Fourth Open Scientific EIP Symposium

Lonza

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

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

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

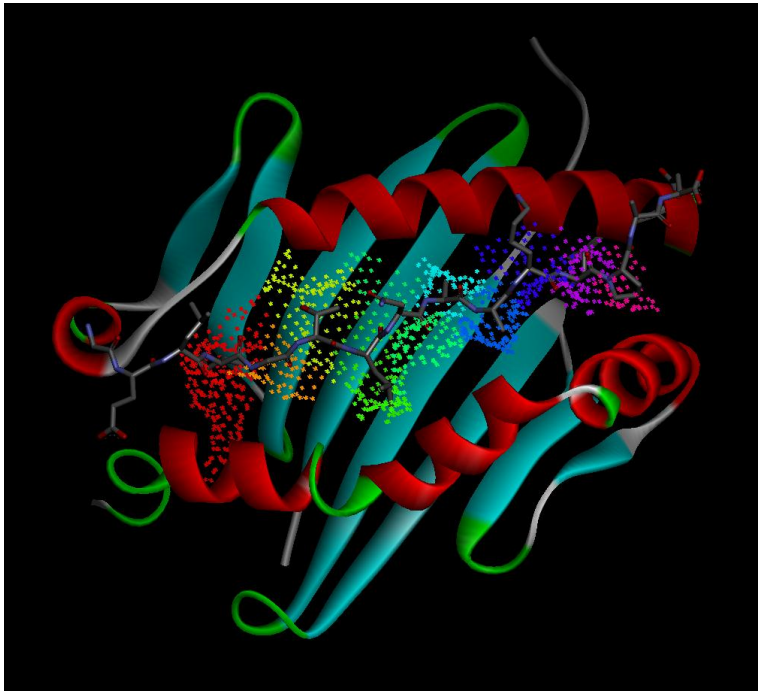
Immunogenicity ranking of

- 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



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

Method

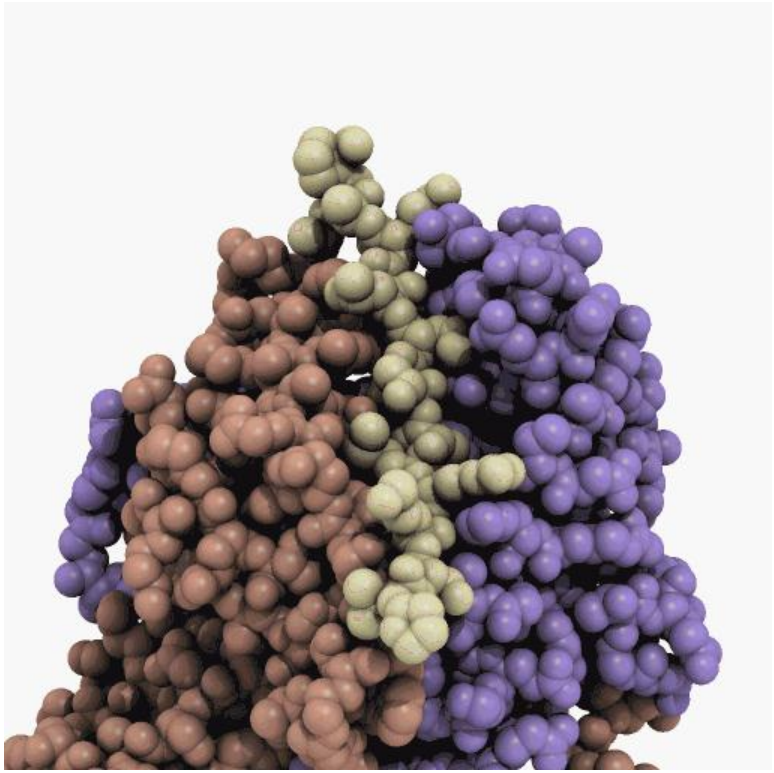
- Peptide/HLA binding – necessary condition for T-cell activation
- Uses structural characteristics of the HLA receptor
  - Estimation of binding affinity using Pepscope technology*
- Statistical layer based on experimentally determined binding affinities of peptides

