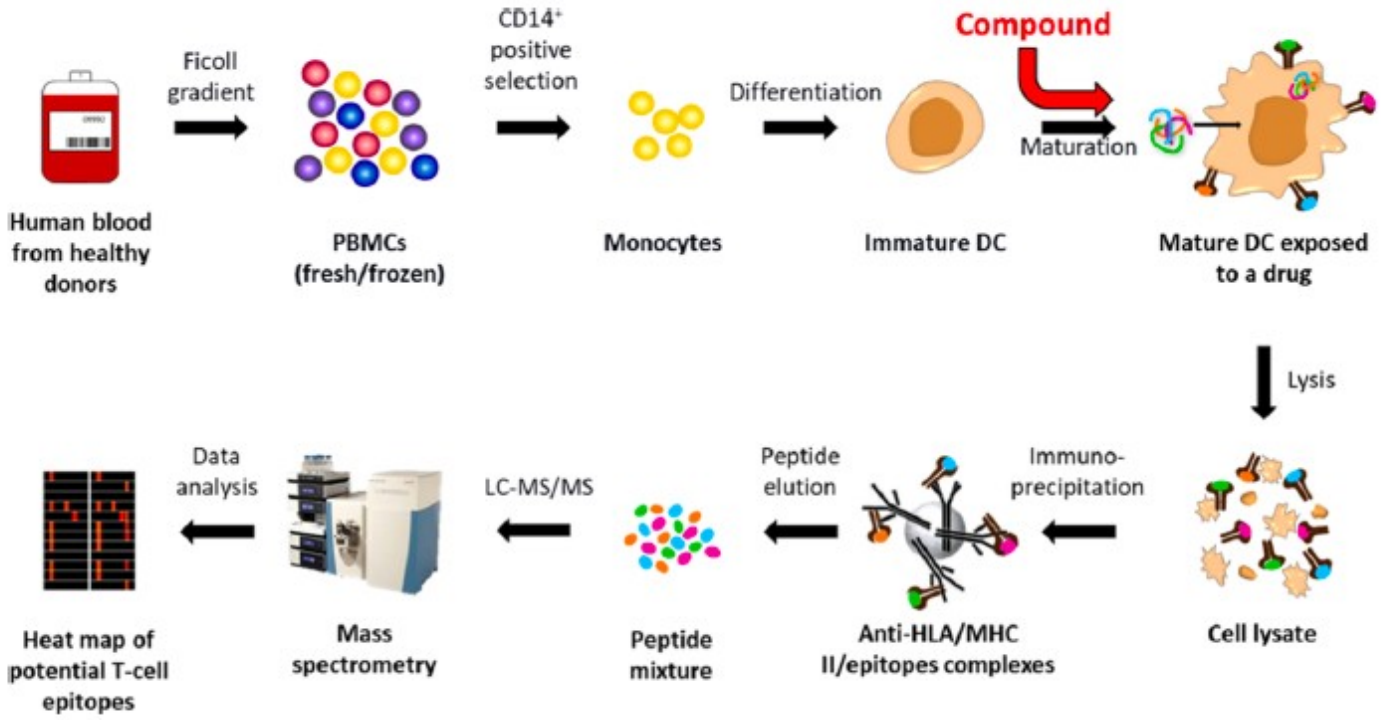
- 6 different protein therapeutics plus KLH
  - Therapeutics had known immunogenic potential based on clinical data.
- 4 different *in vitro* T cell assays.
  - T cell assays were used “as is”, without optimization.
- No good correlation in terms of ranking between results from assays.
  - Some assays failed to predict high risk.
  - Mechanism of action must be taken into account.
- Established reference standards and controls would help to ensure comparable performance of such assays.

Assessment of Immunogenicity Risk for Protein Therapeutics





MHCII Associated Peptide Proteomics (MAPPs) Workflow



Steiner et al 2020

MAPPs, dataMAPPs and heatMAPPs

MAPPs utility for immunogenicity risk assessment depends on:

- technical stability
- robustness
- ability to compare results across experiments and donors.

	Donor 1	Donor 2	Donor 3
Day 1	√√√	√√√	√√√
Day 2	√√√√	√√√	√√√
Day 3	√√√√	√√√	√√√

dataMAPPs - specialized MAPPs data processing pipeline.

