# The ABIRISK integrated approach to identify and evaluate predictive markers of immunogenicity

Sophie Tourdot, PhD

BioMedicine Design

*"Predictive immunogenicity for better clinical outcomes"*
*Sliver Spring, 2018*

Pfizer WORLDWIDE RESEARCH & DEVELOPMENT


# Anti-Biopharmaceutical Immunization: Prediction and analysis of clinical relevance to minimize the Risk

Pfizer WORLDWIDE RESEARCH & DEVELOPMENT
BioMedicine Design

# A European IMI-funded project

Public Private Partnership

innovative medicines initiative

efpia [1]

**37 partners**

9 EFPIA companies

25 academic institutes

3 SMEs

**Project Coordinator**

Sebastian Spindeldreher, **Novartis**

Dan Sikkema, **GSK** (2012-2016)

**IMI JU Managing Entity**

Marc Pallardy, INSERM

**6 YEARS**

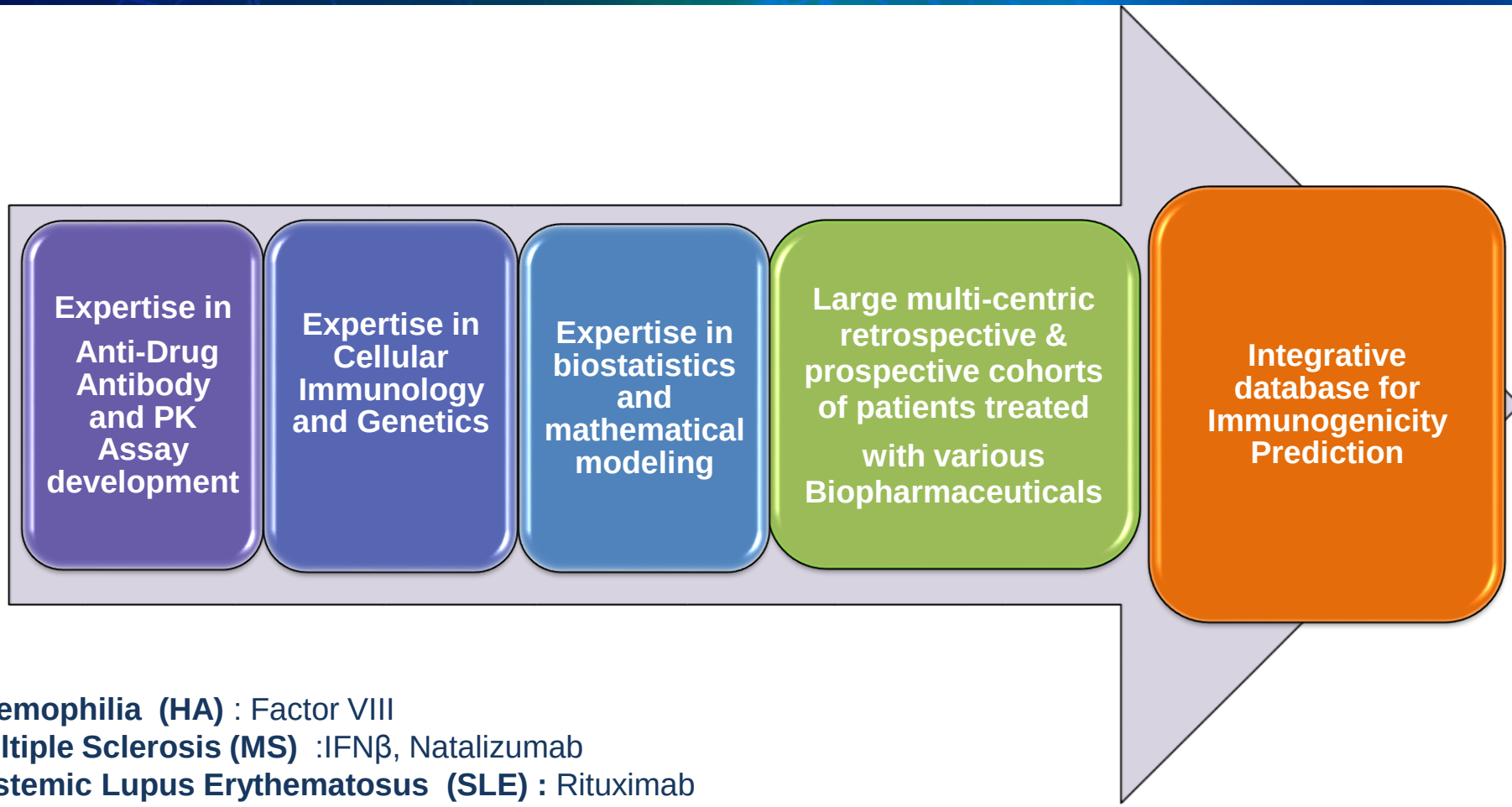
March 2012- February 2018

Total budget €34.9 million

Pfizer WORLDWIDE RESEARCH & DEV
BioMedicine Design

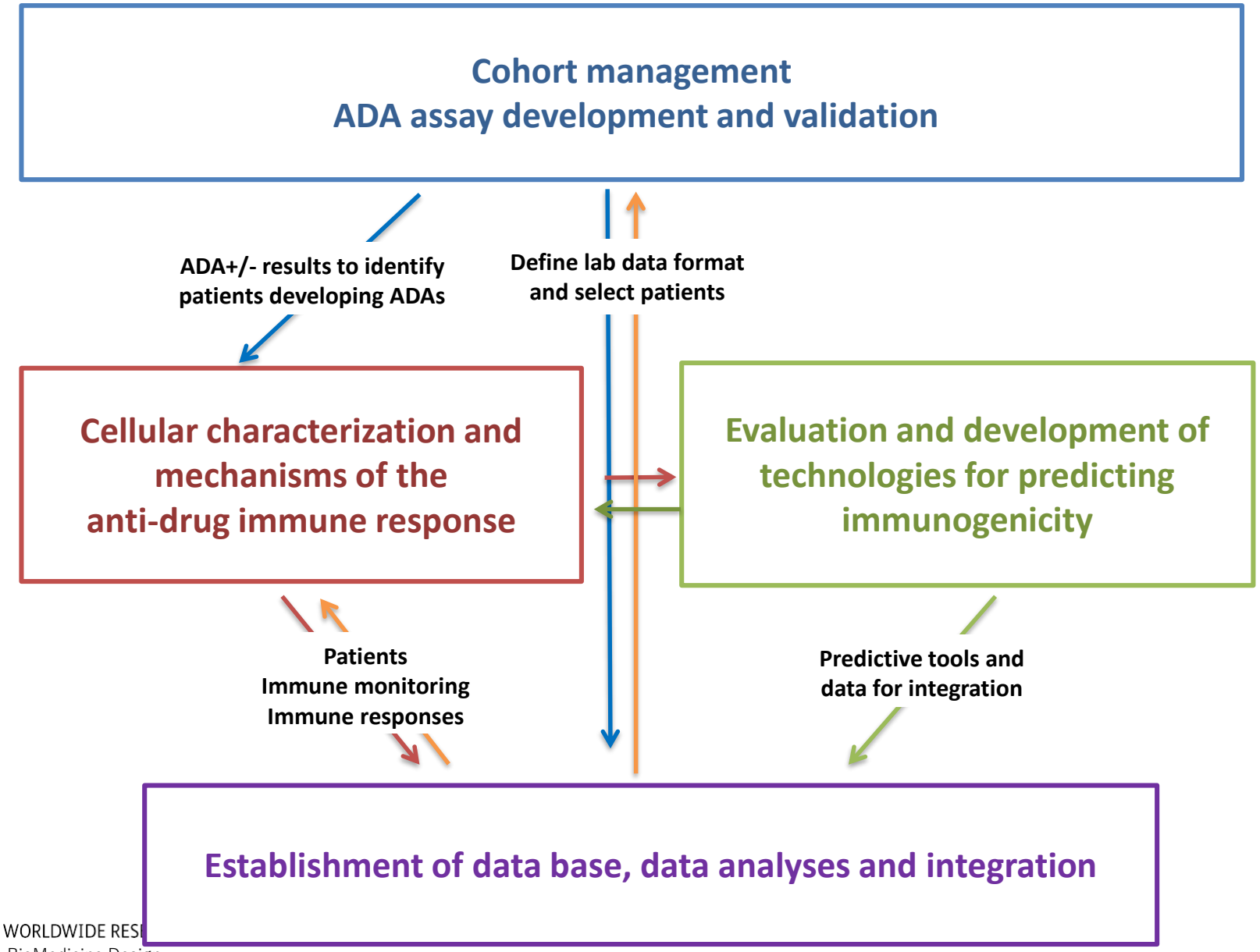
[1]***EFPIA***= *European Federation of Pharmaceutical Industries and Associations*

