

Fourth Open Scientific EIP Symposium

**Lonza**

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**Using Predictions of Peptide – MHC Class II  
Binding in Immunogenicity Ranking During  
Early Stage Biotherapeutics Development**

Olga Obrezanova / Lonza Biologics plc / 8 February 2012

# Immunogenicity Screening Preclinical Strategies

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## *In vivo* strategies

- Animal studies exploring ADA response
- Transgenic animal studies exploring T-cell responses
- Tolerised animal models/humanised animals

## *In silico* strategies

- T-cell epitope mapping tools

## *In vitro* strategies

- T- cell epitope binding assays (HLA binding assays)
- T-cell and B-cell activation and proliferation assays

# Outline

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Epibase™ *in silico* predictive platform for mapping of T-cell epitopes

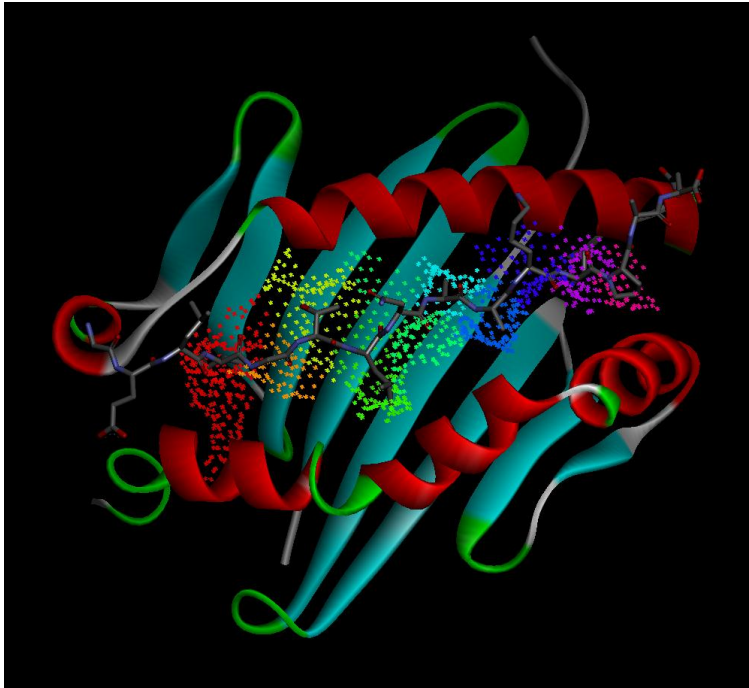
Immunogenicity ranking of

- proteins to enable lead selection
- potential T-cell epitopes to guide protein re-engineering (deimmunisation)

Combined use of *in silico* and *in vitro* tools to reduce potential immunogenicity

- Case study – deimmunisation of armed antibody

# Epibase™ *In Silico* Technology



## Method

- Peptide/HLA binding – necessary condition for T-cell activation
- Uses structural characteristics of the HLA receptor
  - Estimation of binding affinity using Pepscope technology\*
- Statistical layer based on experimentally determined binding affinities of peptides

## To make a prediction

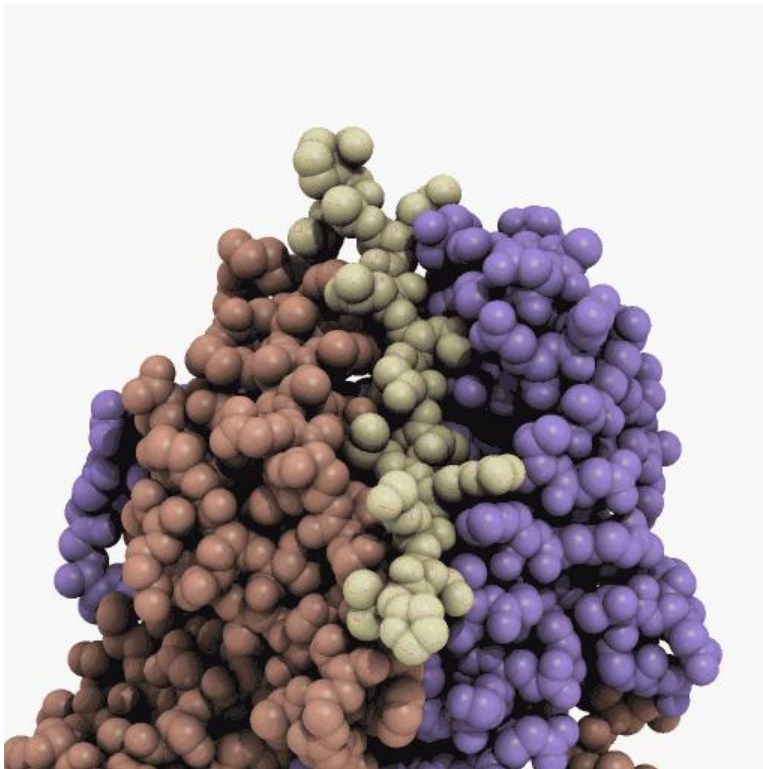
- Protein sequence is cut into 10mer overlapping peptides
- Predicted binding affinity is categorised using allotype specific thresholds

