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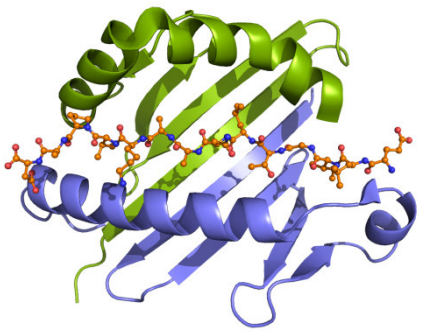
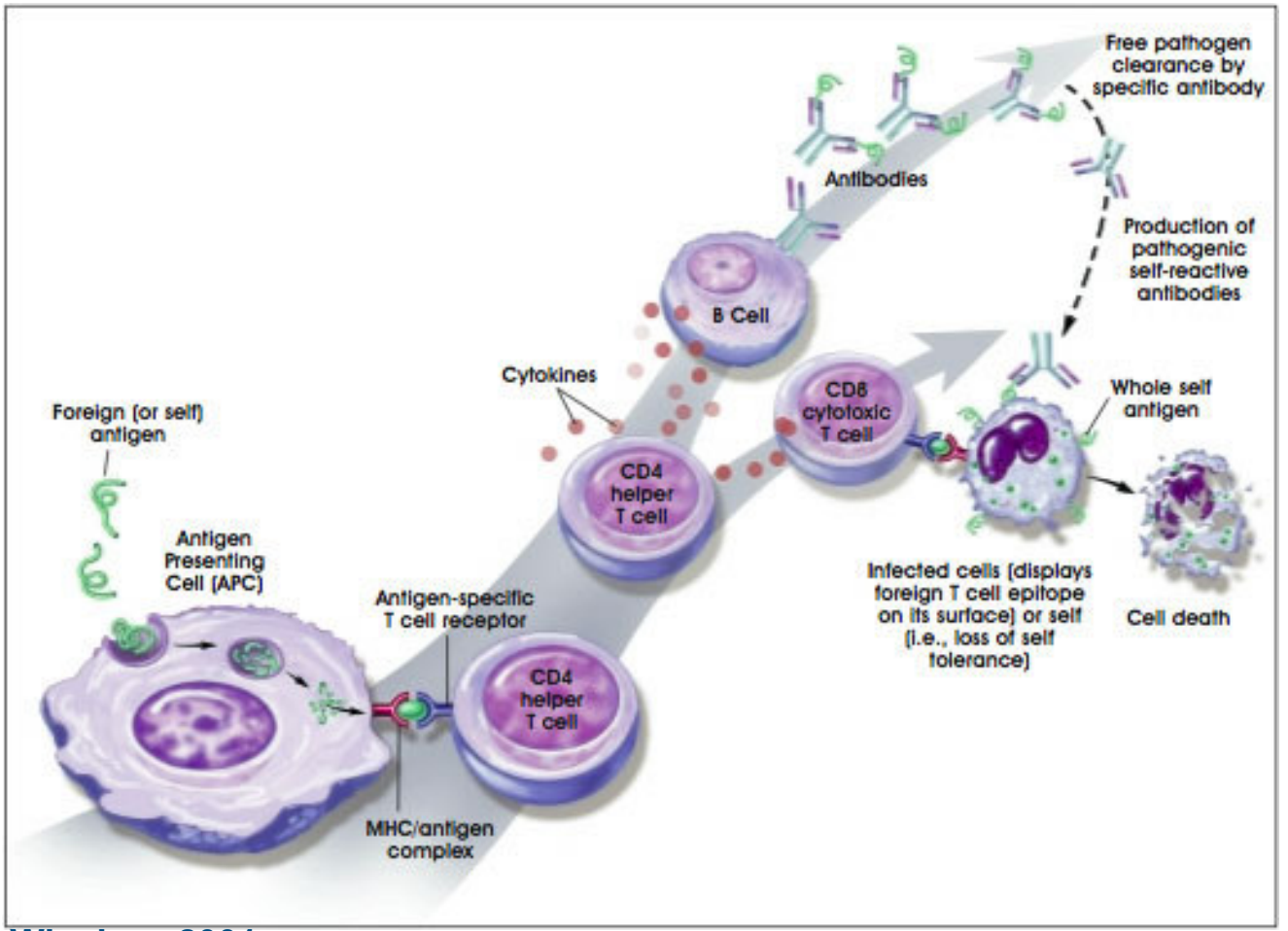


Immunogenicity of biotherapeutics

Immune response



Winslow, 2001

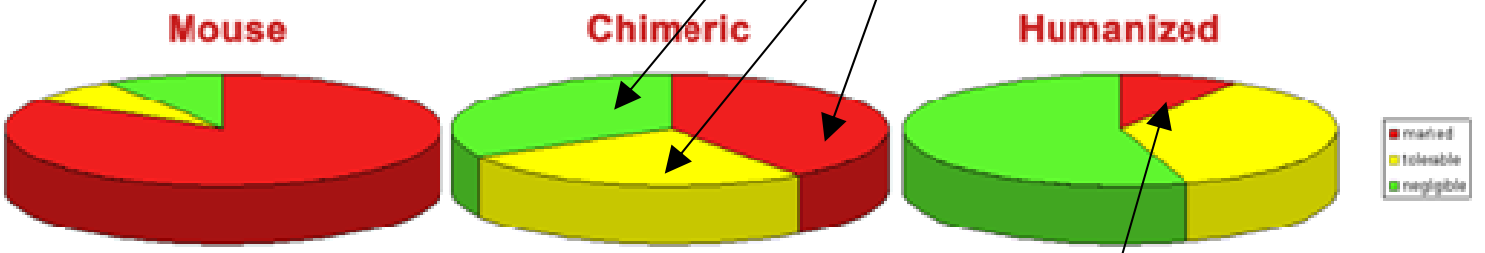
Observed Immunogenicity

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)

