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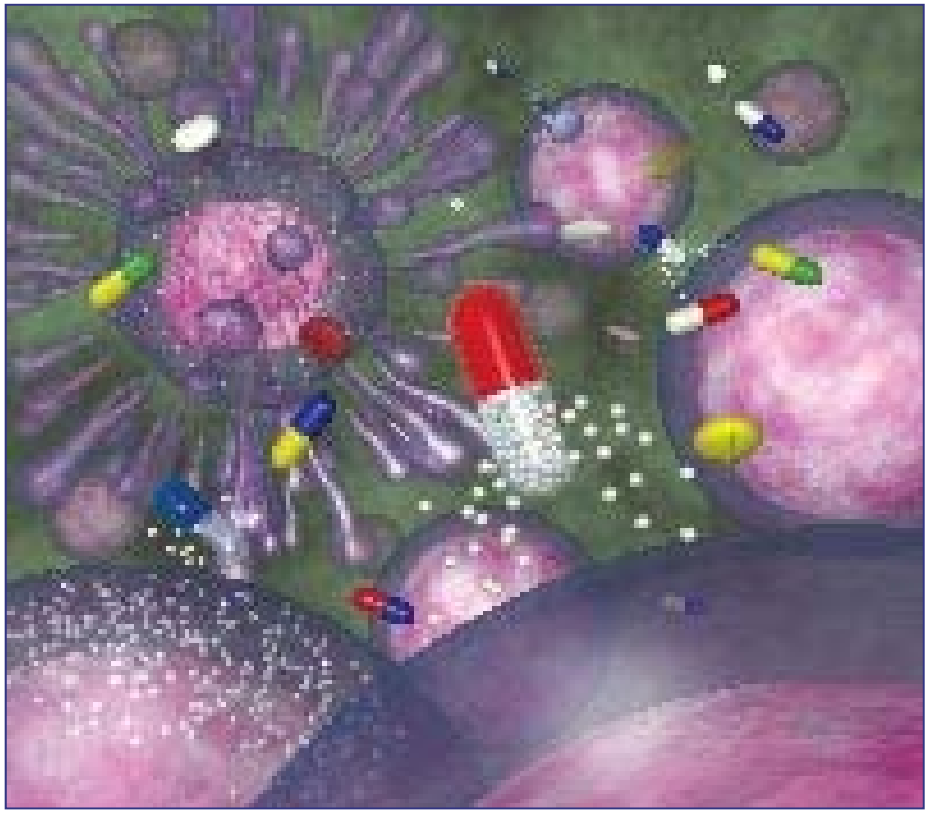
Predictive Immunogenicity in lead discovery

Immunogenicity

'Immunogenicity is the ability of an antigen to provoke an immune response'



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Applied Protein Services

Immunogenicity = The intrinsic capacity of a product to induce an immune response in a target population

Vaccines	Therapeutic proteins
Designed to mount a maximal protective immune response	Designed to exert therapeutic function (biological activity) in vivo
❑ Raise Effector T-cell function (HTL/CTL) for fighting disease	❑ Avoid T-cell activation (HTL) for helping antibody generation
❑ Raise broadly neutralising antibodies to prevent new infection and/or further spread	❑ Avoid neutralising antibodies that prevent the protein from exerting its therapeutic function
WANTED IMMUNOGENICITY	UNWANTED IMMUNOGENICITY

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Observed Immunogenicity

Non-antibody protein therapeutics:

	Abs	Nabs
IFN-α	± 50%	± 25-30%
IL-2	± 50%	± 5%
GM-CSF	± 70-95%	± 0-50%
F-VIII	± 40%	± 30%

anti-idiotypic networks are an example of Abs against self proteins

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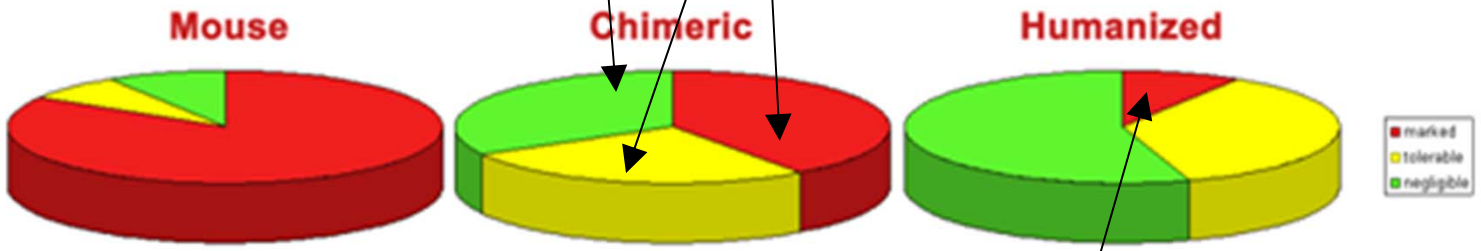
Observed Immunogenicity

Therapeutic protein	Type	Target	Indication	Assay	AR (%)	Pop	Supr
OKT3	murine	CD3	Graft rejection	ELISA	54	82	+
Bexxar/tositumomab	murine	CD20	Non-Hodgkin's lymphoma	ELISA	9	55	+
Reopro/abciximab	chimeric	GPIIb/IIIa	Coronary angioplasty	ELISA	21	500	
Remicade/infliximab	chimeric	TNFα	Crohn's disease		9	199	+
Remicade/infliximab	chimeric	TNFα	Crohn's disease	ELISA	61	125	+
Remicade/infliximab	chimeric	TNFα	Rheumatoid arthritis	ELISA	8	60	+
Rituxan/rituximab	chimeric	CD20	Non-Hodgkin's lymphoma	ELISA	0	37	
Rituxan/rituximab	chimeric	CD20	Systemic lupus erythematosus	ELISA	65	17	+
Rituxan/rituximab	chimeric	CD20	Primary Sjogren's syndrome	RIA	27	15	+
Raptiva/efalizumab	humanised	CD11a	Psoriasis		2.3	501	
Raptiva/efalizumab	humanised	CD11a	Psoriasis		4	292	+
Raptiva/efalizumab	humanised	CD11a	Psoriasis	ELISA	6	1063	
Campath/alemtuzumab	humanised	CD52	Rheumatoid arthritis		63	40	
Campath/alemtuzumab	humanised	CD52	Rheumatoid arthritis		29	31	
Campath/alemtuzumab	humanised	CD52	Rheumatoid arthritis		53	30	
Campath/alemtuzumab	humanised	CD52	B-cell lymphoma		1.9	211	
Humira/adalimumab	human	TNFα	Rheumatoid arthritis	ELISA	5	1062	+

Van Walle et al, Expert Opin. Biol. Ther., 7(3), in press

Therapeutic antibodies

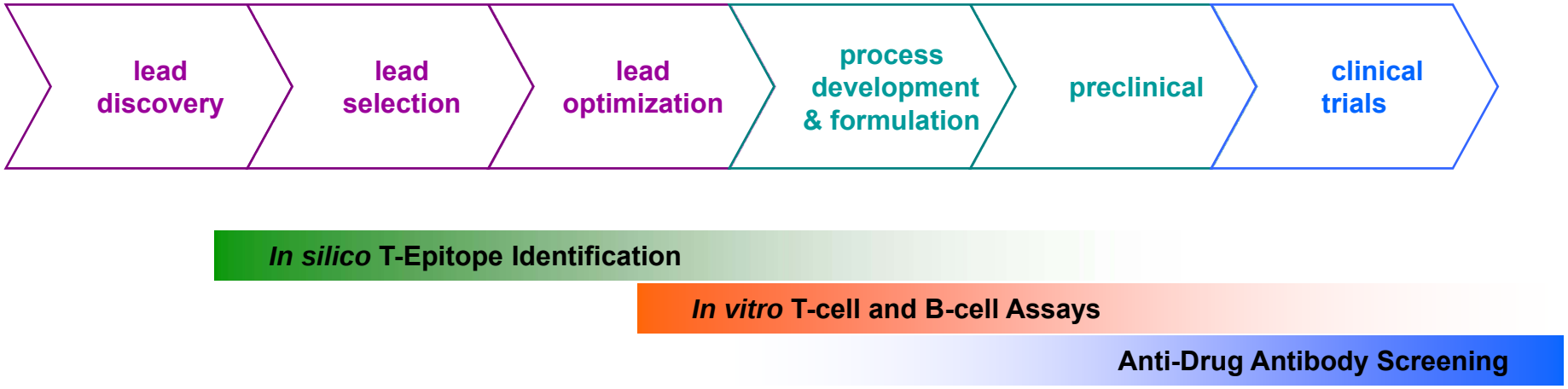
rituximab	chimeric	CD20	NHL	0	37	multiple	iv
rituximab	chimeric	CD20	SLE	65	17	multiple	iv
rituximab	chimeric	CD20	RA	27	15	multiple	iv



Hwang & Foote, 2005

adalimumab	human	TNFa	RA

Early Stage Immunogenicity Assessment



Generating a risk profile of the potential immunogenicity

- Analysis of the probability to observe immunogenicity
- Analysis of the severity of the observed immunogenicity

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According to the EMEA …

European Medicines Agency

London, 13 December 2007
Doc. Ref. EMEA/CHMP/BMWP/14327/2006

COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)

GUIDELINE ON IMMUNOGENICITY ASSESSMENT OF BIOTECHNOLOGY-DERIVED THERAPEUTIC PROTEINS

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Preclinical Immunogenicity Assessment

GUIDELINE ON IMMUNOGENICITY ASSESSMENT OF BIOTECHNOLOGY-DERIVED THERAPEUTIC PROTEINS

Doc. Ref. EMEA/CHMP/BMWP/14327/2006

4.2 Non-clinical assessment of immunogenicity and its consequences

Therapeutic proteins show species differences in most cases. Thus, human proteins will be recognized as foreign proteins by animals. **For this reason, the predictivity of non-clinical studies for evaluation of immunogenicity is considered low.**

Non-clinical studies aiming at predicting immunogenicity in humans are normally not required.

However, ongoing consideration should be given to the use of emerging technologies (novel *in vivo, in vitro* and *in silico models),* which might be used as tools.

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Preclinical Strategies

In vivo strategies

- Animal studies exploring ADA response
- Transgenic animal studies exploring T-cell responses
- Tolerized animal models/humanized animals

In vitro strategies

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

In silico strategies

- T-cell epitope mapping tools

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Preclinical Immunogenicity screening

- In vivo strategies
  - Animal studies exploring ADA response
  - Animal studies exploring T-cell responses
  - Tolerized animal models
- In vitro strategies
  - T-cell epitope binding assays
  - T-cell activation and proliferation assays
- In silico strategies
  - T-cell epitope mapping tools

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In-vivo methods

- Increasingly used to study immunogenicity
  - as predictive tools to assess immunogenicity
  - in studying the mechanisms underlying immunogenicity
- What needs to be predicted

Brinks et al, Pharm. Res (2011) 28:2379

  - neo-epitopes on modified proteins,
  - relative immunogenicity between products
  - breaking of tolerance,
  - immunogenicity in patients,
  - incidence of immunogenicity in patients restricted
  - clinical consequences of antibody development
- However, animal models needs critical evaluation.
  - species differences,
  - predictive value of such models is limited,
  - mechanistic studies can be

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In vivo Predictive methods

- Difficult to map observed immunogenicity in animal studies to results in human
- Murine and primate models different from humans
- HLA transgenics
- Evolution towards using mouse models through the grafting of hematopoietic stem cells in immunodeficient mice.
  Models in Rag2-/- γc-/- and NOD/SCID/IL2r γc-/- mice demonstrated to develop of human DC, B- and T-cells.
- Breaking-tolerance: humanized and/or transgenic mice.

