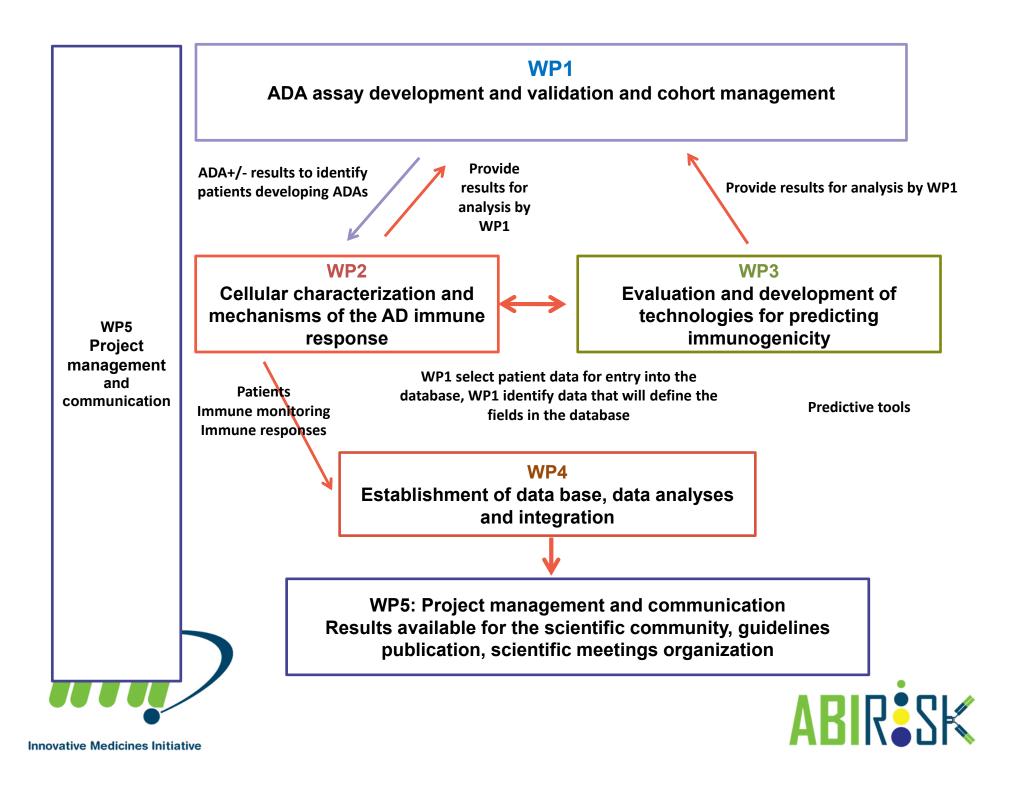
ABIRISK WP2 Scientific update WP Co-leaders: Claudia Mauri, Tim Hickling, Vincent Mikol EIP, Vilamoura, Portugal 24th February 2016



ABIRSK

Innovative Medicines Initiative



Scientific Milestones

	-	
Characterization of ADA 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
	- 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

• WP2.1: Cellular mechanisms of Immunogenicity

- 'Signature' of biomarkers for cellular mechanisms identified for IFNβ responses
- T cell responses to Infliximab and IFNβ 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