\* Desmet et al., Proteins 2002  
Desmet et al., Proteins 2005

# Epibase™ *In Silico* Technology

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Prediction of binding affinities of peptides for HLA class II molecules



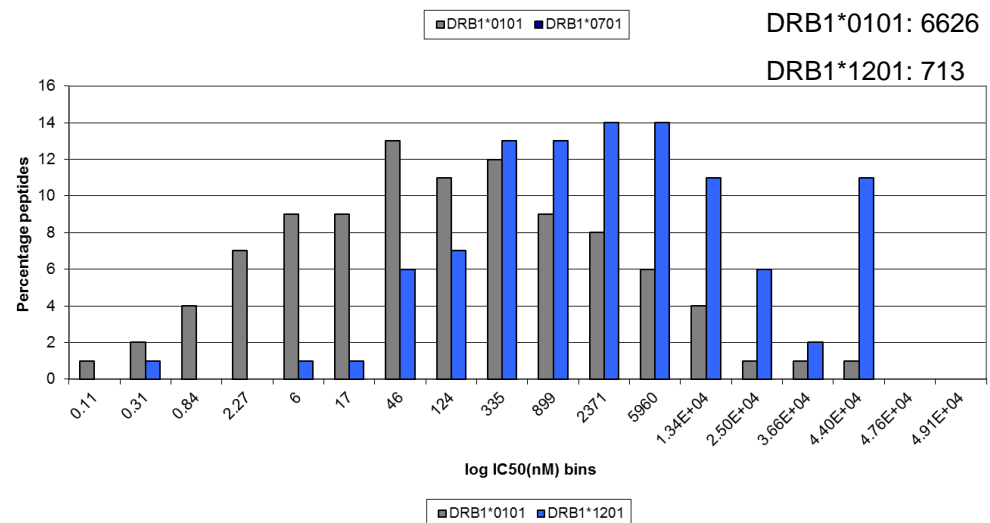
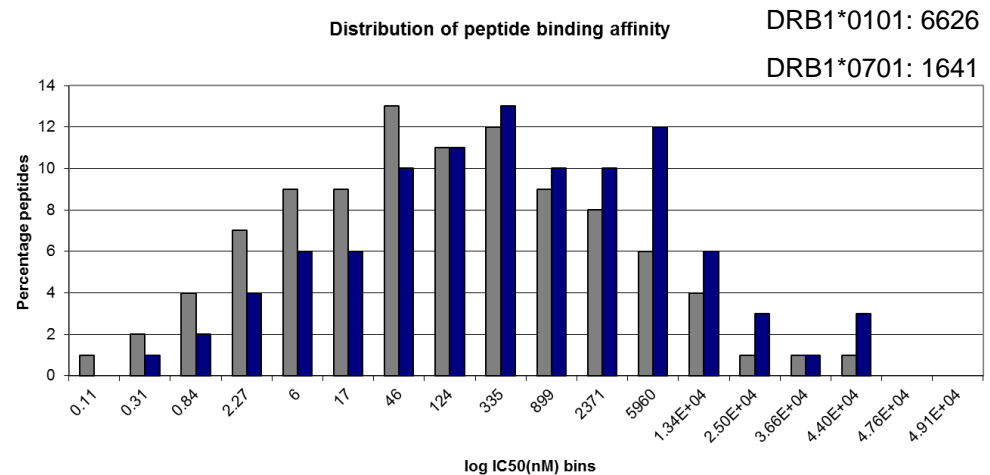
Epibase™ version 3.0  
new features:

- Incorporation of new experimental and structural data improved accuracy of predictions
- Use of allotype specific thresholds to separate binders and non-binders
- New allele frequencies for HLA class II allotypes, global frequencies

# Allotype Specific Thresholds

- Different HLA molecules bind peptides with different strengths
  - X.Rao et al. J.Immunology 2009, 182, p.1526
- Peptides  $IC_{50} < 50nM$  -
  - DRB1\*01:01 – 30%
  - DRB1\*12:01 – 5%
- Thresholds\*:
  - DRB1\*01:01 – 100 nM
  - DRB1\*07:01 – 200 nM
  - DRB1\*12:01 – 800 nM

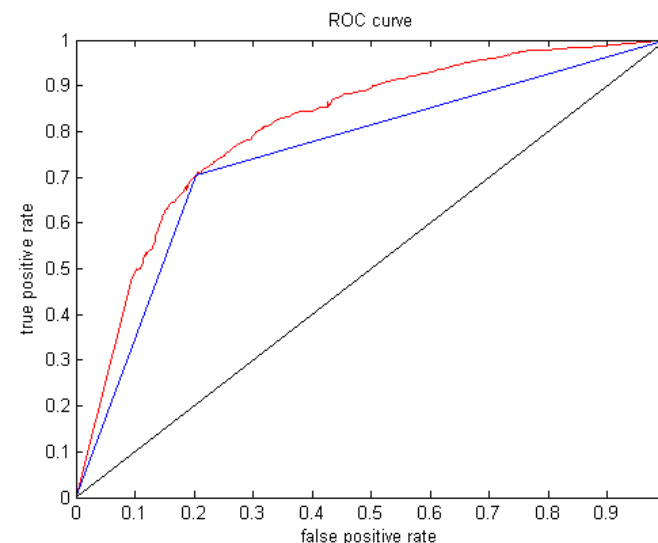
\* New in version 3.0



# Allotype Specific Thresholds

Thresholds are chosen by maximising AUROC measure on training sets

- Optimal separation between binders and non-binders
- Best balance of accuracies in classes
- Threshold is set between medium binder and non-binder
- Threshold between strong and medium binders – about 10 times less than medium thresholds



Allotype specific binding energy thresholds

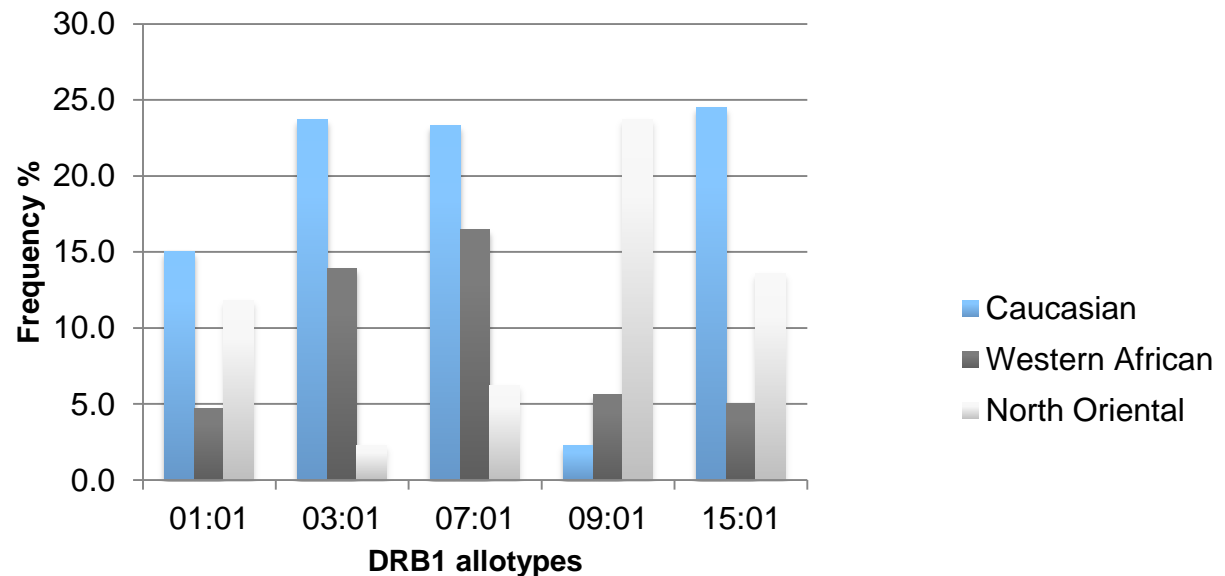
- Improve identification of top binders for all allotypes
- Provide better differentiation of non-binders from binders