Therapeutic antibodies

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



Hwang & Foote, 2005

Humira	human	TNFa	RA

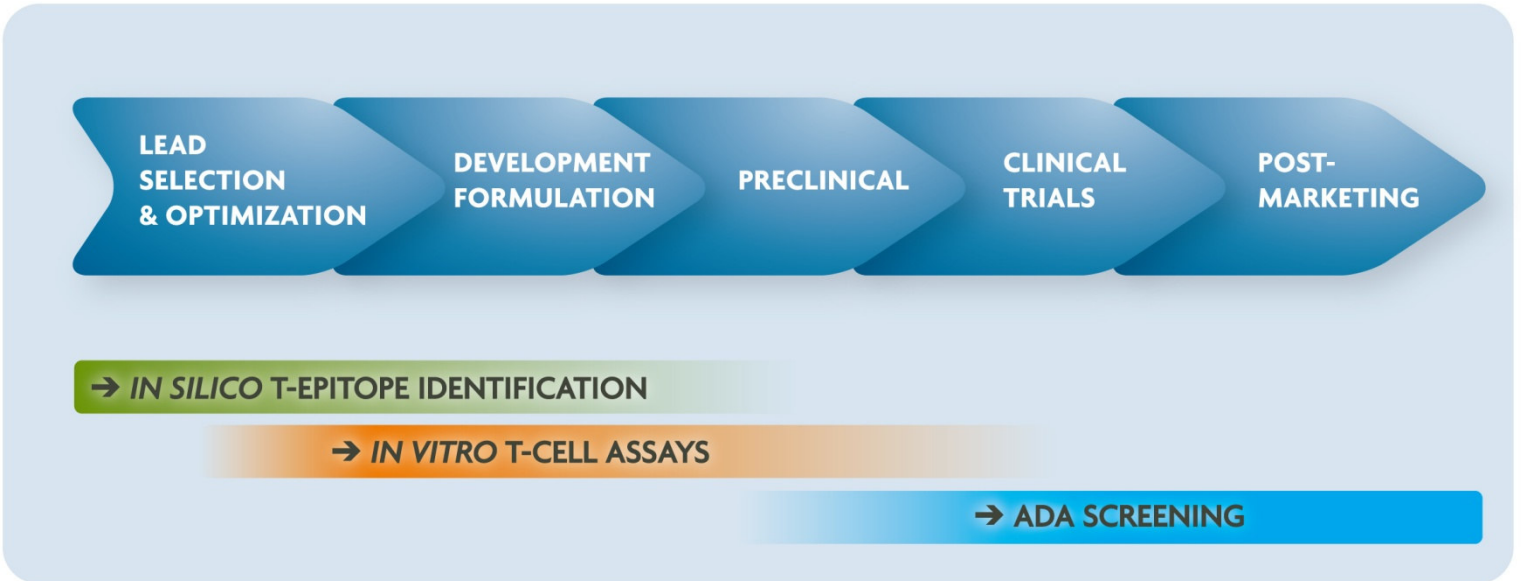
European Medicines Agency

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

Immunogenicity assessment



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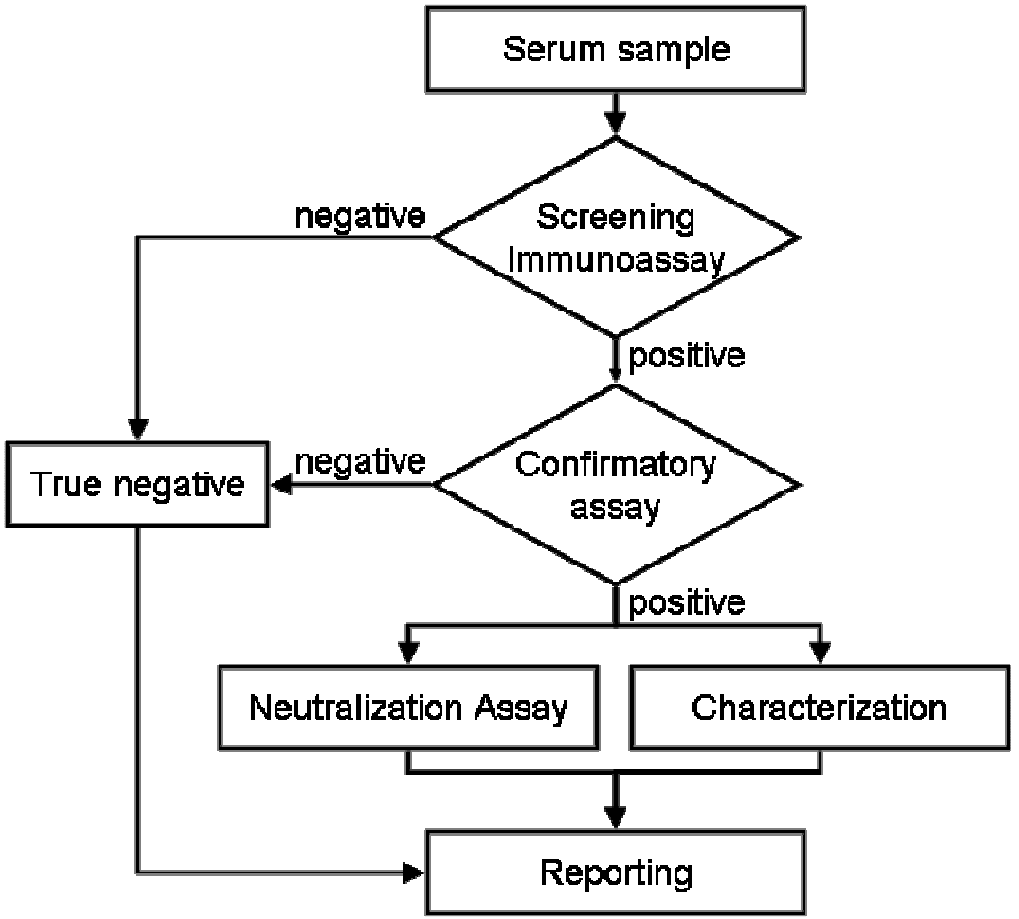
ADA screening strategy



