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WP3

Evaluation and development of technologies for predicting immunogenicity

Co-leaders

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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.'

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Objectives of WP3

Objectives	Work package sub-tasks addressing the aims
Aim 1 Evaluate clinical relevance and gain a greater understanding of technologies of prediction of immunogenicity.	✓ 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
Aim 2 Develop and assess novel prediction methods.	✓ In vitro modulation of dendritic cell function and activation by BP ✓ Evaluation of the Artificial Lymph Node system ✓ Relevance of innovative animal models
Aim 3 Assess effects of aggregation on immunogenicity.	✓ Generation of post-translational modifications and aggregates and their characterization ✓ Test modified BPs for their effect in established and newly developed prediction models

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Biopharmaceuticals

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

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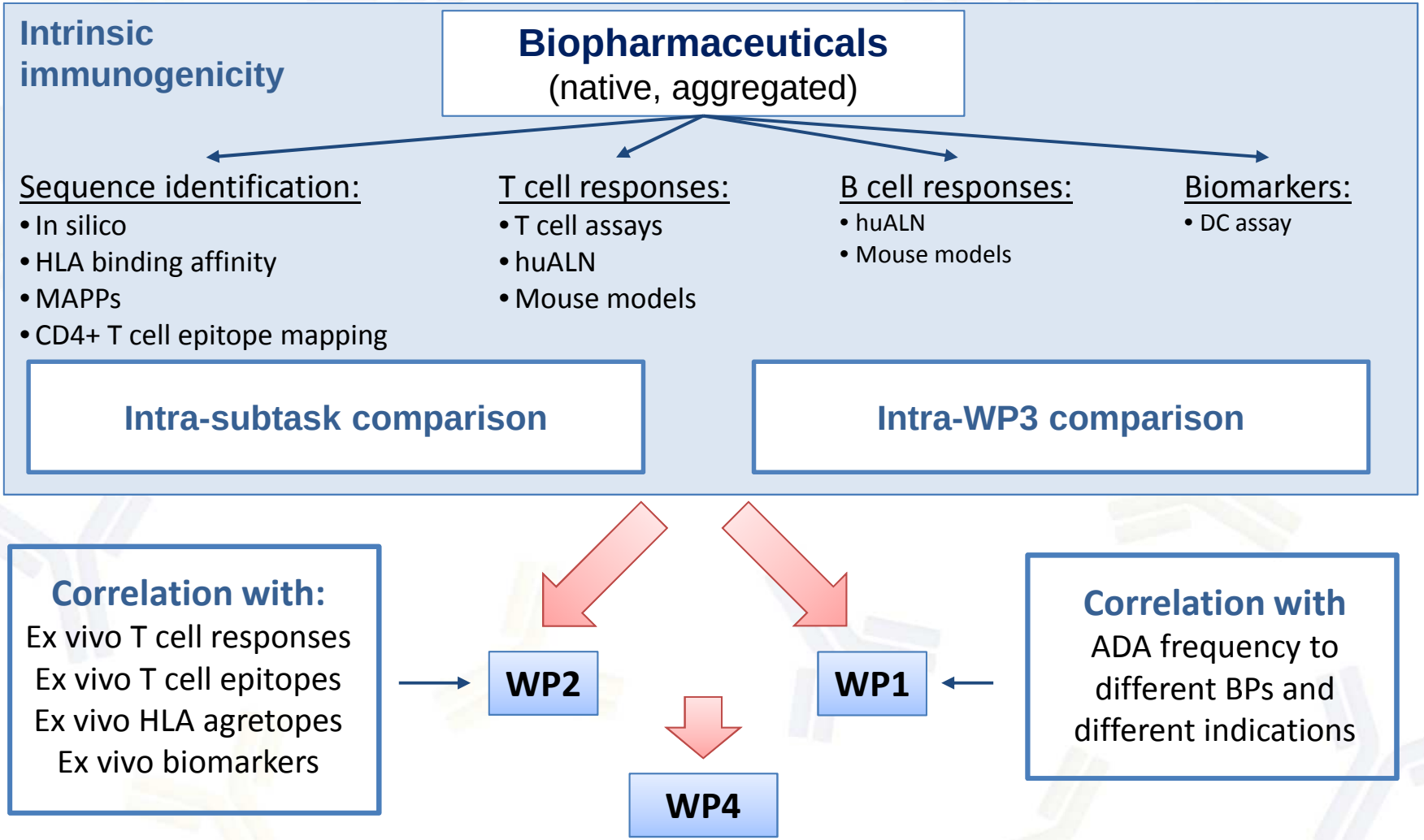
Data collection and comparison