# Allele Frequencies in Populations

To assess the impact of potential epitopes on specific populations utilise allele frequencies

- Global (weighed averages using data from the major groups)
- Weight is the proportion of an ethnic group in the world population
- Caucasian, Western African, Eastern African, Oriental, North Oriental, Mestizo, Indo-European, Austronesian

New global frequencies in version 3.0





# Broad Population Coverage

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Epibase™ covers 97% of world population

Allotype group	Global	Caucasian
DRB1	43	15
DRB3/4/5	8	6
DQ	22	12
DP	12	7
Total	85	40

## Caucasian allotype set

- all allotypes present in >3% of Caucasian population

## Global allotype set

- all DRs present in >3% of major ethnic groups
- all DQs apart from 3
- most important DPs

# Relative Ranking Criteria - Epitopes

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## Individual epitopes (Deimmunisation context)

- Binding strength
- Filtered out as self- peptides or not
  - E.g. human antibody germline, AIRE promoted thymus proteins
- Promiscuity
  - Binders to multiple HLA allotypes contribute more to the immunogenic potential than binders who affect only a single allotype
- Importance of affected allotypes in population
  - Epitopes affecting high frequency allotypes contribute more to the immunogenic potential
  - Allotype group: DRB1 – primary focus, DQ and DP –lower expression levels

## DRB1 score provides useful ranking

- combining all above criteria

# Example: D1.3 Antibody

Potential epitopes for Caucasian population

Only DRB1 allotypes are shown

Allotype	01:01	01:02	03:01	04:01	04:04	07:01	08:01	11:01	11:04	12:01	13:01	13:02	14:01	15:01	16:01	DRB1 score	Self-peptides filter
Frequency	15	4	24	16	6	23	5	12	6	3	11	8	5	25	5		
YNSALKSRLS	M					M		S	M	M			M			6.4	-
FLKMNSLHTD	S	M		M		M		M					M			7.5	-
VAPSQSLSIT			M			S					M	M				6.6	-
VQLQESGPGL	M	M		S										S	S		IGHV4
WVRQPPGKGL	S	M		M	M												IGHV3
MNSLHTDDTA					M					M						0.9	-
LHNHHTTKSF					S								M			1.1	-

# Deimmunisation Heat Map

Assessment of an increase/decrease in immunogenicity potential due to a single mutation in protein sequence

Can guide protein engineering efforts to remove T-cell epitopes

		Critical epitope count difference (WT: 124)																			
Pos	Res	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
8	G	1	0	0	0	1	0	0	1	0	1	1	1	0	0	0	0	1	1	1	1
9	P	2	1	0	0	2	2	1	3	2	2	3	1	0	2	2	1	1	4	1	3
10	G	1	0	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
11	L	0	-1	-1	-1	2	0	0	1	1	0	1	1	-1	0	2	0	-1	1	1	2
12	V	-1	-3	-4	-3	0	-1	-3	0	-2	0	0	-2	-3	-2	0	-1	-1	0	-1	0
13	A	0	-1	-1	-2	0	-2	0	0	0	1	0	-1	-1	-2	1	0	-1	0	0	0
14	P	1	0	0	1	2	1	-1	2	1	2	2	1	0	1	1	1	1	2	2	2
15	S	1	-1	-1	-1	1	0	0	1	1	2	1	-1	0	-1	1	0	0	1	1	1
16	Q	2	1	-1	-1	1	1	2	1	2	1	1	1	1	0	2	1	1	2	1	1
17	S	2	0	-2	-2	2	0	0	3	1	3	3	2	1	-1	1	0	0	3	1	3
18	L	-1	-1	-1	-1	-1	-2	-2	0	-1	0	0	-2	-2	-2	0	-1	-2	0	-1	-1

