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FDA Workshop: Non-clinical Immunogenicity Assessment of Generic Peptide Products: Development, Validation, and Sampling

Session 3: Assays monitoring antigen-specific T cell activation: technical challenges and validations

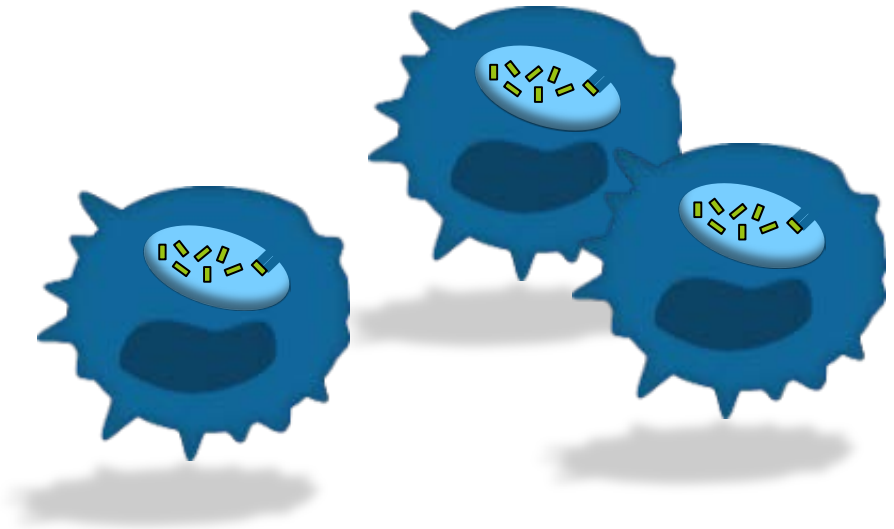
Ex vivo Immunogenicity Assays – Landscape and Limitations

Campbell Bunce

26th Jan 2021

Ex vivo Immunogenicity Assays – Landscape and Limitations

- Drivers of immunogenicity risk and the pre-clinical tools to assess this
- Assay methodologies and readouts
- Case studies



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Drivers of Immunogenicity Risk

Product Related

Sequence
- Self or non-self
- T or B cell epitopes

Structural
- Post translational modifications
- B cell Epitopes
- Cryptic T cell epitopes

Formulation
- Excipients
- Contaminants
- Aggregates

Clinical Related

Pharmacology
- Target (cell vs soluble)
- Immunomodulation

Dosing
- Route of administration (*sc* vs *iv*)
- Frequency

Genetic Profile
- Haplotype (e.g. HLA)
- Other genetic factors (e.g. SNPs)

Patient History/Status
- Co-meds (immune suppressants)
- Previous exposure to drug
- Acute or chronic disease

Drivers of Immunogenicity Risk & Evaluation Tools

Product Related

Sequence
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- T or B cell epitopes

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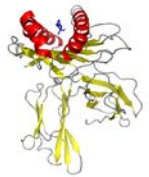
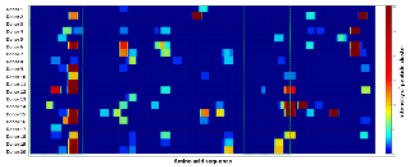
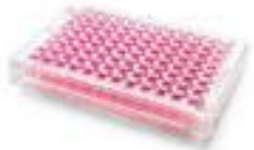
Preclinical Evaluation Tools

in silico





proteomics



ex vivo/in vitro

in vivo

Clinical Related

Pharmacology
- Target (cell vs soluble)
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- Route of administration (*sc* vs *iv*)
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Genetic Profile
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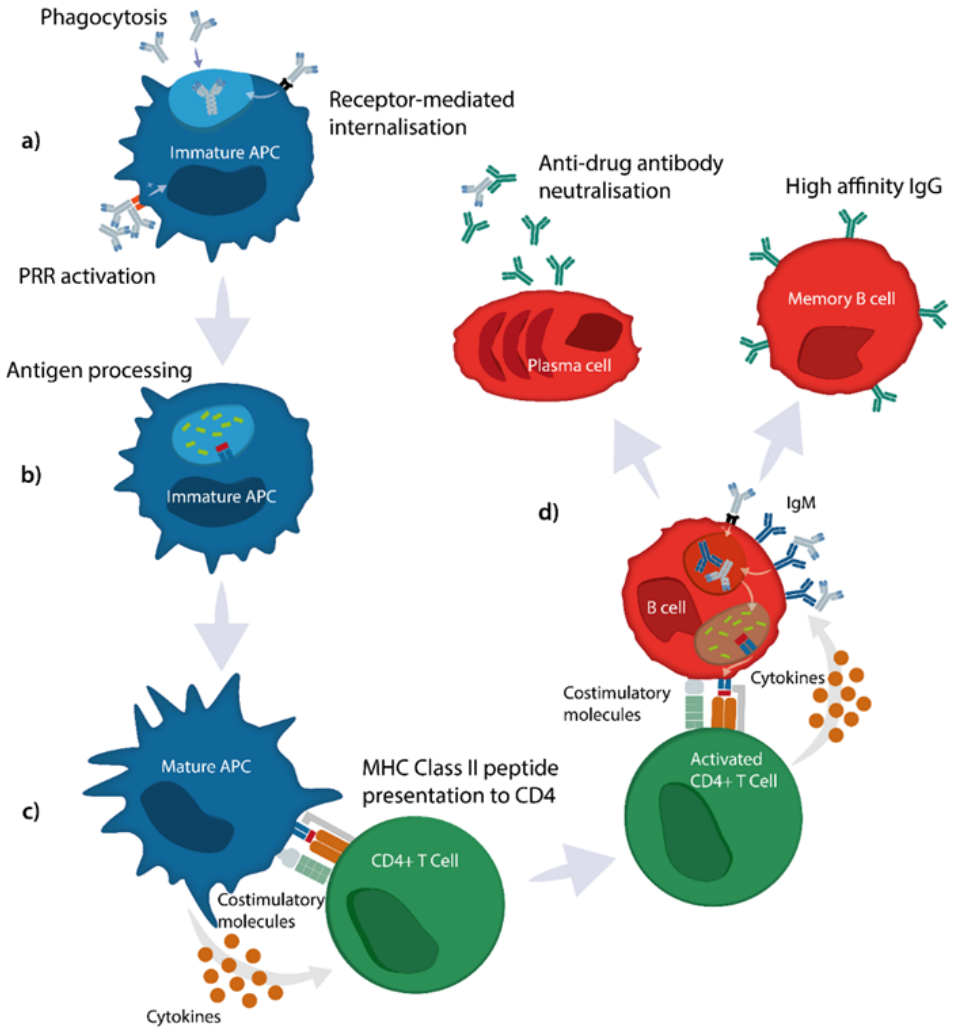
Using This Suite of Immunogenicity Risk Assessment Tools