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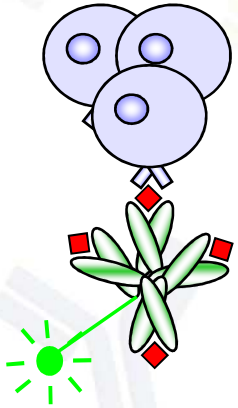
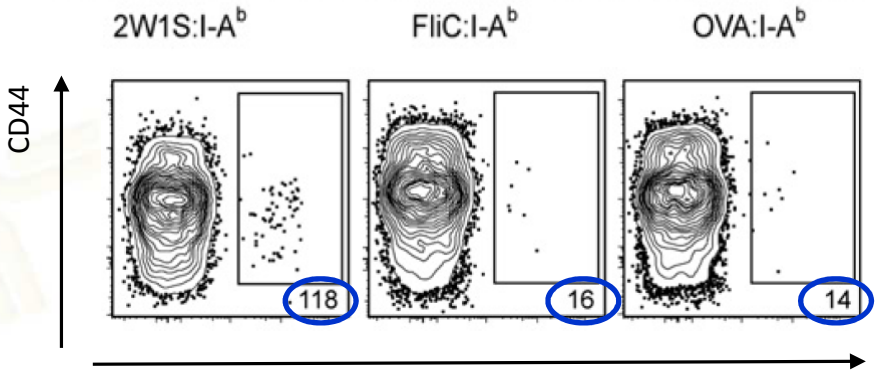
Aim 1 : T cell assays and naive T cell repertoire

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

Moon et al, Immunity, 2007

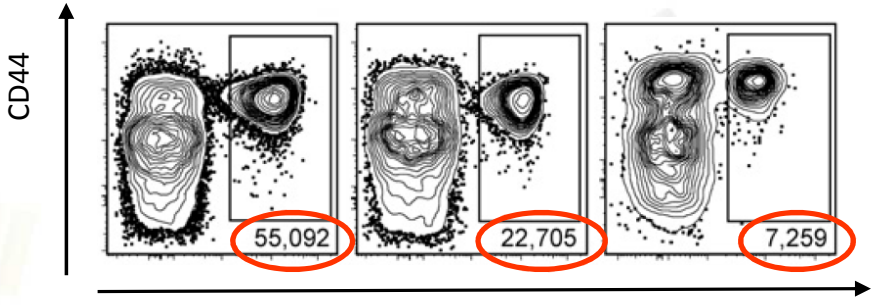


Naive Mice



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

Immunized Mice



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

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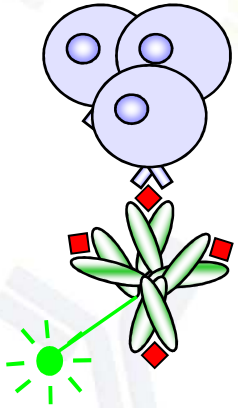
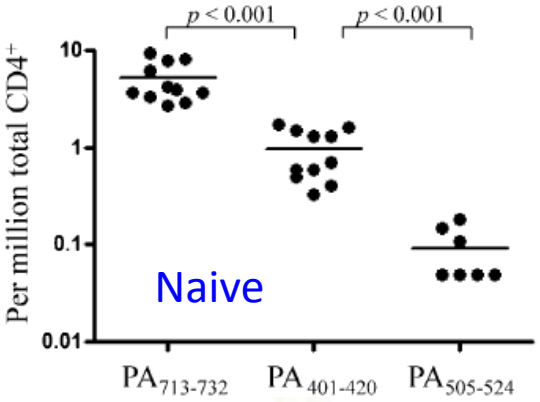
Aim 1 : T cell assays and naive T cell repertoire