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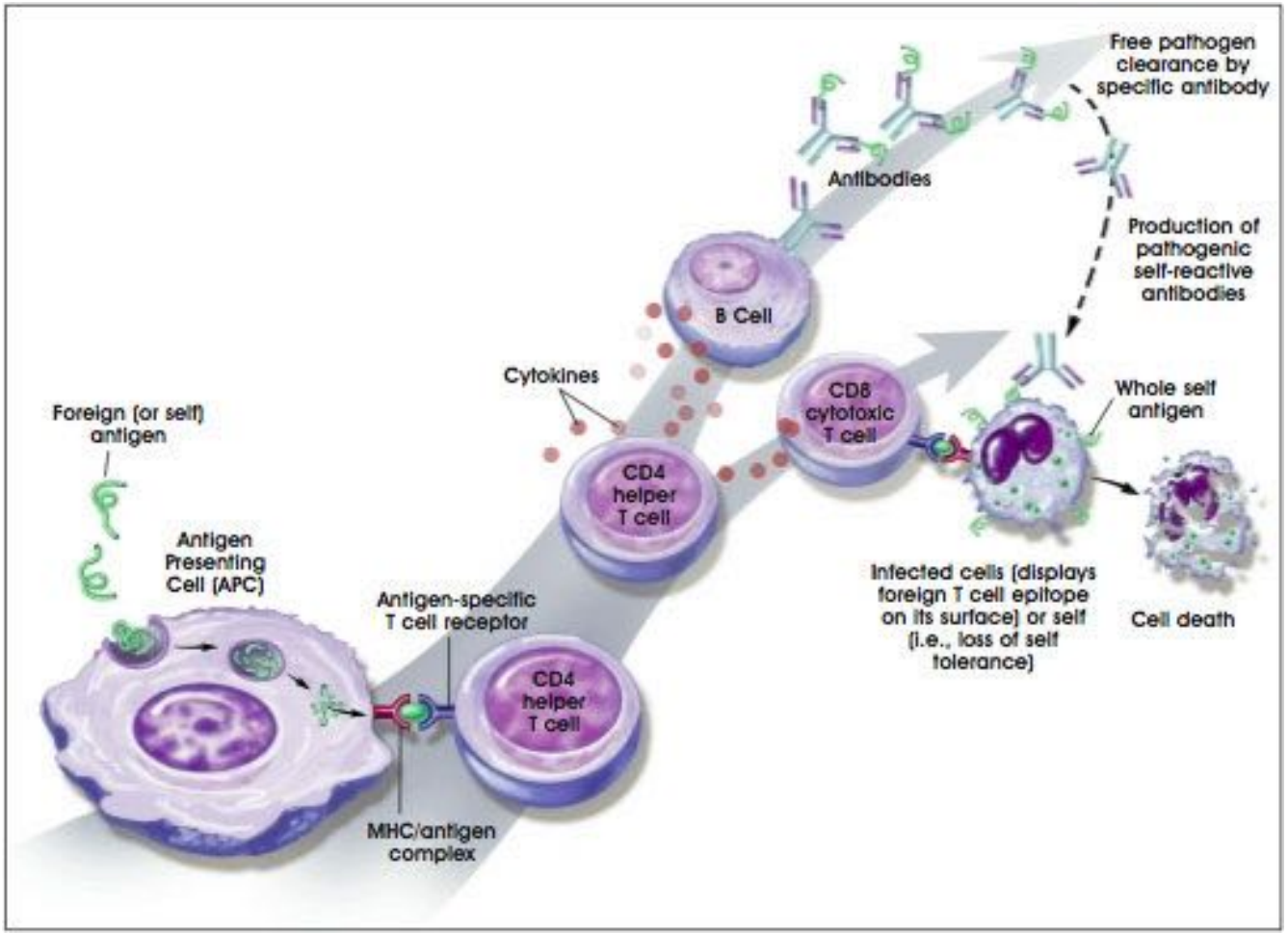
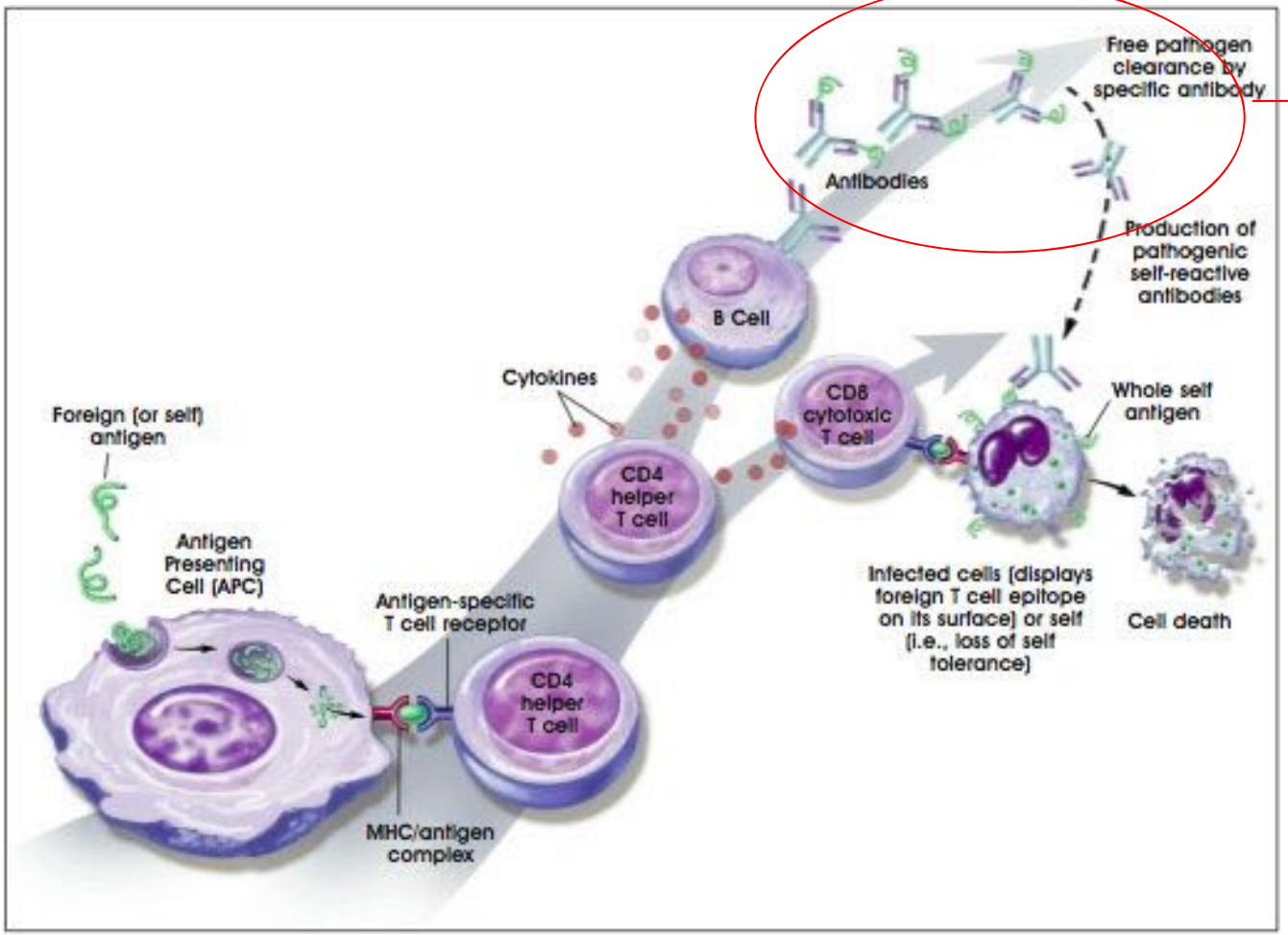
Preclinical Immunoprofiling