Select assays based on what you want to know and do:

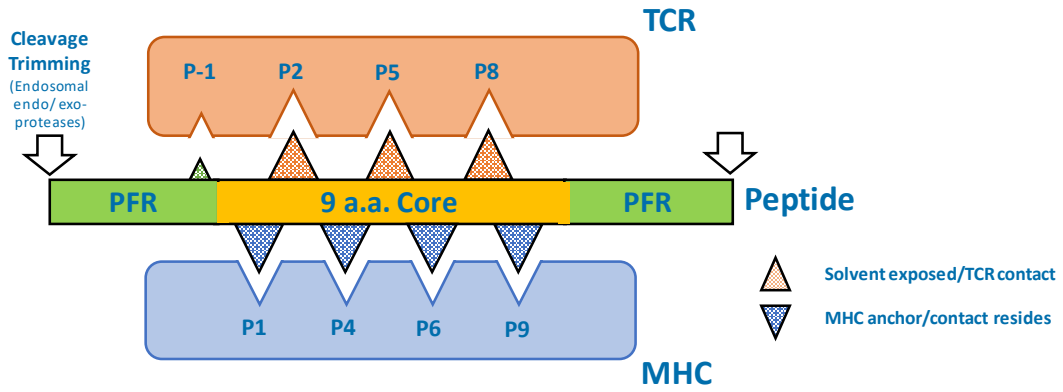
- Identify specific sequence liabilities and engineer these out of the drug design
- Compare or rank against similar product candidates to select the best lead candidate for development
- Identify drug product components or contaminants that may promote ADA responses
- Pre-inform a clinical safety management plan if suspect immunogenicity risk

The Immune Axis to ADA Responses



- Uptake of drug by APCs (a)
- Linear peptides derived from drug during antigen-processing form complexes with MHC class II and activate T cells (b-c)
  Note: peptide drugs (linear) could bypass processing by APCs and form complexes directly with MHC II molecules
- T cell help ($CD4^+$ T cells) → high affinity, isotype switched anti-drug antibody (ADA) responses (d)
- ADA responses can:
  - neutralise drug activity
  - reduce half-life by enhancing clearance
  - promote injection site/allergic reactions
  - cross-react with endogenous counterparts to result in 'autoimmune'-like reactions

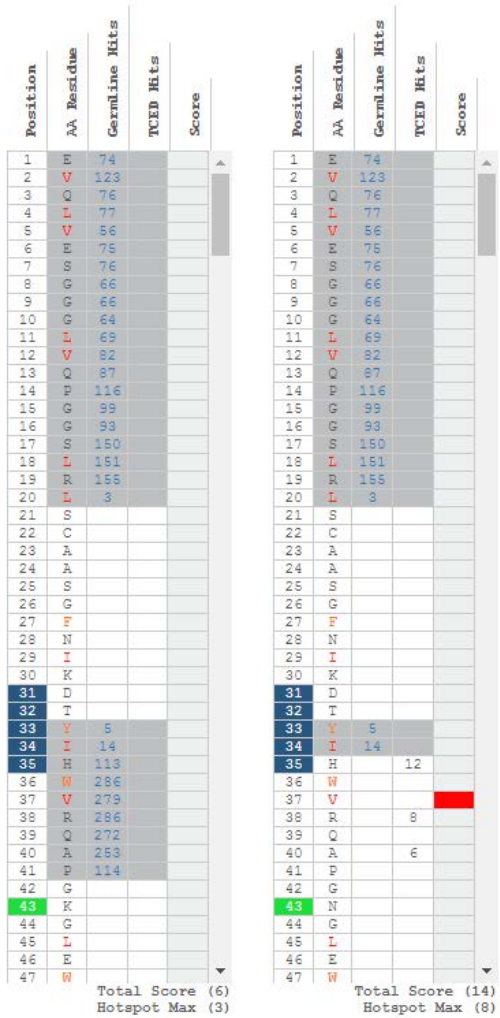
In silico Immunogenicity Risk Evaluation