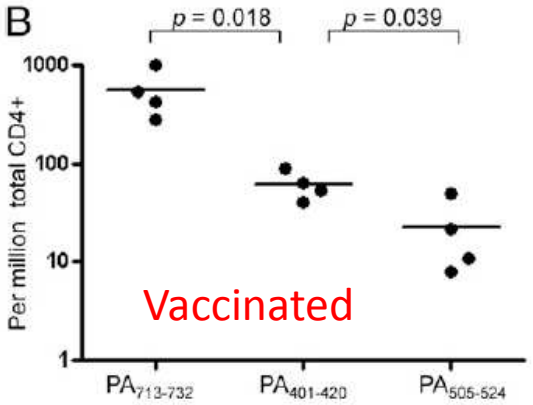
Frequency of Epitope-Specific Naive CD4$^+$ T Cells Correlates with Immunodominance in the Human Memory Repertoire

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

[a]Frequency per million total CD4$^+$ T cells.

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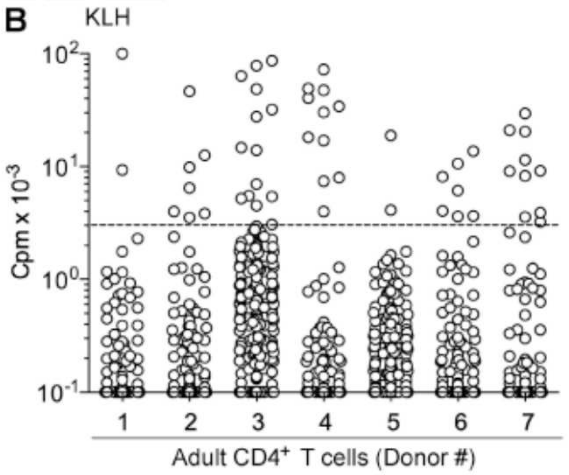
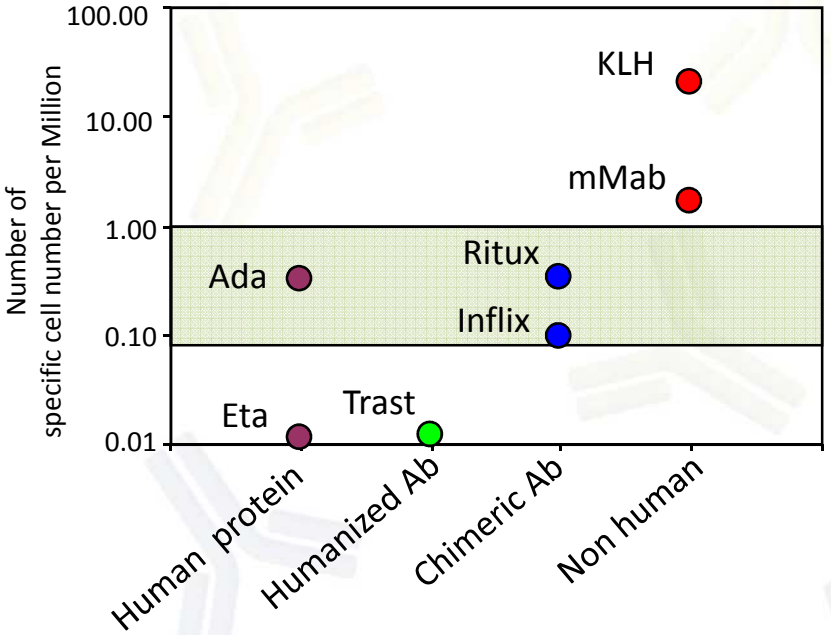
Aim 1 : T cell assays and naive T cell repertoire

