

WP3

Evaluation and development of technologies for predicting immunogenicity

Co-leaders

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Objectives	Work package sub-tasks addressing the aims
<p>Aim 1 Evaluate clinical relevance and gain a greater understanding of technologies of prediction of immunogenicity.</p>	<ul style="list-style-type: none"> ✓ Evaluation of different T cell assay approaches ✓ Evaluation of different in silico prediction methods ✓ Identification naturally processed HLA peptides by MAPPs ✓ Mapping of CD4+ T-cell epitopes ✓ Peptide affinity for HLA class II
<p>Aim 2 Develop and assess novel prediction methods.</p>	<ul style="list-style-type: none"> ✓ In vitro modulation of dendritic cell function and activation by BP ✓ Evaluation of the Artificial Lymph Node system ✓ Relevance of innovative animal models
<p>Aim 3 Assess effects of aggregation on immunogenicity.</p>	<ul style="list-style-type: none"> ✓ Generation of post-translational modifications and aggregates and their characterization ✓ Test modified BPs for their effect in established and newly developed prediction models

Selection aligned with WP1 and WP2

- Therapeutic Antibodies

Chimeric: Infliximab, Rituximab

Humanized: Natalizumab

Fully human: Adalimumab

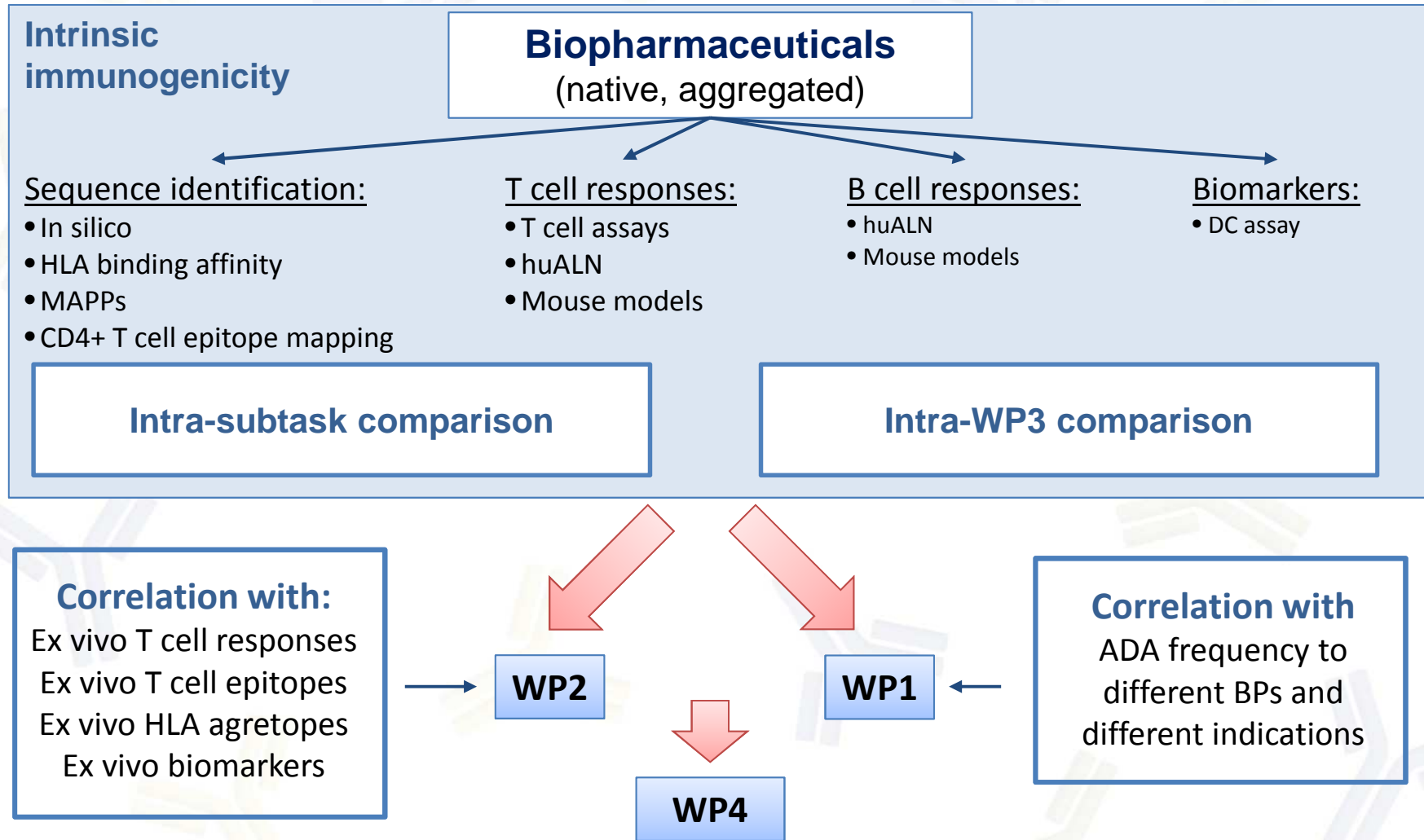
- Cytokine: (endogenous counterpart)