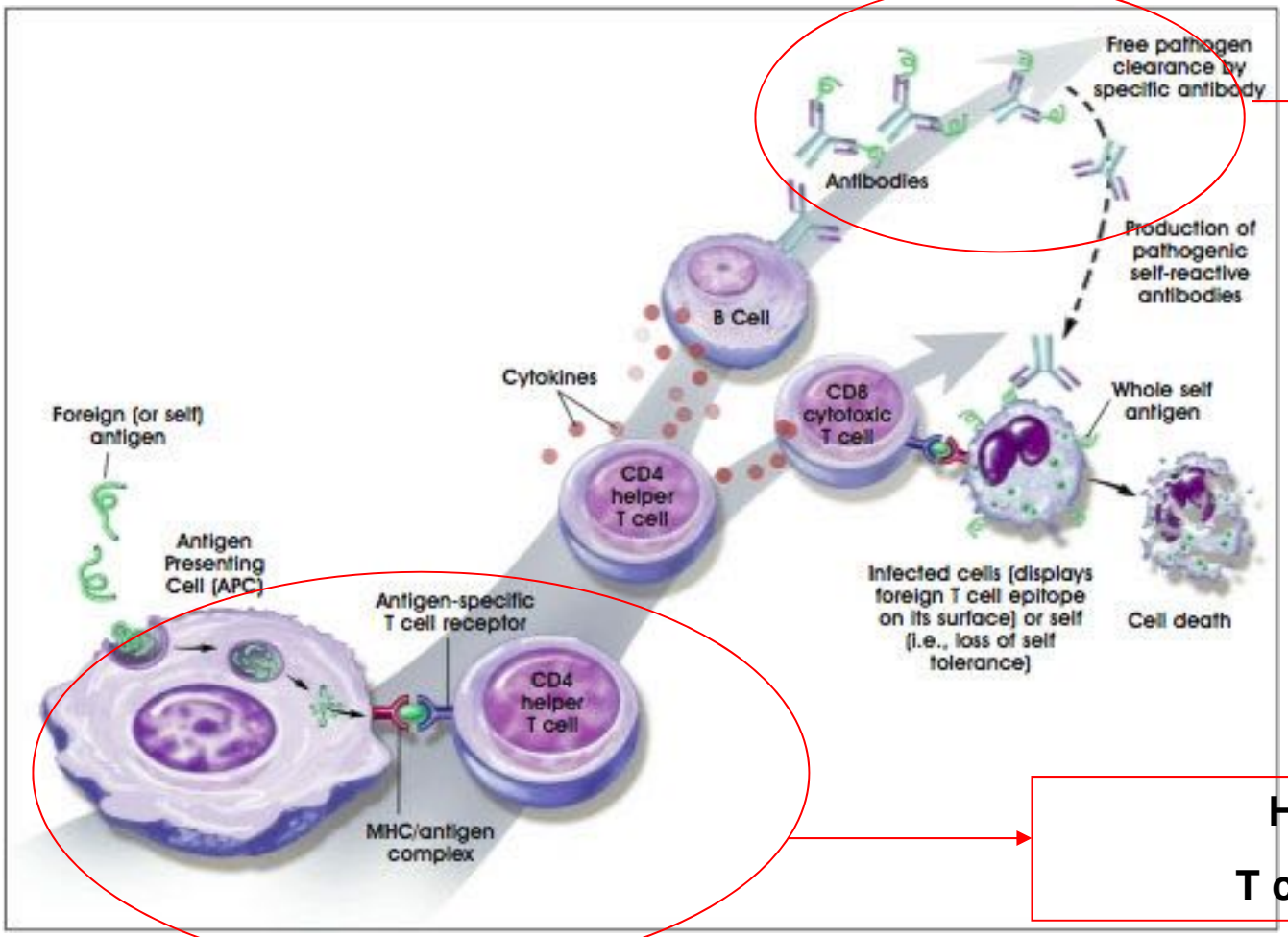


Preclinical Immunoprofiling



Clinical trials: ADA responses

Preclinical Immunoprofiling

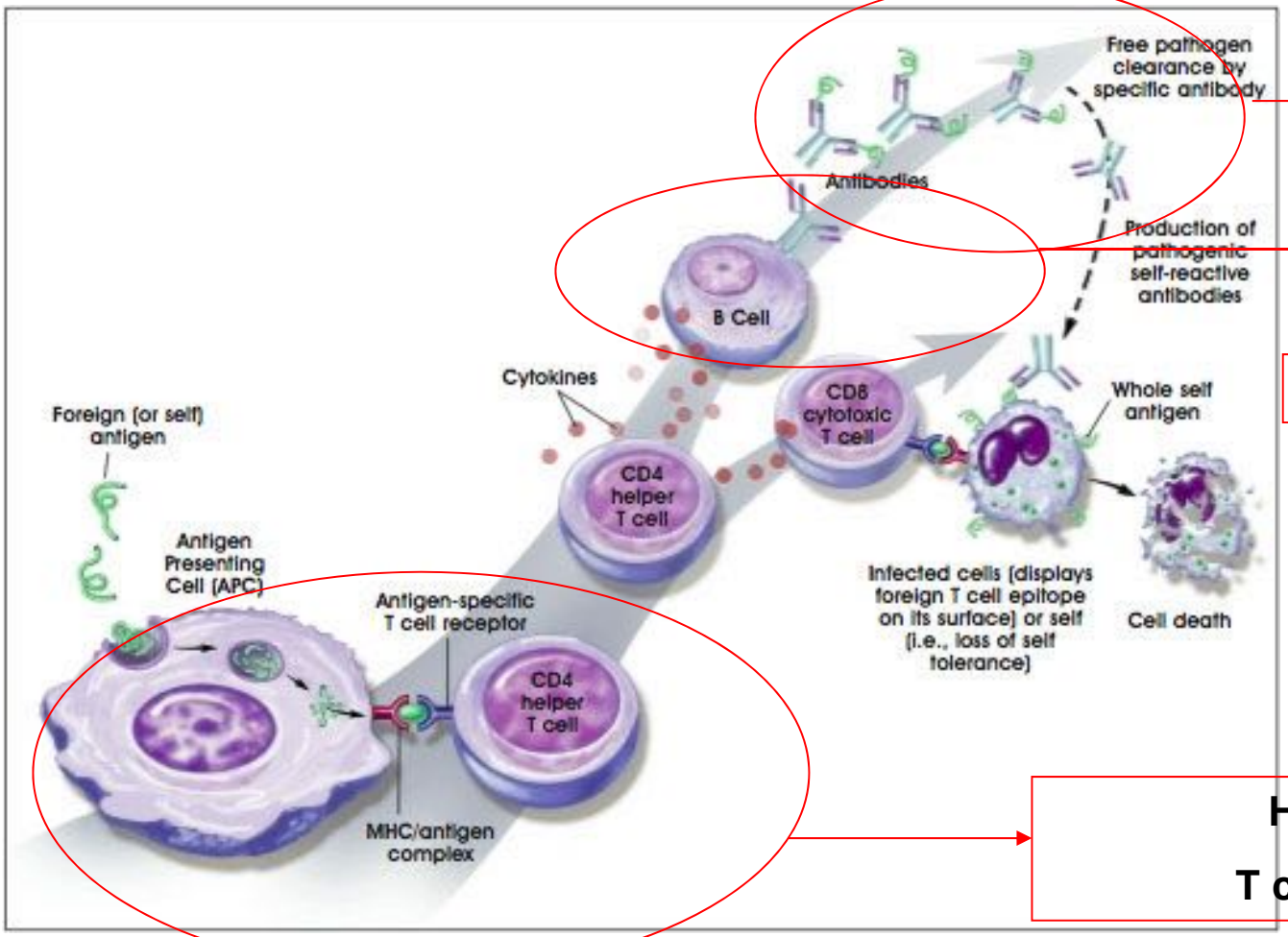


Clinical trials: ADA responses

HLA binding

T cell activation

Preclinical Immunoprofiling



Clinical trials: ADA responses

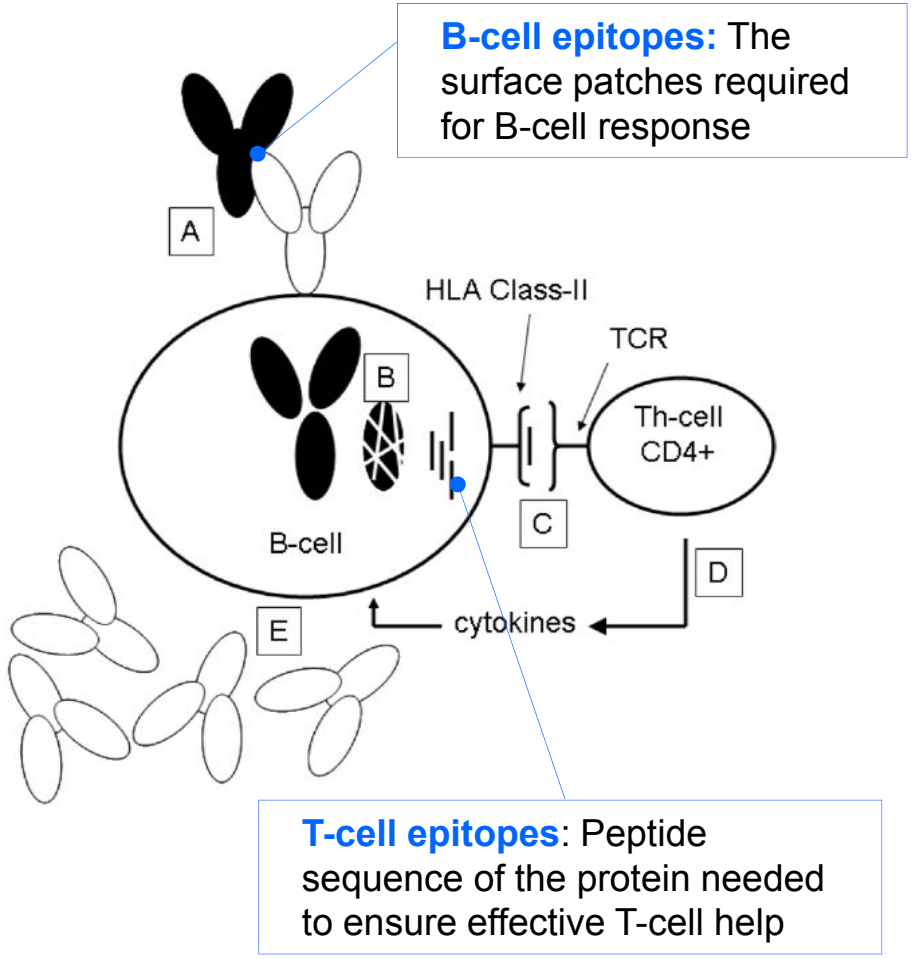
B cell analysis

HLA binding

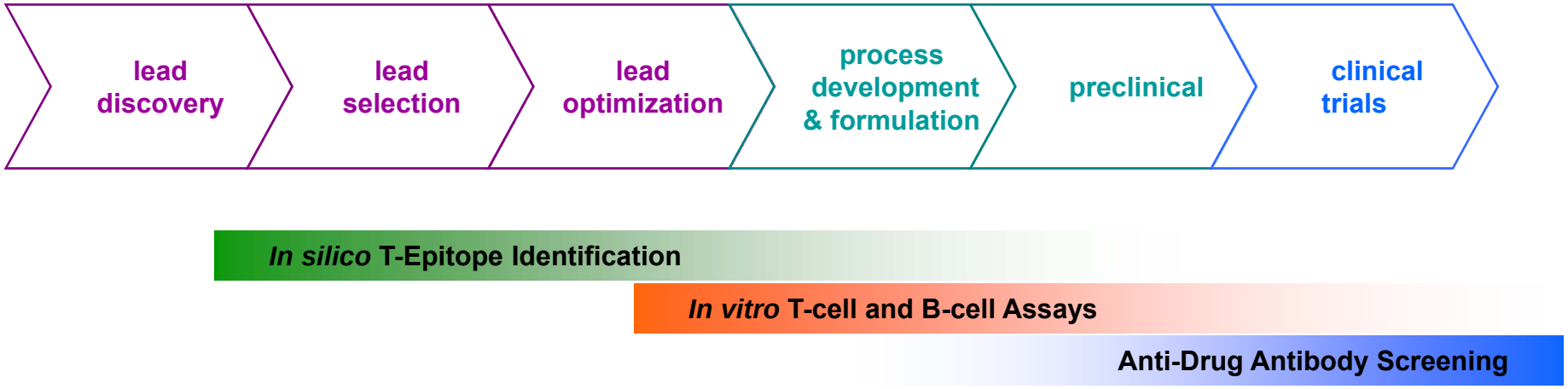
T cell activation

Predict and Reduce Immunogenicity?

- B cells need T-cell help to produce high affinity antibodies
- Eliminate T-helper epitopes will potentially reduce / diminish immunogenicity



In Silico Immunoprofiling



T-cell Epitope Prediction

- Lead ranking and selection
- Deimmunization in combination with the support of protein modelling

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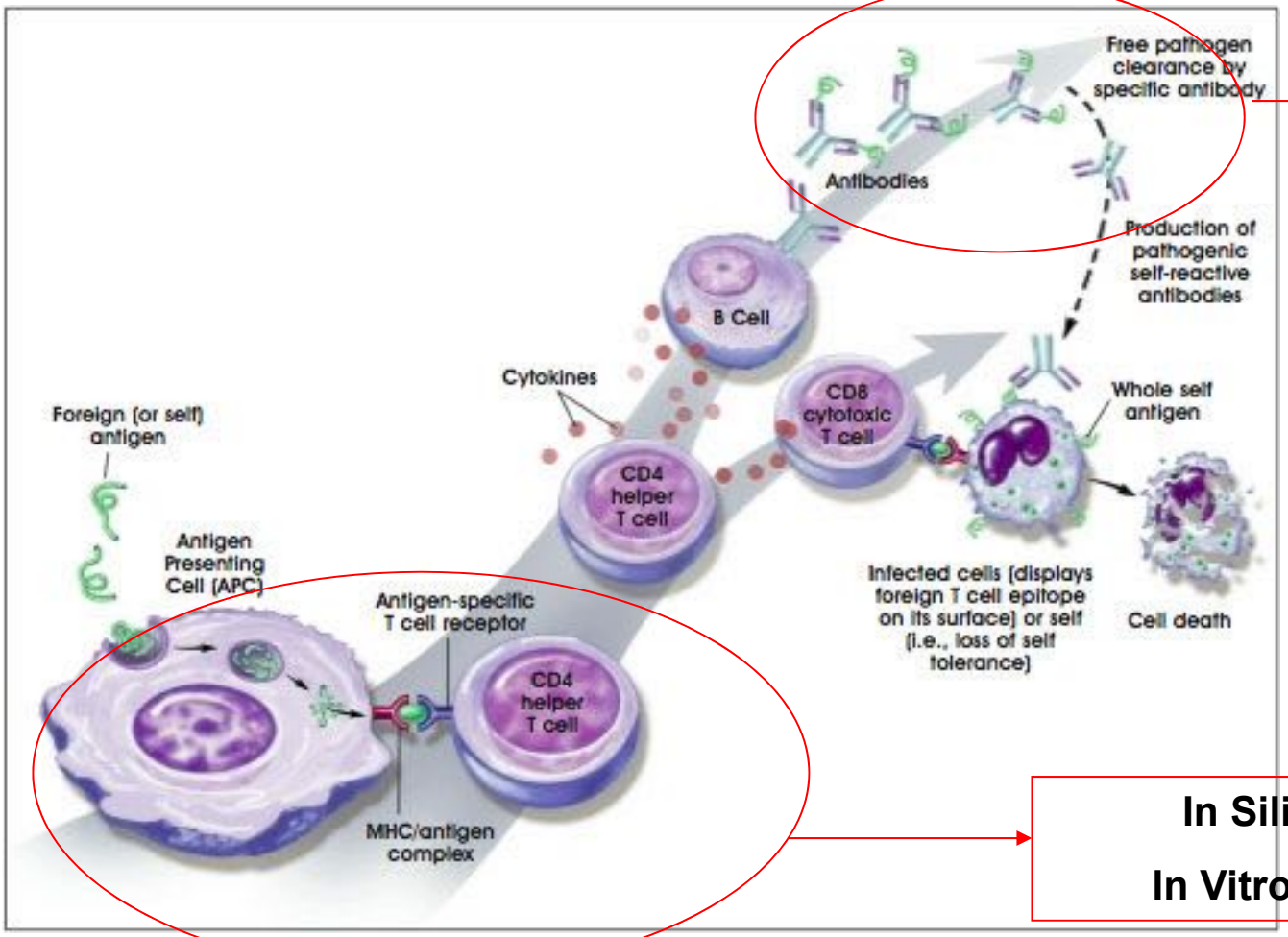
In Silico Immunoprofiling

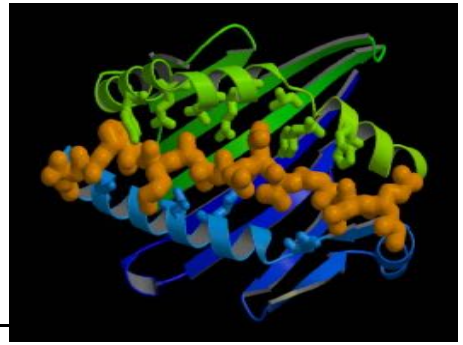


Clinical trials: ADA responses

In Silico:HLA binding

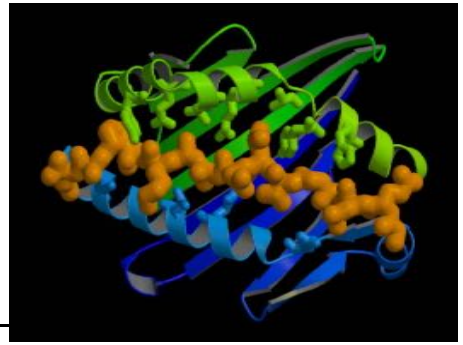
In Vitro :T cell activation

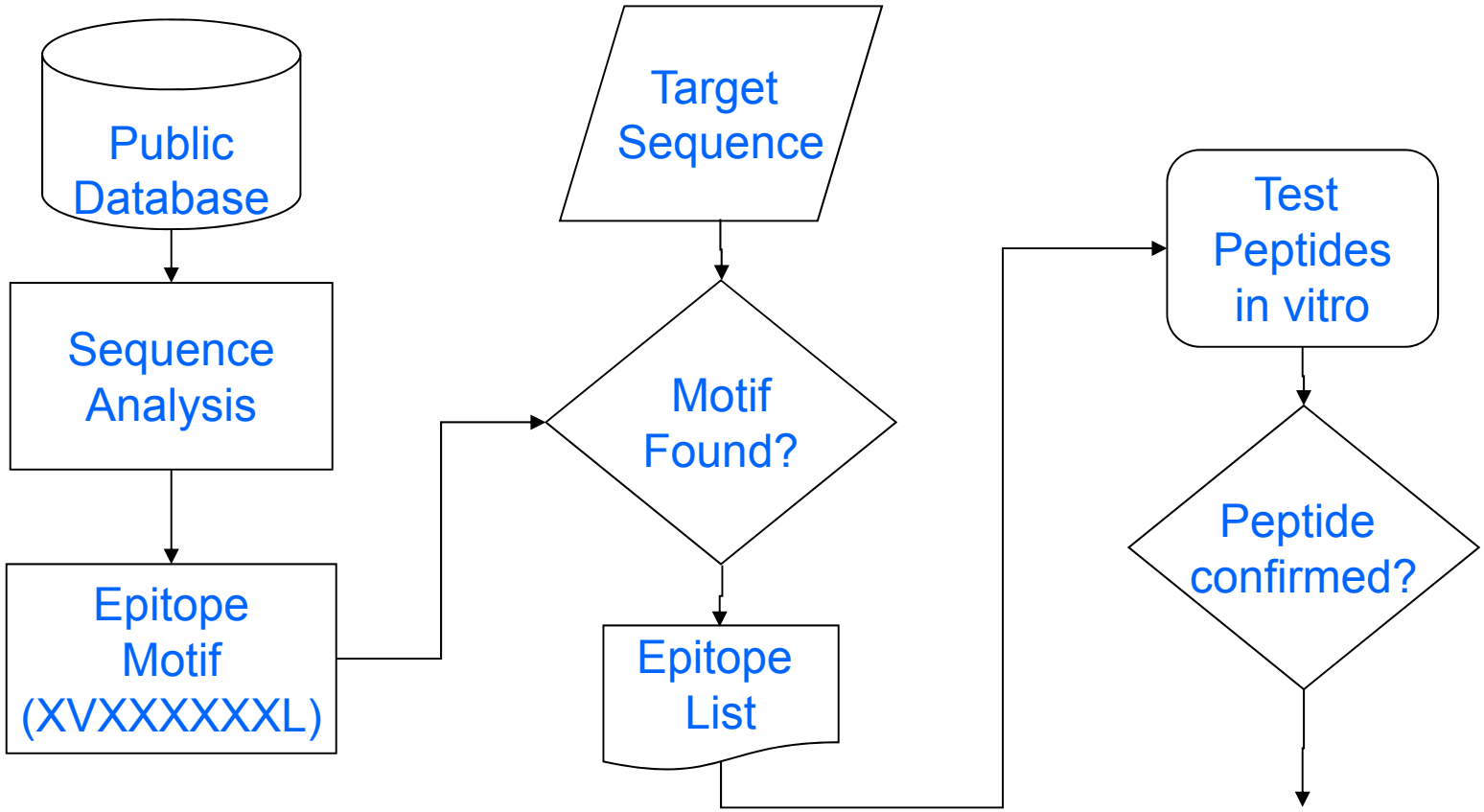
T-cell epitope identification



- *In silico* methods
  - Low cost
  - High througput
- Previous generation methods:
  - methods by inference
  - Sequence based methods
    - Based on known epitopes and sequences comparison
    - Use of different kinds of learning based algorithms
    - Bias towards known “peptide motives” and “anchor residues”
- Inference based methods tend to become better as more experimental data exists and fail on less studied HLA subtypes