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

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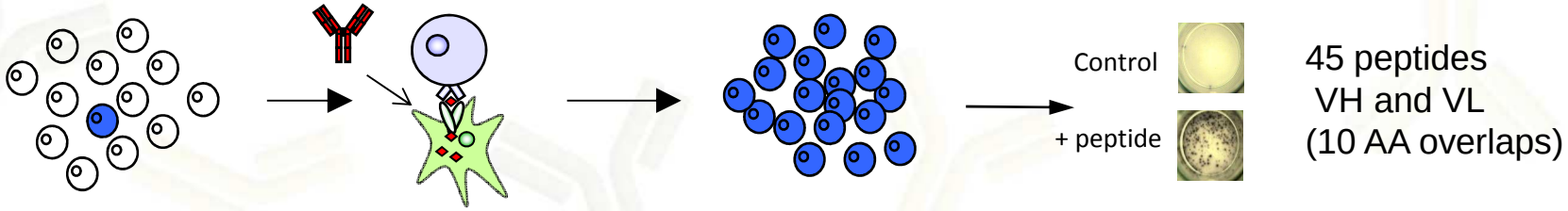
Aim 1: T cell epitope mapping

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

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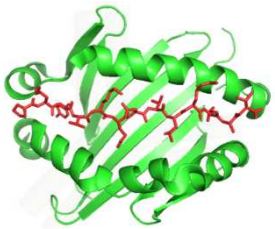
Aim 1: T cell epitope mapping (2)



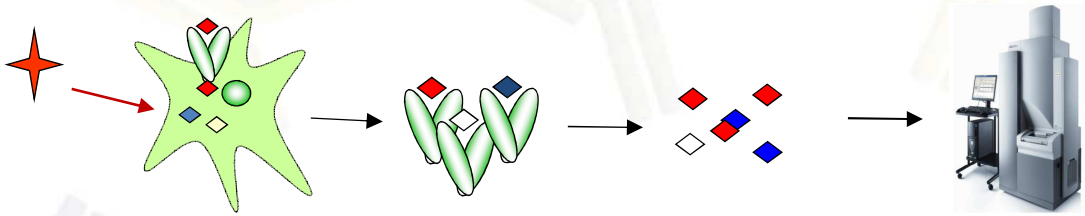
Identification of immunogenic sequences (CD4 T cell epitopes)

- *In silico* prediction

  IEDB : SMM, ARB, TEPITOPE, NetMHCIIpan, ANN, Consensus
  Merck Serono : Lexitope, Antipred

- MAPPS assays

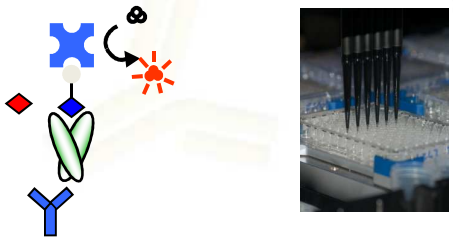
  MHC-associated Peptide Proteomics



Identification of peptides displayed by DC

Effect of aggregation

- Binding assays



Evaluation of peptide affinity for HLA-DR molecules

10 HLA-DR allotypes

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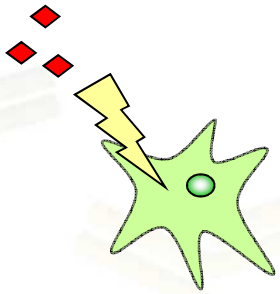
Aim 2: New approaches

- 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

Giese et al, J Bioetchnology, 2010

Bioreactor for long-term cell culture

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

T and B cell activation

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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$^{-/-}$ ß2m$^{-/-}$: Thymus education

  CD34+ cells from HLA-DR1-typed cord blood

- FVIII-deficient HLA-DR1-transgenic mice

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Moderate heamophilia A in FVIII deficient mice

FVIII immunogenicity in Heamophiliacs

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

$HLA\text{-}A2^{+/+}\ DR1^{+/+}$

$IA\beta^{-/-}\beta2m^{-/-}$

$FVIII^{-/-}$

iv administration of therapeutic FVIII

Tolerance?

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Conclusions

- 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

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