To make a prediction

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

Epibase™ *In Silico* Technology

Prediction of binding affinities of peptides for HLA class II molecules



Epibase™ version 3.0 new features:

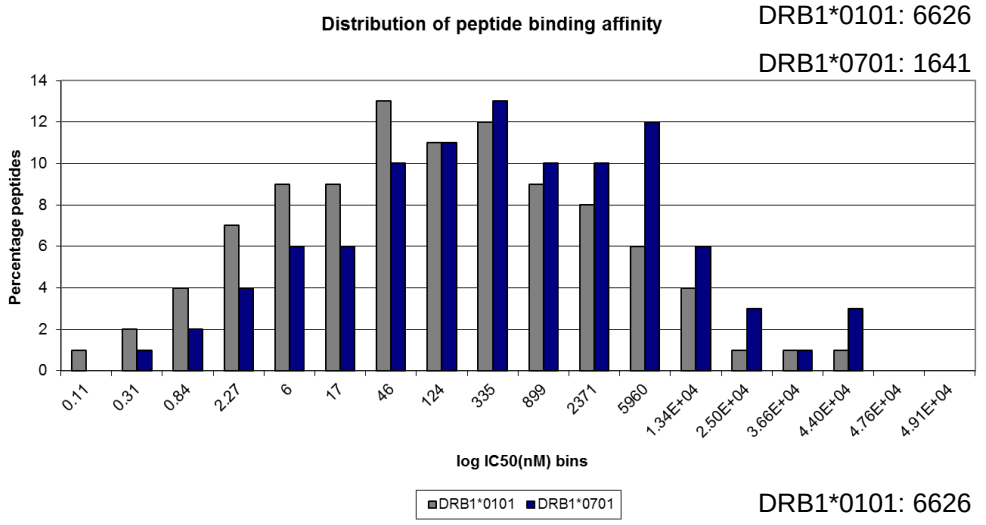
- Incorporation of new experimental and structural data improved accuracy of predictions
- Use of allotype specific thresholds to separate binders and non-binders
- New allele frequencies for HLA class II allotypes, global frequencies

Allotype Specific Thresholds

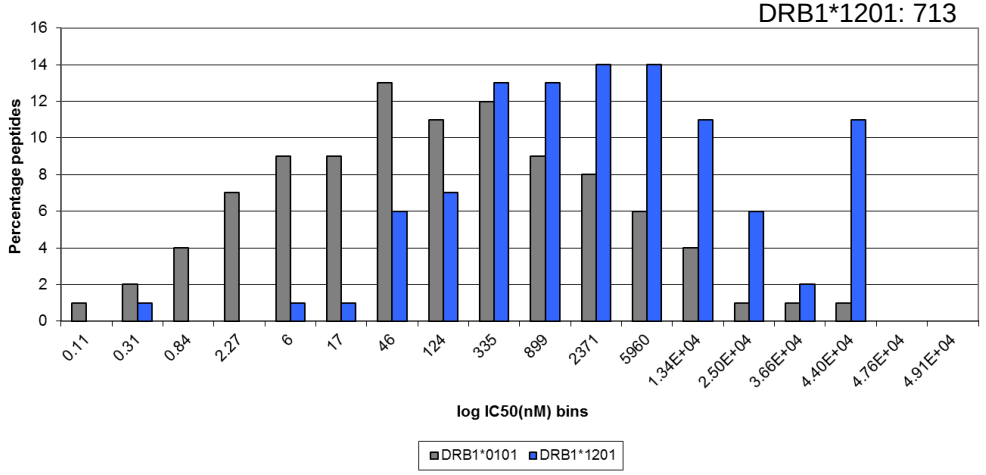
- Different HLA molecules bind peptides with different strengths
  - X.Rao et al. J.Immunology 2009, 182, p.1526
- Peptides IC_{50} < 50nM -
  DRB1*01:01 – 30%
  DRB1*12:01 – 5%
- Thresholds*:
  DRB1*01:01 – 100 nM
  DRB1*07:01 – 200 nM
  DRB1*12:01 – 800 nM