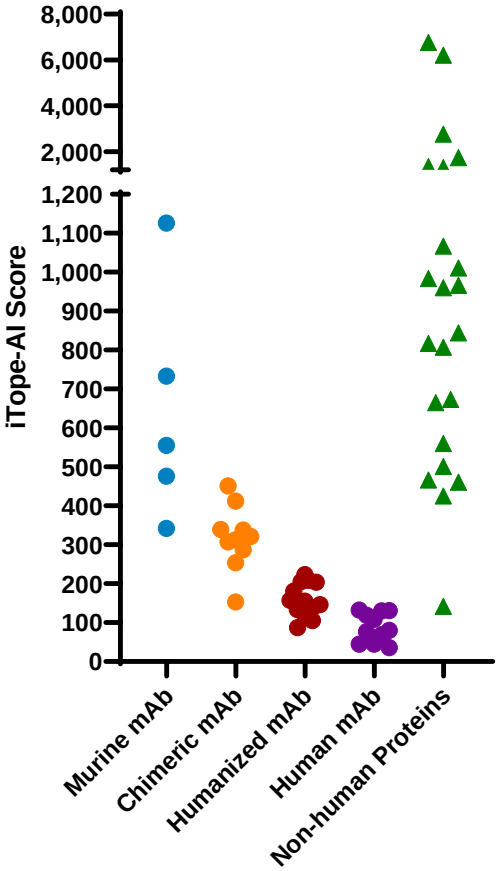


- Algorithms have been established predicting strength of peptide binding across the four major MHC class II pockets (HLA-DP, DQ and DR allele coverage)
- MHC-peptide binding strength has been shown to correlate with peptide immunogenicity

Limitations and Solutions

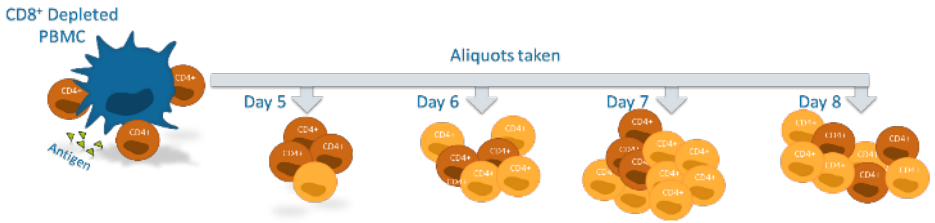
- Prospective machine learning
- Can be over-predictive → combine with other assay systems (e.g., proteomics)



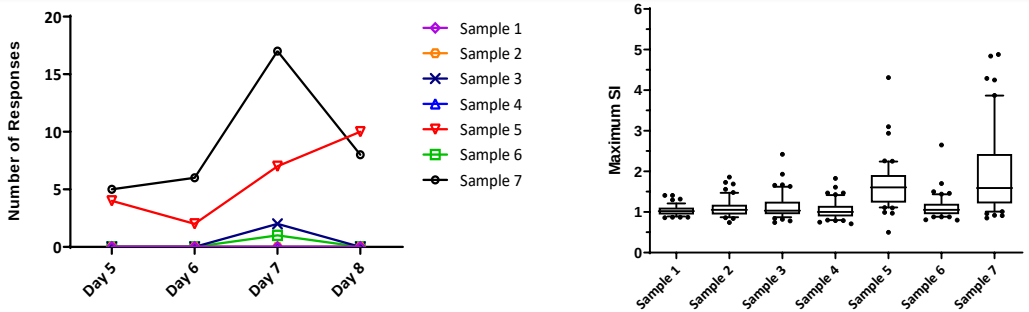


EpiScreen™ Time Course and DC:T Assays

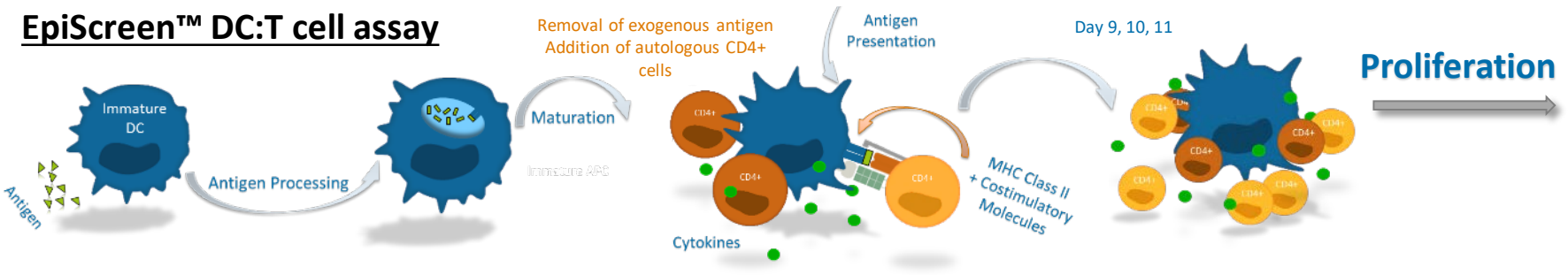
EpiScreen™ Time Course Assay



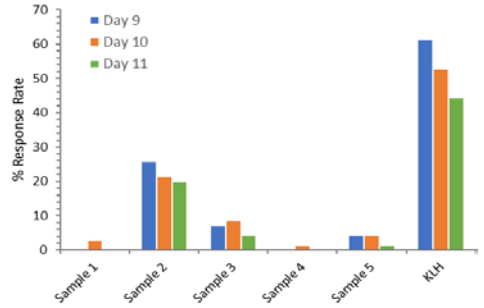
Proliferation



EpiScreen™ DC:T cell assay



Proliferation



Assay readouts:

- Proliferation (% response rates & magnitude)
- Cytokine release
- Cell phenotyping (activation markers)

Limitations and Solutions:

- Drug pharmacology e.g., immune regulator → alternative assay
- Different protocols between labs → standardize methods
- Different controls for benchmarking against clinical data → harmonize through same controls and source

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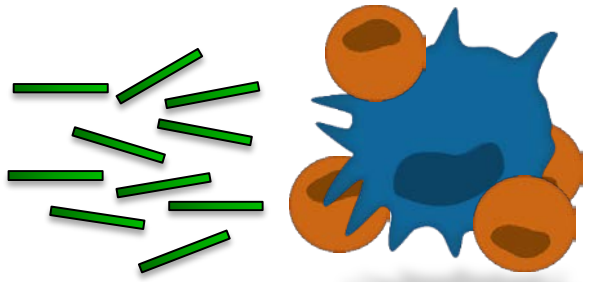
EpiScreen™ T cell Epitope Mapping (TCEM)

Peptide cluster identified in MAPPs

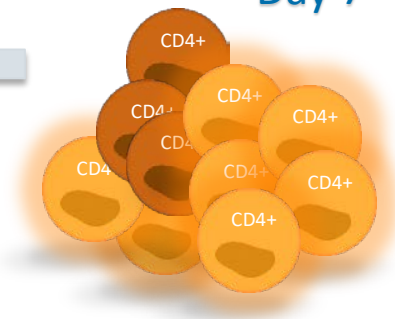
AATAGNMLHWWFDQNKDQIKRYLE
AATAGNMLHWWFDQN Peptide 1
AGNMLHWWFDQNKDQ Peptide 2
MLHWWFDQNKDQIKR Peptide 3
WWFDQNKDQIKRYLE Peptide 4



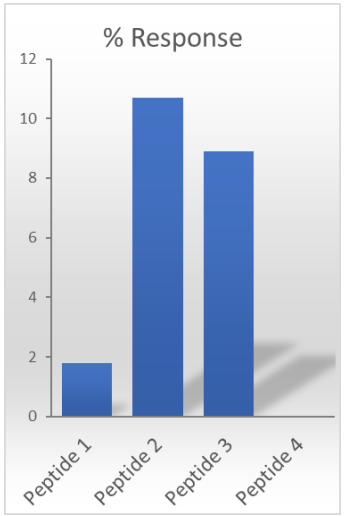
Peptides

CD8⁺ Depleted PBMC

Day 7



Proliferation



AGNMLHWWFDQNKDQ Peptide 2
MLHWWFDQNKDQIKR Peptide 3

iTope-AI Identification of core 9mer

Precise region of molecule identified for modification

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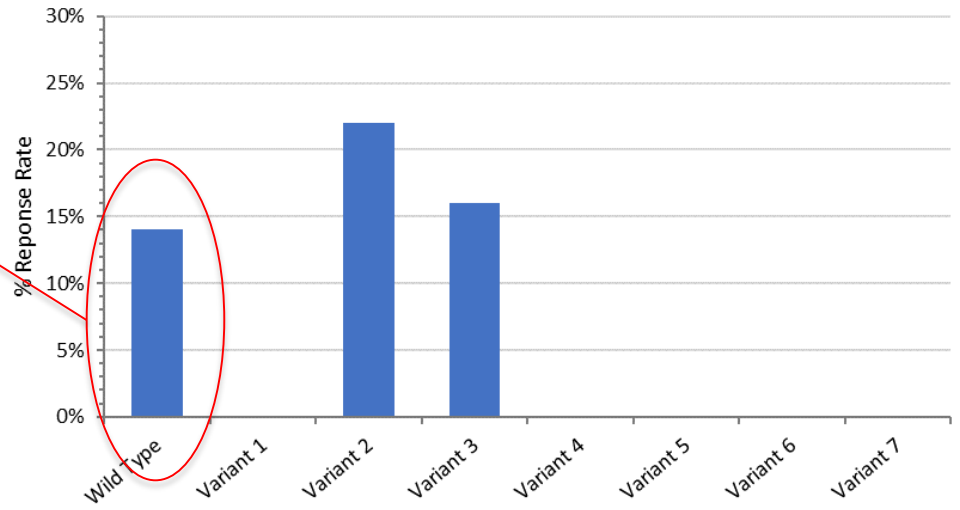
Designing Out Peptide Binding

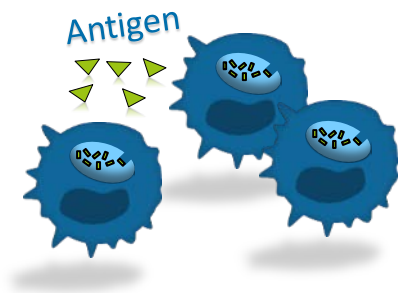
- TCEM identified a T cell epitope in wt sequence
- Variants designed based on in silico evaluation (iTopeAI) of this region
- Variants peptides synthesized and assessed in TCEM
- Removal of T cell epitope from wt in variants 1, 4, 5, 6 & 7

Note: careful not to engineer out function.