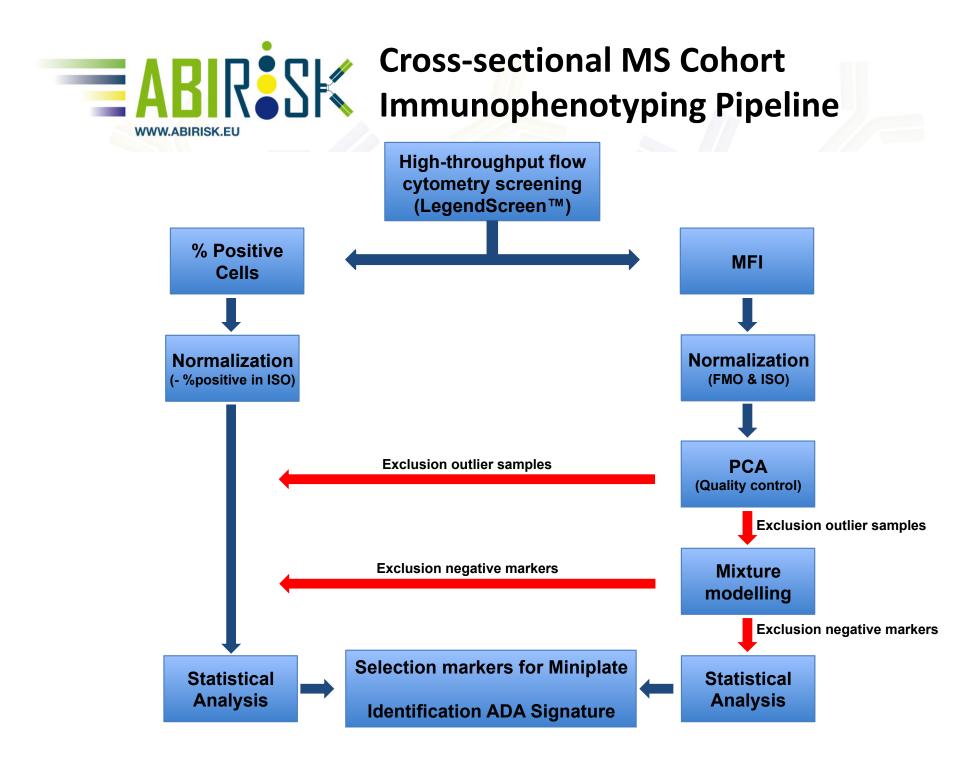
 D2.4 Identification of markers correlating with ADA status in MS patients receiving IFNβ (markers exclusive from disease and treatment)

- '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

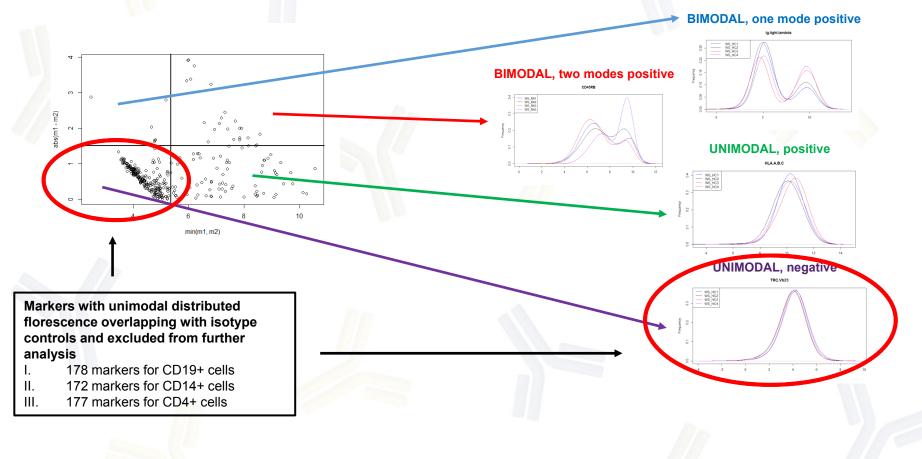
D2.23 Clonality analysis and epitope mapping of T- and B-cell AD responses from patients with RA