* New in version 3.0

DRB1*0101: 6626
DRB1*0701: 1641



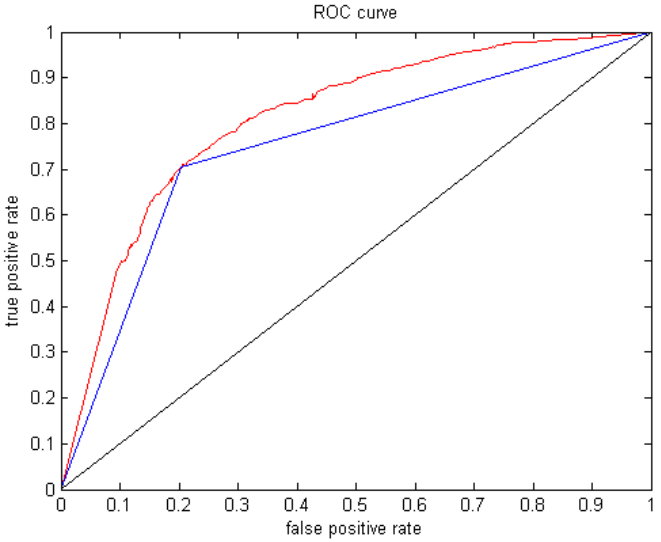
DRB1*0101: 6626
DRB1*1201: 713



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

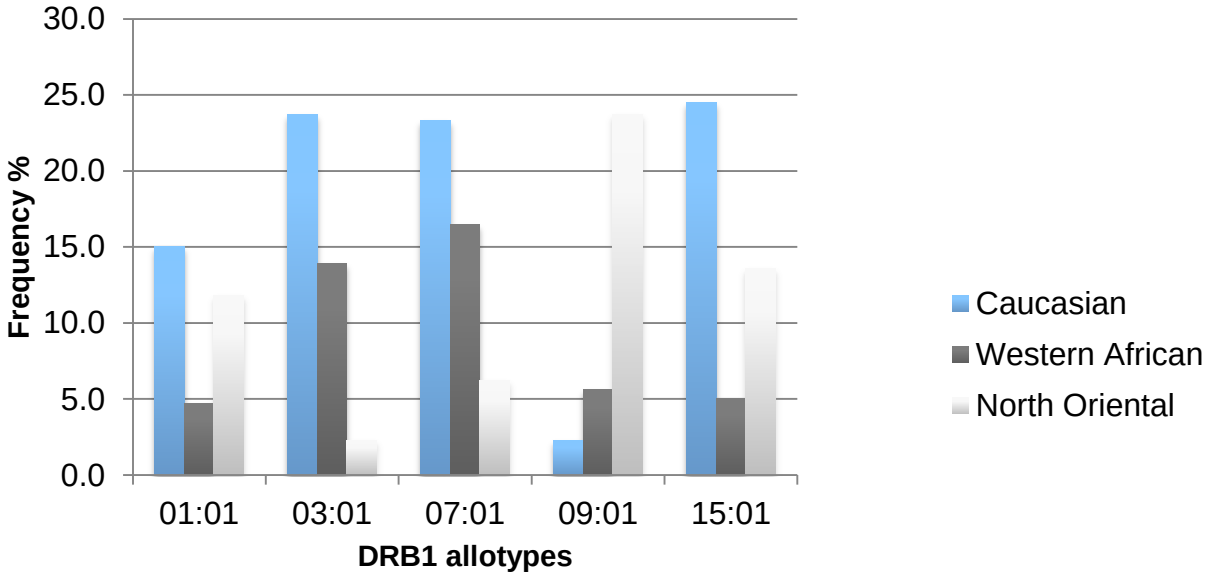
- Improve identification of top binders for all allotypes
- Provide better differentiation of non-binders from binders