Interferon beta

- Coagulation factor

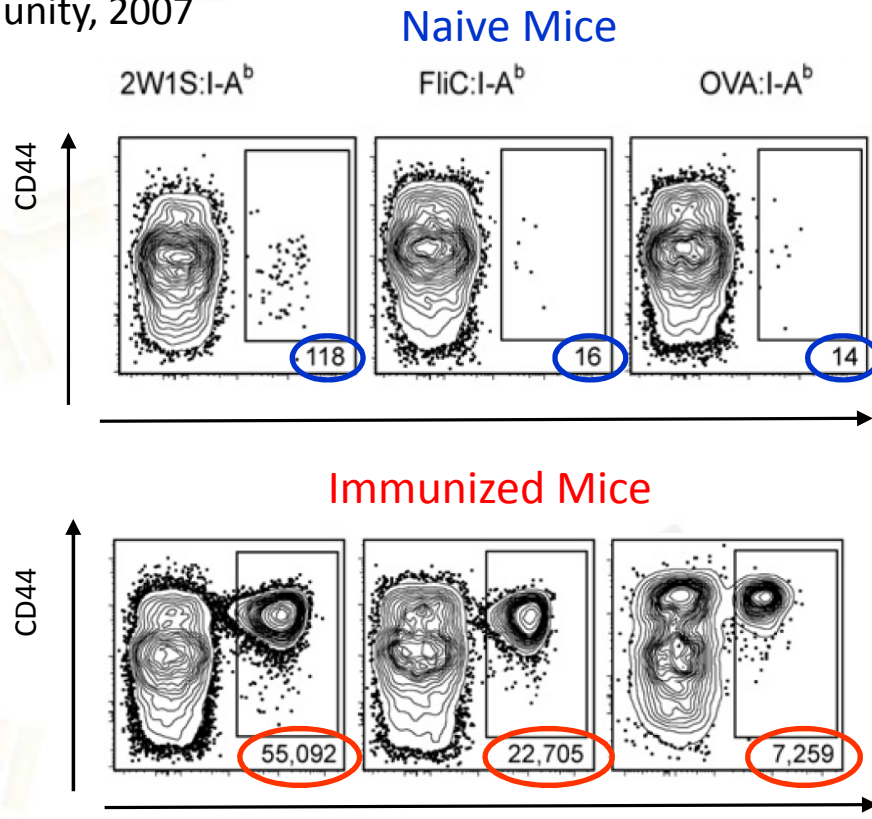
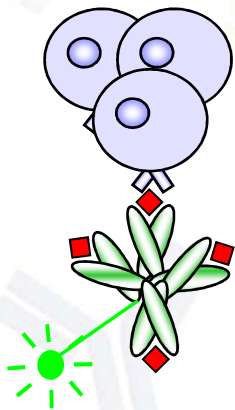
(Replacement protein: no endogenous/altered counterpart)