- 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





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



Cyprien Mbogning

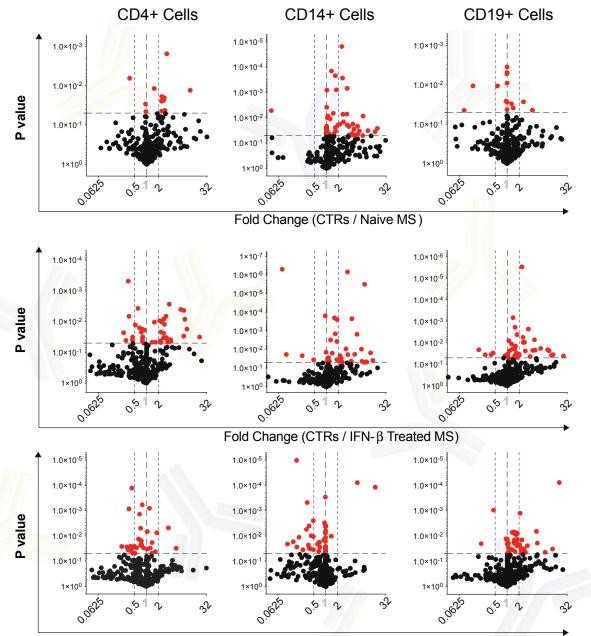


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The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement nº [115303], resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution.' www.imi.europa.eu

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Global phenotyping of immune cells reveals markers on distinct cell types associated with MS and IFNβ treatment

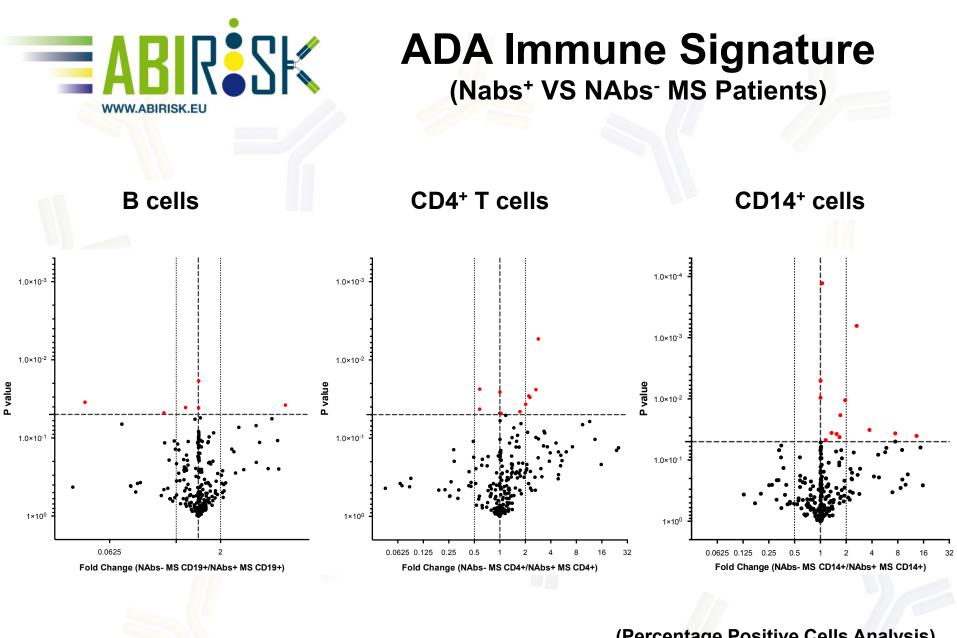


Fold Change (Naive MS / IFN-β Treated MS)

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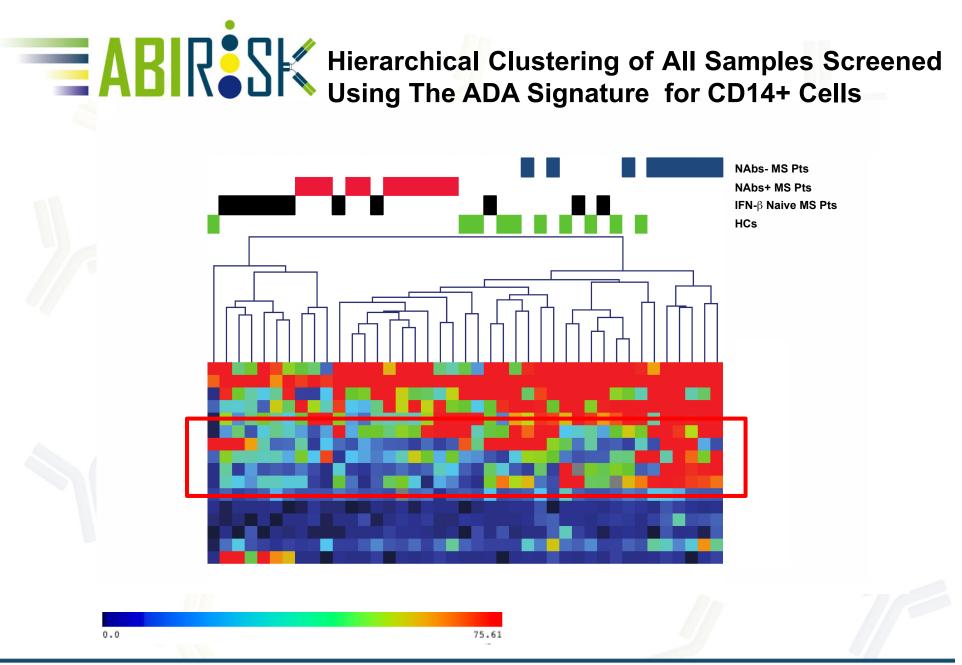
efpia