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

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

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

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

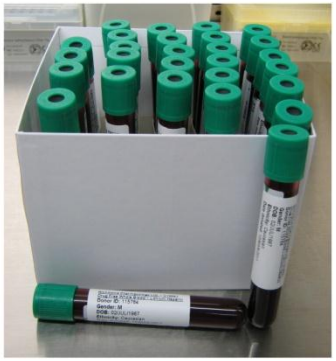
Example: Therapeutic Antibodies

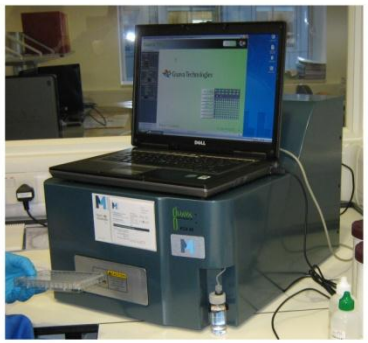
Assessment of potential immunogenicity for Caucasian population

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



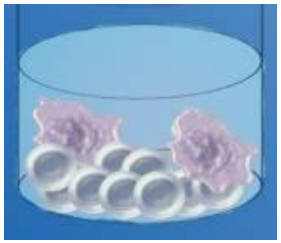


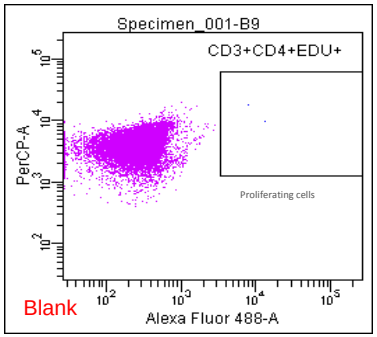


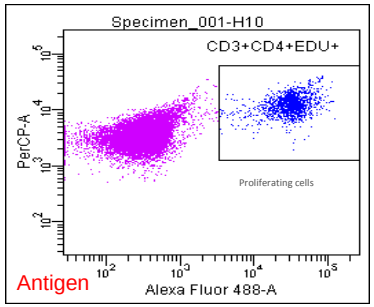
Blood collection	PBMC isolation	Counting	Freezing	Vapour phase/liquid N2 storage
50-600ml blood volume	within 4 hours of blood collection	Automated cell counting by Guava	Automated cell freezing	Sample storage management in Itemtracker

- 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)

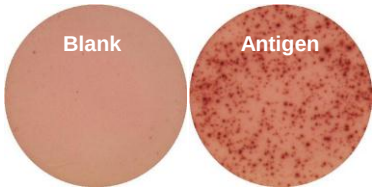
Epibase™ *In Vitro* Immunogenicity Assessment







- 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®

Background

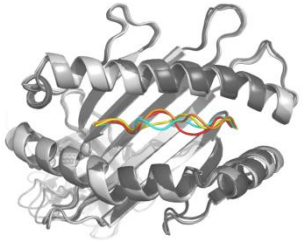
- Viventia's anti-EpCAM recombinant immunotoxin
  - Humanised Fab fragment fused to a deimmunised toxin (bouganin)
- Targets and mediates cell death in EpCAM-positive solid tumors
- First-in-man Phase I trial assessed the safety of VB6-845 in 13 patients with various EpCAM-positive cancers
  - Low or 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®

In silico deimmunisation

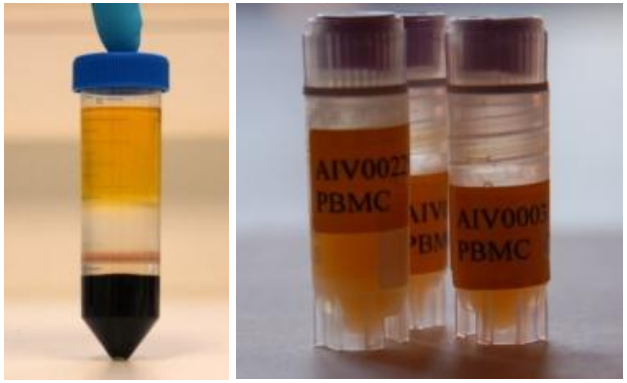


- Screening for T-cell epitopes using Epibase™
- Antibody structure modelling
- Substitutions to eliminate T-cell epitopes while retaining affinity for target and structural integrity
- Proposed changes:
  - 19 mutations (11 in VH and 8 in VL) removed critical epitopes or decreased the affinity of remaining 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:

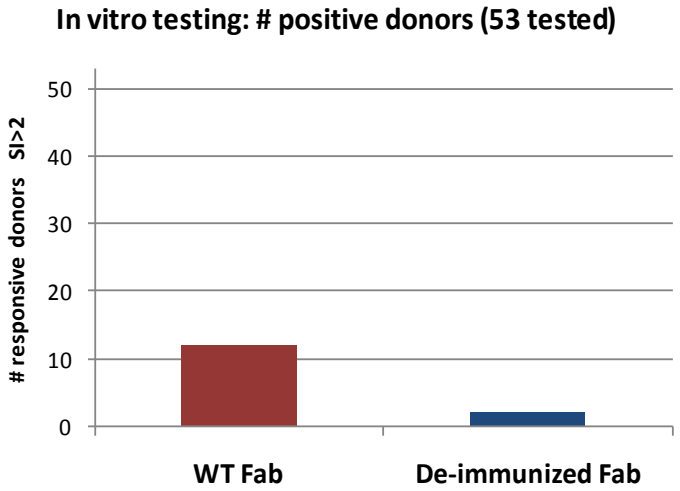
- De-Fab: KD = 1.31x10-9
- WT : KD = 1.56x10-9

Case Study: Deimmunisation of VB6-845®

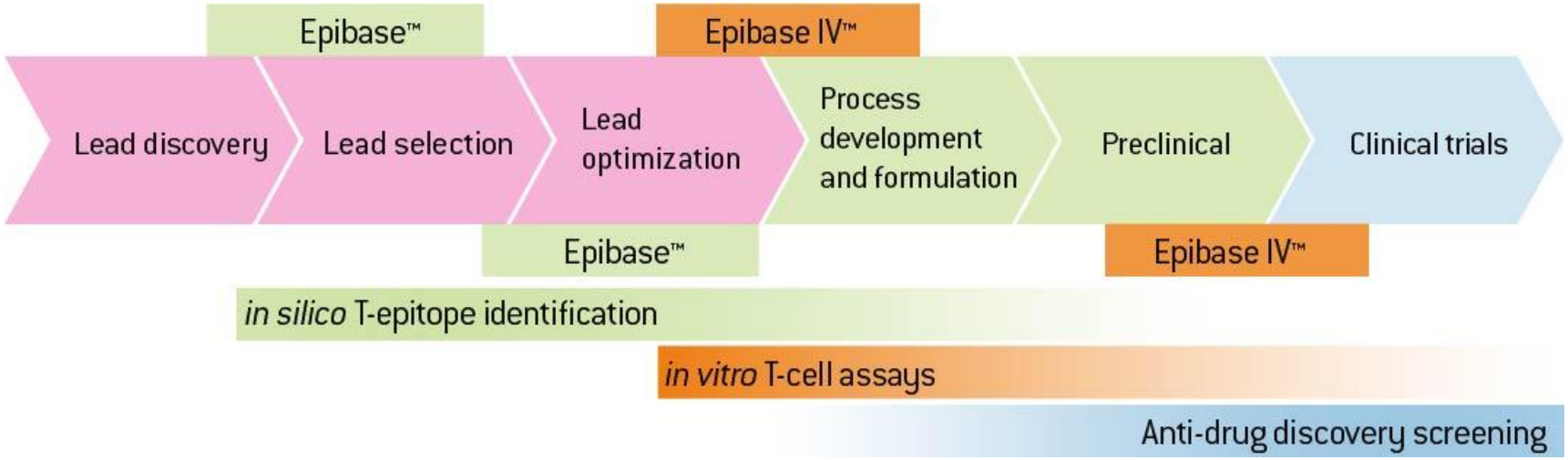
In vitro testing – single donor and population level

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

VIVENTIA

Biotechnologies Inc.

Glen MacDonald

CSO and V.P. Operations

Jeannick Cizeau

Director of Research

Joycelyn Entwistle

Director Preclinical Development

Lonza

Yvette Stallwood

Sofie Pattijn

Lee Larcombe

Philippe Stas

Andreas Arnell

Thomas Gallagher

Yannick Gansemans

Younes Mokrab

http://www.lonza-aps.com