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 recognised 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 Immunogenicity Assessment

CONCEPT PAPER ON IMMUNOGENICITY ASSESSMENT OF MONOCLONAL ANTIBODIES INTENDED FOR IN VIVO CLINICAL USE

Doc. Ref. EMEA/CHMP/BMWP/114720/2009 (DRAFT)

1. Discussion

Recently developed combinations of in-silico and T-cell based procedures are showing promise for predicting potential immunogenicity with some biologicals including mAbs. Identification of epitopes associated with induction or suppression of immune responses has been possible.

4. Recommendations

The main topics to be addressed include: (6 topics)

…

- **Approaches which may be helpful in predicting unwanted immunogenicity of mAbs.**

...

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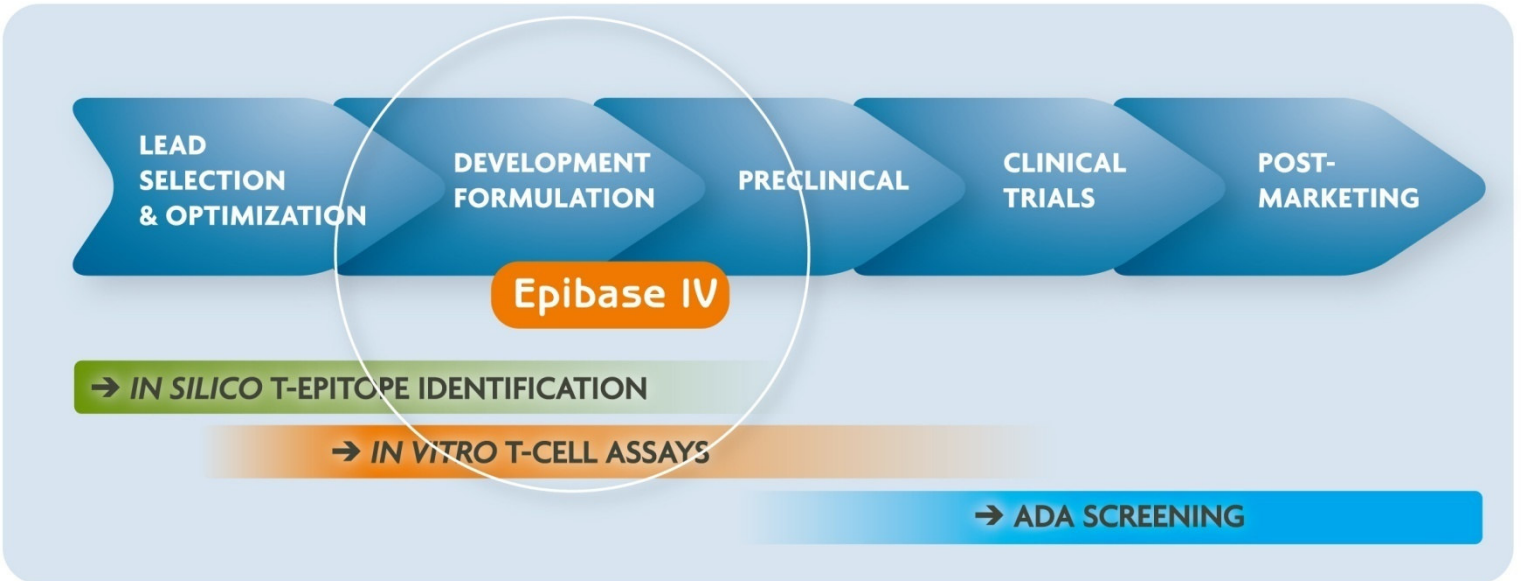
Immunogenicity assessment