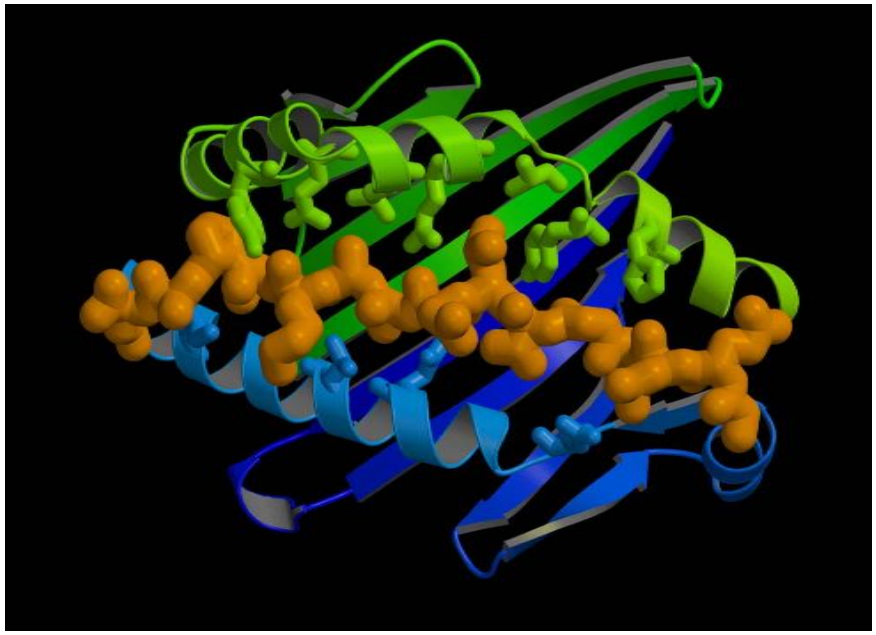
Previous Generation tools





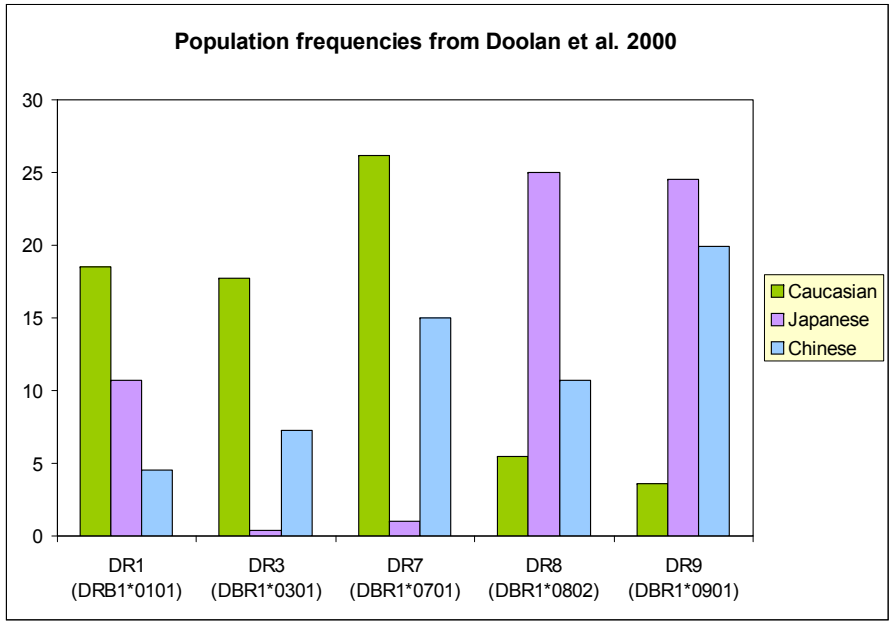
Epibase™ for epitope prediction

- Analyze the antibody sequence
- Explore whether 10-mer peptides can bind to the MHC receptor
- Predictive tool driven by structural bioinformatics in conjunction with experimental data



- Usage:
  - Project Basis: compare a limited set of lead candidates in a program to explore which drug to proceed.
  - Compute server: to screen libraries or high volume selection techniques

Epibase™ and MHCII Population Frequencies



	Epibase™ Global	Epibase™ Caucasian	Epibase™ Asian	Epibase™ Hispanic	Epibase™ Afro-Am.
DR	45	27	29	26	24
DQ	23	14	14	N/A	N/A
DP	10	7	8	N/A	N/A

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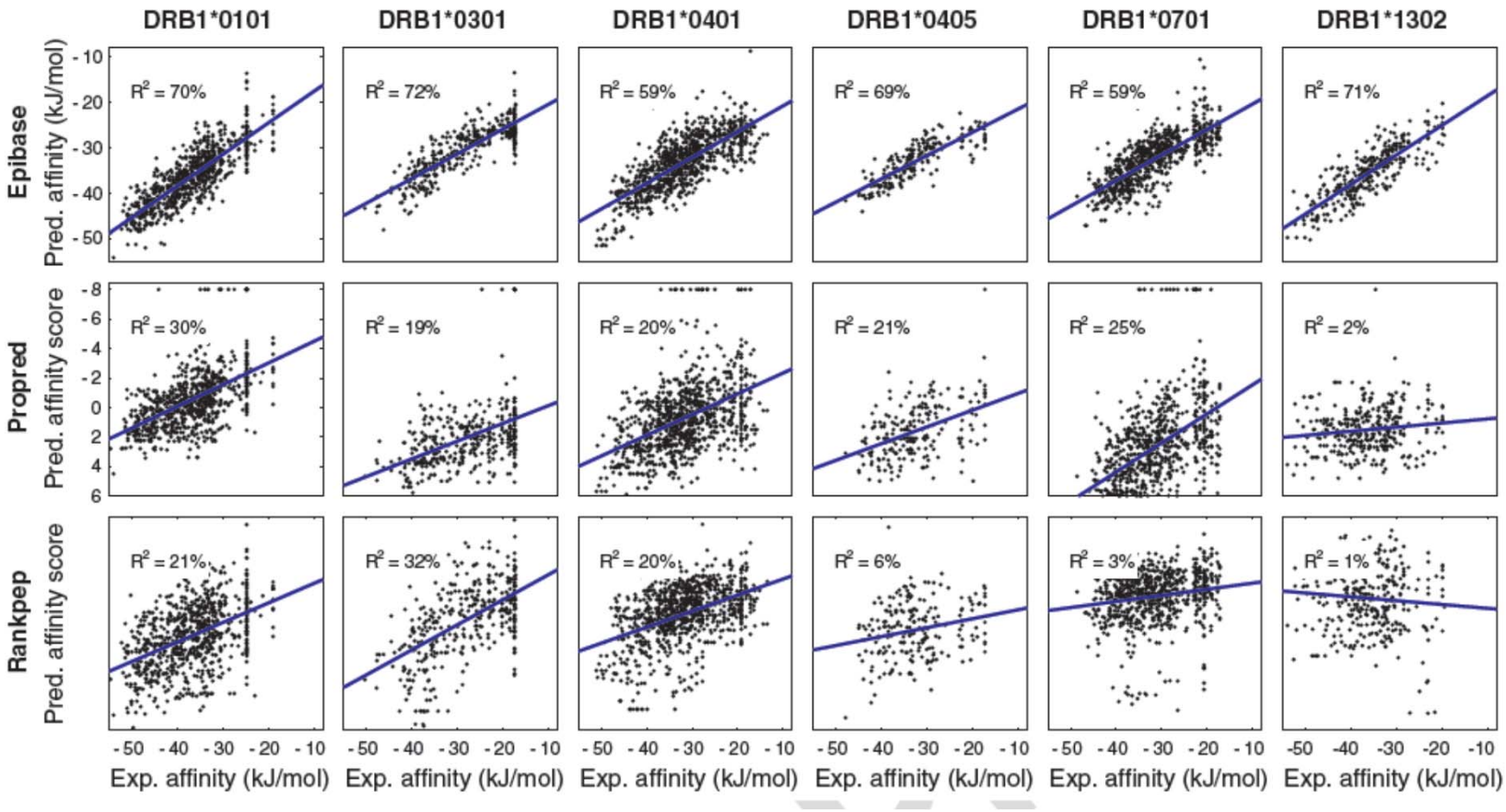

DRB1*0101
DRB1*0301
DRB1*0401
DRB1*0405
DRB1*0701
DRB1*1302
Epibase
Propred
Rankpep
Pred. affinity (kJ/mol)
Pred. affinity score
Pred. affinity score
R² = 70%
R² = 72%
R² = 59%
R² = 69%
R² = 59%
R² = 71%
R² = 30%
R² = 19%
R² = 20%
R² = 21%
R² = 25%
R² = 2%
R² = 21%
R² = 32%
R² = 20%
R² = 6%
R² = 3%
R² = 1%
-10 -20 -30 -40 -50
-8 -6 -4 -2 0 2 4 6
-50 -40 -30 -20 -10
Exp. affinity (kJ/mol)

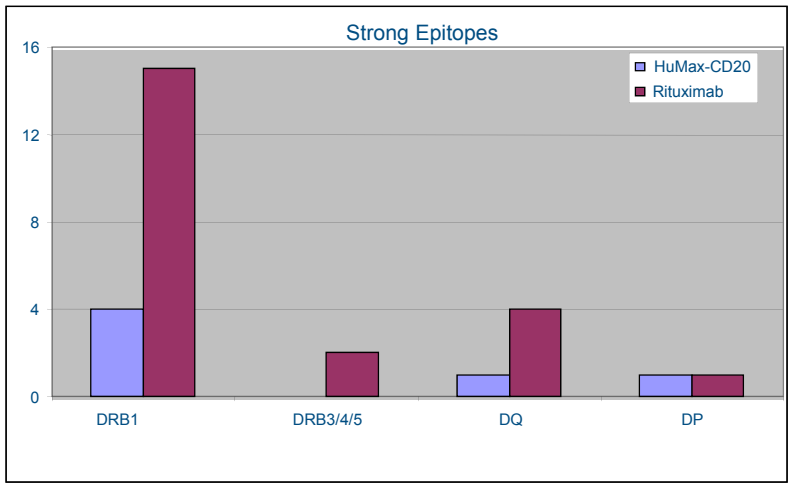
Case Study 1: Ofatumumab and Rituximab

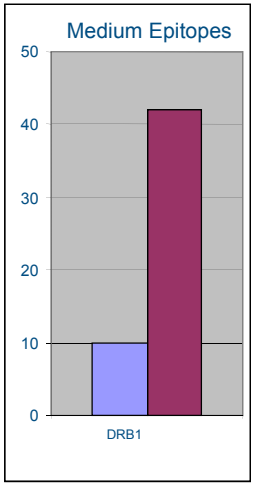
- Targeting CD20, a B-cell differentiation antigen
- Treatment of
  - Cancer: e.g. Follicular lymphoma.
  - Inflammatory disease: e.g. Rheumatoid arthritis, SLE
- Observed immunogenicity of Rituximab
  - <1% in B-CLL
  - 35-60% in SLE
  - 4.3-23% in RA
  - Chimeric antibody
- Ofatumumab
  - BLA in B-CLL
  - Phase III in RA
  - Fully human antibody

Immunoprofile: Ofatumumab and Rituximab

- Ofatumumab is very clean in epitopes as compared to rituximab

- Ofatumumab contains no epitopes for HLA allotypes associated with RA





HLA class II gene	RA Risk ratio	Epitopes in rituximab	Epitopes in ofatumumab
DRB1*0401	1 in 35	2 strong	no
DRB1*0404	1 in 20	no	no
DRB1*0101	1 in 80	4 strong	no
0401 and 0404	1 in 7	2 strong	no

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Case Study 2: Adalimumab

- Human antibody recognizing TNF-α isolated by phage-display technology
- 109 RA patients enrolled for the study (collaboration with Sanquin and Genmab)
  - Patients were tested for:
    - HAHA response (low, high)
    - determined from the binding of the Humira Fab fragment to protein A absorbed patient IgG
    - DQ, DR high resolution typing
    - no DP typing was done as no strong epitopes were identified by Epibase®

Immunoprofiling of Adalimumab

- Epibase profiling
  - Epitope identification on full sequence
  - Removal of epitopes present in the human germline
  - Critical epitopes are identified as the strong and medium binders to DRB1, and the strong binders to DRB3/4/5, DQ and DP

- 7 strong epitopes found
  - 5 strong epitopes in the VH
    - 2 in the FwR2-HCDR2 region
    - 3 in the FwR3-HCDR3 region
  - 2 strong epitopes in the VL:
    - LCDR1 and FwR3-LCDR3

Patient Data

- Level of HAHA response
  - 19 patients show a HAHA response, i.e. 17.6% of the patients are HAHA +

- RA associated HLA allotypes:

Allotype	Caucasian	RA group
DRB1*0101	17.2%	28.4%
DRB1*0401	9.8%	52.3%
DRB1*0404	5.9%	9.2%

Epitopes and HAHA response

- The 7 strong epitopes explain 17/19 HAHA+ patients
- Epitopes are directed against the RA associated allotypes

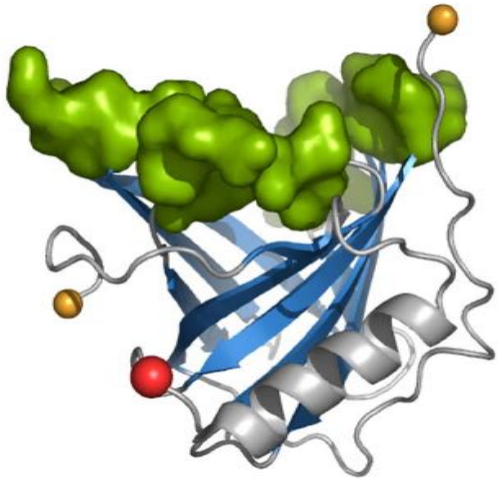
Epitopes	Region	HLA allotypes	HAHA+ patients
1	FwR2-HCDR2	DRB1*0701	1
2	FwR2-HCDR2	DQA1*0201|DQB1*0303	1
		DQA1*0401|DQB1*0402	
		DQA1*0501|DQB1*0301	3
		DRB1*0101	4
		DRB1*0401	7
		DRB1*0405	1
		DRB1*0407	
		DRB1*0901	1
3	FwR3-HCDR3	DRB5*0101	5
4	FwR3-HCDR3	DRB1*0407	
5	FwR3-HCDR3	DRB1*0801	
6	LCDR1	DQA1*0501|DQB1*0201	3
7	FwR3-LCDR3	DRB5*0101	5