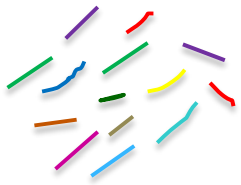
Peptide	% Response Rate
Wild type	14%
Variant 1	0%
Variant 2	22%
Variant 3	16%
Variant 4	0%
Variant 5	0%
Variant 6	0%
Variant 7	0%

Identification of ‘hot spots’: MHC-Associated Peptide Proteomics (MAPPs)



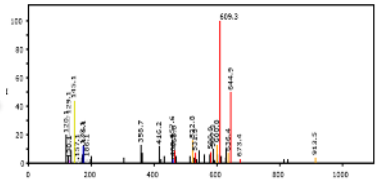
Antigen or drug-exposed monocyte derived DCs

Class II (HLA-DR) isolated from cells



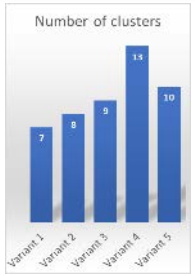
Peptide elution

Orbitrap-MS analysis of isolated peptides



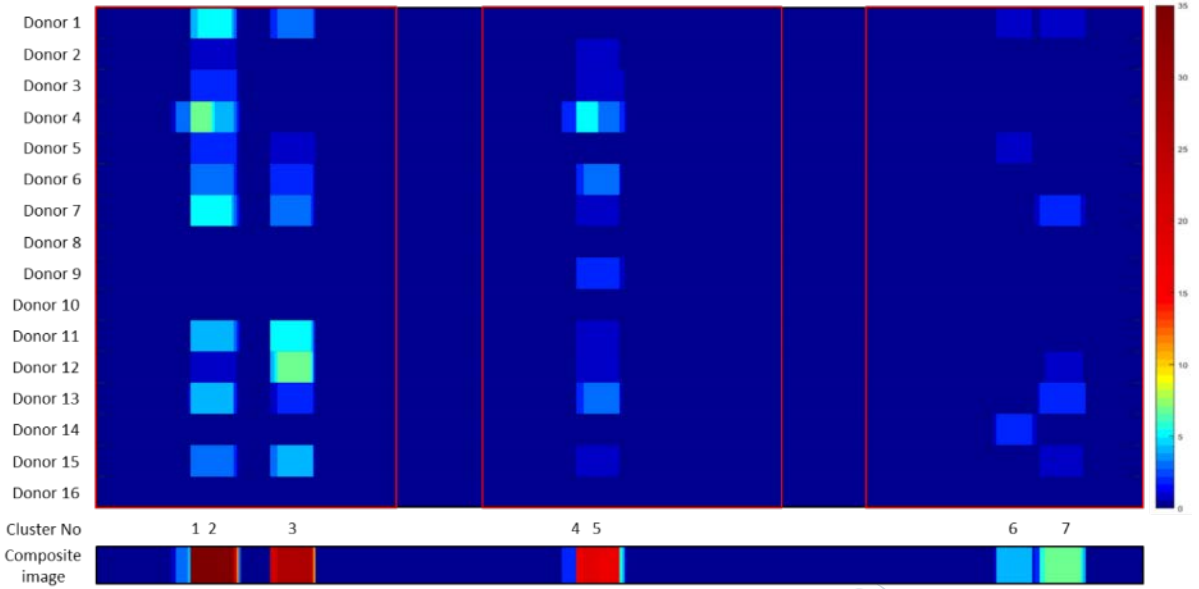
Bioinformatics analysis

Ranking of variants based on antigen presentation profile



T Cell Epitope Mapping of peptide clusters to identify precise regions for modification

i-Tope-AI



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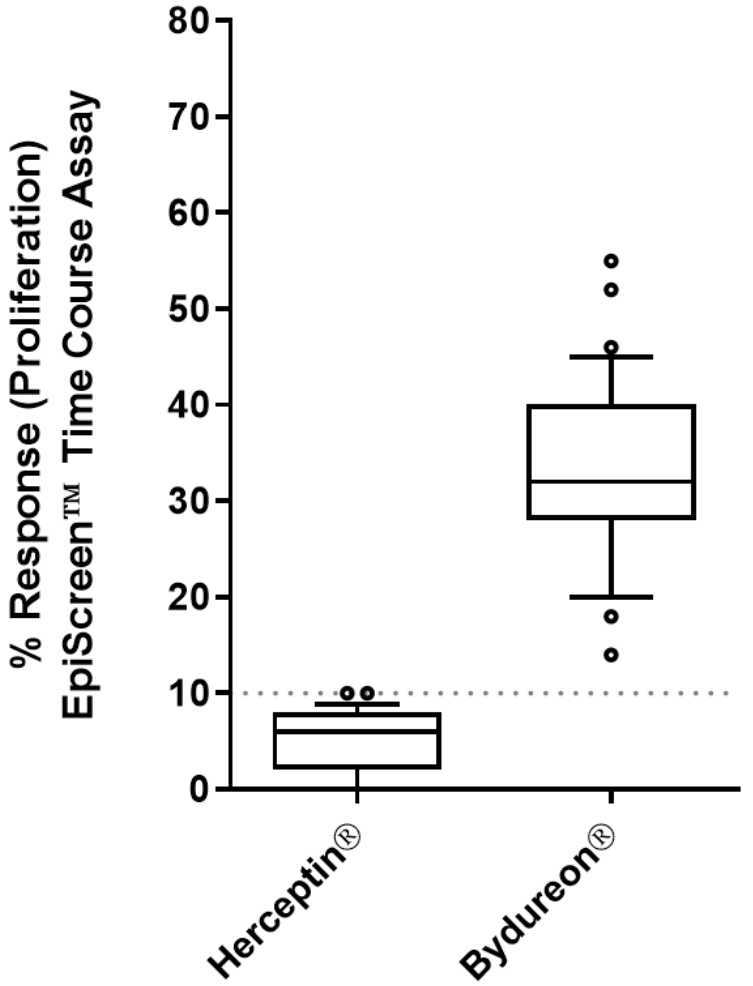
Case Study 1: Immunogenicity of Bydureon