# Relative Ranking Criteria - Proteins

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Complete proteins (Lead selection/ profiling context)

- Critical epitope counts
- Promiscuity – number of affected allotypes
- Importance of affected allotypes (frequency and allotype group)

Rank – reflects potential risk

- based on critical epitopes and frequencies of affected allotypes

# Example: Therapeutic Antibodies

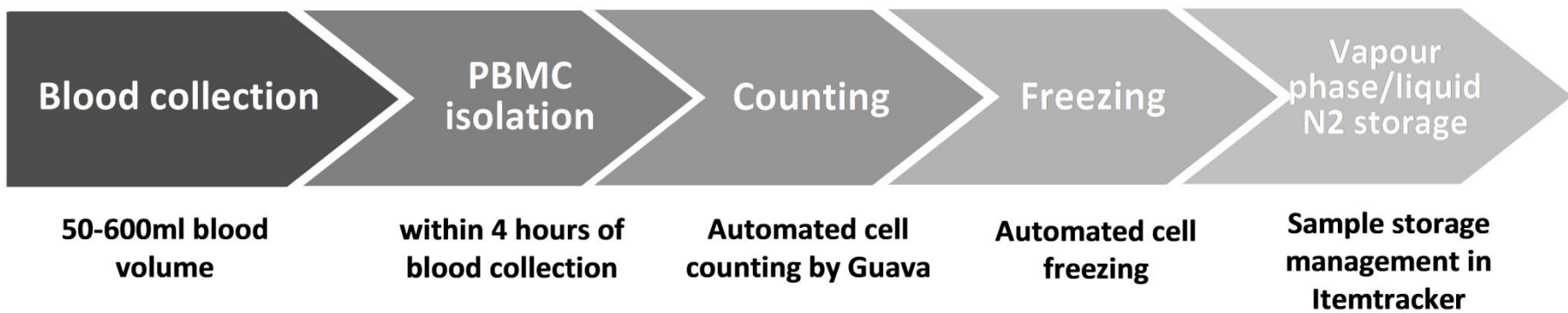
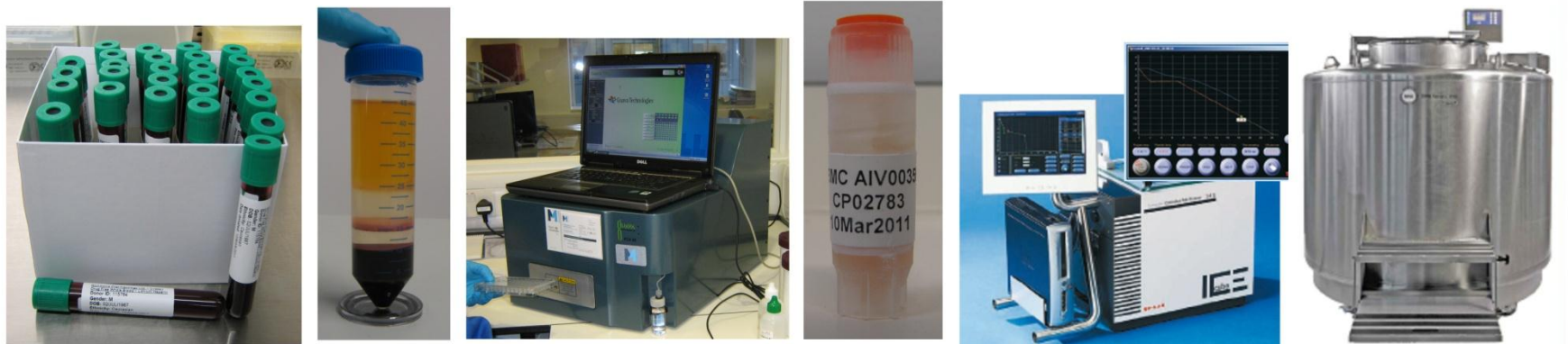
Assessment of potential immunogenicity for Caucasian population

