

# **ABIRISK WP2**

## **Scientific update**

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Vincent Mikol

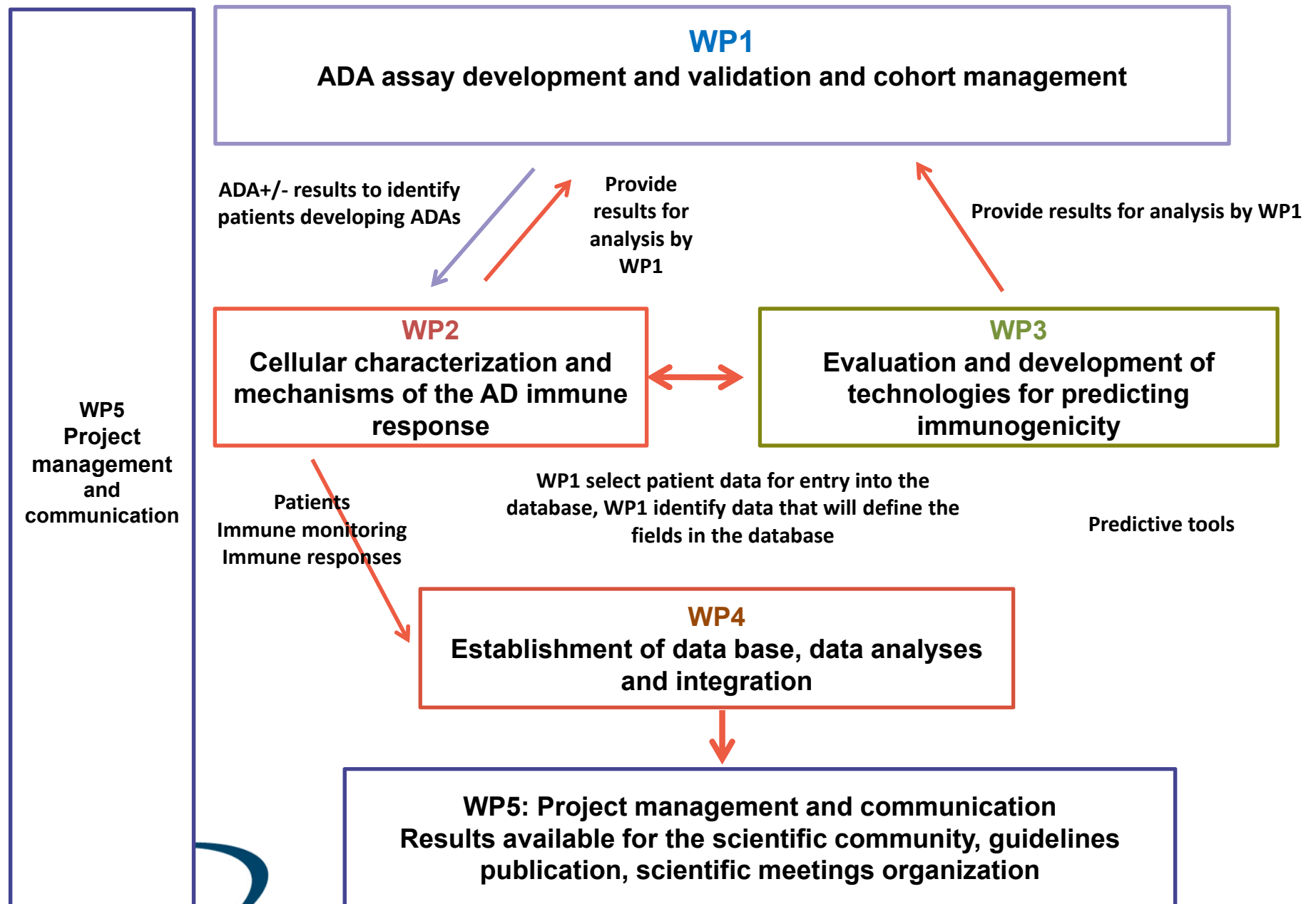
**EIP, Vilamoura, Portugal**

24<sup>th</sup> February 2016



Innovative Medicines Initiative





# Scientific Milestones

Cellular mechanisms	M2.3	Feasibility of the MAPPs assay in patient samples.
	M2.5	Pilot experiments to assess the role of Bregs and Tregs in AD immunogenicity
	M2.9	Pilot experiments to assess the role of Tfh cells in AD immunogenicity
	M2.10	Feasibility of the PBMC assay in patient samples
Characterization of ADA	M2.2	Generation of ADA-specific B cell clones
	M2.6	Feasibility of determination of the glycosylation pattern of FVIII-specific ADA.
	M2.7	Predictive value of BAB for NAB development Patients inclusion completed
	M2.11	Crystal structure determination for FVIII- specific ADAs in complex with FVIII