Case study 3: Anticalin®

- Anticalins® are engineered human proteins with prescribed binding properties derived from the lipocalin fold

- Lipocalins
  - Highly-conserved family of structural proteins
  - Optimized by evolution to perform diverse binding and physiological functions
  - Function in human tissues and body fluids in the presence of the human immune system
  - Low molecular weight, non-glycosylated, monomeric human proteins

- Pieris AG has pioneered the design of Anticalins® from lipocalins by advanced protein engineering



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Potential immunogenicity of anticalins

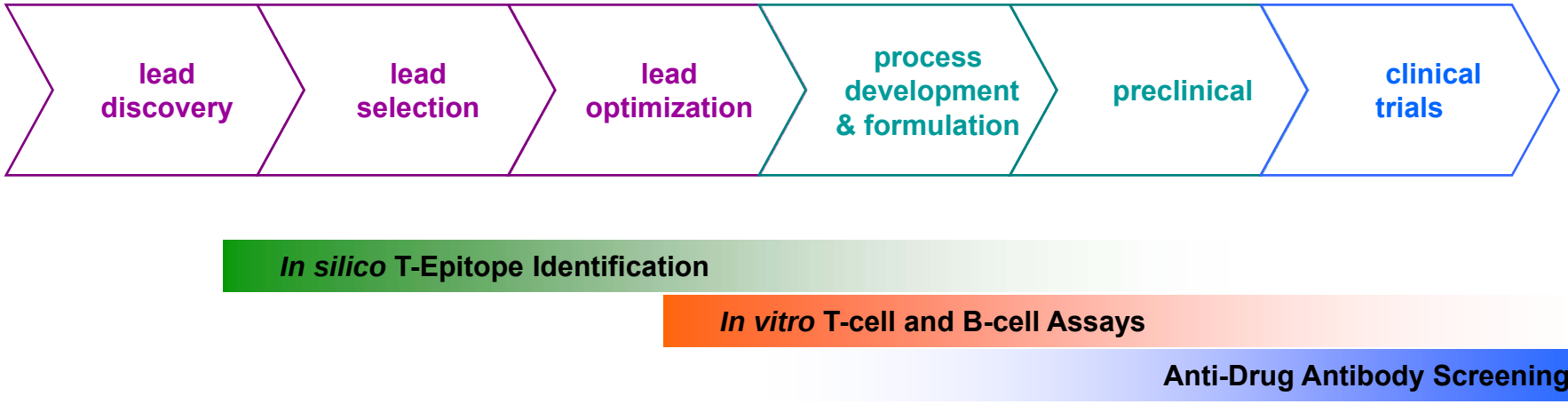
- Epibase® profiles were generated for a human lipocalin and four derived, target-specific Anticalin® lead candidates

Results:

- Number of mapped epitopes in lipocalin and Anticalins® ery limited

	DRB1		DRB 3/4/5	DP	DQ
	Strong	medium	strong	strong	Strong
lipocalin	5	26	0	2	4
Anticalin® 1	7	33	2	2	2
Anticalin® 2	6	31	2	2	2
Anticalin® 3	6	37	2	2	2
Anticalin® 4	9	34	2	2	3
Anticalin® 5	4	34	2	0	2

In Vitro Immunoprofiling



An Indication of External Factors on Drug Immunogenicity

- Formulation
- Aggregates
- Degradation products
- Production contaminants
- Biosimilar / Innovator comparisons

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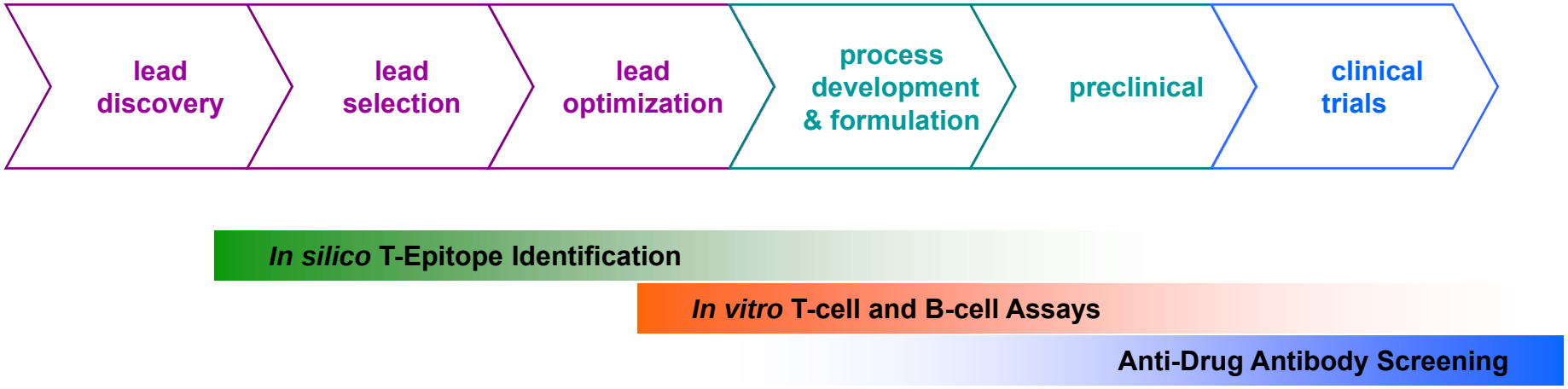
In Vitro Immunoprofiling



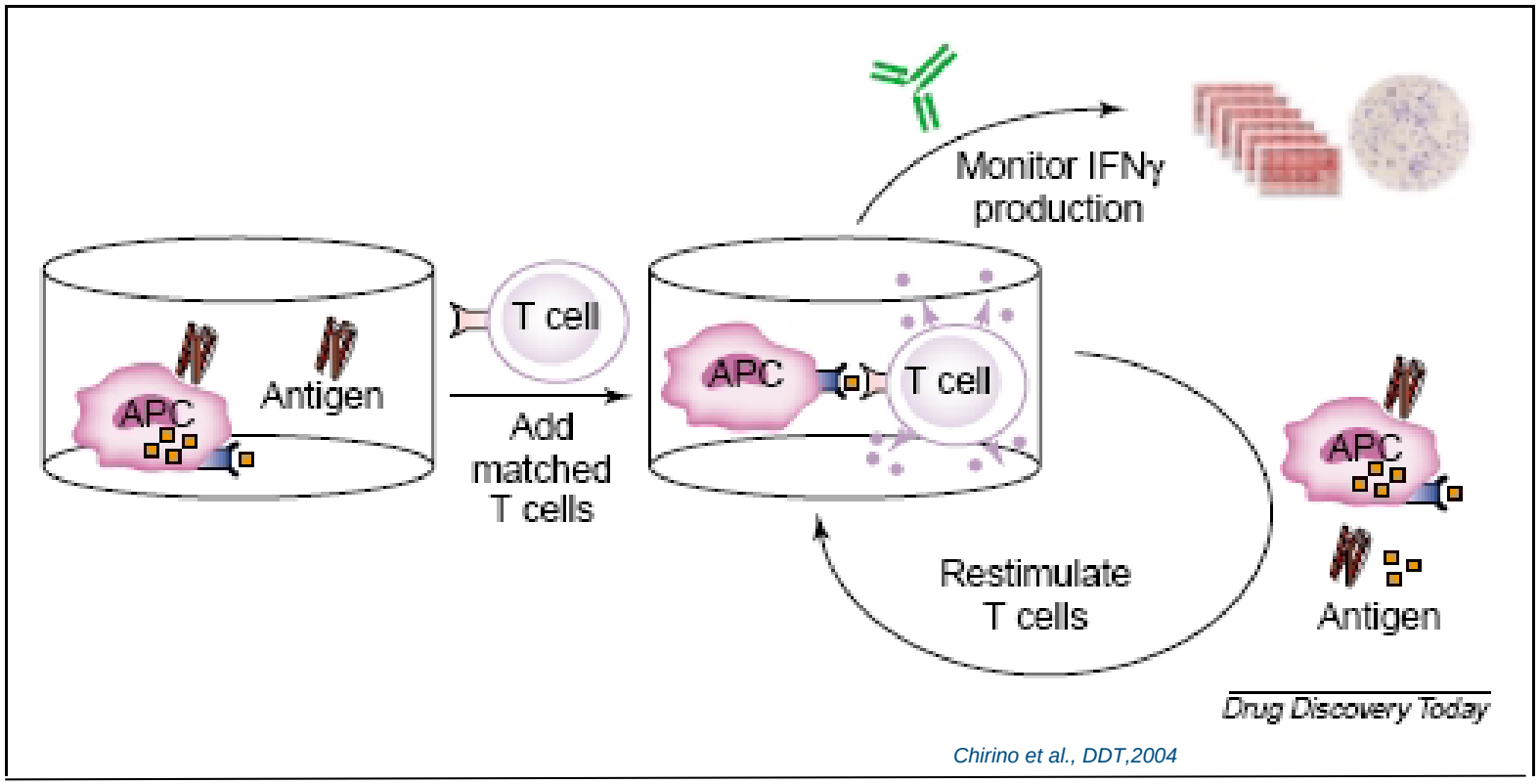
Characterize T cell epitopes guided by in silico

- At individual donor and population level

Comparison of immunogenicity between biosimilar/second generation products and reference products