# ABIRISK - Assets and driving force




- **Haemophilia (HA)** : Factor VIII
- **Multiple Sclerosis (MS)** :IFNβ, Natalizumab
- **Systemic Lupus Erythematosus (SLE) :** Rituximab
- **Inflammatory Bowel Disease (IBD) :** Infliximab, Adalimumab
- **Adult and Juvenile Rheumatoid Arthritis (RA)**: Infliximab, Adalimumab, Rituximab, Etanercept, Tocilizumab

# ABIRISK work packages & workflow



# ABIRISK Main Objectives

**Describe** the natural history of anti-drugs antibodies (ADA) occurrence using **validated and harmonized assays**

Identify disease-specific and drug-specific **biomarkers associated with immunogenicity, including markers of prediction**

Provide insight into the basic mechanisms by which **therapeutic proteins drive immune cell activation**

Evaluate existing and new tools for **immunogenicity risk assessment,** including animal models

Develop **mathematical models** to **predict** :

- the occurrence of ADA
- the occurrence or absence of subsequent clinical outcomes

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# Assay harmonization

## Validated Assays

| Biological Product | ADA Assay | PK Assay |
|---|---|---|
| Adalimumab | ✓ | ✓ |
| Infliximab | ✓ | ✓ |
| Etanercept | ✓ | ✓ |
| Rituximab | ✓ | ✓ |
| Natalizumab | ✓ | ✓ |
| IFNβ 1-a, 1-b | ✓ | - |
| FVIII | ✓ | - |

## Human Positive Controls

NIBSC Confidence in Biological Medicines

World Health Organization

| Targeted Biopharmaceutical | Monoclonal Antibody | Type (Bab/Nab*) | Isotype | |
|---|---|---|---|---|
| Rituximab | RXA1 | Nab | IgG1 | κ |
| | RXA3 | Nab | IgG2 | κ |
| | RXA10 | Nab | IgG1 | κ |
| Natalizumab | NAA32 | Non-NAb | IgG1 | κ |
| | NAA80 | Nab | IgG1 | λ |
| | NAA84 | Nab | IgG1 | λ |
| | NAA96 | Non-NAb | IgG3 | λ |
| Infliximab | INA29 | Nab | IgG1 | κ |
| | INA62 | Nab | IgG4 | κ |
| | INA79 | Nab | IgG4 | κ |
| | INA85 | Nab | IgG4 | λ |
| Adalimumab | ADA19 | Nab | IgG1 | κ |
| | ADA23 | Nab | IgG1 | λ |
| | ADA44 | Nab | IgG1 | κ |
| | ADA39 | Nab | IgG1 | κ |
| Interferon-β (IFNβ 1-a, Rebif®) | sa01.53 | Nab | IgG2 | k |
| | sa01.54 | Nab | IgG4 | k |
| | sa01.71 | Nab | IgG1 | λ |

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# Patient/treatment associated risk factors – Hemophilia A

## Microsatellite polymorphism promoter at the HMOX1 is associated risk of ADA development to FVIII in severe hemophilia patients