- Bydureon† – slow release exenatide (39aa peptide)
- Improve glycaemic control in Type 2 diabetic patients
- 45% of patients are ADA positive
- Associated with increased injection site reactions
- ADAs do not significantly cross-reactive with endogenous peptides (e.g.,GLP-1)
- Ex vivo immunogenicity risk assessment aligned with clinical ADA data



† *EMA: Byrudeon EPAR – appendix 1*
Exenatide: synthetic peptide (39 aa), sequence derived from lizard salivary hormone (exendin-4) with antihyperglycemic activity (mimics glucagon-like peptide-1).

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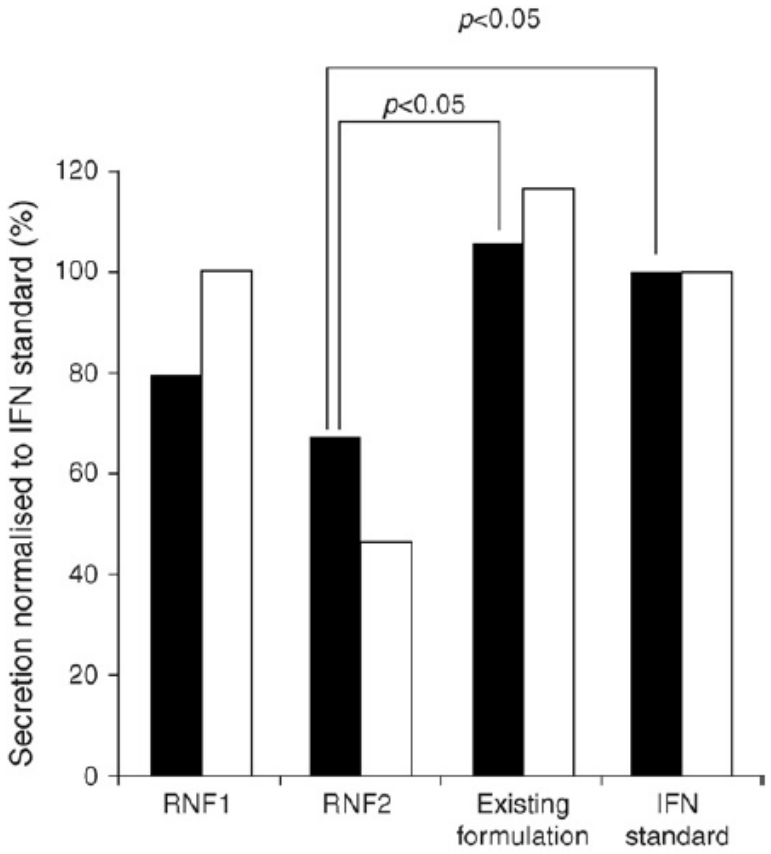
Case Study 2: Impact of Formulation on Immunogenicity Risk

	RNF1	RNF2	Current IFN beta-1a formulation	IFN beta standard
Mean cpm	41,903	35,719	55,612	55,012
Mean SI	0.9*	0.7*,†	1.4	1.6
Normalised SI[a] (%)	59.65	47.67	90.83	100

cpm, counts per minute; IFN, interferon; KLH, keyhole limit haemocyanin; RNF, Rebif® New Formulation; SI, stimulation index.
[a] SI were normalised to SI of IFN beta standard.
* $p < 0.05$ vs. the IFN beta standard.
† $p < 0.05$ vs. the current IFN beta-1a formulation.