# WP2: Summary

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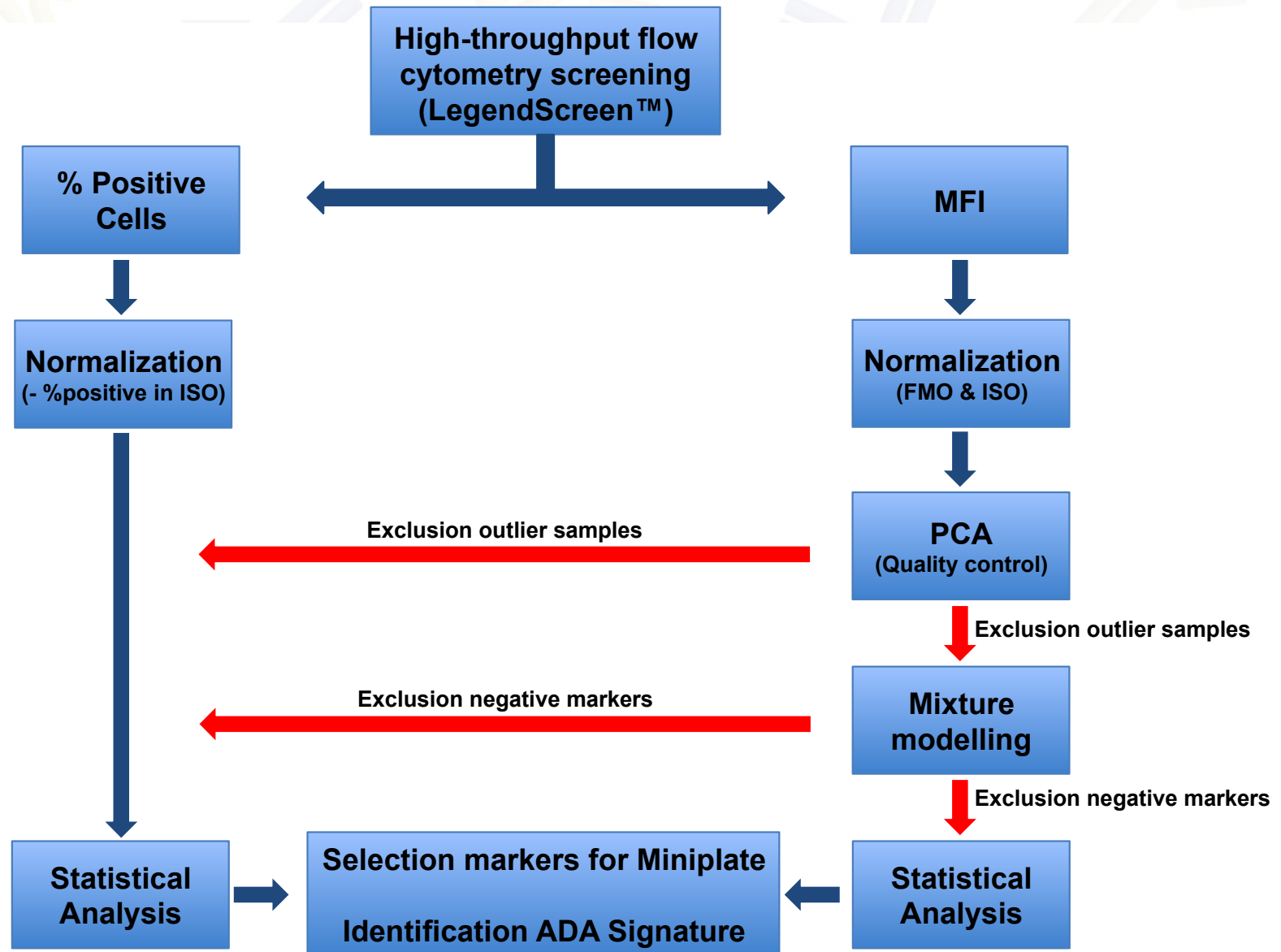
- **WP2.1: Cellular mechanisms of Immunogenicity**
  - 'Signature' of biomarkers for cellular mechanisms identified for IFN $\beta$  responses
  - T cell responses to Infliximab and IFN $\beta$  characterized
- **WP2.2: Characterization of ADA**
  - Glycosylation of anti-FVIII antibodies
  - ADA cloned for 5/6 drugs
  - Crystal structures of ADA-drug complexes being assessed
- **WP2.3: Genetic pre-disposition**
  - Concordance across cohorts/drugs
    - Previously reported associations
    - GWAS
  - Risk: Low numbers for some cohorts

# WP2.1 Cellular Mechanisms

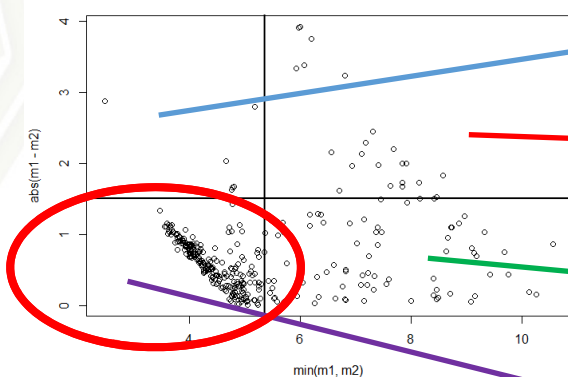
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- **D2.4 Identification of markers correlating with ADA status in MS patients receiving IFN $\beta$  (markers exclusive from disease and treatment)**
  - 'Signature' identified.
  - Similar approach for RA and Lupus initiated.
- **D2.6 Infliximab-specific T cells are detectable in treated patients,**
  - mainly in ADA+ patients who have developed HR and out of therapy.
  - IFX induced IL-10 production may impair the detection of immune response to IFX itself
  - Evaluation of proliferative response (even with peptides) underestimates the exact incidence of CD4+ T cells sensitization in treated patients
- **D2.26 SLE - Characterised potential exhausted B cell state**
  - Further evidence for defective gut homing in SLE which appeared to be restored after RTX treatment only in patients w/o adverse reaction and responding
  - New markers for immature B cells and IL-10+ B cells; new phenotype for T1 and T2 cells
- **D2.23 Clonality analysis and epitope mapping of T- and B-cell AD responses from patients with RA**
  - Can specificity can be connected to BCR/TCR repertoire?
- **D2.25 MAPPs in patient samples**
  - Sequences to be investigated in short-term B cell response