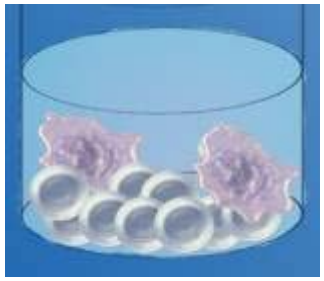
- Healthy population/Patient population

Cellular Immunoprofiling

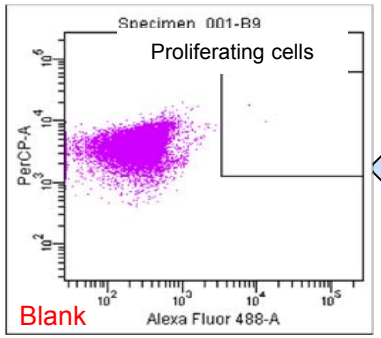


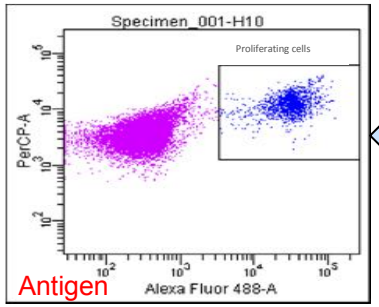
Chirino et al., DDT,2004

Identify T-Cell Responses: Overview Read Out Parameters



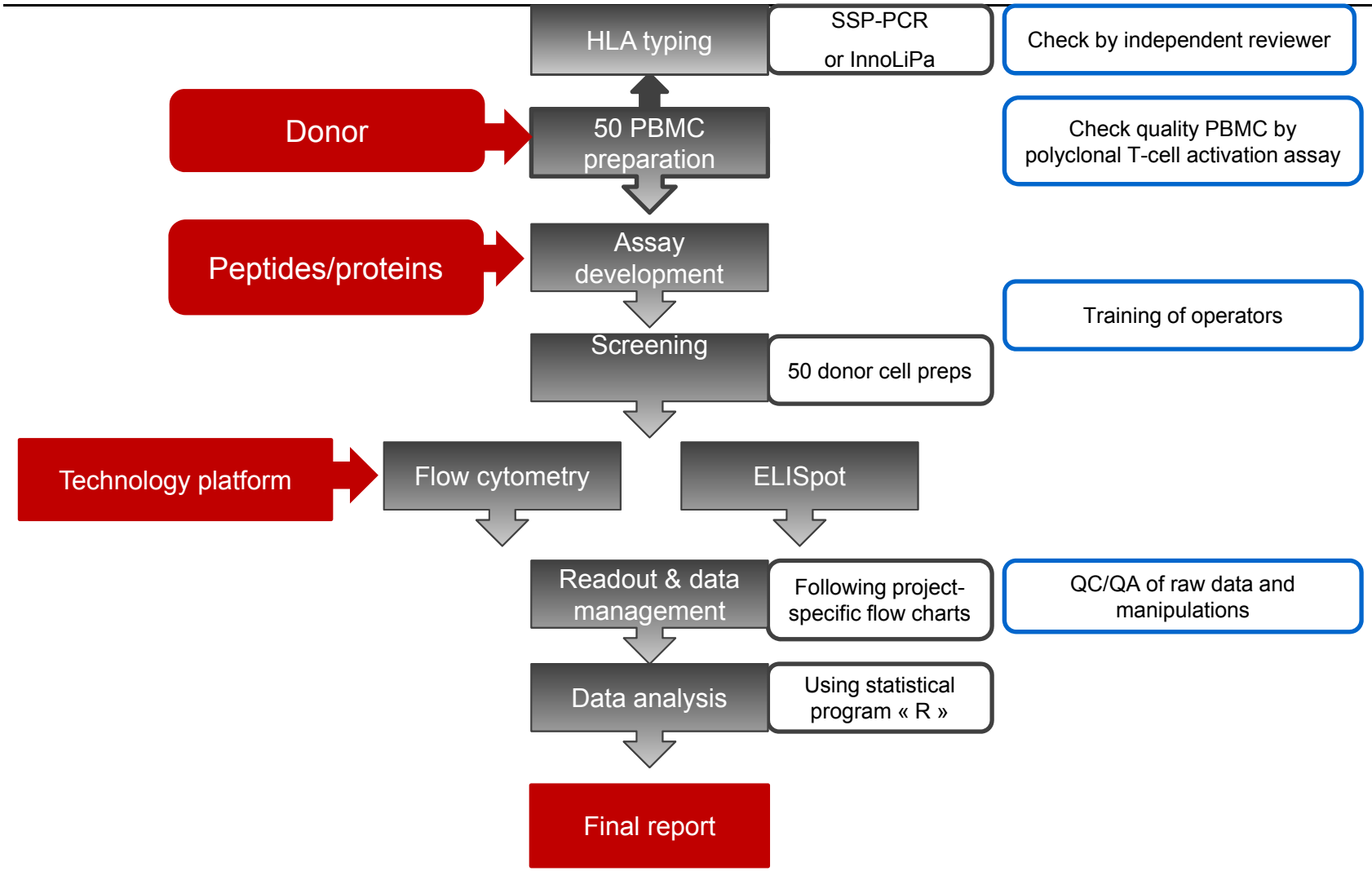
CD4/CD8 T-cell ELISPOT



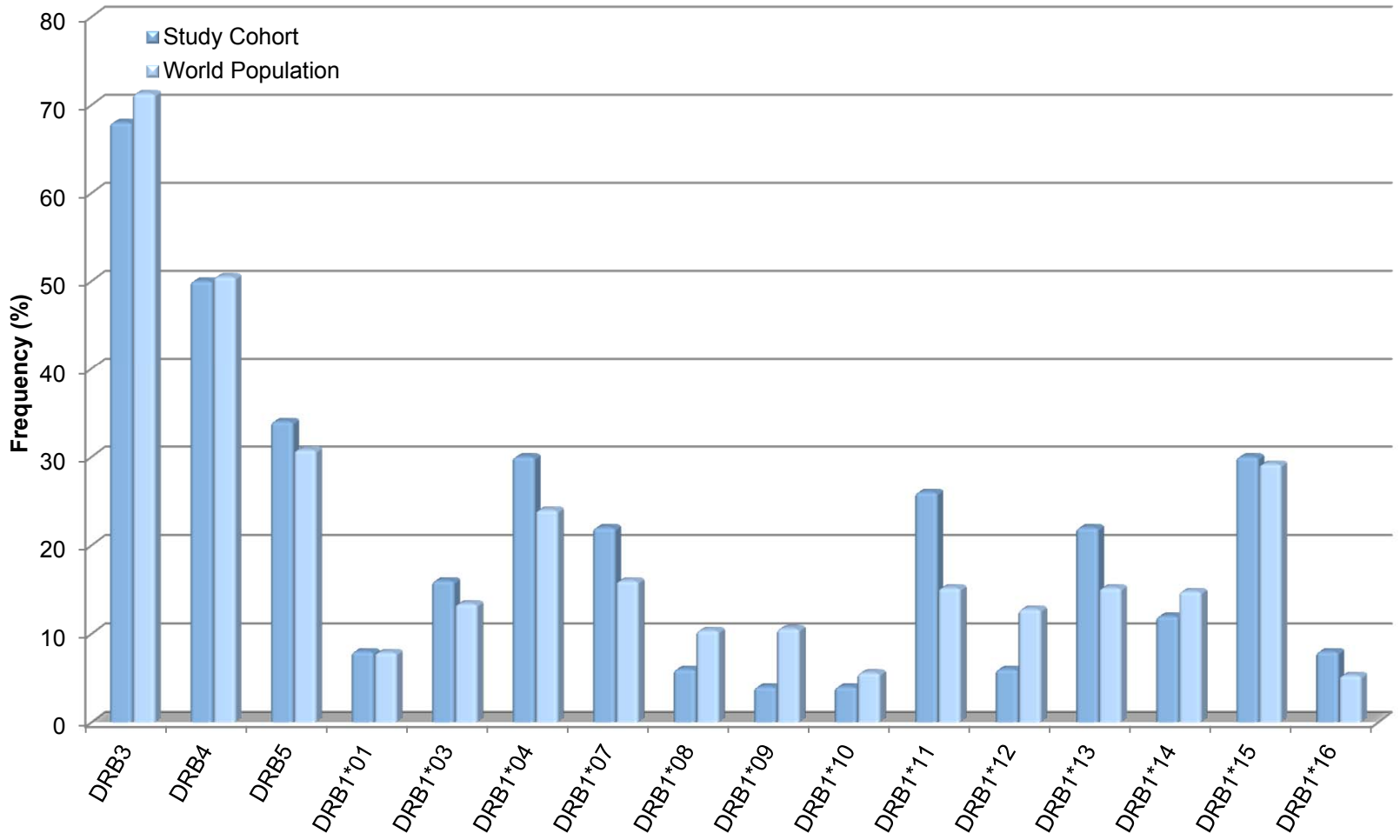


- Cell surface markers (CD3/CD4)
- **Proliferation (EDU)**
- Cytokine analysis
  - Intracellular cytokine staining
  - CBA
  - ELISA
- Antigen-specific T cells
  - HLA class I tetramers
  - HLA class II tetramers

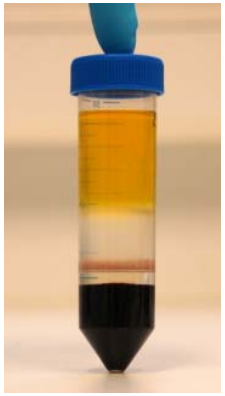
Screening Strategy



Donor Population



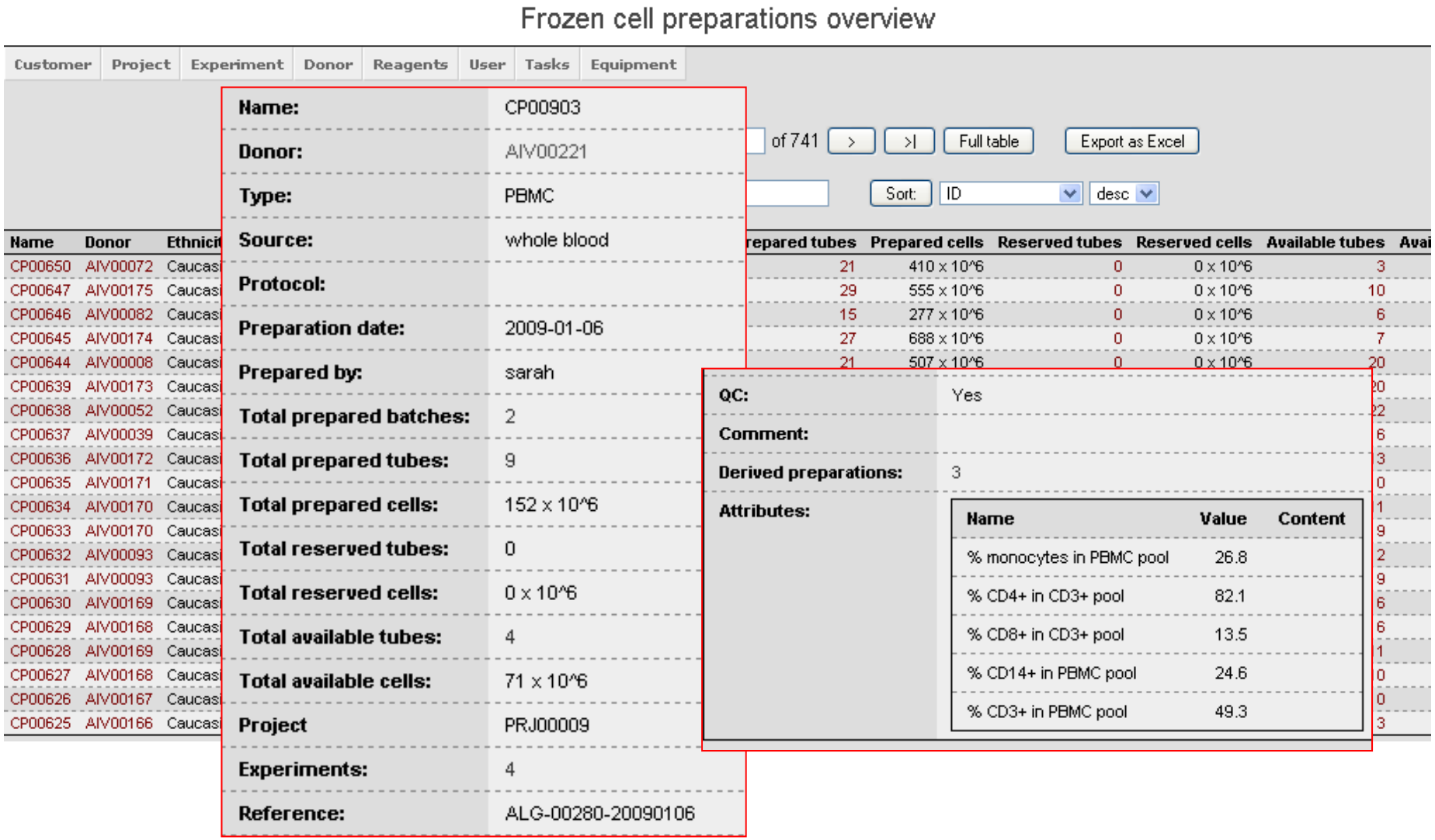
PBMC Quality Control



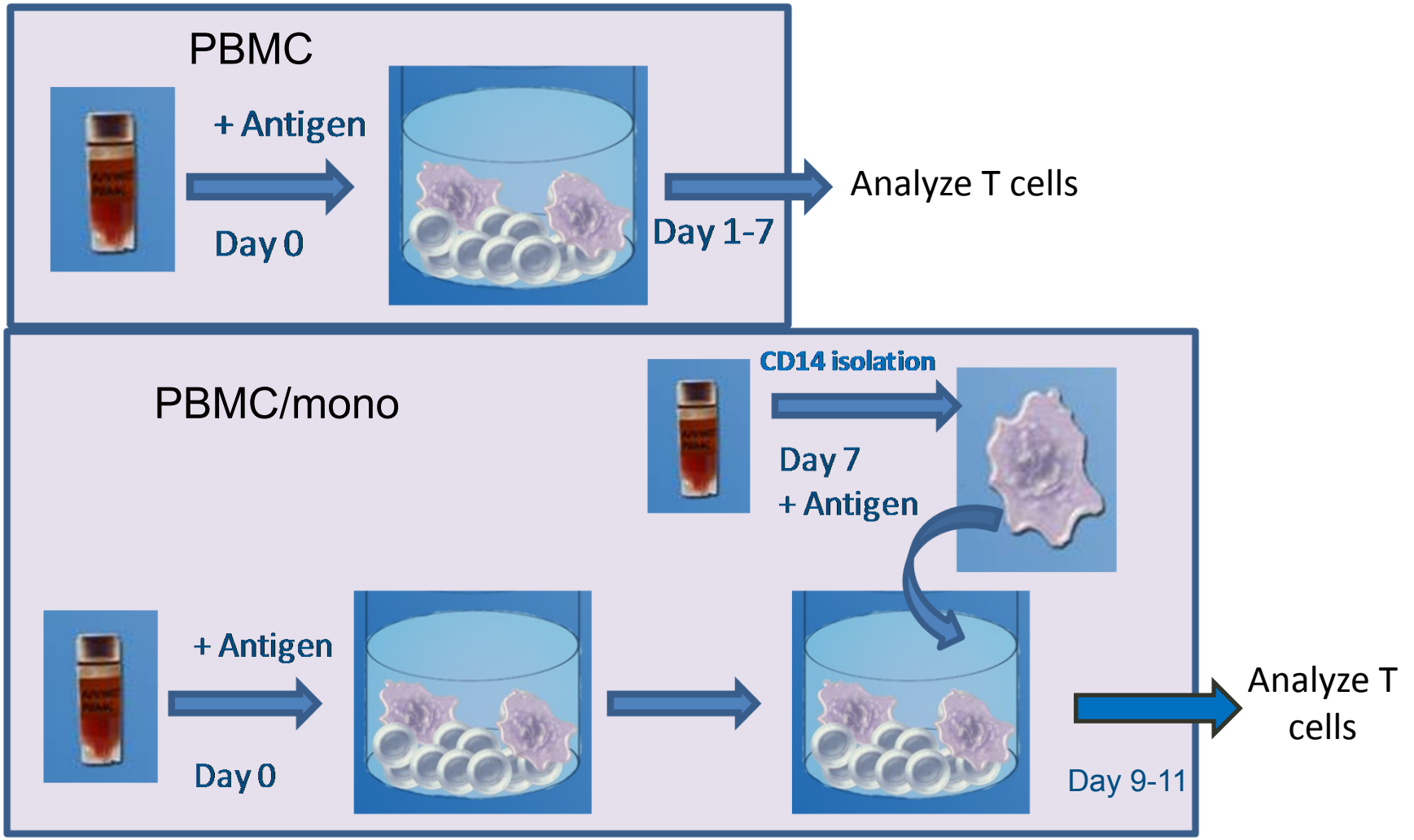
Blood collection → PBMC isolation → Counting → Freezing → Liquid nitrogen storage

- PBMC isolation: within 3 hours after collection
- Counting: Automated cell counting by Guava
- Freezing: Automated cell freezing by Nicool+
- Liquid nitrogen storage: Sample storage management in LIMS