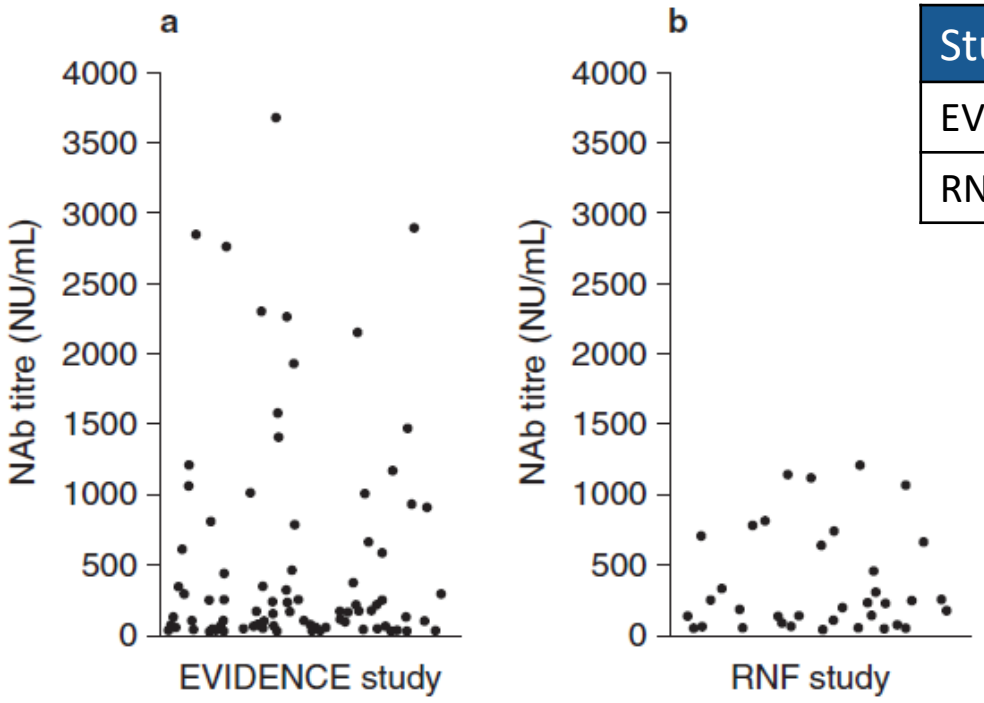
- EpiScreen™ was utilised in the programme to identify less immunogenic formulations of Rebif.
- HSA suspected to be involved in aggregation of IFN and replace with alternative stabilising excipients.
- Multiple formulation parameters were screened for physicochemical stability prior to progressing leads for immunogenicity assessment (RNF 1 and 2).



Jaber & Baker 2007 *J. Pharm Biomed Anal* 43:1256

Case Study 2 (cont.): Ex vivo Immunogenicity Aligned With Clinical Immunogenicity

- Reduced levels of neutralising antibodies with new formulation
- The safety profile was also improved.



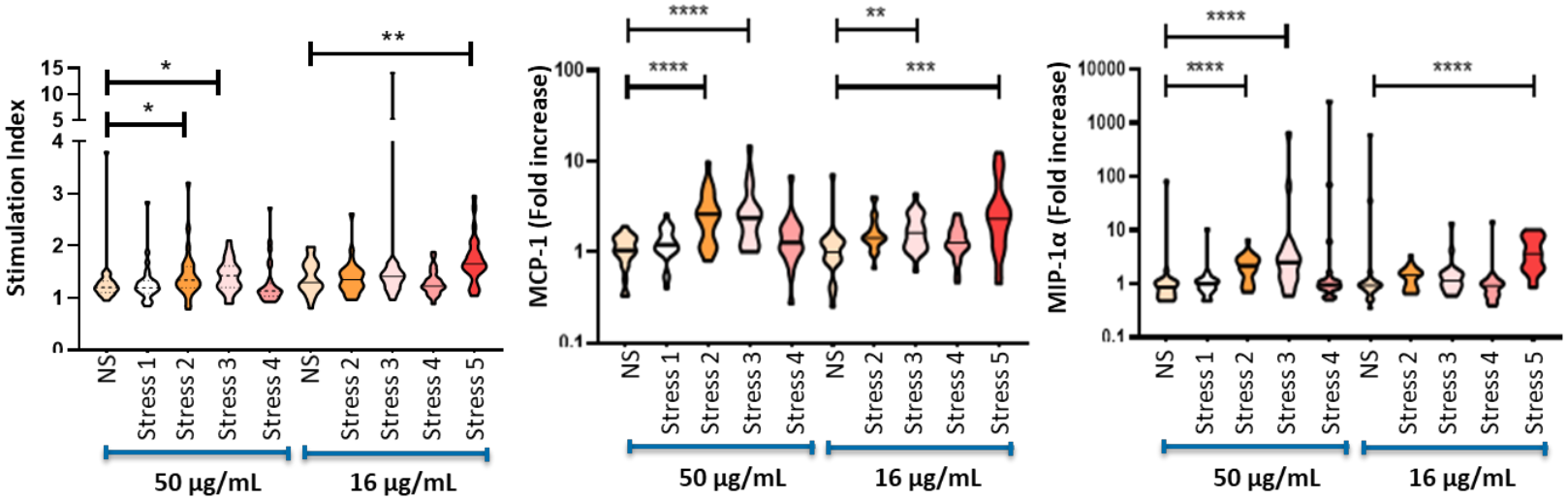
Study	Persistent Nab Response	Injection Site reactions
EVIDENCE	14% patients / high titres	84% patients
RNF2	2.5% patients / low titres	30% patients