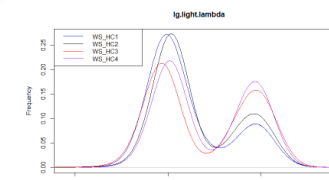
# Cross-sectional MS Cohort Immunophenotyping Pipeline



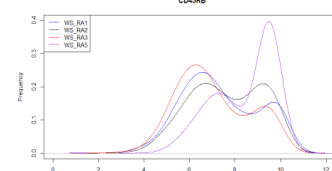
# Mixture Modelling: Selection of markers expressed in the different PBMC subsets



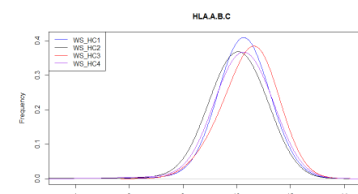
BIMODAL, one mode positive



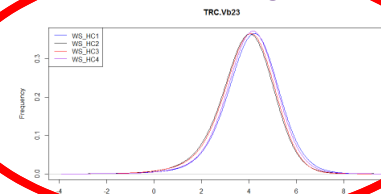
BIMODAL, two modes positive



UNIMODAL, positive



UNIMODAL, negative

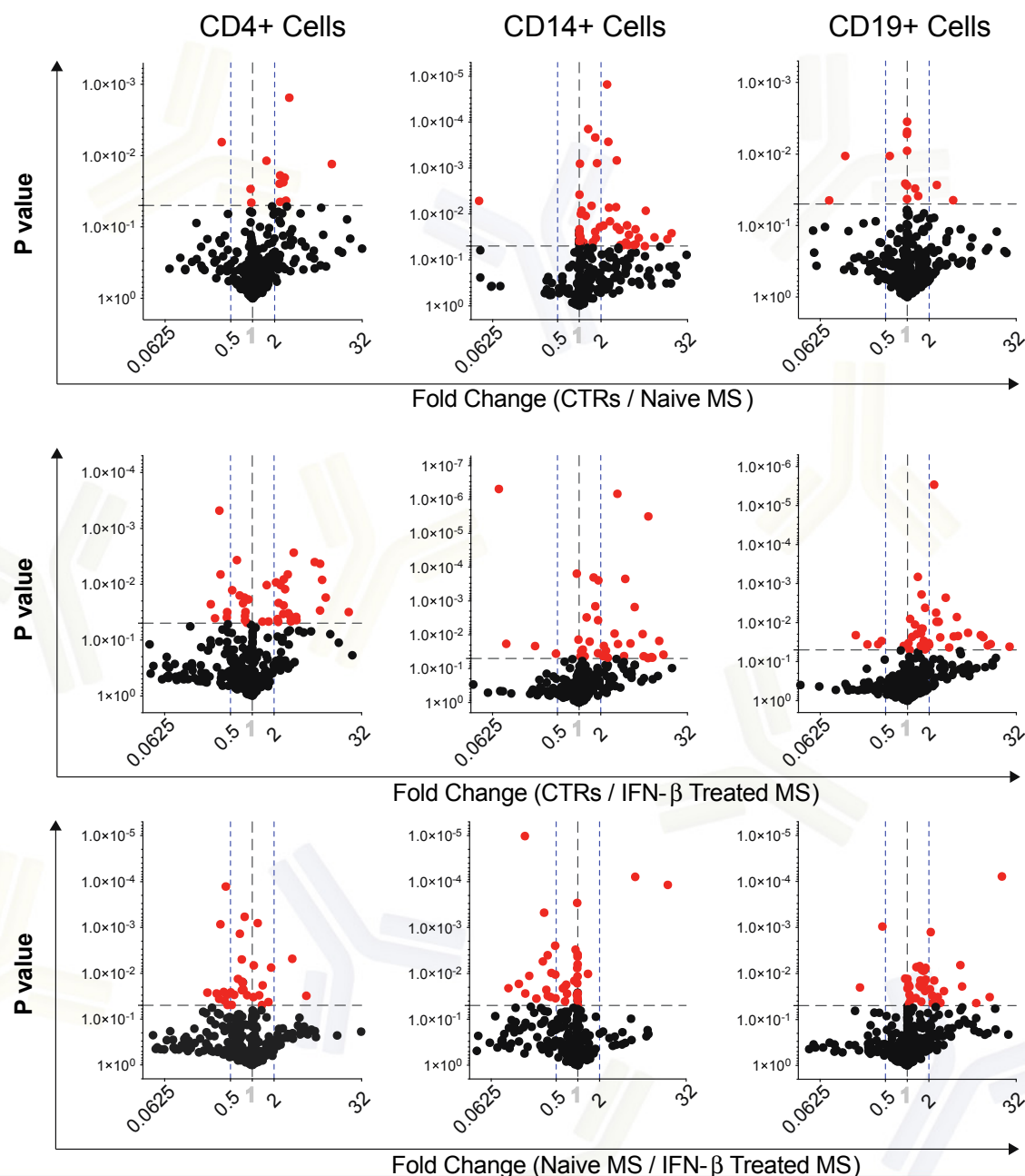


Markers with unimodal distributed florescence overlapping with isotype controls and excluded from further analysis