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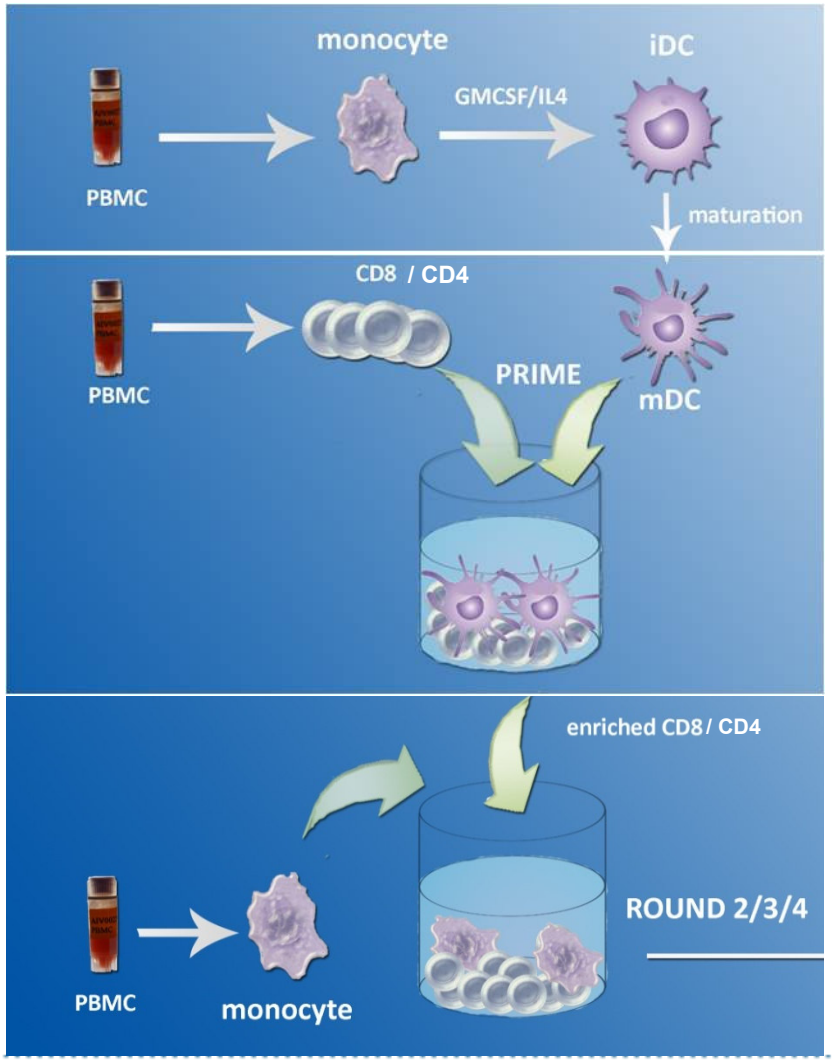
Adaptive immunity : Induction of naïve T-cell responses



- Generation/maturation of dendritic
- QC of dendritic cells

- Antigen loading of DC
- co-culture DC + T-cells

- Antigen-loaded $CD14^+$
- co-culture $CD14^+$ enriched T-cells

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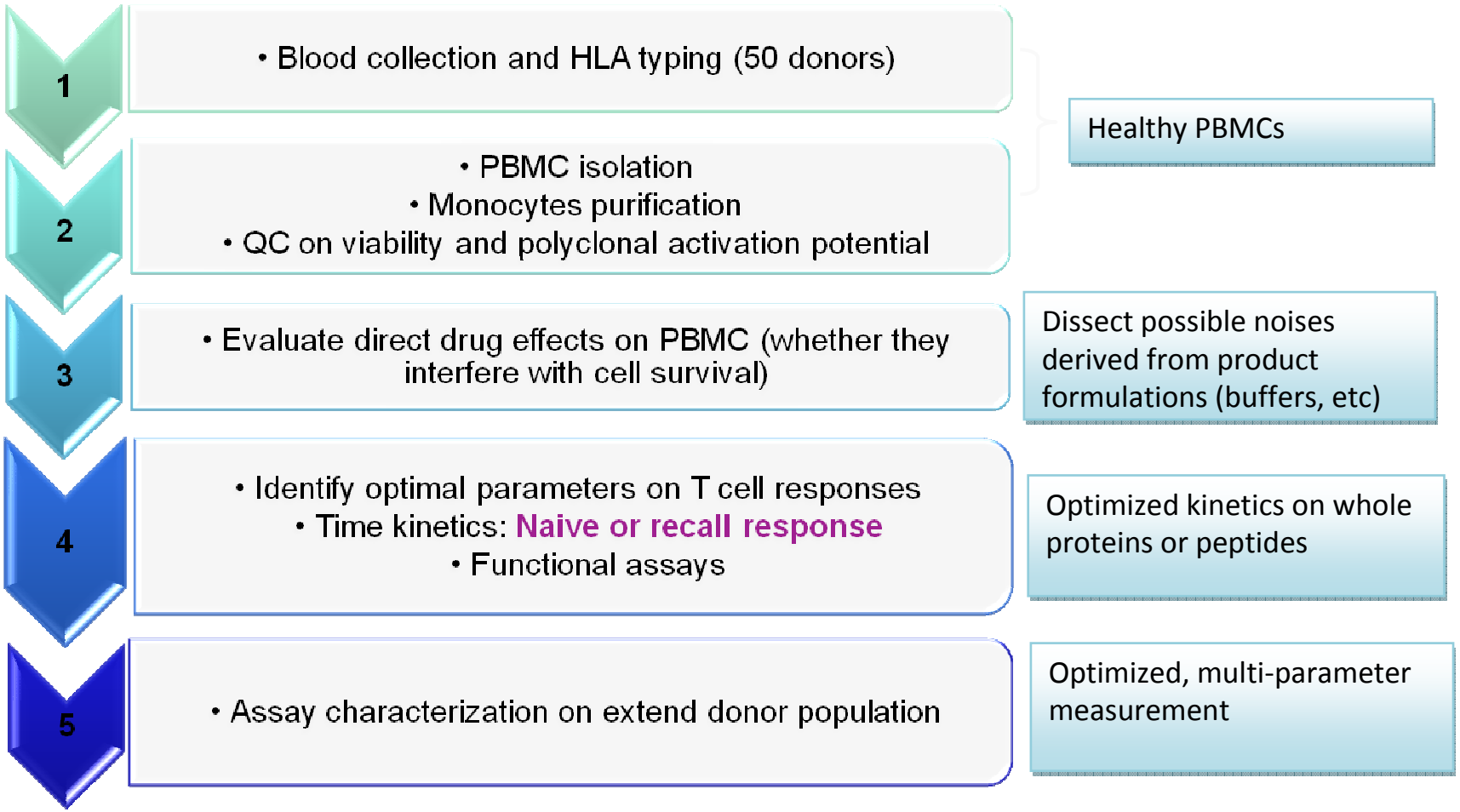
in vitro T cell assays:



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Response

Assessment of recall responses to peptides (CEFT) qualifies the multi-parameter flow cytometry assay and enriched IFN-g Elspot for determining the recall responses to vaccins.

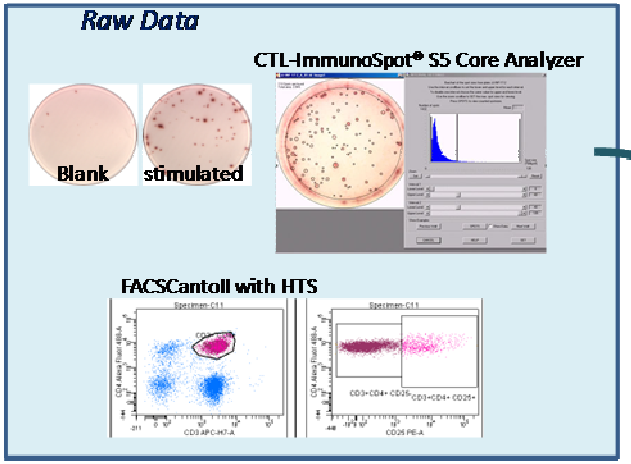
Analysis parameter : % responsive donors in a set of 15 healthy community donor samples

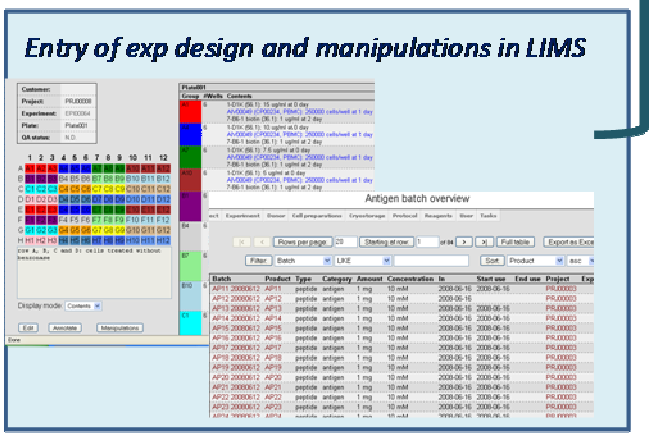
IFN-γ spotforming units PBMC, Δ frequency of activated wells (FCM) $CD3^+CD4^+$

	Peptide (properties)	Elispot IFN-γ	Flow cytometry
Negative contol peptides	AP3 (non-binding self)	0%	6%
Positive control peptides	CEFT (binding – non self – recall)	100%	94%

Multiparameter Measurements

EPIBASE IV Data flow





Upload data in LIMS database to link experimental design and manipulations to the raw data