(Percentage Positive Cells Analysis)



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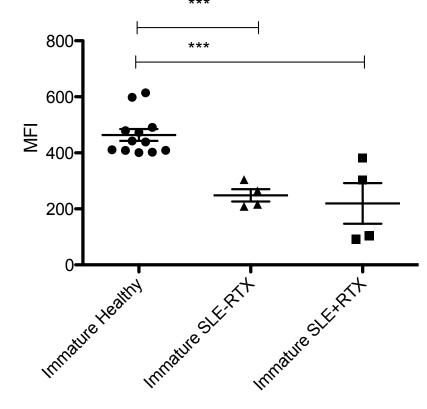
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B cell markers

Altered B cell markers (UCL)

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



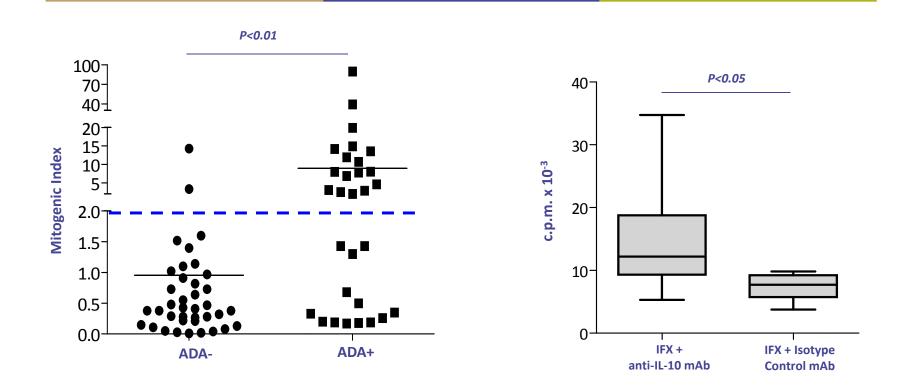


T cell responses to therapeutic proteins

- 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





Mapping T cell responses in IFN-beta:

- Strong T cell responses to IFN beta protein and peptides are found in MS patients
- Several immunodominant epitopes have been identified that elicte 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.



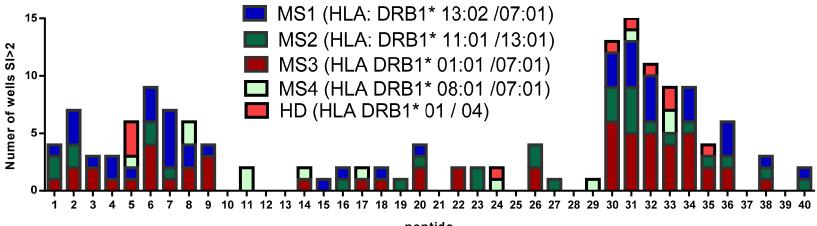
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T cell response against Interferon beta in MS patients who develop neutralizing antibodies