- I. 178 markers for CD19+ cells
- II. 172 markers for CD14+ cells
- III. 177 markers for CD4+ cells

**Cyprien Mbogning**

# Global phenotyping of immune cells reveals markers on distinct cell types associated with MS and IFN $\beta$ treatment





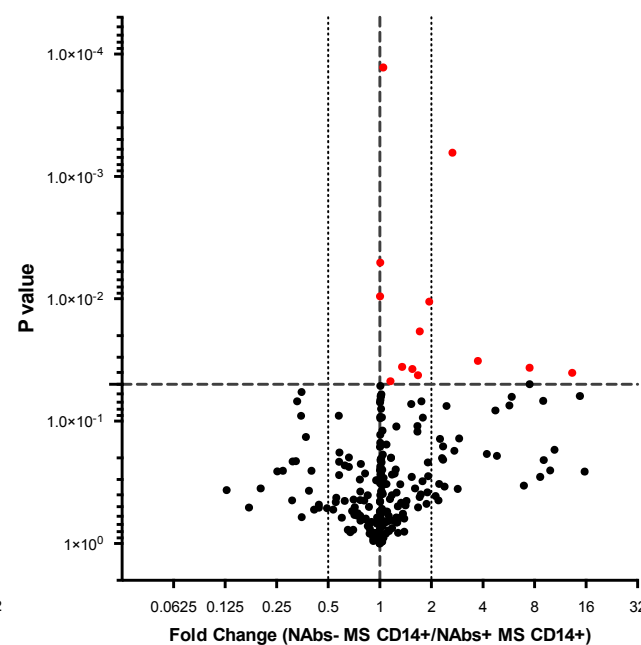
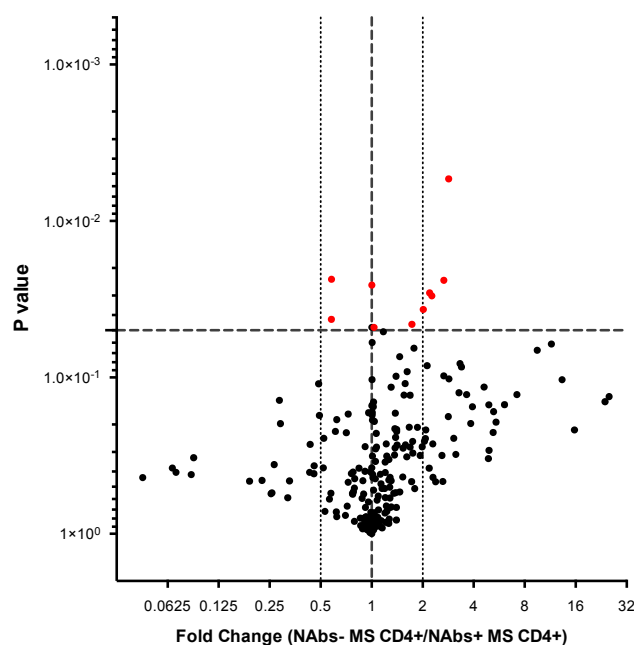
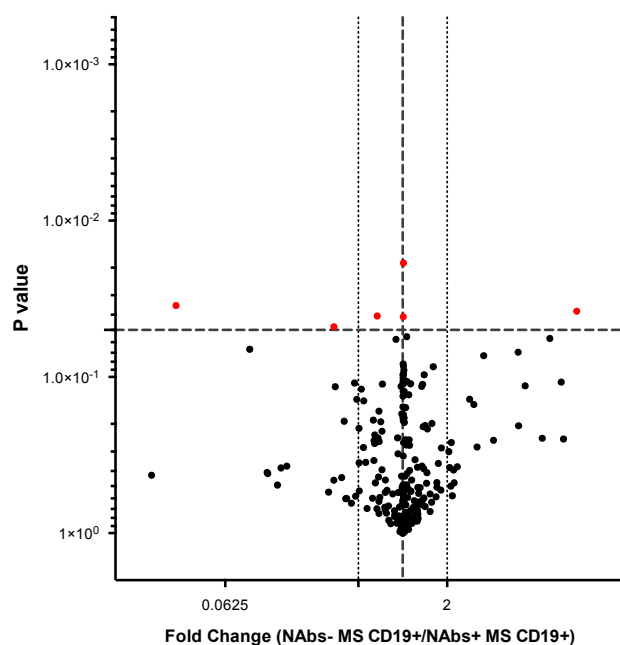
# ADA Immune Signature

(Nabs<sup>+</sup> VS NAbs<sup>-</sup> MS Patients)

