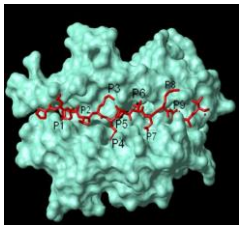
Institute of Biology and Technologies
Service of molecular engineering of proteins
Saclay, France



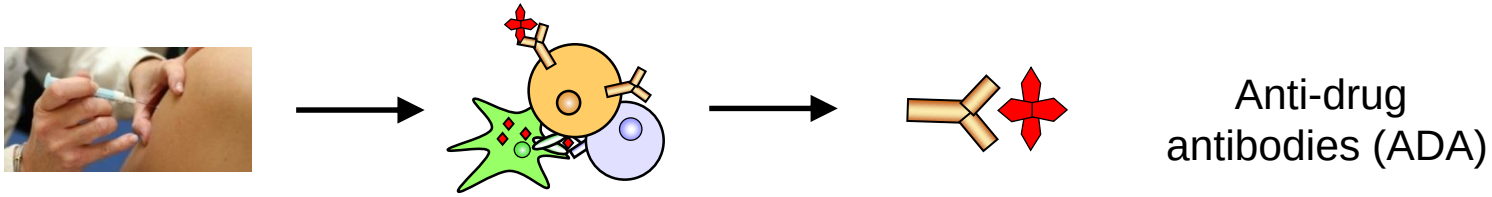
Immunogénicité des protéines thérapeutiques : impact et anticipation

B. Maillere, PhD

Contact: bernard.maillere@cea.fr

Risk of immunogenicity of therapeutic proteins

- Immunogenicity: capacity to elicit a specific immune response



Undesirable effects

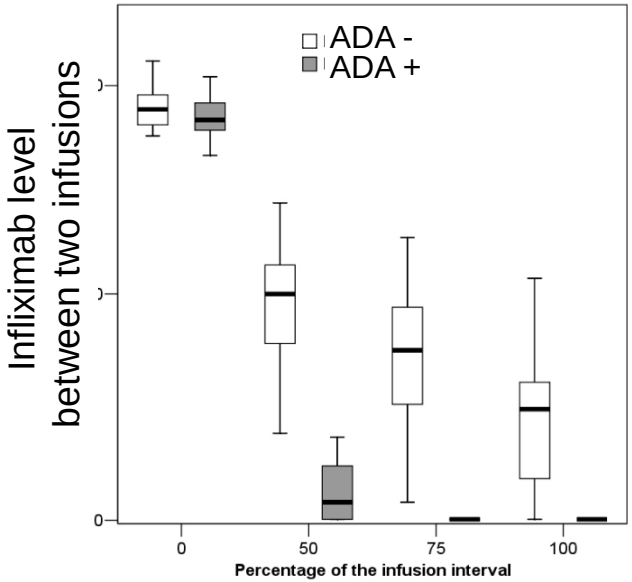
- No effect
- PK alteration : clearing or sustaining antibodies
- Resistance to the treatment : Neutralizing antibodies
  FVIII, Anti-TNFα , IFNβ
- Safety issues
  - Autoimmune symptoms (endogenous counterpart) Epo
  - Allergic symptoms Cetuximab Infliximab
  - Cytokine storm TGN1412