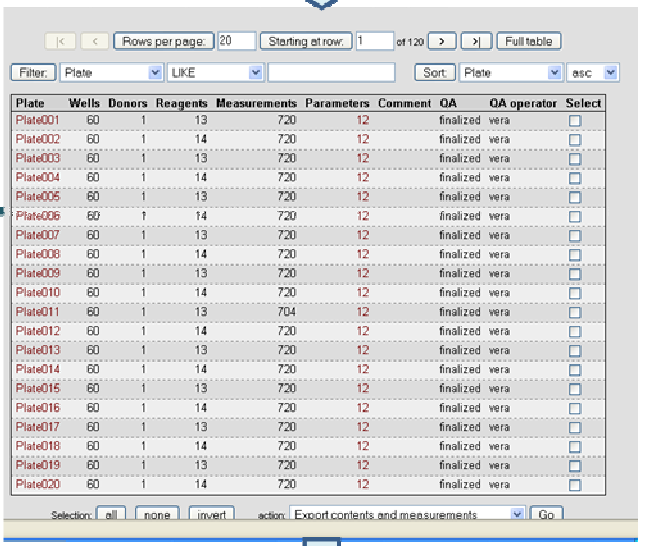


Plate	Wells	Donors	Reagents	Measurements	Parameters	Comment	QA	QA operator	Select
Plate001	60	1	13	720	12		finalized	vera	
Plate002	60	1	14	720	12		finalized	vera	
Plate003	60	1	13	720	12		finalized	vera	
Plate004	60	1	14	720	12		finalized	vera	
Plate005	60	1	13	720	12		finalized	vera	
Plate006	60	1	14	720	12		finalized	vera	
Plate007	60	1	13	720	12		finalized	vera	
Plate008	60	1	14	720	12		finalized	vera	
Plate009	60	1	13	720	12		finalized	vera	
Plate010	60	1	14	720	12		finalized	vera	
Plate011	60	1	13	704	12		finalized	vera	
Plate012	60	1	14	720	12		finalized	vera	
Plate013	60	1	13	720	12		finalized	vera	
Plate014	60	1	14	720	12		finalized	vera	
Plate015	60	1	13	720	12		finalized	vera	
Plate016	60	1	14	720	12		finalized	vera	
Plate017	60	1	13	720	12		finalized	vera	
Plate018	60	1	14	720	12		finalized	vera	
Plate019	60	1	13	720	12		finalized	vera	
Plate020	60	1	14	720	12		finalized	vera	

Generates csv file for statistical analysis

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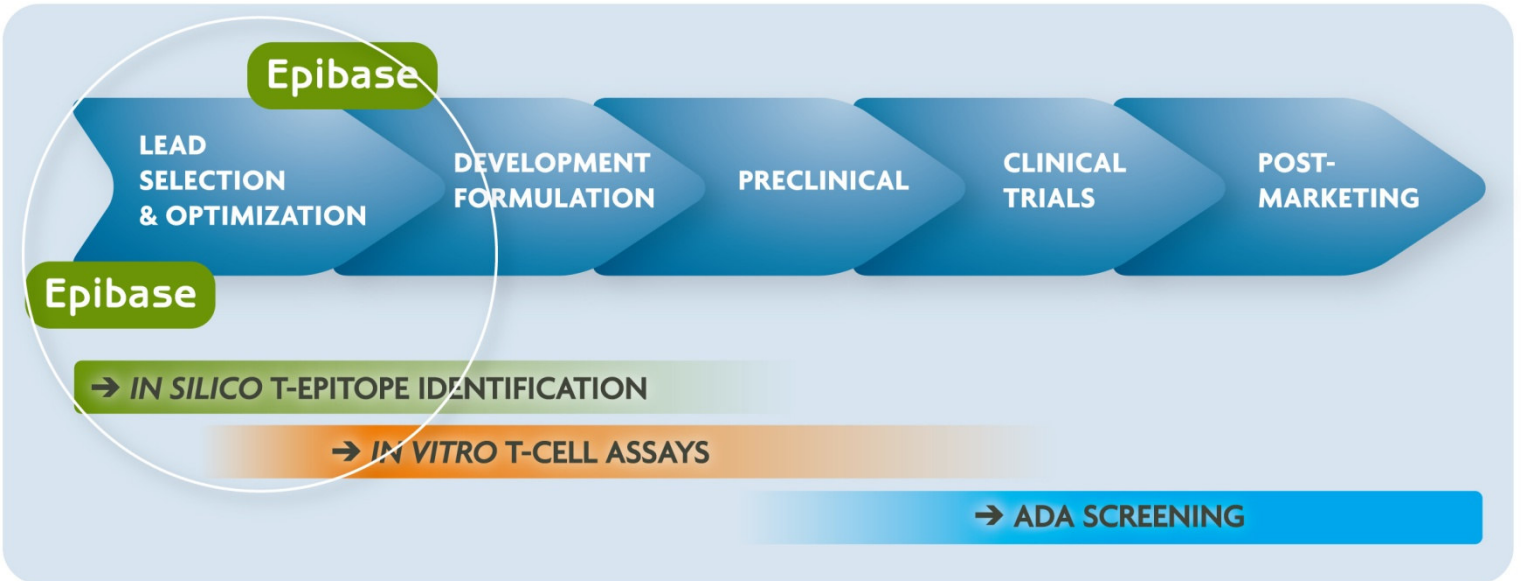
Immunogenicity assessment