## B cells

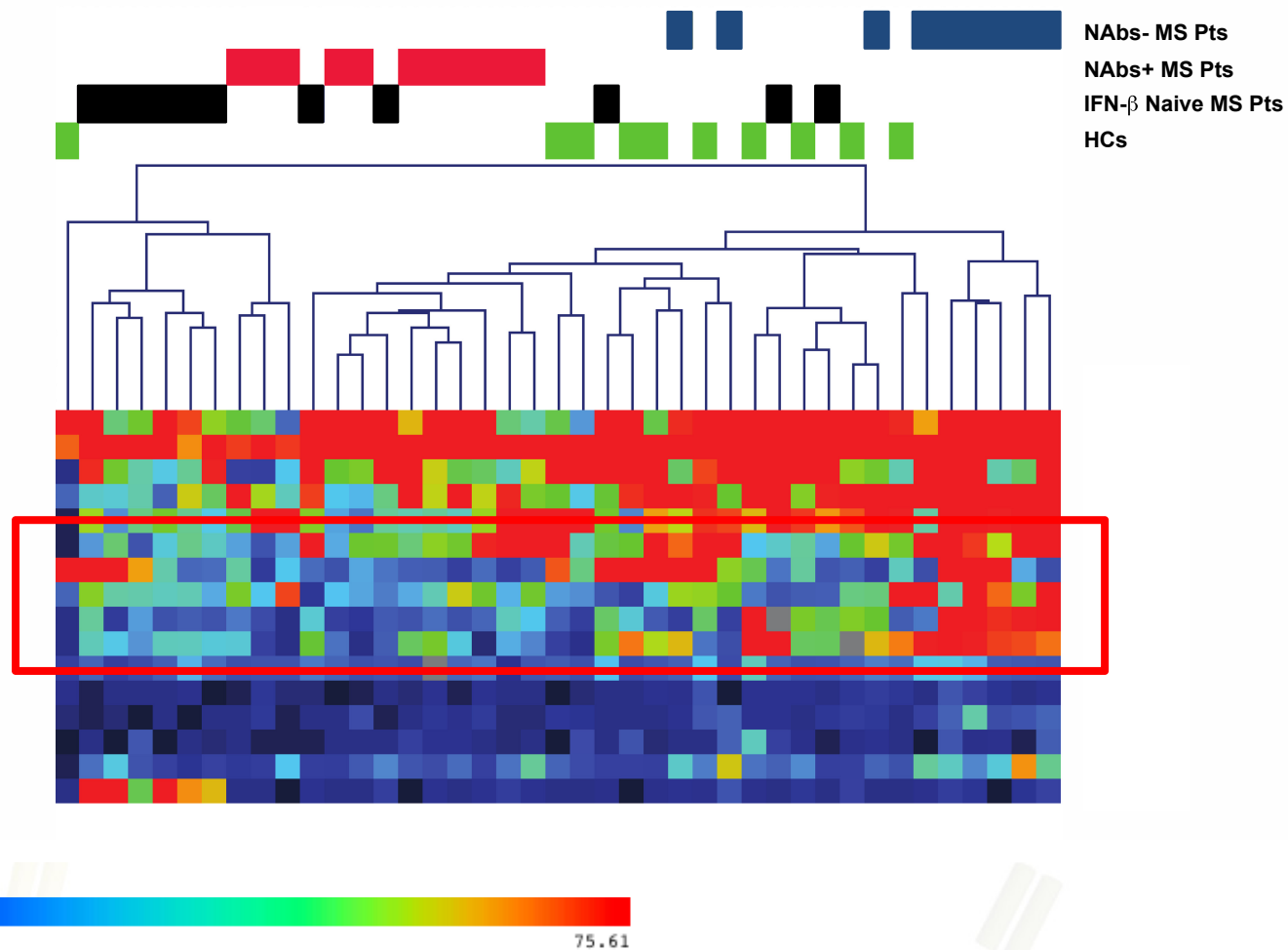
## CD4<sup>+</sup> T cells

## CD14<sup>+</sup> cells



(Percentage Positive Cells Analysis)

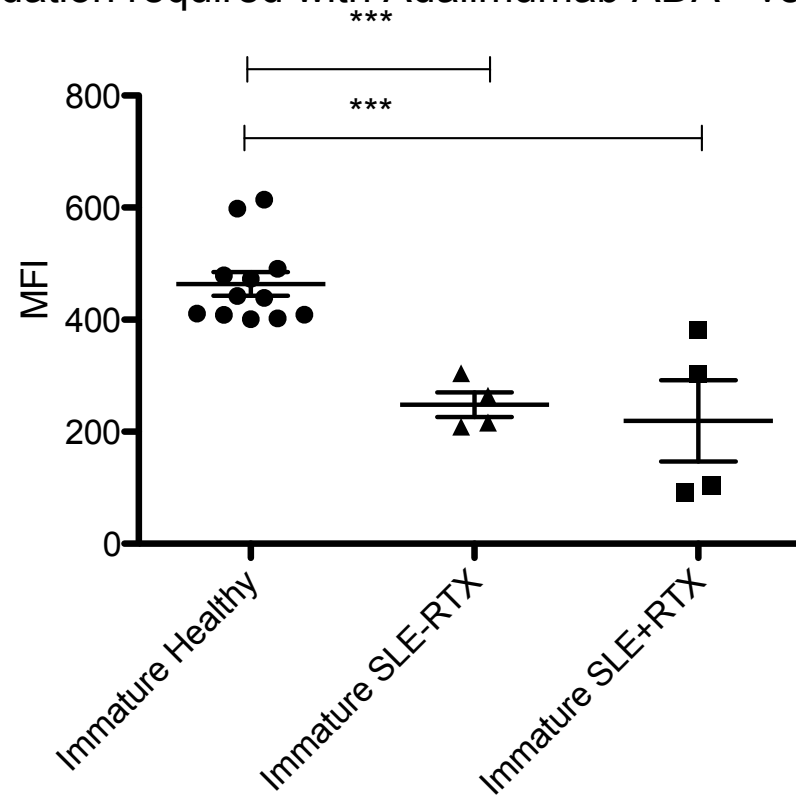
# Hierarchical Clustering of All Samples Screened Using The ADA Signature for CD14+ Cells