PK alteration

- Clearing antibodies

  Examples: Therapeutic antibodies

  Formation of large multivalent complexes,
  Fast clearance

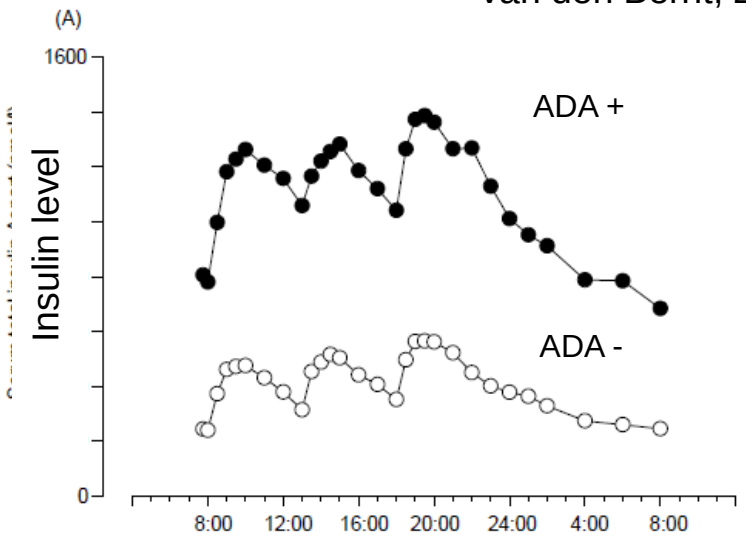


Van den Bernt, 2011

- Sustaining antibodies

  Examples: Insulin, IL-2, IL-3, IL-7

  Small monovalent complexes
  Low clearance

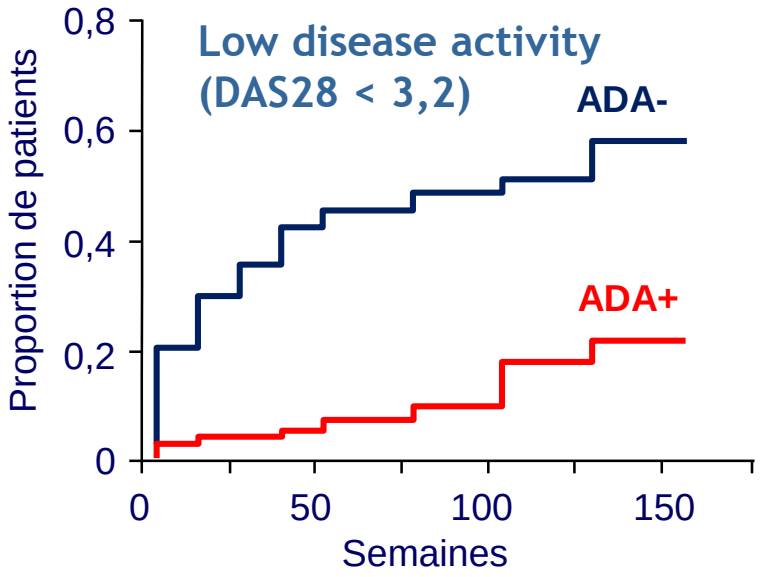


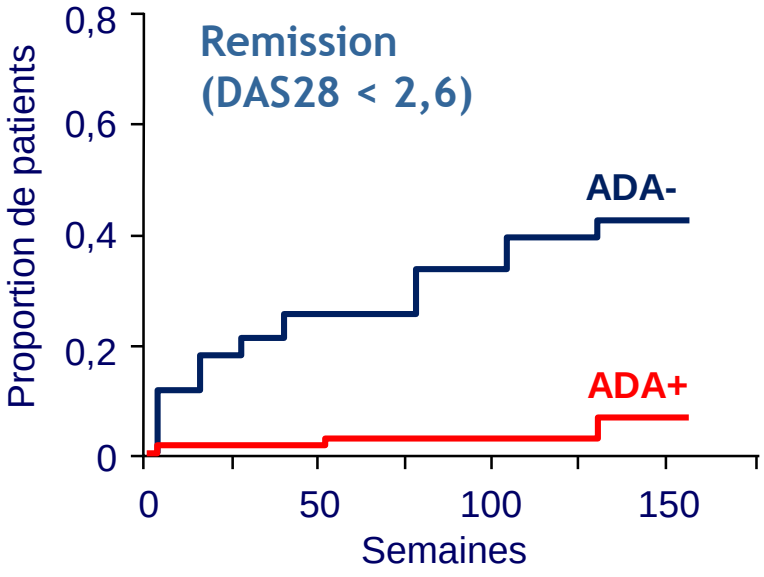
Chen, 2005

Resistance to the treatment

- Progressive loss of the therapeutic efficacy
  Neutralizing antibodies
  Examples: anti-TNFα (RA), IFNα (HCV), IFNβ (MS), FVIII (Haemophilia)

- Resistance to adalimumab treatment in RA patients





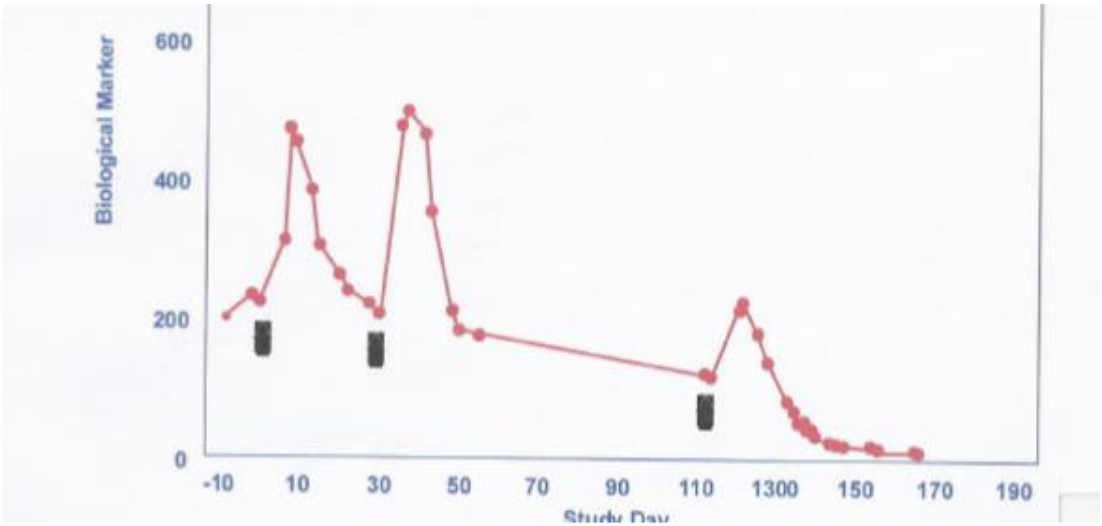
Bartfelds JAMA, 2011

Autoimmune symptoms

- Antibodies induced by the recombinant protein neutralize the endogenous form
  Examples: Thrombopoietin and Erythropoietin (EPO)

- Pure Red Cell Aplasia (PRCA) :
  Deficiency in mature erythroid progenitors,
  Rare event
  Can result from antibody response to injected recombinant EPO

- In the late 90s, sudden increase in cases of PRCA
  Changes in the formulation and injection mode
  Due to anti-Epo neutralizing antibodies



Allergic symptoms

- Allergic reactions mediated by specific IgE **induced by repeated injections** of therapeutic proteins

Acute and delayed hypersensitivity reactions to infliximab and adalimumab in a patient with Crohn's disease

Casper Steenholdt [a,*], Morten Svenson [b], Klaus Bendtzen [b,c], Ole Østergaard Thomsen [a], Jørn Brynskov [a], Mark Andrew Ainsworth [a]

Steenhold et al, 2012

- Allergic reactions mediated by specific IgE **pre-existing before injection** of therapeutic proteins

  - ✓ Crossreactive antibodies elicited by foreign antigens
  - ✓ Anaphylactic shock (IgE mediated)

Cetuximab-Induced Anaphylaxis and IgE Specific for Galactose-α-1,3-Galactose

Cheung, NEJM, 2008

Pre-existing antibodies to Cetuximab

- Cetuximab and allergic symptoms

  - ✓ A chimeric Mab anti-EGFR: colorectal and head and neck cancer
  - ✓ Severe hypersensitivity reactions in 3% of patients (up to 22%)
  - ✓ Pre-existing antibodies: symptoms at the first injections of Cetuximab

- The antibodies are specific for galactose-α-1,3-galactose (α-Gal)

  - ✓ α-Gal : present in the Fab part of the cetuximab heavy chain
  - ✓ abundantly expressed on cells tissues of nonprimate mammals (SP2)
  - ✓ IgE result from allergy to tick bites or to meat (Beef, pork)

	Type of Cetuximab†		
Hypersensitivity reaction	SP2/0‡	CHO‡	α-gal
Anaphylaxis related to cetuximab			
1	41.6	0.35	13.8
2	38.8	0.35	35.2
3	20.2	0.35	12.6
4	11.1	0.35	2.9
5	4.9	0.35	2.0
6	4.2	0.35	2.7