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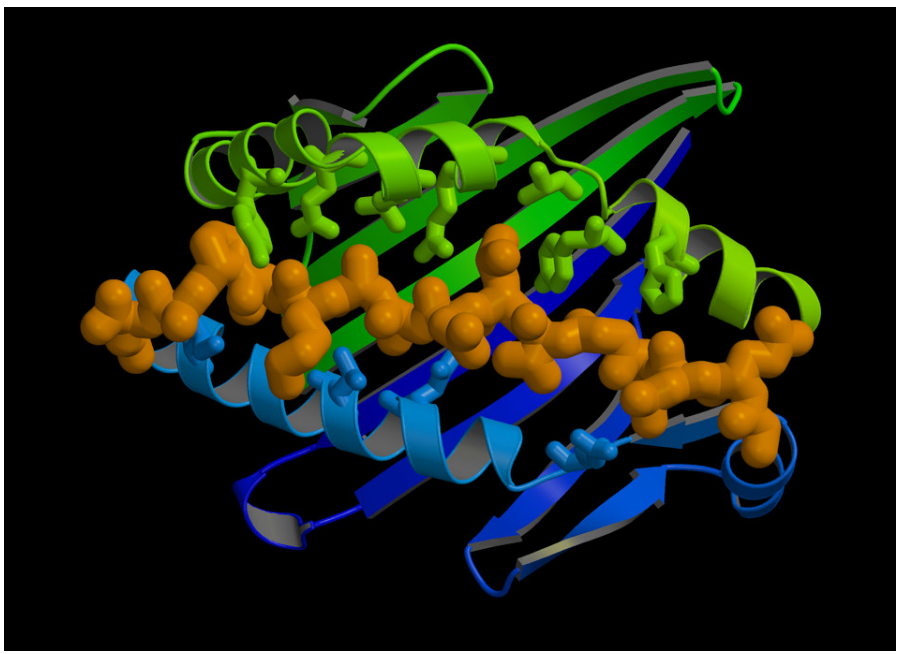
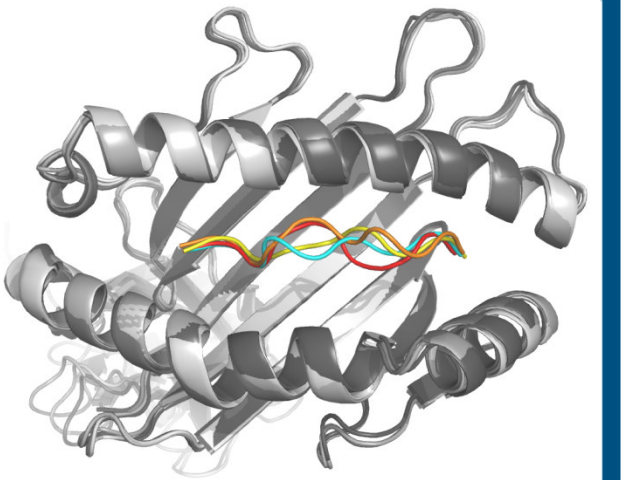
Classical Predictive Methods

- **Position matrices:**
  - Score each position in the peptide for "likelihood" to fit in "pockets"
  - Sum those scores => epitope vs. non--epitope



Epibase®



1. Model building

- Template identification: retrieve HLA subtypes of known 3-D structure that are at least 50% identical to a given HLA subtype
- Build a 3-D structure

2. Run the proprietary FASTER algorithm

- Select relevant part of the receptor
- Include the flexibility of side chains

EP 1226528, Proteins, 2002

3. Determine

- Binding affinity
- Promiscuity

Proteins, 2005

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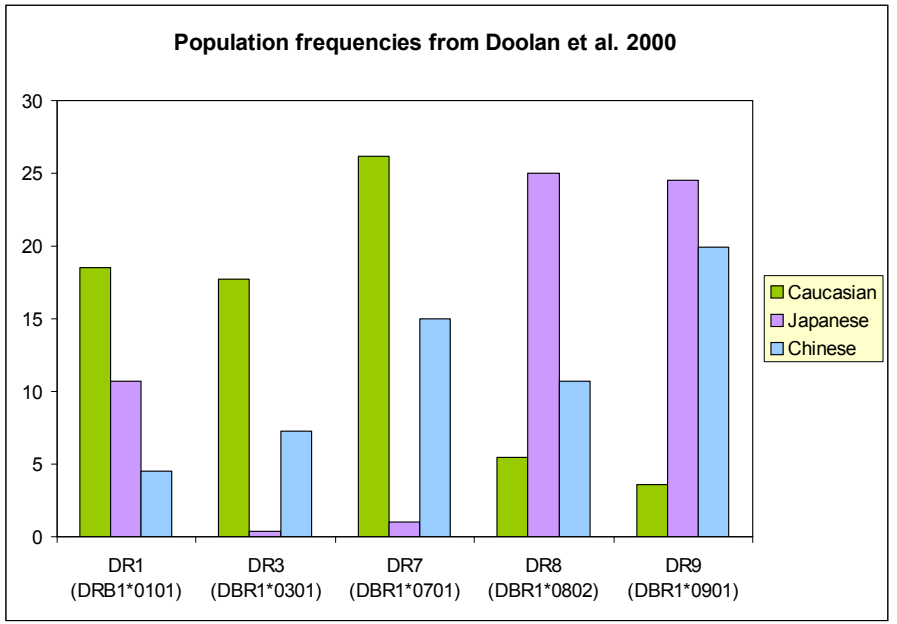
MHCII population frequencies