# B cell markers

- **Altered B cell markers (UCL)**

- Initial experiments have identified 5 specific markers
- Further validation required with Adalimumab ADA +ve and –ve patient samples

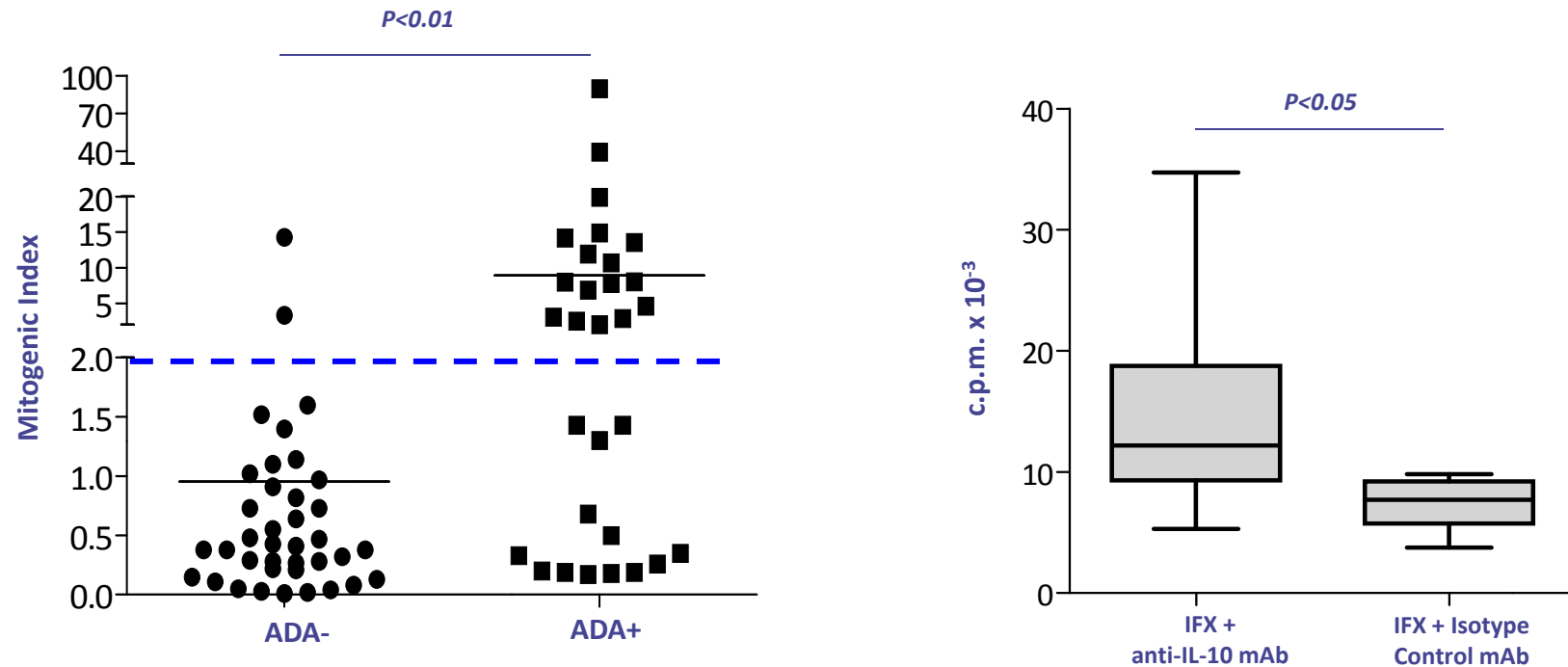


# T cell responses to therapeutic proteins

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- **Infliximab-specific T cells are detectable in treated patients,**
  - mainly in ADA+ patients
  - who have developed HR and out of therapy.
- **IFX induces IL-10 production both *in vitro* and *in vivo* (confirmed at clonal analysis) also in T cells that may impair the detection of immune response to IFX itself**
- **the only evaluation of proliferative response (even with peptides) underestimates the exact incidence of CD4+ T cells sensitization in treated patients**

# The T cell response to Infliximab correlates with ADA



IFX-induced proliferative response is impaired by IL-10



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DEGLI STUDI  
FIRENZE  
DIPARTIMENTO DI  
MEDICINA SPERIMENTALE  
E CLINICA

(Vultaggio A et al, submitted)



# Mapping T cell responses in IFN-beta:

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- Strong T cell responses to IFN beta protein and peptides are found in MS patients
- Several immunodominant epitopes have been identified that elicit T cell responses in patients and control (aa 31-60, aa 141-170)
- Preliminary experiments suggest a higher T cell response to IFN-beta proteins and peptides in NABs+ versus NABs- patients.