Cheung, NEJM, 2008

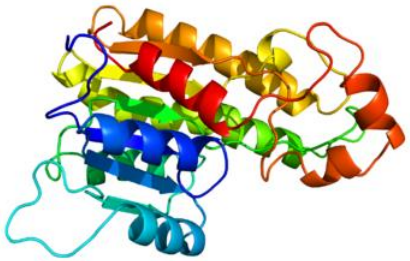
Cytokine Release Syndrome

- Origins of CRS
  - ✓ **Massive and transient release of TNF-α, IL-2 and IFN-γ**
  - ✓ Peak serum TNF at 1 hr
  - ✓ Peak serum IFN at 4 hr
- Muromonab (anti-CD3)
  - ✓ In 1988, description of a reversible clinical syndrome observed in patients treated with Muromonab (anti-CD3)
- The dramatic first clinical trial with TGN1412
  - ✓ Humanized Anti-CD28 superagonist, stimulates Tregs in rats
  - ✓ I.V. injection in 6 volunteers March 2006
  - ✓ followed by a systemic inflammatory response: headache, myalgias, nausea, hypotension, lung injury, renal failure, acute respiratory distress syndrome

Symptoms not observed in animal models

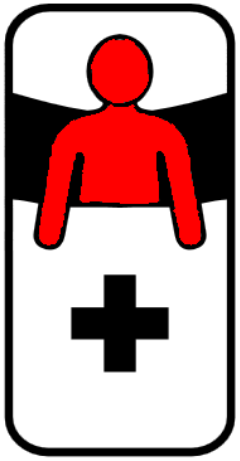
- ✓ Effective dose is very low in humans in contrast to animal models including NHP
- ✓ Injected doses : very high for humans

Aims of immunogenicity prediction



Technologies of immunogenicity prediction

PATIENTS



ADA:
Frequency?
Intensity?
Neutralizing?

Limitations of animal models

- EMEA/CHMP/BMWP/14327/2006

  Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins

  4.2 NON-CLINICAL ASSESSMENT OF IMMUNOGENICITY AND ITS CONSEQUENCES

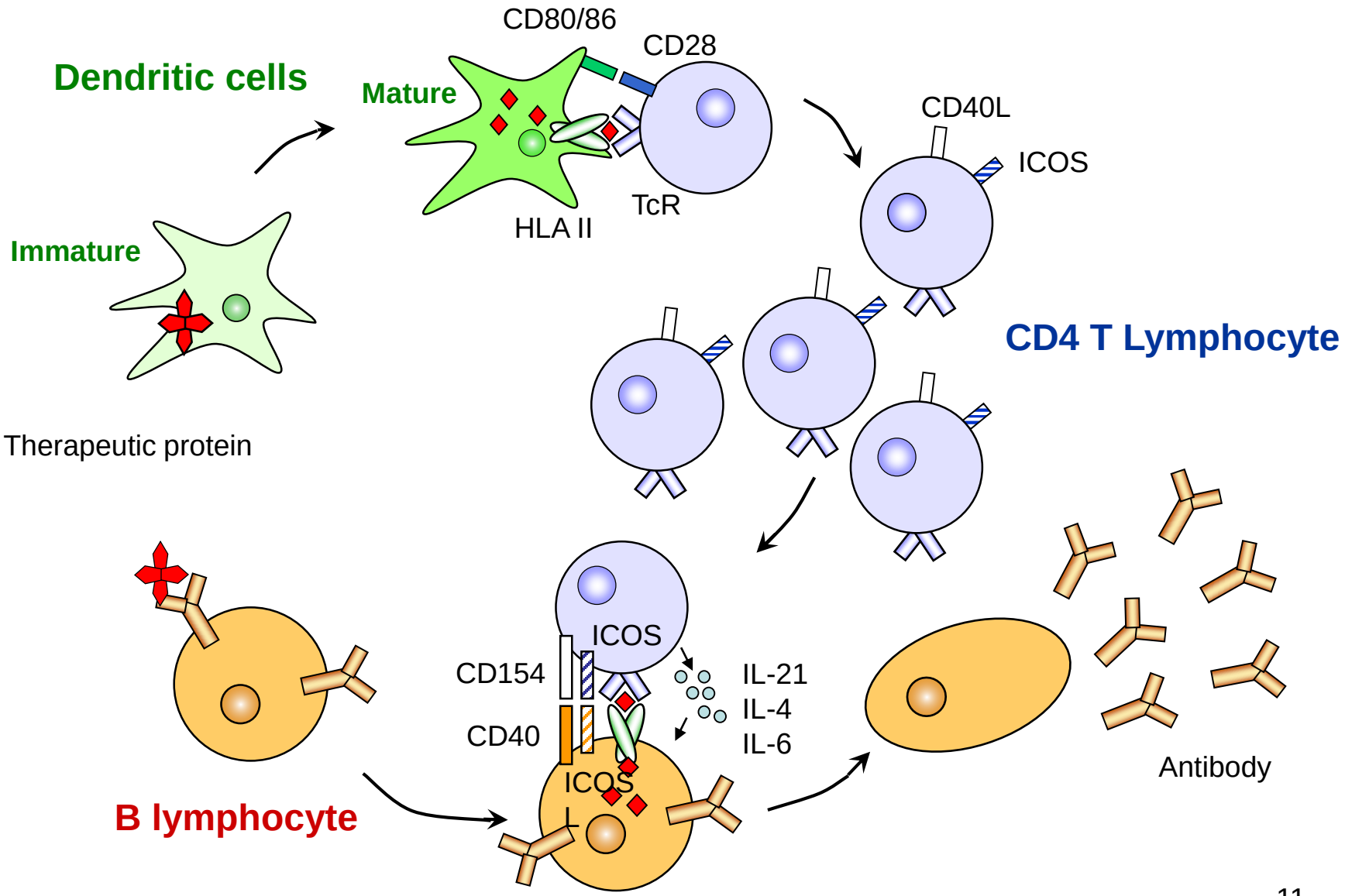
  Human proteins will be recognised as foreign proteins by animals. For this reason, the predictivity of non-clinical studies for evaluation of immunogenicity is considered low.