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



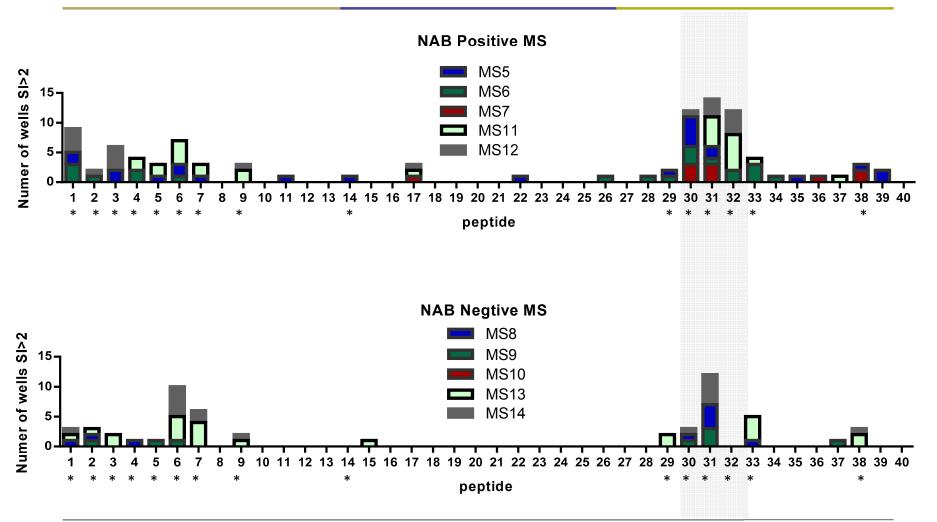
peptide



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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

- 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

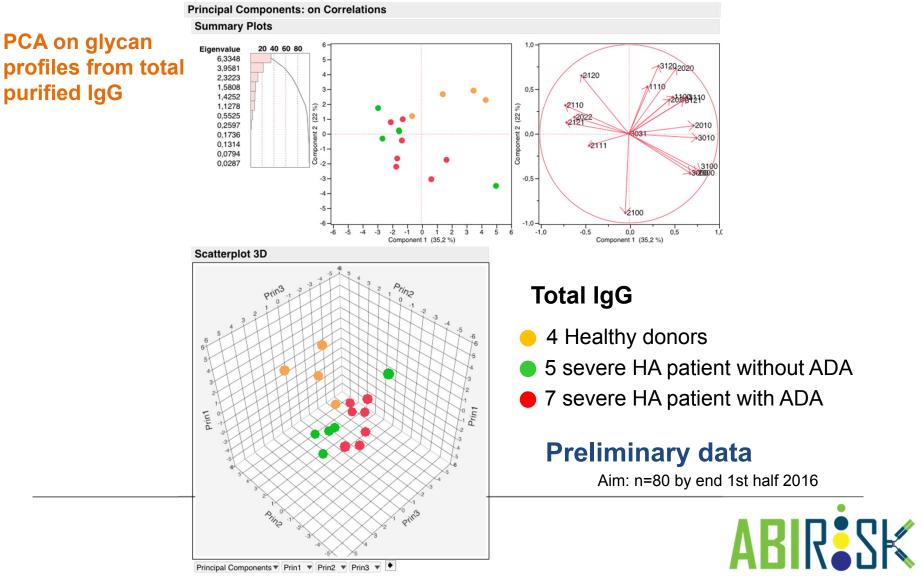
- Identify epitopes and biochemical properties of ADA
- Cloning of anti-BP antibodies (IRB)
 - Natalizumab 31 NAbs cloned 4 produced in larger scale
 - Infliximab 12 NAbs cloned
 - Rituximab 3 NAbs cloned
 - IFN-beta 5 NAbs cloned
 - Adalimumab 30 ADA cloned
 - FVIII no NAbs cloned

Crystallization of ADA with drug underway (Sanofi)



Glycosylation of anti-FVIII antibodies (INSERM)

Glycan profiles differ between ADA +va and –ve patients



Principal Components V Prin1 V Prin2 V Prin3 V

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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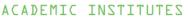
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