Jaber et al 2007 *Drug RD 8 (6):335*

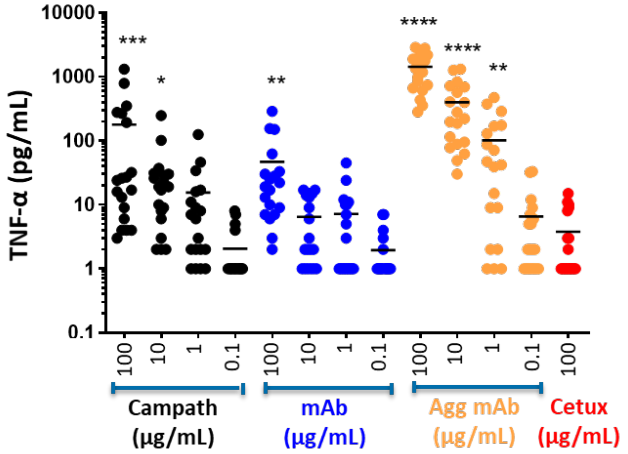
Case Study 3: Effect Of Aggregation On Immunogenicity

- Stress induced aggregate formation (antibody) – different particle size.
- Aggregation induced significant increase in CD4⁺ T cell responses in Episcreen assay
- Aggregates promoted cytokine responses in whole blood

CD4⁺ T cell Proliferation & PBMC Responses



Cytokine Screen™ (Whole Blood)



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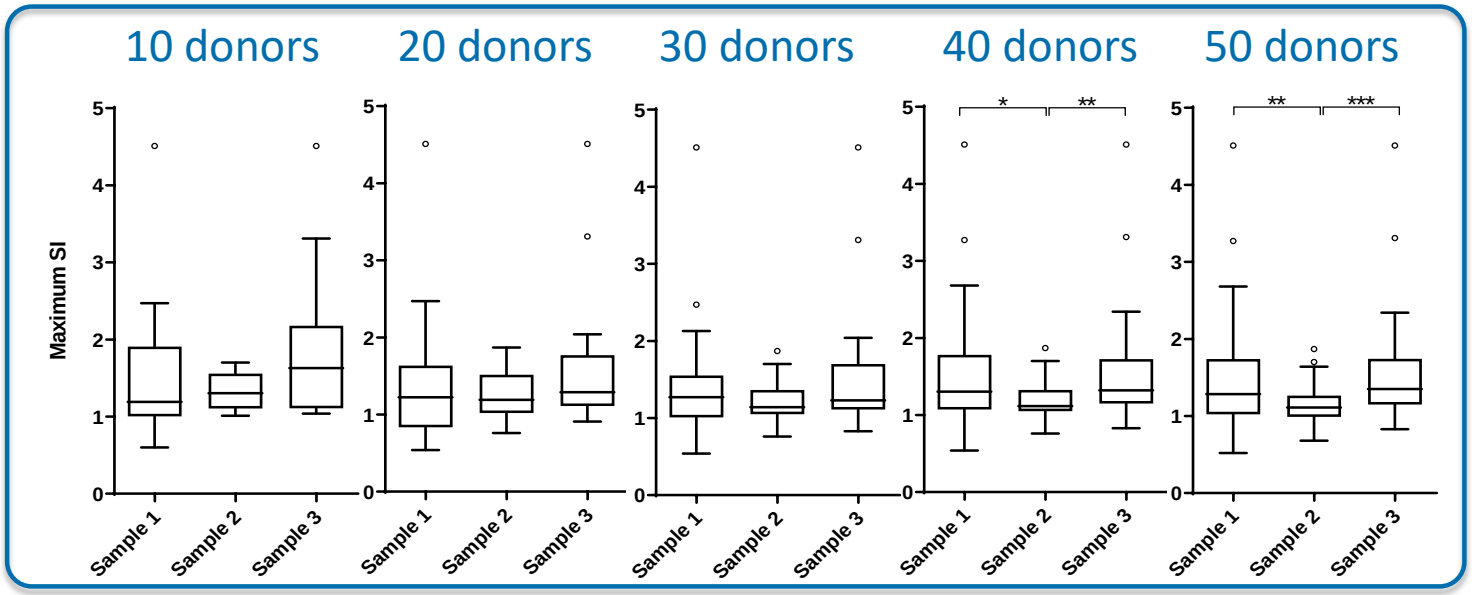
Case Study 4: Ex vivo Assay Donor Cohort Size Comparisons

Study comparing results from testing 10, 20, 30, 40 and 50 donors in the Episcreen™ Time Course Assay

SI indicates stimulation index and graphs show maximum SI over the 4-day time course



Number of donors	Sample 1	Sample 3
10	ns	ns
20	ns	ns
30	ns	ns
40	*	**
50	**	***

Significance against Sample 2 using repeated measures one-way ANOVA (Friedman's test) using Dunns post-test pairs comparison

Number of donors	Sample 1	Sample 2	Sample 3
10	20	0	20
20	15	0	15
30	13	0	10
40	20	0	15
50	20	0	14

% response rate using different donor cohort numbers

- Overall percentage response rate is similar
- Increased cohort size strengthens significance – from 40 donors

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Summary

- Many factors can contribute to immunogenicity with CD4+ T cell epitopes critical drivers of ADA induction.
- Other factors that can promote immunogenicity risk include excipients, contaminants and aggregates.
- Many pre-clinical assessment tools available to evaluate potential immunogenicity risk
- These can be used individually or in combination to fully interrogate what components of the drug are the drivers of immunogenicity risk.
- Limitations and appropriate powering of assays important considerations when interpreting the data

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