- Example: Etanercept

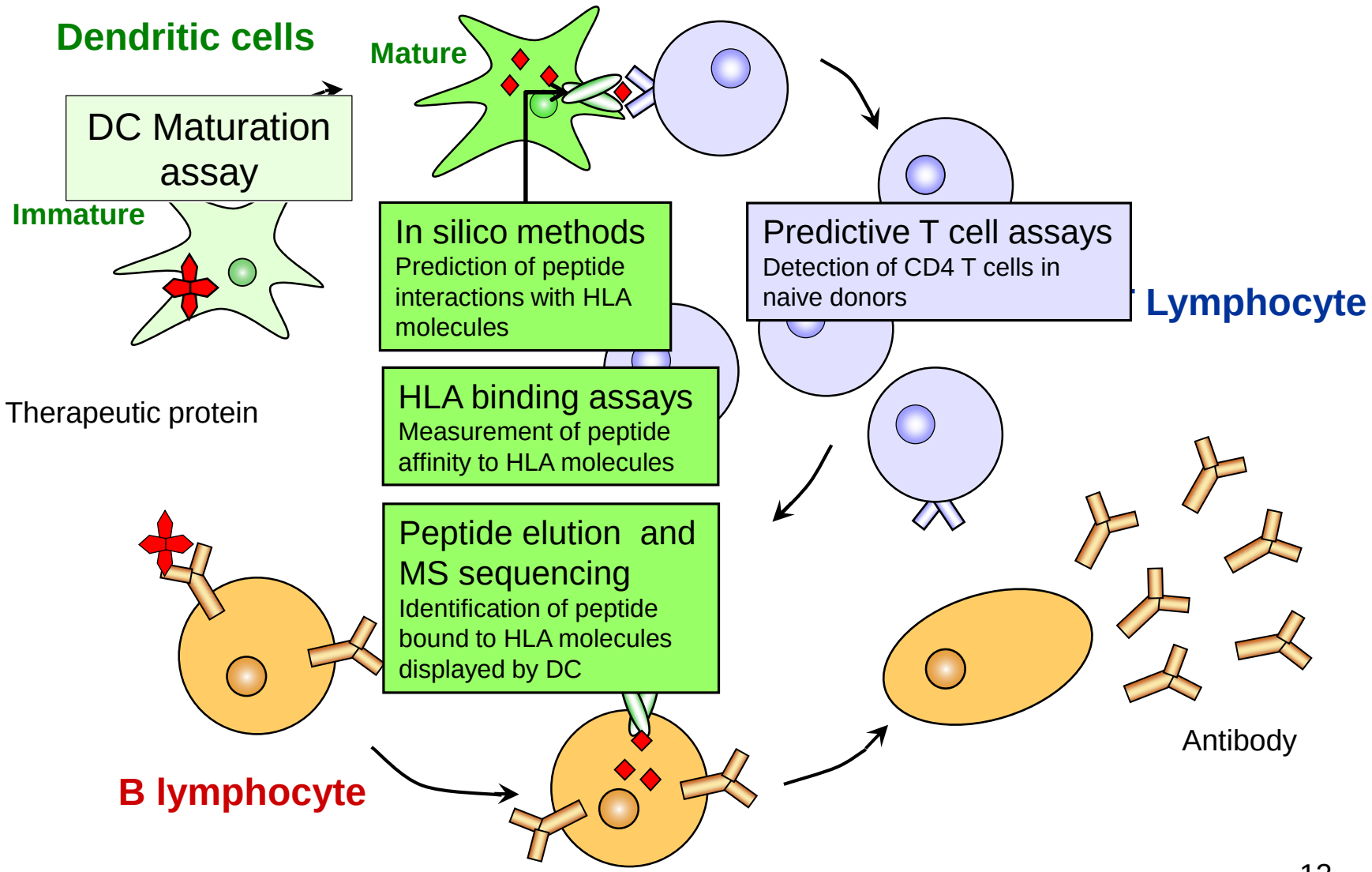
  Following twice weekly s.c. administration, the majority of mice, rats and rabbits developed neutralizing antibodies prior to week 4 (EMA, Ref: WC500027358)

Indication	Nb of patients	Nb of injections	Ab response (%)	References
Rheumatoid arthritis	212	24	5	Dore et al, 2007
Psoriasis	611	Up to 96	18	Tyring et al, 2007
Psoriasis	486	24 to 60	2	Leonardi, et al 2003
Ankylosing spondylitis	53	48	0	de Vries et al, 2009

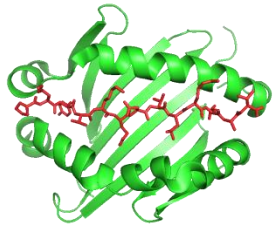
Cellular mechanisms of antibody response



Methods of prediction of immunogenicity



In silico methods



- **Objective**

To predict the peptide interactions with HLA molecules

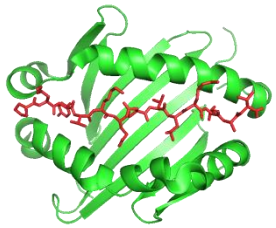
- **Method principles**
  - Peptide alignments (motif): SYFPEITHI, RANKPEP
  - Scoring matrices: ARB (IEDB), SMM-Align, PROPRED (TEPITOPE), DP4predict
  - Structural analysis
  - Learning algorithms (NetMHCpan)

- **Availability**
  - Easy-to-do, not expensive, Web resources IEDB www.immuneepitope.org
  - Proprietary resources

- **Achievements** (Wang et al, Plos, 2008)
  - Prediction of binders: very good but allele dependent
  - Prediction of CD4 T cell epitopes: overpredictive

Commonly used in early steps of drug development as preliminary immunogenicity assessment and for T cell epitope mapping

HLA Class II binding assays



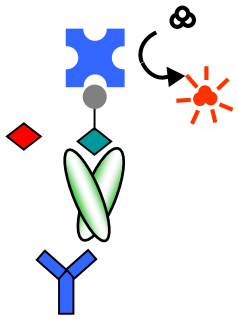
- **Objective**

  To evaluate the affinity for multiple HLA II molecules

- **Method principles**

  Competitive ELISA assay, RIA
  Direct assay

- **Particularities**
  - Experimental data of affinity
  - high throughput
  - need to purify HLA class II molecules
  - limited to preponderant alleles



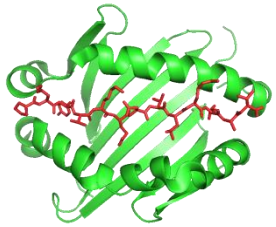
HLA II alleles	Frequency
DRB1*0101	9.3
DRB1*0401	5.6
DRB1*1101	9.2
DRB1*0701	14.0
DRB1*0301	10.9
DRB1*1301	6.0
DRB1*1501	8.0
DRB5*0101	7.9
DRB3*0101	9.2
DRB4*0101	28
DPB1*0401	40
DPB1*0402	11

(Texier *et al* . J Immunol. 2000;
Texier *et al*. Eur J Immunol. 2001
Castelli *et al*. J Immunol. 2002)