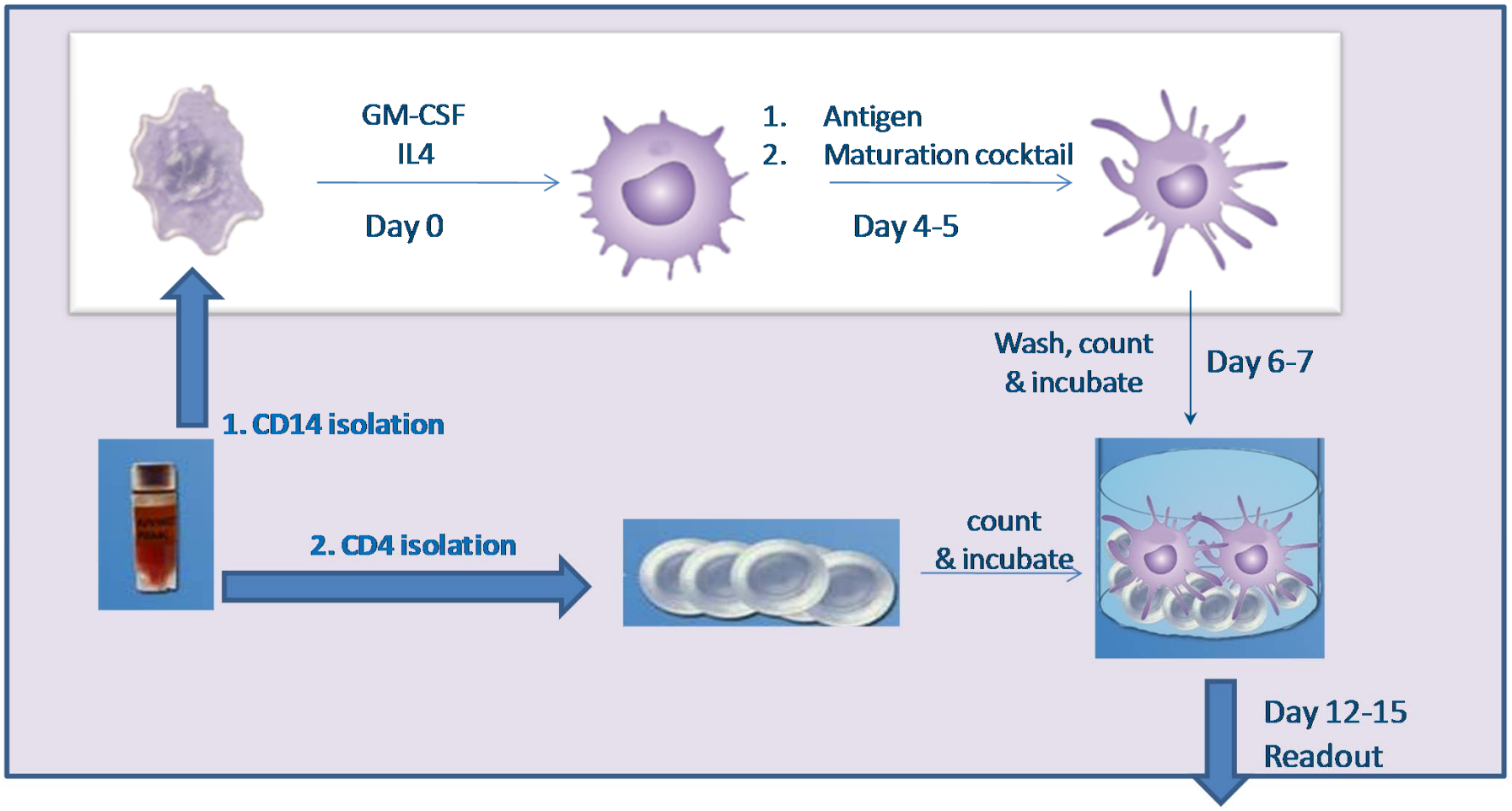
Epibase IV : PBMC Data Recording



T Cell Assays: whole PBMC formats



T Cell Assays: DC/CD4+ format

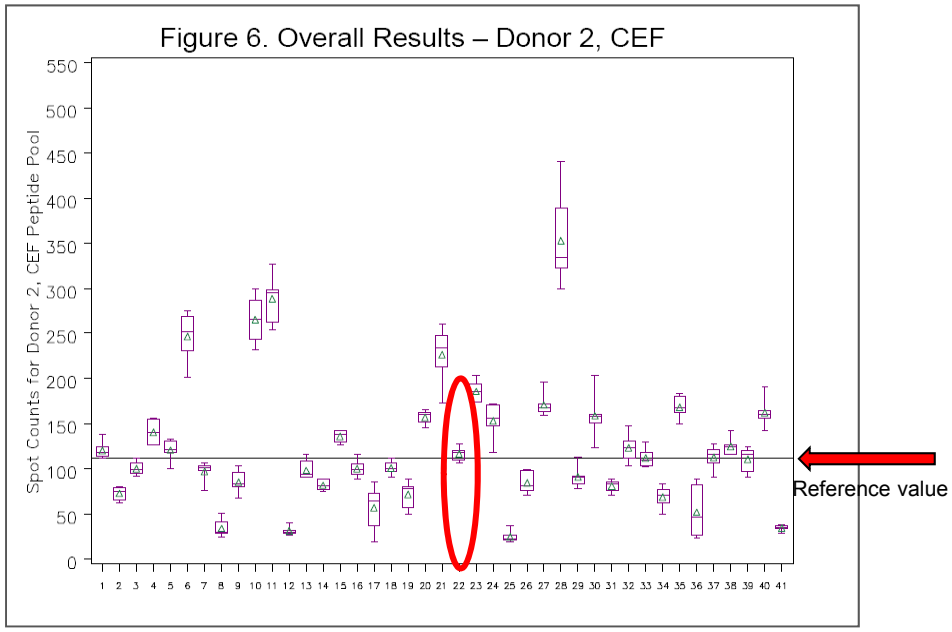


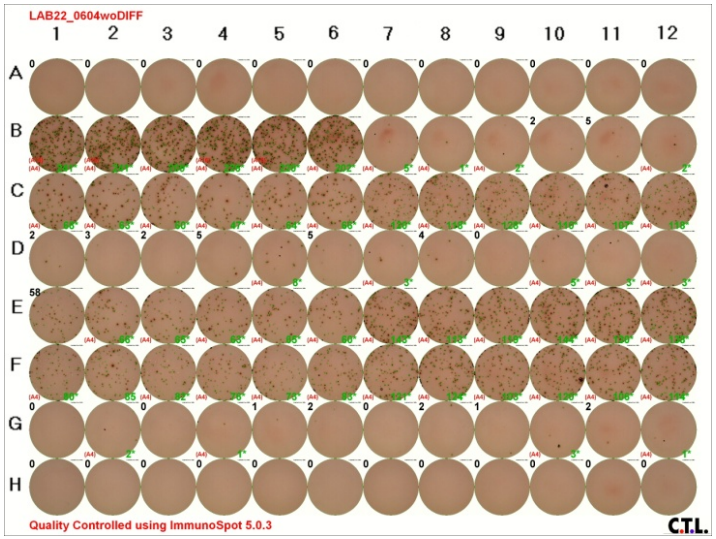
Analyze T cells

Analyze T Cells : ensure quality

PARTICIPATE IN PROFICIENCY PANELS

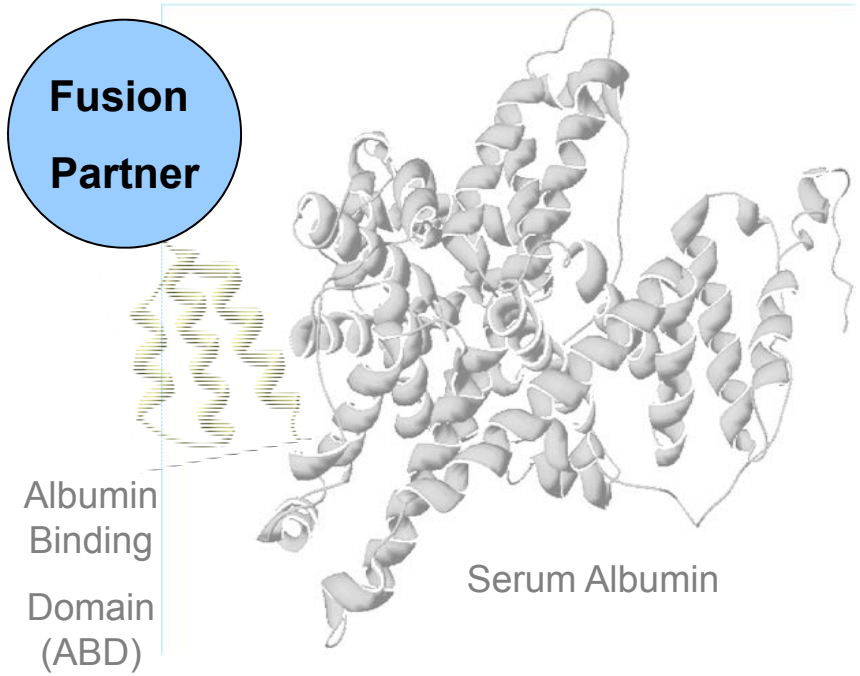
ELISPOT PROFICIENCY PANEL IV CVC-2009, REPORT FOR LAB 22, DATE: September 20, 2009





Case Study : Background

Half-life extension by Albumin binding



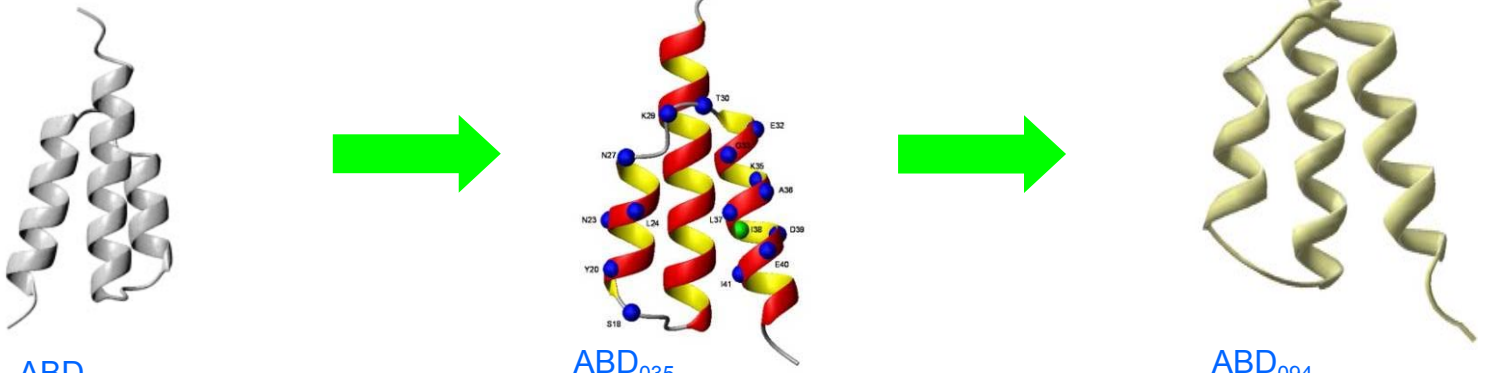
Serum albumin an ideal carrier

- Long half-life (17-19 days)
- Favorable dosing
- Convenience
- Safety
- Wide distribution
- Present in plasma (40%) and tissues (60%)

- Albumin Binding Domain (ABD)
  - high affinity for HSA
  - Small size (5 kDa)

Case Study : objective

- Originally naturally occurring bacterial protein: ABD_{wt} with known T-cell epitope (Goetsch 2003)
  - Affinity maturation to femtomolar affinity (Jonsson 2008)
    - Structure analysis and modelling of ABD variants
- Protein engineering for stability, expression yield and reduced immunogenicity
  - Validation by Algonomics/Lonza
    - T-cell epitope mapping in silico
    - T-cell proliferation assay of selected deimmunized mutants



ABD_{wt} Bacterial Johansson02

ABD_{035} Affinity matured Jonsson08

ABD_{094} Deimmunized unpublished

Epibase™ *In Silico* Profile of ABD and variants

- **Epibase™ screening**
  - In silico T cell epitope mapping and ranking of 131 variants, selected based on their stability, affinity and predicted antigenicity / immunogenicity
  - Rational selection of best candidate for in vitro testing

Epibase™ *In vitro* Testing of ABD and variants

Compare the immunogenic potential of wild type ABD and variants based on:

- number of responsive donors
- mean SI over the population
- Relative response

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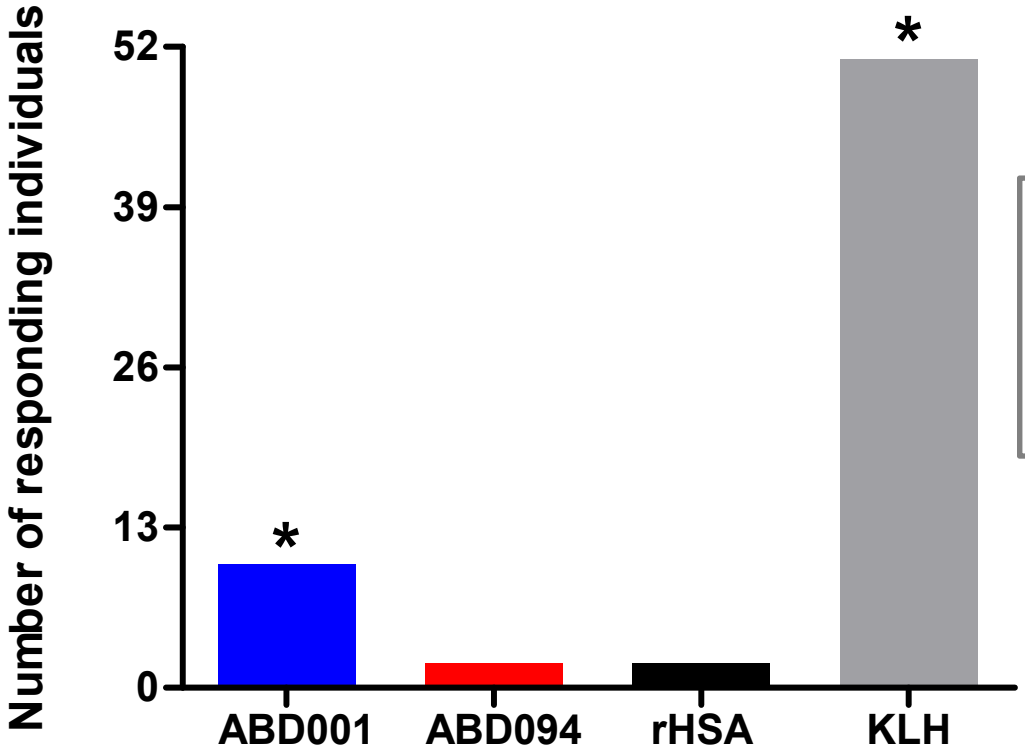
Epibase™ *In vitro* Testing of ABD and variants: results



No significant immunogenicity was detected with ABD094

** Significantly different compared to buffer alone.*

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Conclusion

- In contrast to wildtype ABD, no significant immunogenicity was detected with ABD094
- Combined *in silico* and *in vitro* approach used for testing of mutants allowed for discrimination of molecules differing in only one amino acid
- In silico mapping provides a cost effective and rapid solution to further reducing or avoiding potential immunogenicity risk of therapeutic proteins