FVIII



Naive CD4⁺ T Cell Frequency Varies for Different Epitopes and Predicts Repertoire Diversity and Response Magnitude

Moon et al, Immunity, 2007



The number of epitope-specific naive CD4 T cells is **variable**

Tet⁺

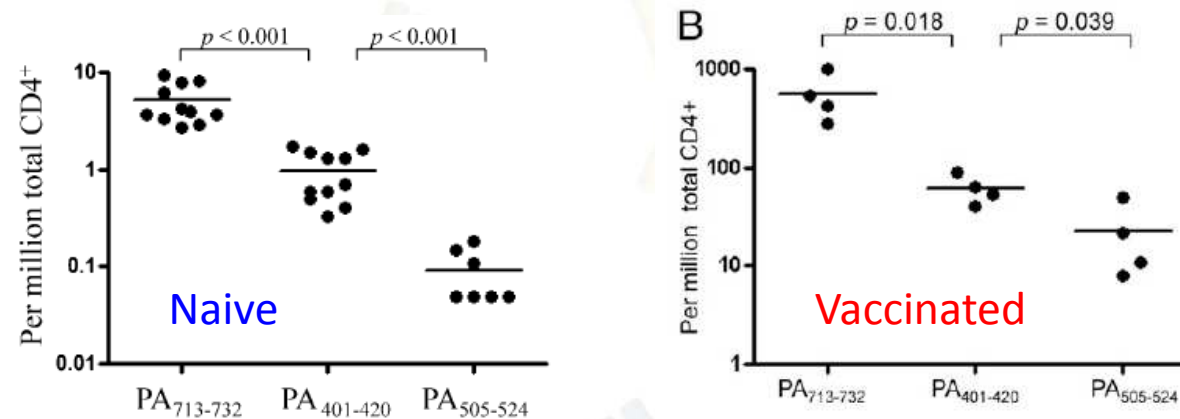
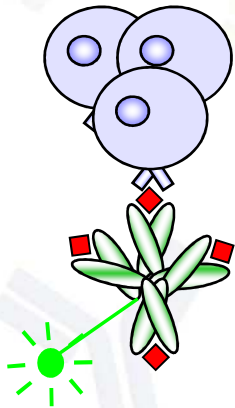
Amplitude of the T cell response is related to the number of epitope-specific naive CD4 T cells

Tet⁺

Frequency of Epitope-Specific Naive CD4⁺ T Cells Correlates with Immunodominance in the Human Memory Repertoire

Kwok, J Immunol, 2012

- T cell response to protective antigen of *Bacillus anthracis*
- Three HLA-DRB1*01:01 restricted T cell epitopes

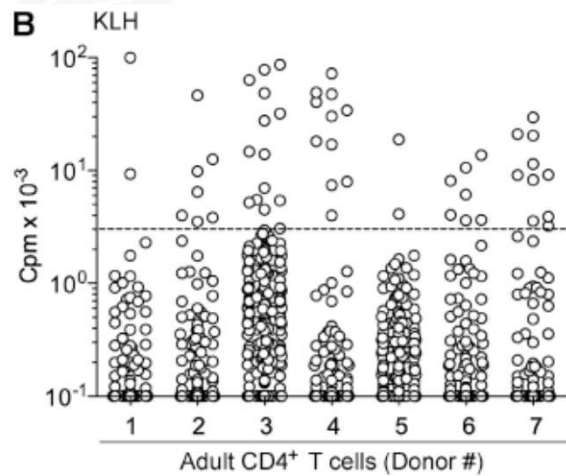


Epitope	Vaccinated	Unvaccinated	Approximate Expansion
PA ₇₁₃₋₇₃₂	500 per million ^a	5 per million	100-fold
PA ₄₀₁₋₄₂₀	60 per million	1 per million	60-fold
PA ₅₀₅₋₅₂₄	22 per million	0.1 per million	200-fold