- **Achievements**
  - Over-predictive
  - Many T cell epitopes identified

Therapeutic proteins: FVIII, Mab, IFN, Epo
Allergens: cat, dog, cow, birch, house dust mite, food
Virus: HCV, HIV, Vaccinia, HSV, HBV
Tumour antigens: Survivin, TRAG, NY-ESO, cyclin B1

Peptide elution and MS sequencing

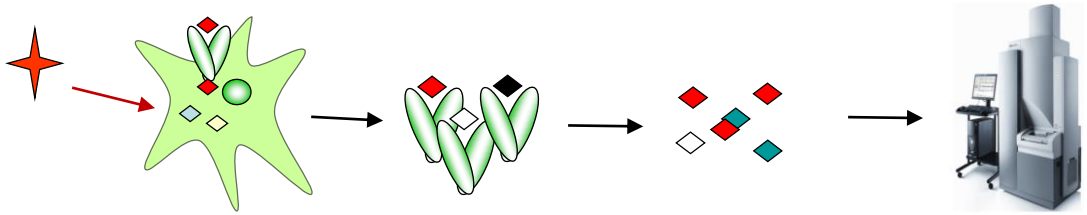


- **Objective**

  To identify naturally processed peptides bound to HLA molecules displayed by DC

- **Principle**

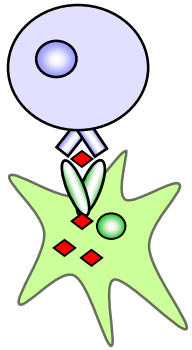
(also called MAPPS assay MHC-associated peptide proteomics)



- **Particularities**
  - Experimental data of peptides displayed by the DC
  - High throughput (panel of donors)
  - Effect of aggregation, formulation on peptide presentation

- **Achievements**
  - Prediction: under investigation
  - Differences between native and aggregated antibodies
  - Expected to be overpredictive

Predictive T cell assays



- **Objective**

  To evaluate the capacity of therapeutic proteins to elicit a CD4 T cell response in humans

- **Common principles**
  - Naive donors (no previous contact with the therapeutic protein)
  - HLA class II molecules representative of the population diversity
  - Activated T cells are detected after a culture phase with the protein

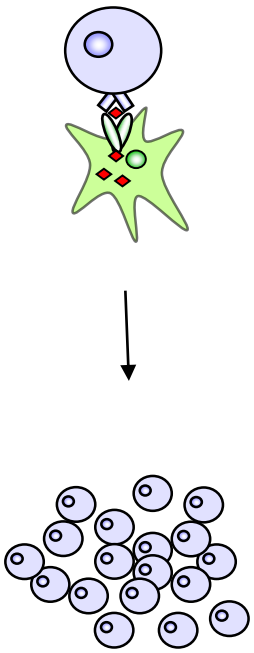
- **Multiple assays formats**
  - Different experimental procedures
    - Culture conditions
    - Number of stimulations
    - Read-out (proliferation, Elispot, ICS)
  - Number of donors – HLA coverture
  - Relative or absolute values (number of pre-existing T cells)

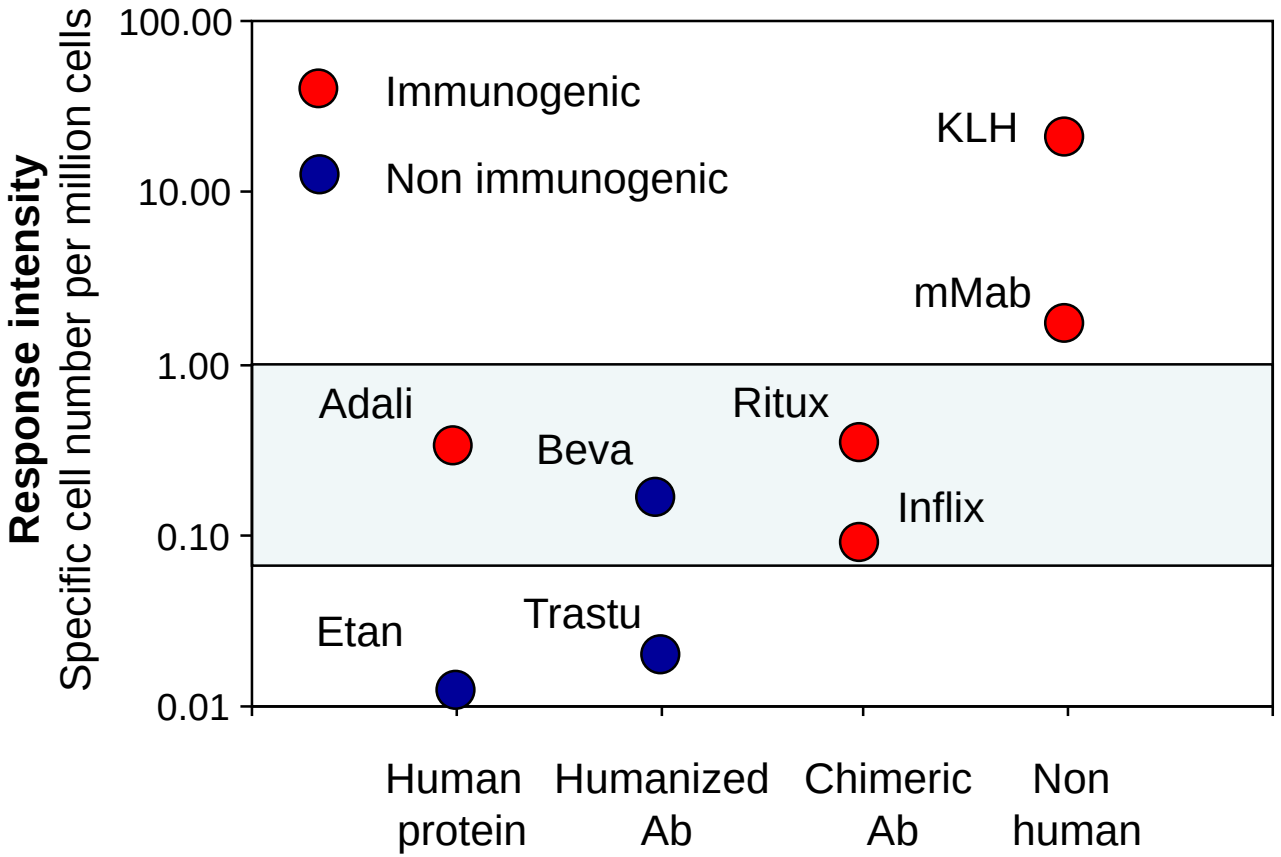
- **Achievements**
  - Existence and size of a pre-existing CD4 T cell repertoire specific for a protein
  - Identification of immunogenic regions (T cell epitopes)