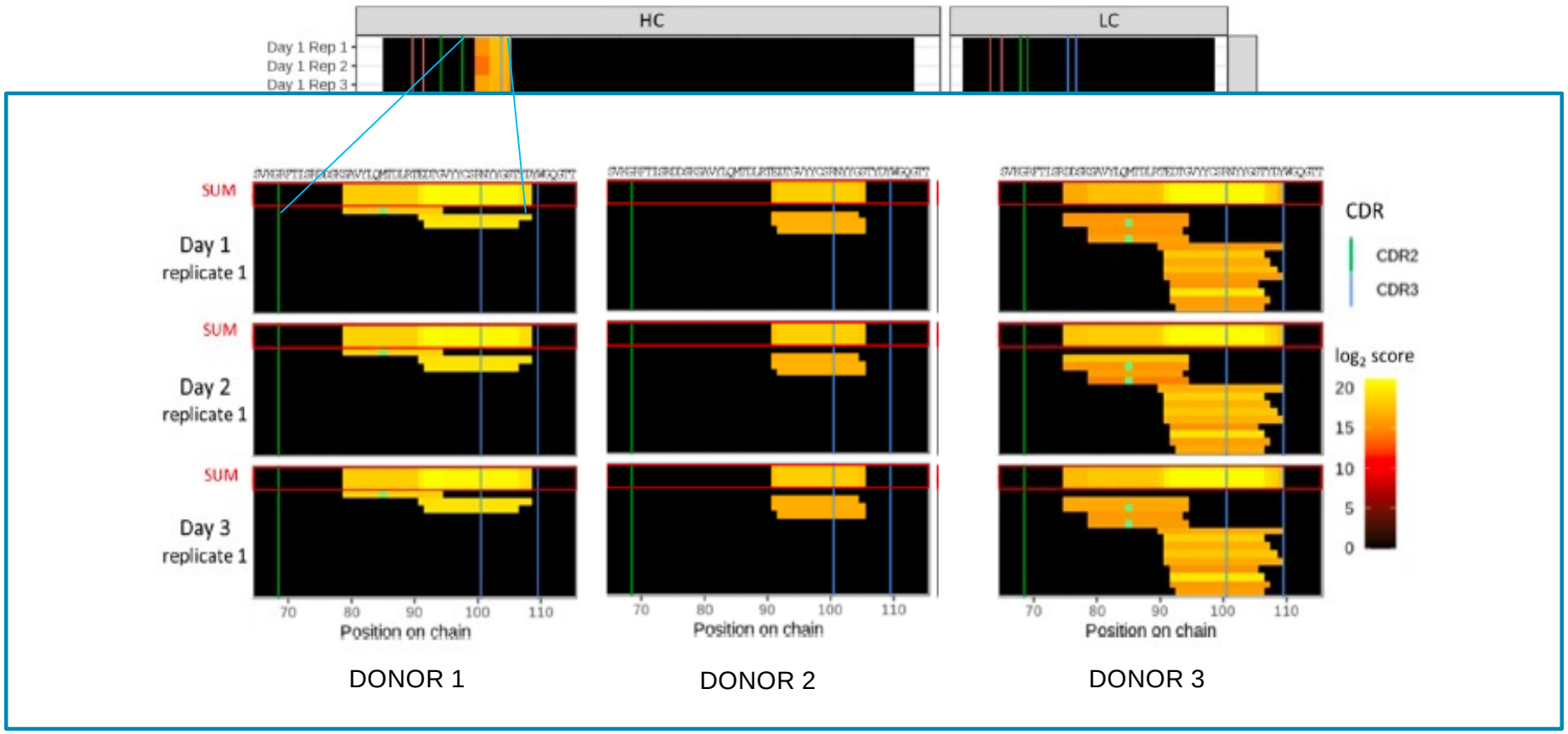
heatMAPPs – complex MS data sets can be displayed as heat maps.

Customized normalization procedure based on identified endogenous peptides standardizes signal intensities within and between donors and enables cross-experimental comparison.

Systematic biological differences across donors outweigh technical sources of variation.

Steiner, et al 2020

dataMAPPs accelerates comparative analysis of MAPPs data:
MAPPs data for 1 benchmark mAb using PBMCs from 3 donors across 3 days



Steiner et al 2020

Parallel Generation of MAPPs and T cell assay data.