## Future Work:

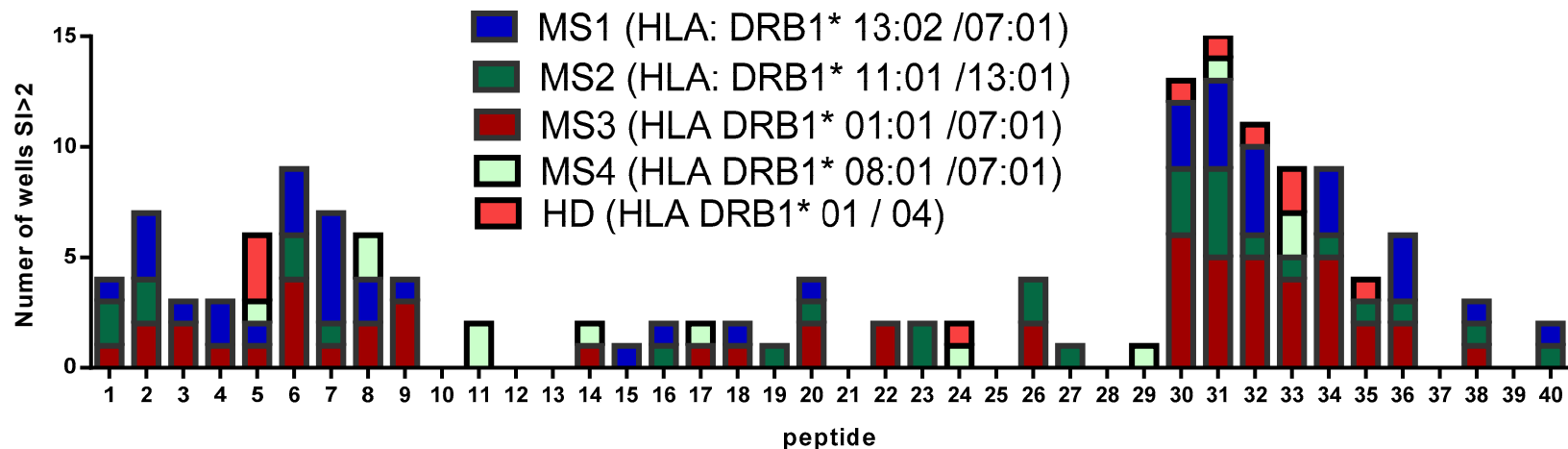
- Investigate more Nabs+ and NABs- patients.
- Demonstrate protein specificity of peptide reactive T cells
- Characterize the cytokine profile of T cells in NAB+ and NABs- MS patients.

# T cell response against Interferon beta in MS patients who develop neutralizing antibodies

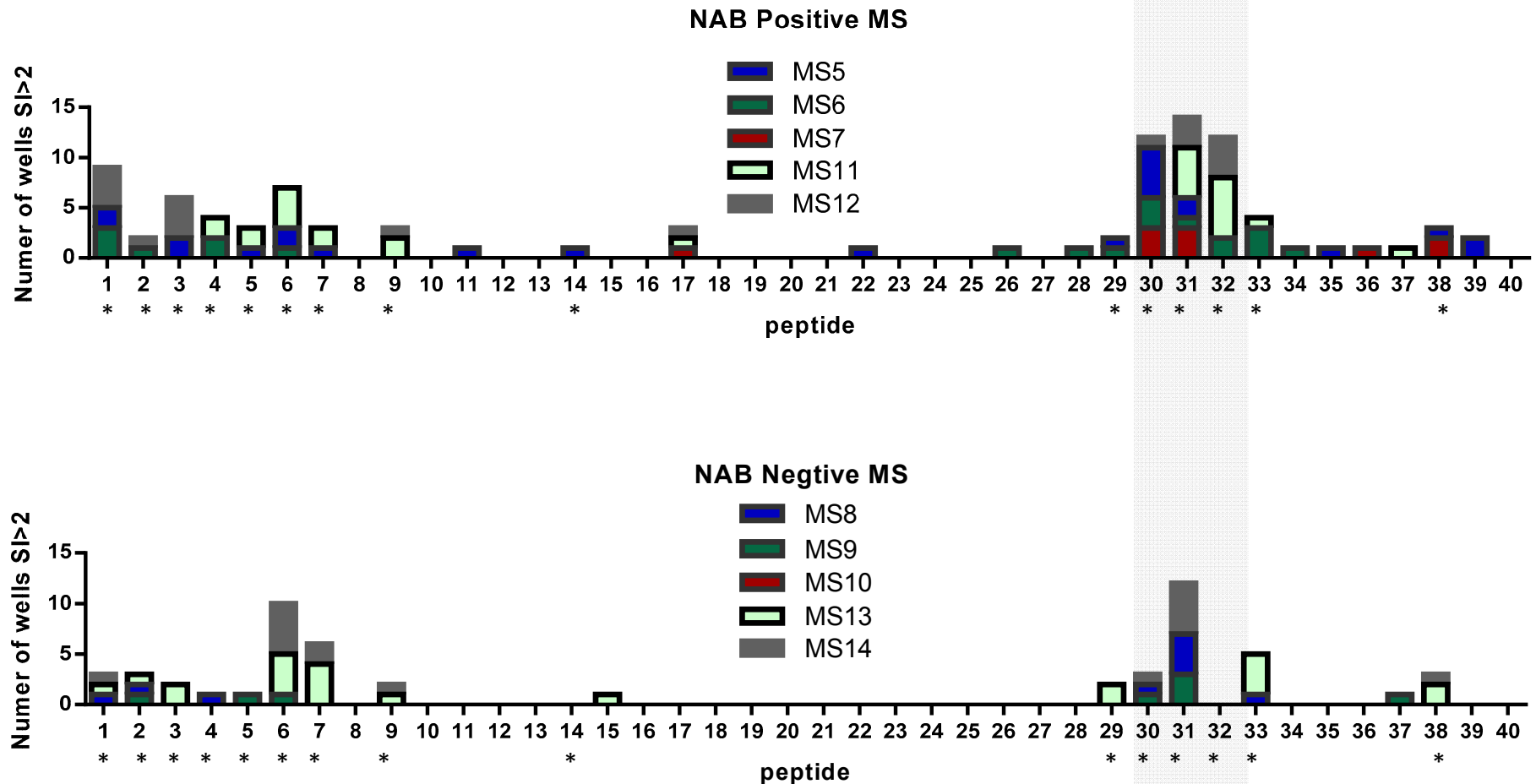
## T cell response to IFN-beta (TUM)