Quantitative analysis of the CD4 T-cell repertoire specific to therapeutic antibodies in healthy donors

(Maillere, FASEB J, 2011)

T cell amplification assay





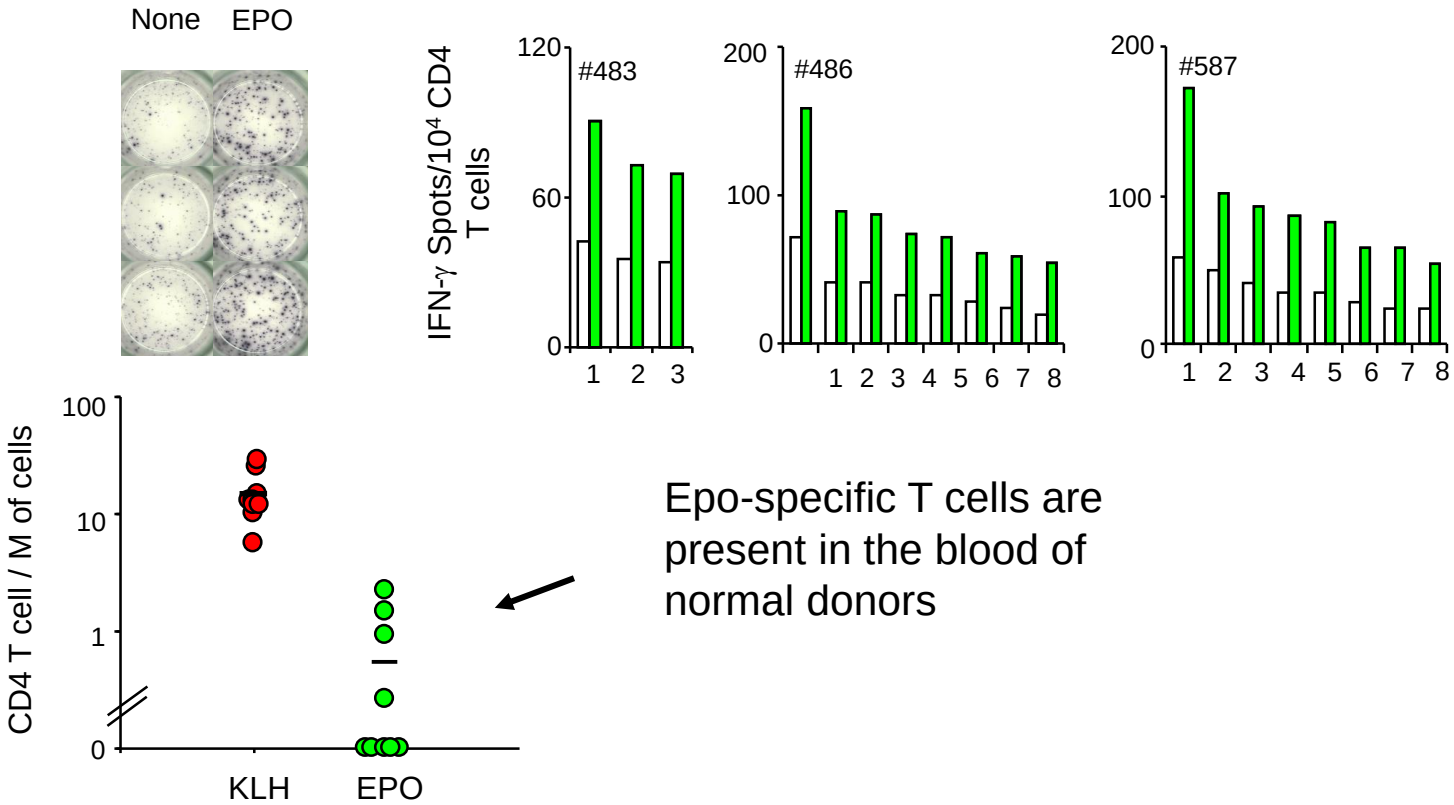
Predictive T cell assays discriminate **non immunogenic** antibodies to **immunogenic antibodies**

(one exception Bevacizumab in cancer patients)

Quantification of the preexisting CD4 T-cell repertoire specific for human erythropoietin reveals its immunogenicity potential

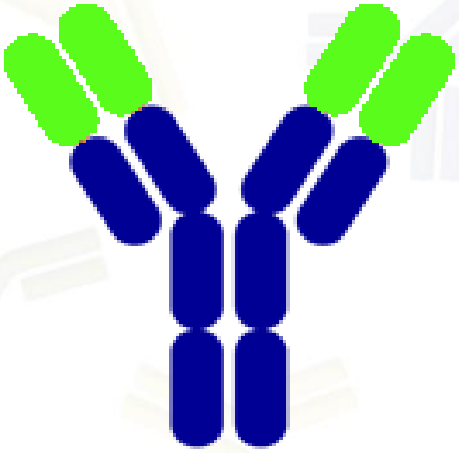
Maillere, Blood, 2010

- Pure Red Cell Aplasia (PRCA): antibody response to injected recombinant EPO.
- In the late 90s. changes in the formulation and injection mode of recombinant Epo were associated with a sudden increase in cases of PRCA.
- CD4 T cell response unknown

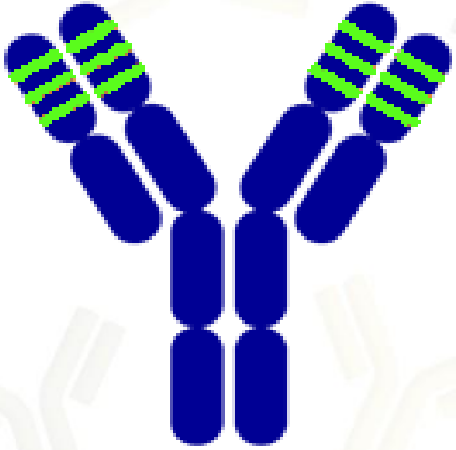


Epo-specific T cells are present in the blood of normal donors

Humanization of antibody sequences



Chimeric



Humanized

Fully human

- **RITUXIMAB**
  Anti-CD20
  Non-Hodgkin lymphoma: 0.6%
  SLE, RA, Sjogren: 17-50%