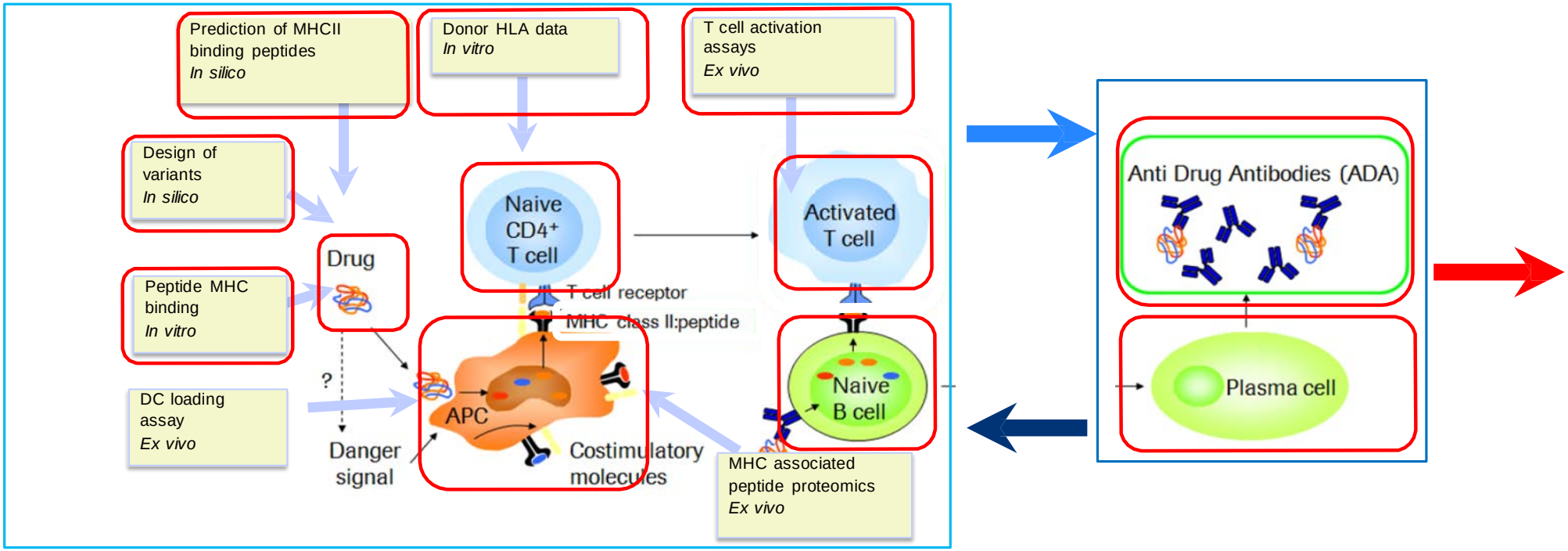
Scale down of PBMC sample requirements and increased MS sensitivity enables parallel generation of T cell and MAPPs data from same PBMC donors.

- HLA DR specific MHCII peptides identified by MAPPs correlate with T cell activating sequences from two therapeutic antibodies.
- One antibody with high clinical ADA incidence had higher number of CD4 T cell reactive peptides identified by MAPPS and T cell activation than an antibody with low ADA incidence.

Roche/Genentech are systematically generating parallel MAPPs and T cell assay datasets from benchmark molecules whose clinical immunogenicity profiles are known.

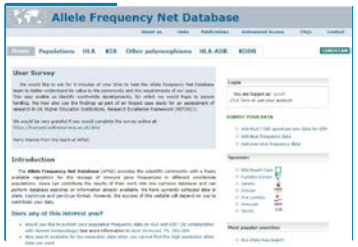
Hamze et al 2017, Spindeldreher et al 2020, Steiner et al 2020

Assessment of Immunogenicity Risk for Protein Therapeutics

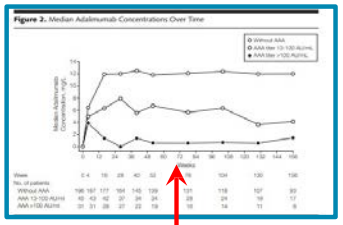


QSP Model for Immunogenicity Risk Assessment will integrate *in vitro* assay data and *in silico* model data with Clinical Data