	Epibase® Global	Epibase® Caucasian	Epibase® Oriental	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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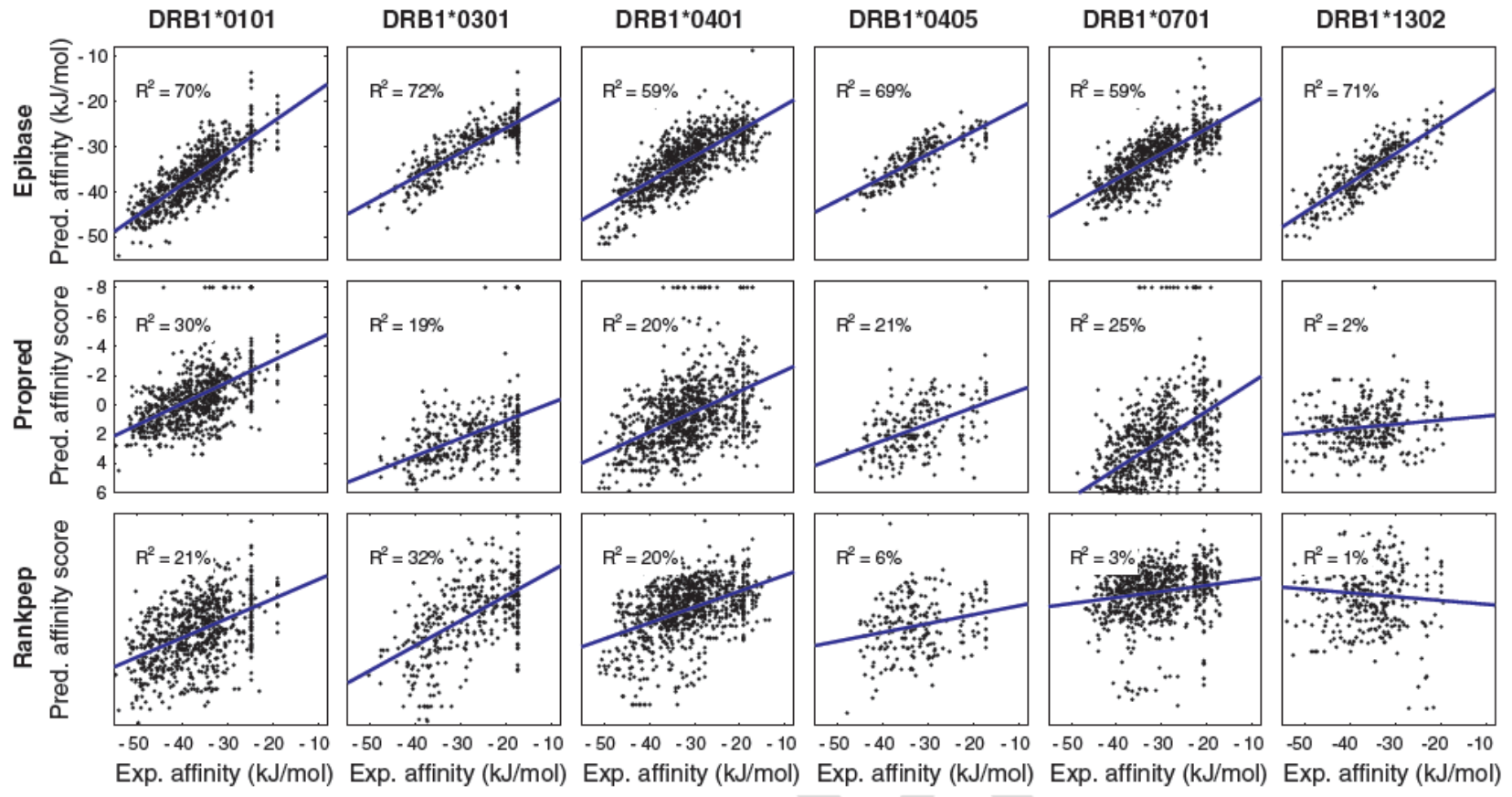


Van Walle et al, Expert Opin. Biol. Ther., 7(3) 2007

Case study: Adalimumab

- Human antibody recognizing TNF-α isolated by phage-display technology
- Study performed in collaboration with Sanquin and Genmab.

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 described:
  - 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

Study design

- 109 RA patient enrolled for the study
- Patients were tested for:
  - HAHA response (low, high)
    determined from the binding of the Humira F(ab')2 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®

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

Conclusions

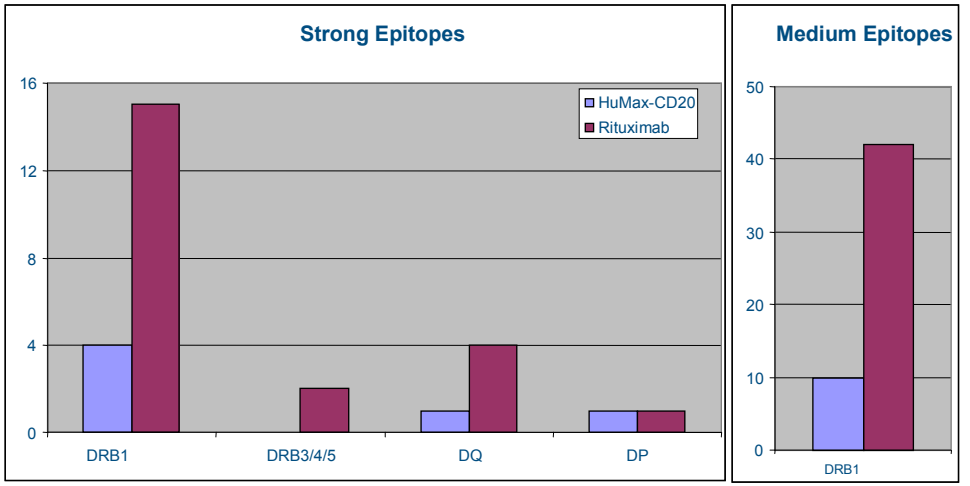
- HAHA+ patients can be explained on basis of the critical epitopes as defined by *in silico* analysis
- RA associated HLA allotypes contribute to HAHA+ response against Humira
- Research project continued to:
  - Measure the epitopes in this patient group using immunological techniques.
  - Measure epitopes in non-MTX population

Case study 2: 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:
  - Phase III 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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