- **INFLIXIMAB**
  Anti-TNFα
  Crohn, RA, SPA: 30-50%

- **NATALIZUMAB**:
  Anti-α4 integrin
  Multiple sclerosis: 6-21%

- **ADALIMUMAB**
  Anti-TNFα
  RA: 30%

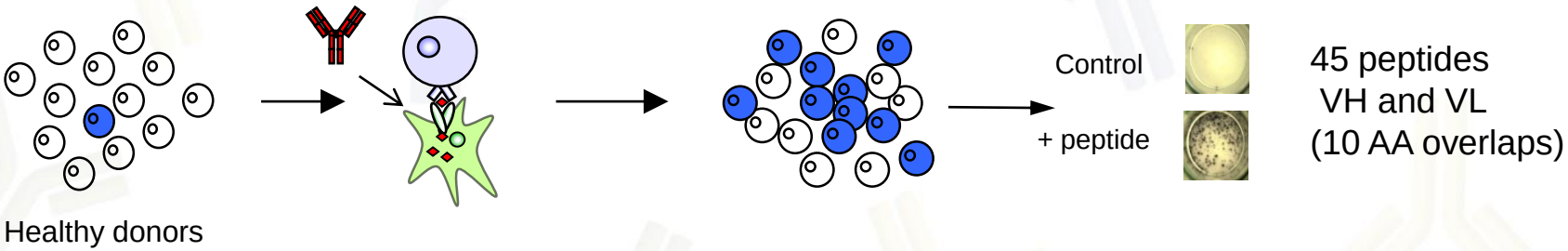
%: taux d'ADA

The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° [115303], resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution.'
www.imi.europa.eu

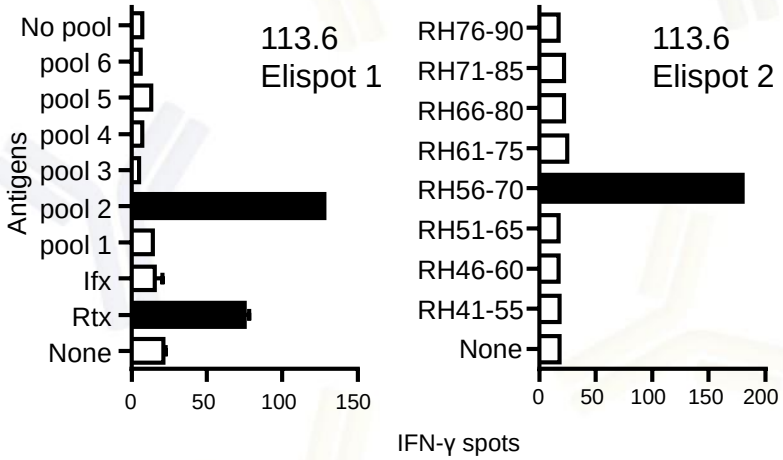
T cell epitope mapping of Rituximab and Infliximab

WWW.ABIRISK.EU

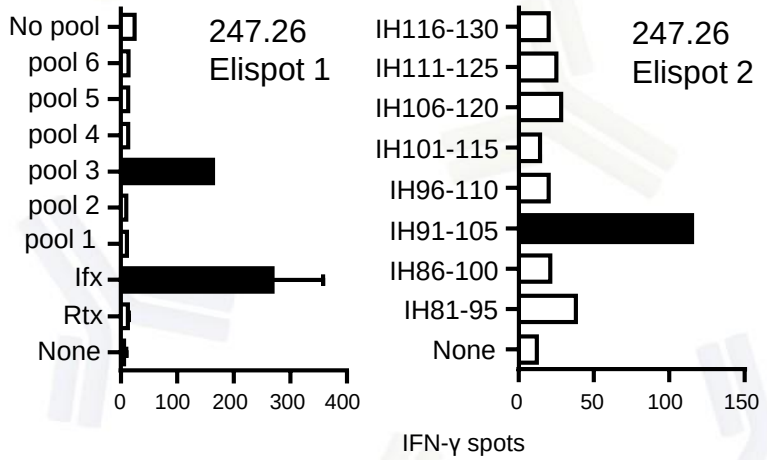
Long-term T cell assays



Rituximab



Infliximab



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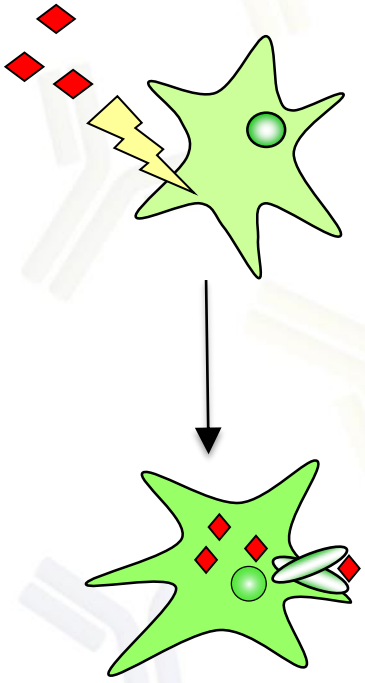
DC maturation assay



- qPCR MSD FACS
- Native antibodies are not active in this assay although they can be immunogenic
- Only artificially aggregated antibodies are active

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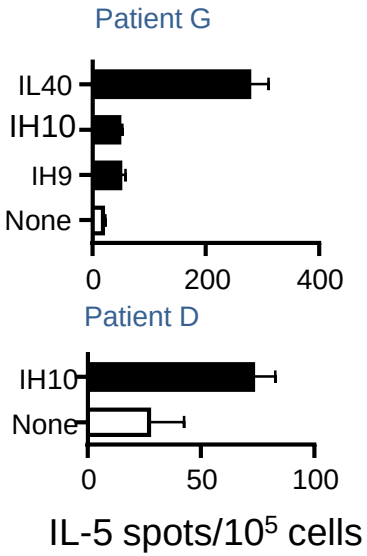
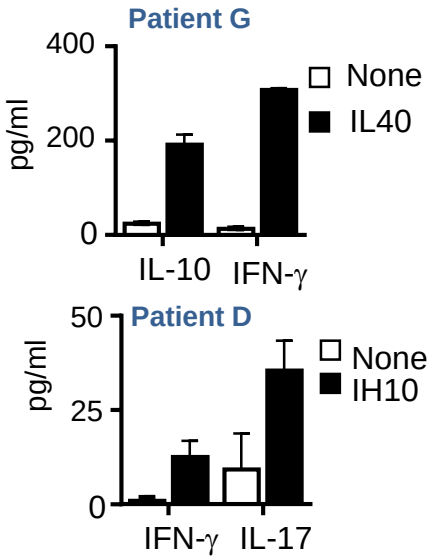
Clinical perspectives