Patient responses differ in SI between ADA +ve and -ve (n = 14)

T cell lines cloned recognize one or two immunodominant peptides



# Response to IFN-beta 1a /1b peptides



\*selected immunodominant peptides  
for 3rd set up (MS patients 11, 12, 13, 14) experiments



## WP2.2 ADA Characterisation

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- **Generated a repertoire of monoclonal ADA specific for biotherapeutic proteins**
  - Sequenced and expressed.
  - NAbs identified
- **Purification and functional characterisation of anti-rituximab Abs (binding and kinetic parameters) progressed**
- **Select mADA bulked for crystallization**
- **Glycosylation profile analysis for anti-FVIII ADA**

# Generation of anti-BP antibodies

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- **Identify epitopes and biochemical properties of ADA**
  - **Cloning of anti-BP antibodies (IRB)**
    - Natalizumab – 31 NAbs cloned – 4 produced in larger scale
    - Infliximab – 12 NAbs cloned
    - Rituximab – 3 NAbs cloned
    - IFN-beta – 5 NAbs cloned
    - Adalimumab – 30 ADA cloned
    - FVIII – no NAbs cloned
  - **Crystallization of ADA with drug underway (Sanofi)**
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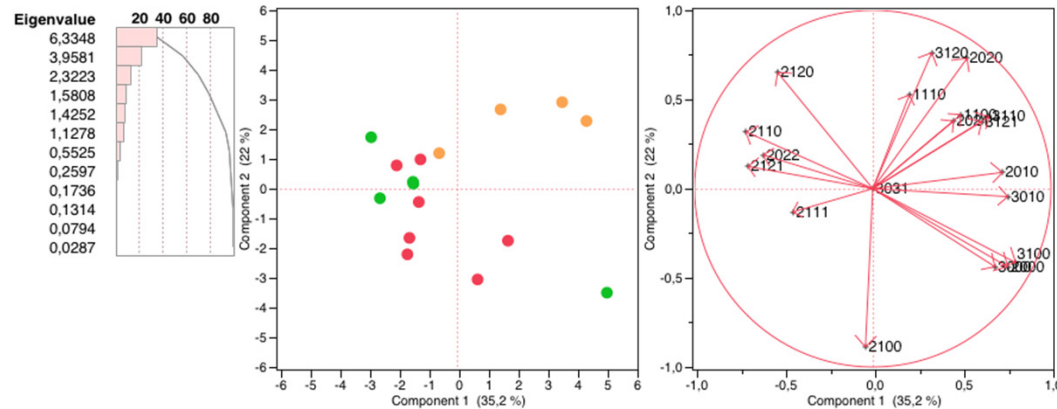
# Glycosylation of anti-FVIII antibodies (INSERM)

Glycan profiles differ between ADA +ve and -ve patients

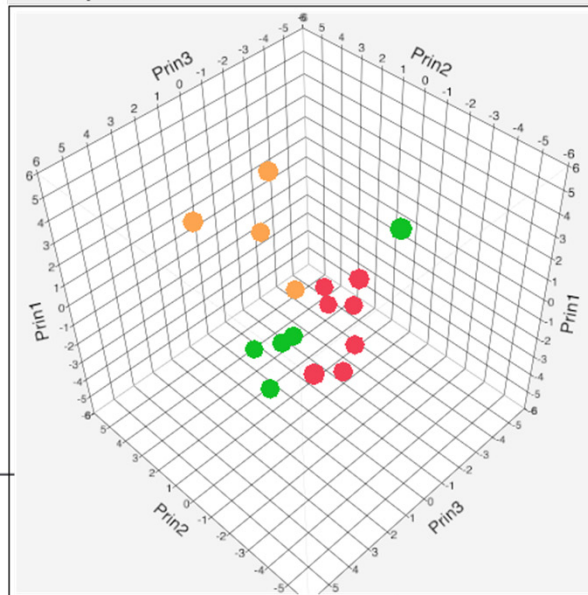
PCA on glycan profiles from total purified IgG

Principal Components: on Correlations

Summary Plots



Scatterplot 3D



**Total IgG**

- 4 Healthy donors
- 5 severe HA patient without ADA
- 7 severe HA patient with ADA

**Preliminary data**

Aim: n=80 by end 1st half 2016

## WP2.3 ABIRISK genetic association

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### **Plans for genetic analysis based on cohort size/sample availability:**

- **MS – advanced in sample attainment and strategy (HLA and GWAS).**
- **HA – advanced in sample attainment with focused genetic analysis.**
  - **Only candidate genes will be investigated.**
- **RA – Sample availability may hinder genetic analysis. Investigating combinations.**
- **IBD – Sample availability may hinder genetic analysis. Investigating targeted analysis, e.g. HLA type.**

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# The EU/IMI consortium

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