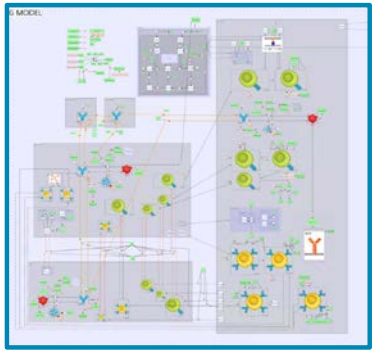
Allele Frequency



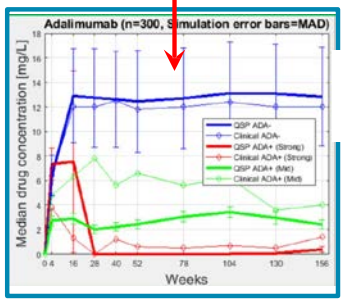
Clinical data



Bioinformatics



Compare results



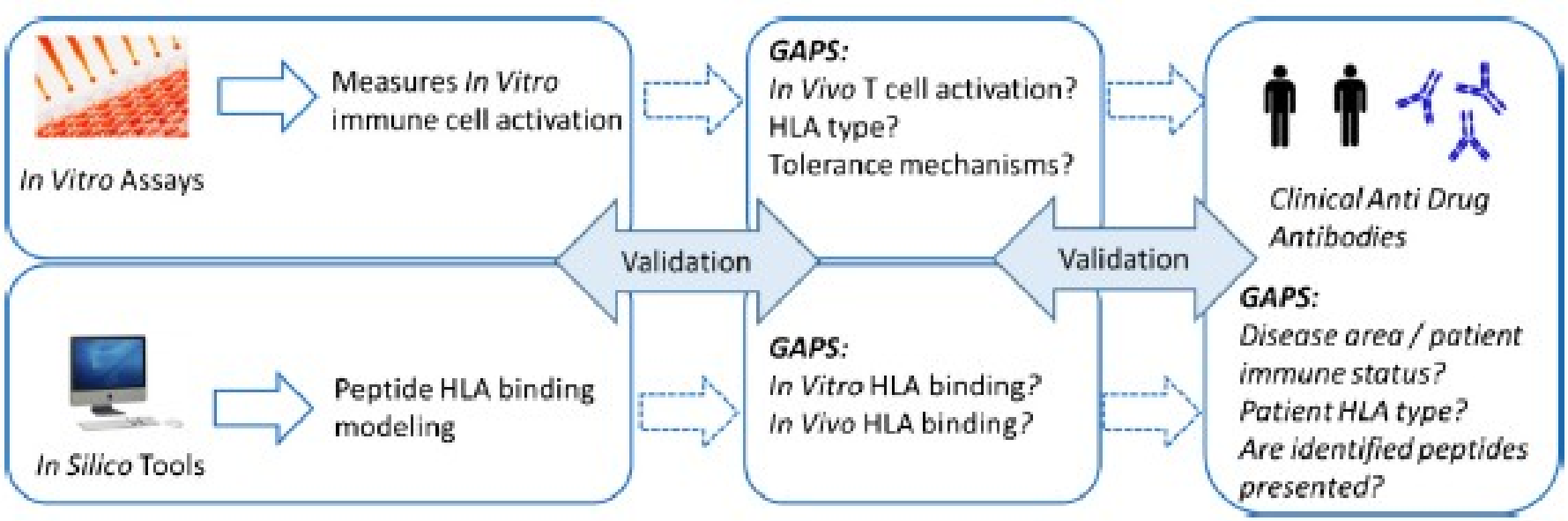
Model predictions

Kierzek 2019

Industry Consortium QSP Model for Immunogenicity Risk Assessment

Kierzek 2019

Immunogenicity Risk Assessment – Mind the Gap(s)…



Gokemeijer 2017

Immunogenicity Risk Assessment - Mind the Gap(s)…

- Immunogenicity assessment strategies assume T cell epitopes play a pivotal role in B cell driven ADA Responses. Confirmatory data on roles played by immune system baseline parameters on ADA development are emerging for:
  - T cell epitope content
  - HLA II
  - Antigen specific T cell numbers

- Our knowledge of patient-related factors influencing the occurrence of ADAs is still limited.
  Roles for some factors are emerging:
  - Autoimmune disease:
    - CRP, ESR, Immunosuppressant use, Antibiotic use, Tobacco smoking, Infections.
  - Oncology:
    - Baseline demographic and disease characteristics that are prognostically related to outcome may also influence ADA development.

Bartelds 2011, Garces 2014, Ducourau 2019, Hassler 2020.

Immunogenicity Risk Assessment – Are We There Yet?

- Strategies assume T cell Epitopes play a pivotal role in B cell driven ADA Responses.
- Multiple in silico and vitro methods can be used for risk assessment.
  - Each method must be carefully implemented:
    - Assay performance parameters should be characterized.
    - Benchmarking molecules can be used to set decision thresholds.
- Data from multiple orthogonal methods should be integrated for a risk assessment.
- Our knowledge of patient-related factors influencing the development of ADAs is still limited….

BioAnalytical Sciences

Thank You!

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