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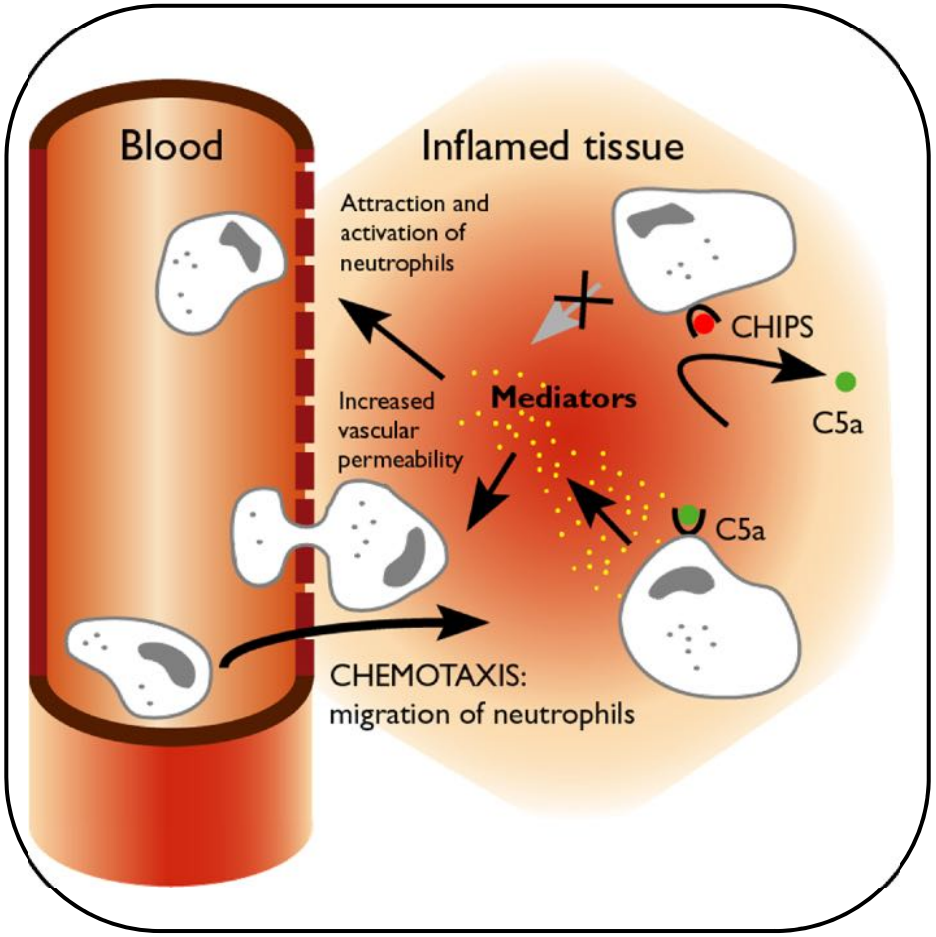
Case study: CHemotaxis Inhibitory Protein



- 14 kDa protein
- High affinity for the C5aR (K_D ~1 nM)
- Blocks the binding of C5a to the C5aR
- Potent inhibitor of C5a-induced activation of phagocytes *in vitro*
- Promising drug candidate for acute indications

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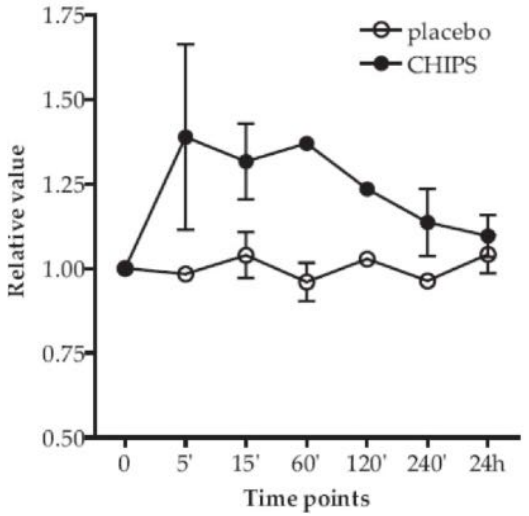
Material of non-human origin

The majority of the human population has circulating anti-CHIPS antibodies



Phase I:

Adverse effects due to immunocomplexes

→ To develop CHIPS as an anti-inflammatory drug, this must be taken into consideration.

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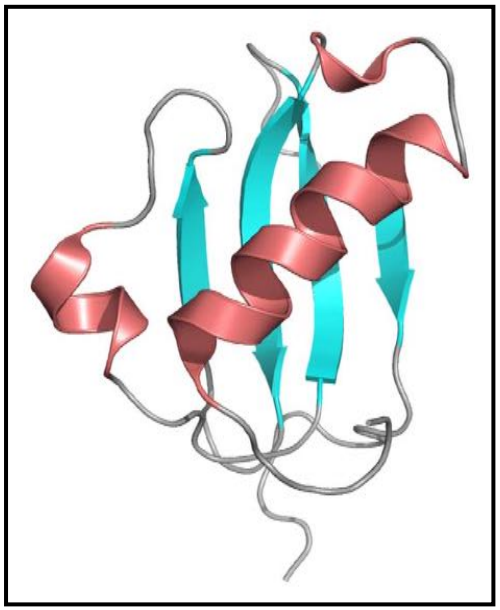
Removal of B cell epitopes



Aim:

Remove antigenic epitopes in CHIPS while retaining function

Methods:

Truncation, random mutagenesis, in vitro evolution, site directed mutagenesis, ELISA using human anti-CHIPS antibodies, Ca+ flux, ELISA using receptor peptides, FACS

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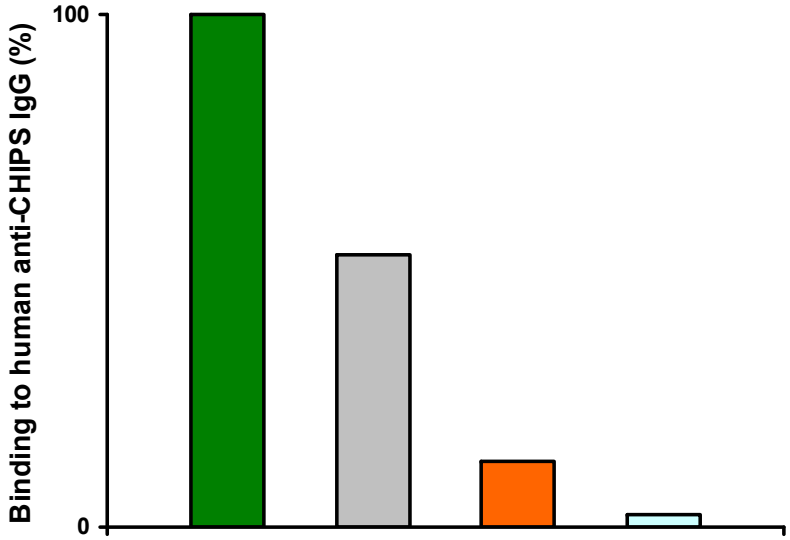
Reduce interaction with pre-existing ADA



CHIPS variants screened for decreased interaction with human anti-CHIPS IgG…..

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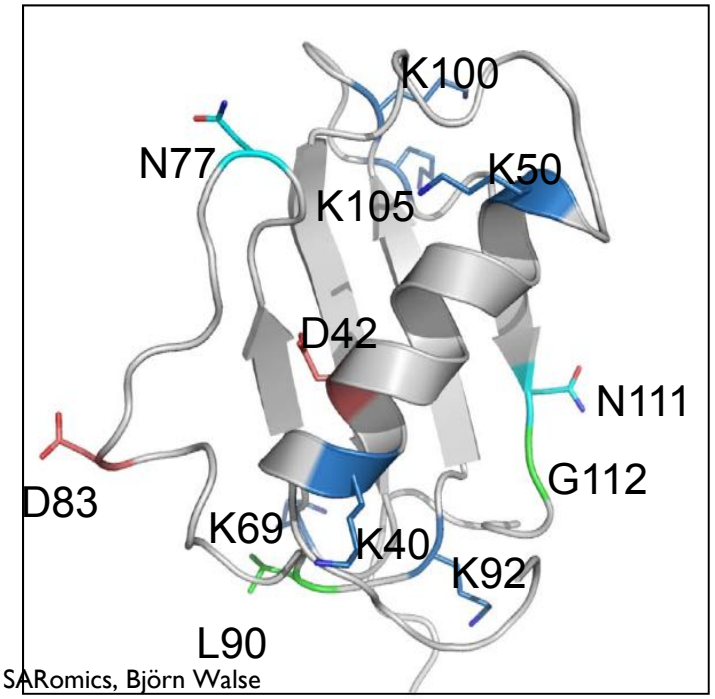
Reduce interaction with pre-existing ADA



The new, less antigenic CHIPS variants are mutated in one or several of the amino acid positions shown.

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Reduce interaction with pre-existing ADA

	DRB1		DRB3/4/5	DQ	DP
	Strong	Medium			
Wt 31-113	6	17	1	1	1
S3.02	7	18	2	1	2
S3.09	7	19	2	1	2
F3.39	6	20	2	1	2
S3.21	6	20	2	1	1
S3.04	7	18	2	1	1
S3.05	7	20	3	1	2
S4.01	6	18	2	1	1
F3.08	7	18	2	1	2
F3.57	6	20	2	1	1
S3.17	5	19	1	2	1
F3.50	5	19	1	1	2
S3.06	7	15	2	2	1
S4.02	6	19	3	1	1
S4.03	6	19	2	2	1
S4.04	6	20	3	2	1

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Reducing Immunogenicity

Study each of the 12 substitution sites

At each site, consider wt and 19 mutants

All strong epitopes overlapping with substitutions can be removed

Only one strong DQ/DR epitope left

DRB1	m	DRB345	DQ	DP
1	17	0	1	0

FEKMVILTEN	59-68	
DQA1*0501/DQB1*0201	DQ2	S
DRB1*0405	I	

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CHIPS: Conclusions

CHIPS variants with drastically decreased interaction with human anti-CHIPS antibodies and with retained function have been identified

No major differences between variants and Wt with respect to T cell epitopes

Optimal clones with respect to minimized T cells epitopes were designed – under evaluation

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Thank you for your attention

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Applied Protein Services

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Yannick Gansemans, Senior Scientist

AFFIBODY

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Lars Abrahamsén

Ingmarie Höidén-Guthenberg

ALLIGATOR bioscience

Christina Furebring

Ann-Kristin Malmborg Hager

Case Study : De-immunization of VB6-845®

Lonza

VIVENTIA

Biotechnologies Inc.

Background

- Viventia's anti-EpCAM recombinant immunotoxin
  - Humanized Fab fragment fused to a deimmunized 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 deimmunized bouganin portion
  - Observed immune response to Fab moiety

Objective

- Minimize the potential immunogenicity risk of the fusion protein by deimmunizing the Fab portion

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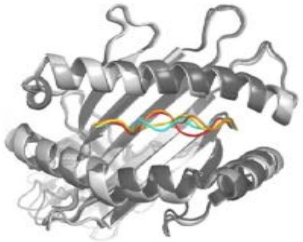
Case Study : De-immunization of VB6-845®



- **In silico de-immunization**
  - Screening for T cell epitopes using Epibase™
  - Antibody structure modelling
  - Substitutions to eliminate T cell epitopes based on structure integrity

- **In Vitro verification and testing of deimmunized protein**
  - Screening for T helper cell responses using PBMCs from healthy donors
  - Individual and population responses

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De-immunization of VB6-845® Fab

- **Epibase™ screening**
  - Epitope identification on Fab sequence
  - Filtering of epitopes present in human germlines
  - Critical epitopes are identified as strong binders to DRB1 and DRB 3/4/5
- **Identification of mutations that remove epitopes**
- **Prevention of novel epitopes**
  - For other allotypes
  - In overlapping frames
- **Respecting structural integrity of the protein**
  - Stability
  - Function (e.g. affinity for ligand)

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De-immunized VB6-845® Fab

- **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

- **De-immunized Fab has a similar binding affinity to wild type**

Binding Affinity

De-Fab: $K_D = 1.31 \times 10^{-9}$

WT : $K_D = 1.56 \times 10^{-9}$

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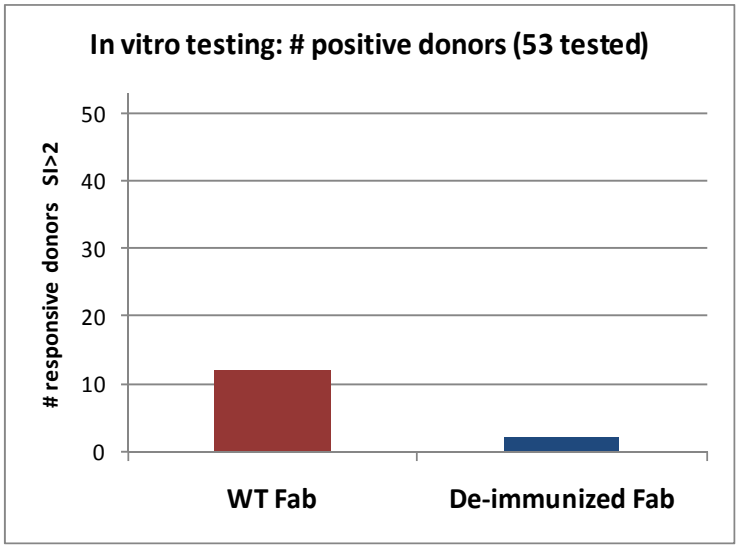
In vitro Testing of De-immunized VB6-845® Fab

Compare the immunogenic potential of de-immunized Fab to wild type based on:

- Number of responsive donors
- Mean SI over the population
- Relative response

In vitro Testing: single donor and population level



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

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Conclusion

- De-immunized anti-EpCAM Fab showed reduced T cell activation potential in vitro, compared to wild type
- A 2nd generation VB6-845 molecule has been engineered and is now ready for testing in Phase I trials
- In silico deimmunization provides a cost effective and rapid solution to further reducing or avoiding potential immunogenicity risk of therapeutic proteins