- Improving immunomonitoring

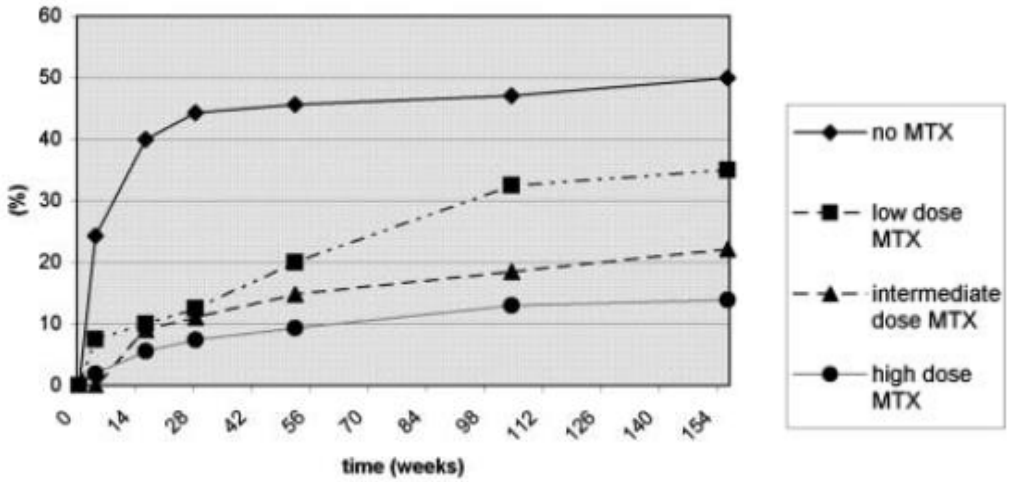
  ADA assays
  T cell immunomonitoring





- Combining with immunosupressive drugs

  Krickaert, 2012

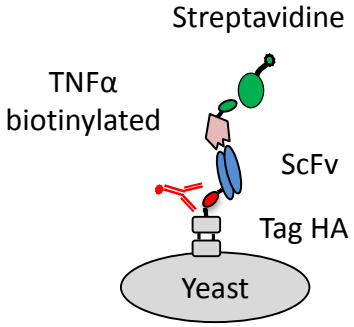


De-immunization of therapeutic proteins

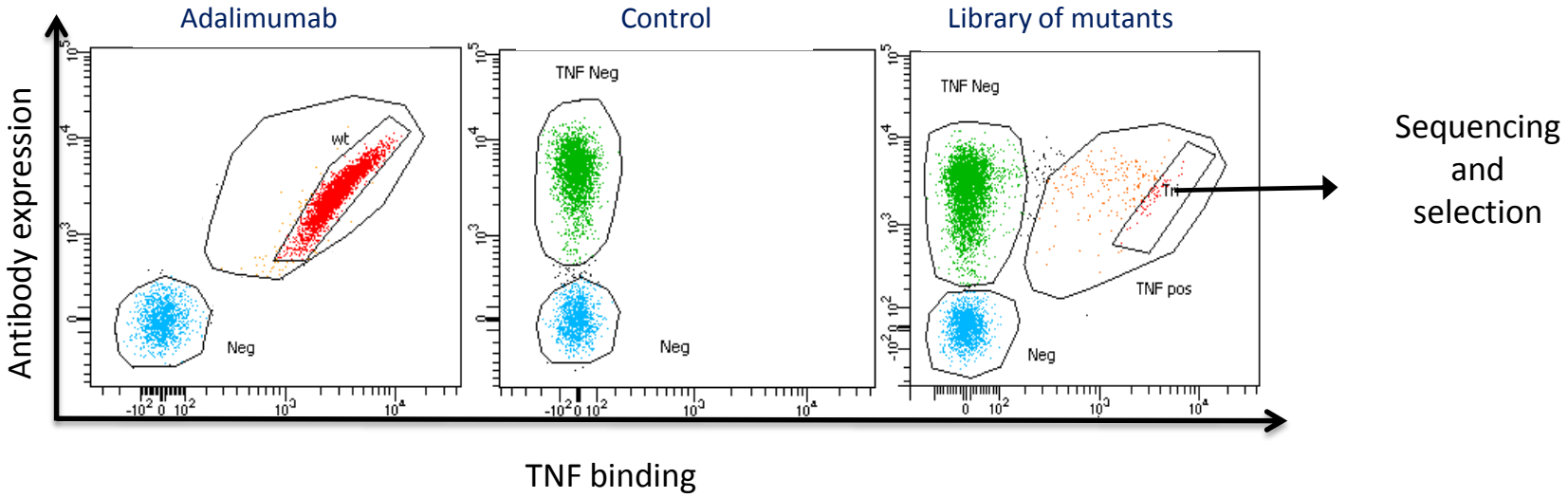
- Humanization is not sufficient
- Removal of T cell epitopes

Yeast display

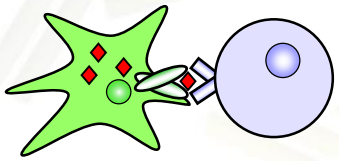
- Generation of libraries
- Active mutants
- Sorting by cytometry

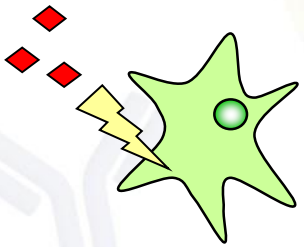


Library of mutants of Adalimumab



Conclusion
Global analysis of immunogenicity risk





- **Immunogenicity :**
  PK, efficacy: Risk for the company
  Allergic, autoimmune, CRS: Risk for the patients
- **Signal 1:** a large toolbox
  In silico, HLA binding assays, MAPPS, T cell assays…
  preliminary assessment, T cell epitope mapping, de-immunization, ranking of molecules.
- **Signal 2:**
  DC maturation, aggregation study
  How to use the provided information? In vivo?
  How to combine with signal 1 data?
- **Assessment of risk immunogenicity**
  - Prediction: focuses on product-related factors
  - Should be included in a global analysis of immunogenicity risk (treatment, patients)

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