Type	Rank	Epitope count			
		DRB1 strong	DRB1 medium	DRB3/4/5 strong	DRB3/4/5 medium
human	0.8	6	17	0	12
humanised A	1	7	25	3	14
humanised B	1.5	14	28	2	26
chimeric	2	15	38	5	24
murine	4.3	31	117	14	69

Immunogenicity risk

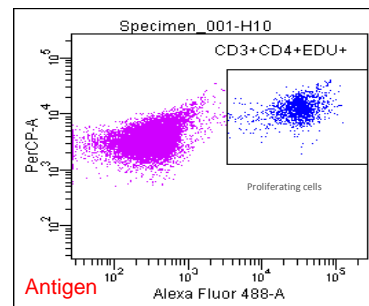
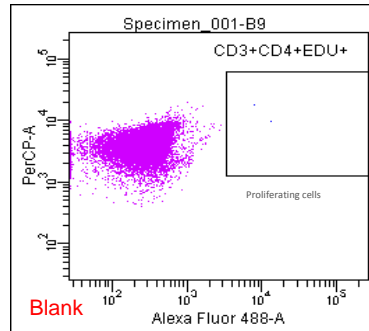
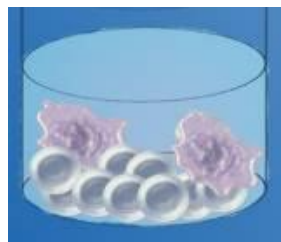


# Epibase™ *In Vitro* PBMC Bank

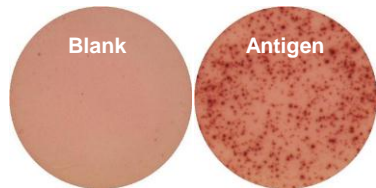


- Access to >100 000 donors
- Ability to sample certain target populations (4-digit HLA-based, ethnicity, elderly, diseased)
- Customer specific sampling/storage (short-long term storage of customer PBMC)

# Epibase™ *In Vitro* Immunogenicity Assessment



- Cell surface markers (CD3/CD4)
- **Proliferation (EdU)**
- Cytokine analysis
  - Intracellular cytokine staining
  - Cytokine Bead Array
  - ELISA
- Antigen-specific T-cells
  - HLA class I/II tetramers



T-cell ELISpot  
B-cell ELISpot



# Case Study: Deimmunisation of VB6-845®

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## Background

- Viventia's anti-EpCAM recombinant immunotoxin
  - Humanised Fab fragment fused to a deimmunised toxin (bouganin)
- Targets and mediates cell death in EpCAM-positive solid tumors
- First-in-man Phase I trial assessed the safety of VB6-845 in 13 patients with various EpCAM-positive cancers
  - Low or no antibody responses against deimmunised bouganin portion
  - Observed immune response to Fab moiety



## Objective

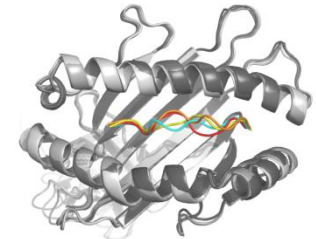
- Minimise the potential immunogenicity risk of the fusion protein by deimmunising the Fab portion

# Case Study: Deimmunisation of VB6-845®

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## *In silico* deimmunisation

- Screening for T-cell epitopes using Epibase™
- Antibody structure modelling
- Substitutions to eliminate T-cell epitopes while retaining affinity for target and structural integrity
- Proposed changes:
  - 19 mutations (11 in VH and 8 in VL) removed critical epitopes or decreased the affinity of remaining epitopes
  - 14 out of 19 proposed mutations (10 in VH and 4 in VL) retained expression and affinity for EpCAM: 74% success rate



## Case Study: Deimmunisation of VB6-845®

*In vitro* verification and testing of deimmunised protein variants

- Screening for T helper cell responses using PBMCs from healthy donors
- Individual and population responses



Selection of the best deimmunised variant

Deimmunised Fab has a similar binding affinity for EpCAM as the wild type:

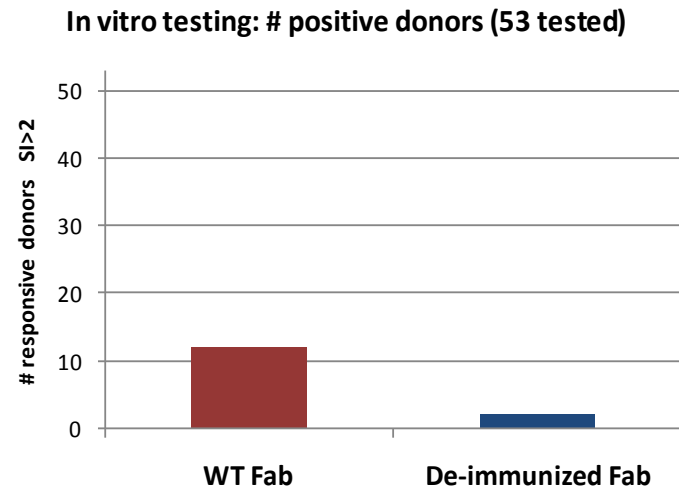
- De-Fab:  $KD = 1.31 \times 10^{-9}$
- WT :  $KD = 1.56 \times 10^{-9}$

# Case Study: Deimmunisation of VB6-845®

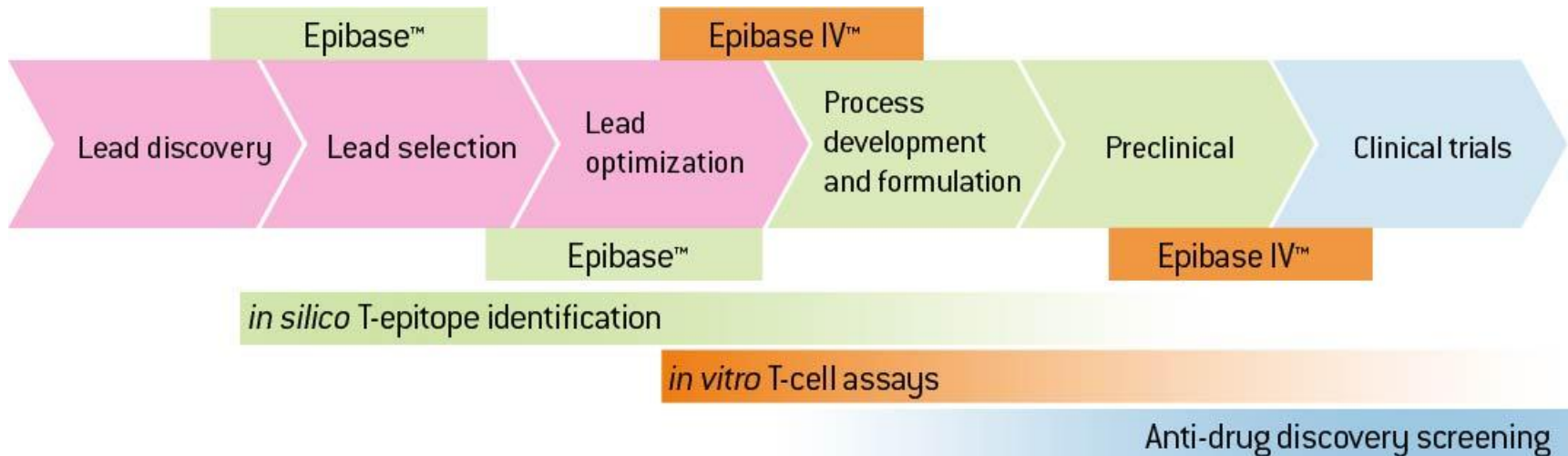
*In vitro* testing – single donor and population level

Deimmunised Fab shows a substantial and significant reduction in its ability to raise T-cell responses

A second generation VB6-845 molecule has been engineered and is now ready for testing in Phase I trials



# Conclusion: Immunogenicity Profiling



Select leads with lower potential immunogenicity early

- Rank leads in combination of properties: activity, immunogenicity, aggregation, stability etc.

Combined *in silico* and *in vitro* testing to reduce immunogenicity risk

- Predict T-cell epitopes, remove epitopes (deimmunisation/humanisation), confirm by *in vitro* assays

# Acknowledgements

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