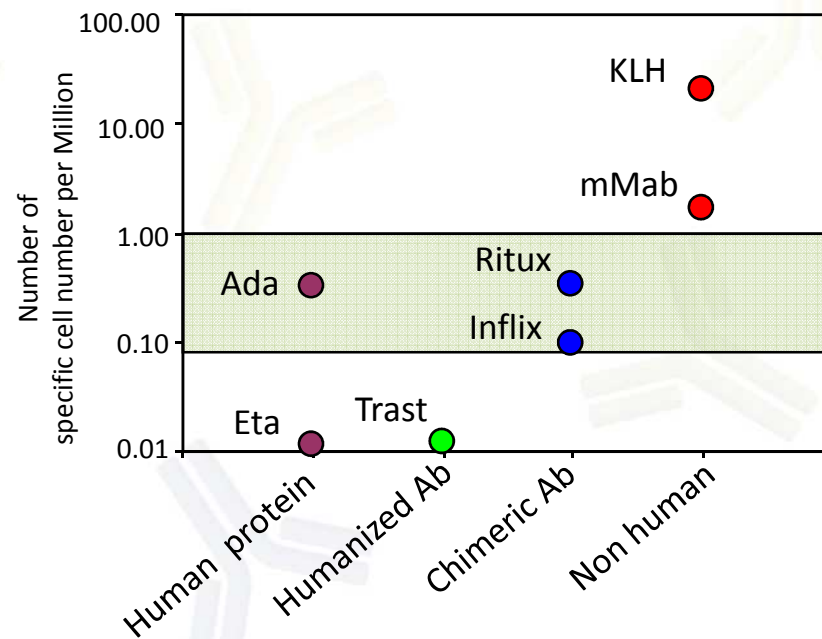
^aFrequency per million total CD4⁺ T cells.

Evaluation of the number of pre-existing T cells (magnitude)

Polyclonal amplification of CD4 T cells
(Geiger et al, JEM, 2009)



Antigen-specific amplification
(Delluc et al, FASEB J, 2011)

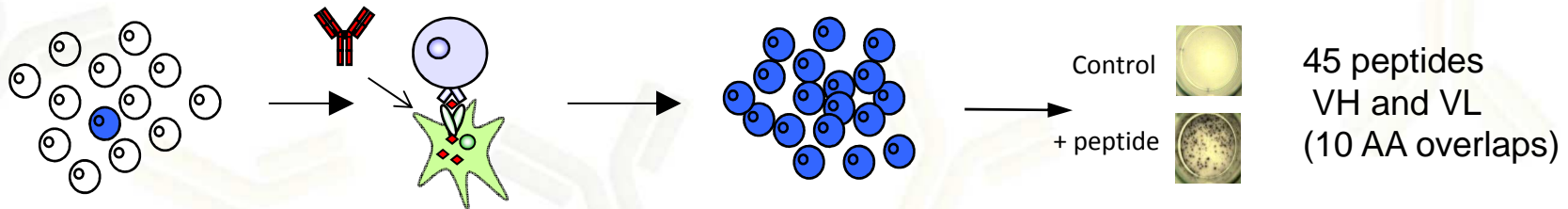


European CROs

Aim 1: T cell epitope mapping

Identification of immunogenic sequences (CD4 T cell epitopes)

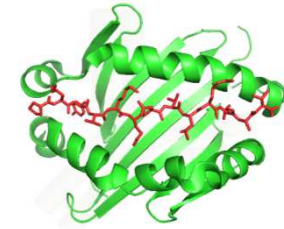
- T cell assay: Naive donors, diverse HLA-DR allotypes



- In vitro T cell response to Rituximab

HLA-typed donors:

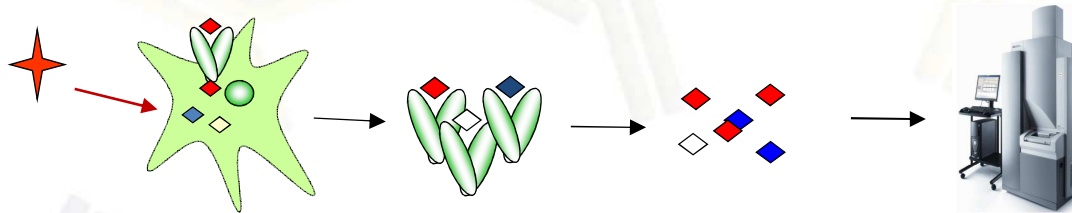
No crossreactivity with Adalimumab



Identification of immunogenic sequences (CD4 T cell epitopes)

- In silico prediction
IEDB : SMM, ARB, TEPITOPE, NetMHCIIpan, ANN, Consensus
Merck Serono : Lexitope, Antipred

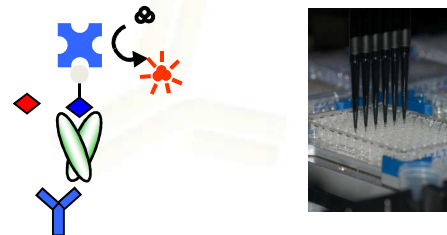
- MAPPS assays
MHC-associated Peptide Proteomics



Identification of peptides displayed by DC

Effect of aggregation

- Binding assays

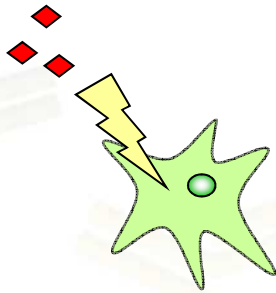


Evaluation of peptide affinity for HLA-DR molecules

10 HLA-DR allotypes

Aim 2: New approaches

- Dendritic cell maturation assay



Potential interactions of BP and aggregates with innate receptors present at the surface of the DC

Standardized conditions (surface biomarkers, gene transcripts)

- Artificial lymph node



Bioreactor for long-term cell culture

Human PBMC-based in-vitro system mimicking human lymph node structure.

T and B cell activation

Giese et al, J Bioethnology, 2010

Aim 2: Humanized mice models



- Immunodeficient mice engrafted with CD34+ human stem cells
 - BRGSF™ : Balb/c Rag2^{-/-} IL2Rγc^{-/-}
Sirpα^{NOD} : Inhibitory signal for murine phagocytes
Flk2^{-/-} : Receptor for Flt3-L, to reduce murine DC development
 - Rag2^{-/-} IL2Rγc^{-/-} /Perf^{-/-}
HLA-A2^{+/+} DR1^{+/+} IAβ^{-/-} β2m^{-/-}: Thymus education

CD34+ cells from HLA-DR1-typed cord blood

- FVIII-deficient HLA-DR1-transgenic mice

FVIII immunogenicity in Hemophiliacs

Large deletion -> 30% antibody response

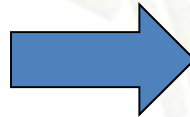
Missense mutations -> generally 5% antibody response

but 15–50% for five highly recurrent missense mutations

(Arg593Cys, Tyr2105Cys, Arg2150His, Trp2229Cys, or Pro2300Leu)

Hydrodynamic injection
of vectors encoding :
FVIII

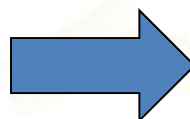
Mutated FVIII



HLA-A2^{+/+} DR1^{+/+}
IAβ^{-/-} β2m^{-/-}
FVIII^{-/-}



iv administration
of therapeutic FVIII



Tolerance?

- Large comparative project
 - Several technologies for the same approach (T cell assays, animal models)
 - Same technologies performed in several laboratories
 - Comparison with data provided by WP1 (ADA+/-) and WP2

- Improvement of immunogenicity prediction
 - Common SOP and standardization
 - Combination of predictive approaches

Immunogenicity = immunogenicity potential + adjuvanticity
In vitro/ in vivo studies

- Biopharmaceuticals : opportunity to address basic immunological issues
 - Well characterized products (homogeneity, few aggregates, no endotoxin), Human sequences, clinical trials and post-marketing observations
 - Immunogenicity of self-proteins and tolerance in humans

WP3 partners

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