Heme oxygenase HO-1 is inducible under inflammatory conditions
Induction of HO-1 before FVIII treatment protect against inhibitor development in FVIII deficient mice

### Genotype frequencies at the polymorphic locus

| Genotype | Patients with severe hemophilia A | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Inhibitor-positive N. (%) | Inhibitor-negative N. (%) | OR | 95% CI | *P* | aOR | 95% CI | *P* |
| L/L+L/M+L/S | 31 (31.3) | 45 (17.1) | 2.21 | 1.30-3.76 | 0.004 | 2.13 | 1.24 - 3.64 | 0.006 |
| S/S+M/S+M/M | 68 (68.7) | 218 (82.9) | | | | | | |

*S, M, L stand for short (<21 GT repeats), medium and long (≥30 GT repeats), respectively. P values were assessed by the two-tailed Fisher's exact test; aOR: odds ratio adjusted on hemophilia-causing mutations; CI: confidence interval.*

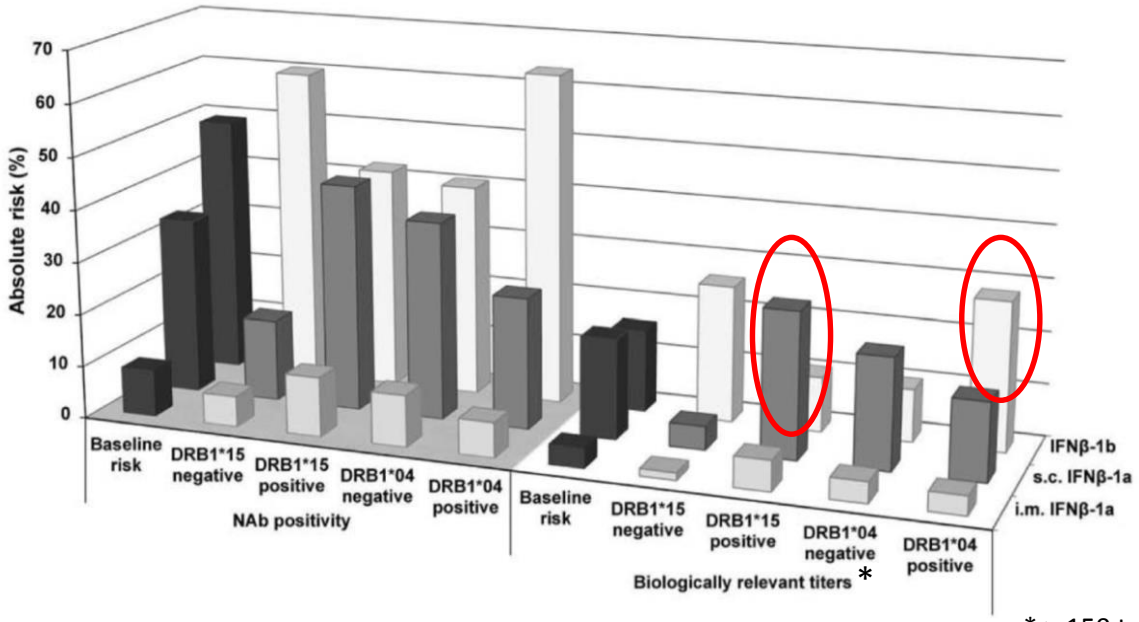
Class L allele (>= 30 GT repeat) subclass genotype is associated with increased inhibitor development in severe hemophilia A patients treated with FVIII



*Repessé et al. 2013*

# Patient/treatment associated risk factors – Multiple sclerosis

## HLA carriage and IFNβ products are associated with increased risk of ADA development in MS patients




* > 150 tenfold reduction unit

- HLA DRB1*15 carriage is associated with a higher risk of ADA development against IFNβ-1a i.m. and s.c.
- HLA DRB1*04 carriage is associated with a higher risk of ADA development against IFNβ-1b
- Choice of INFβ preparation remains the most significant determinant

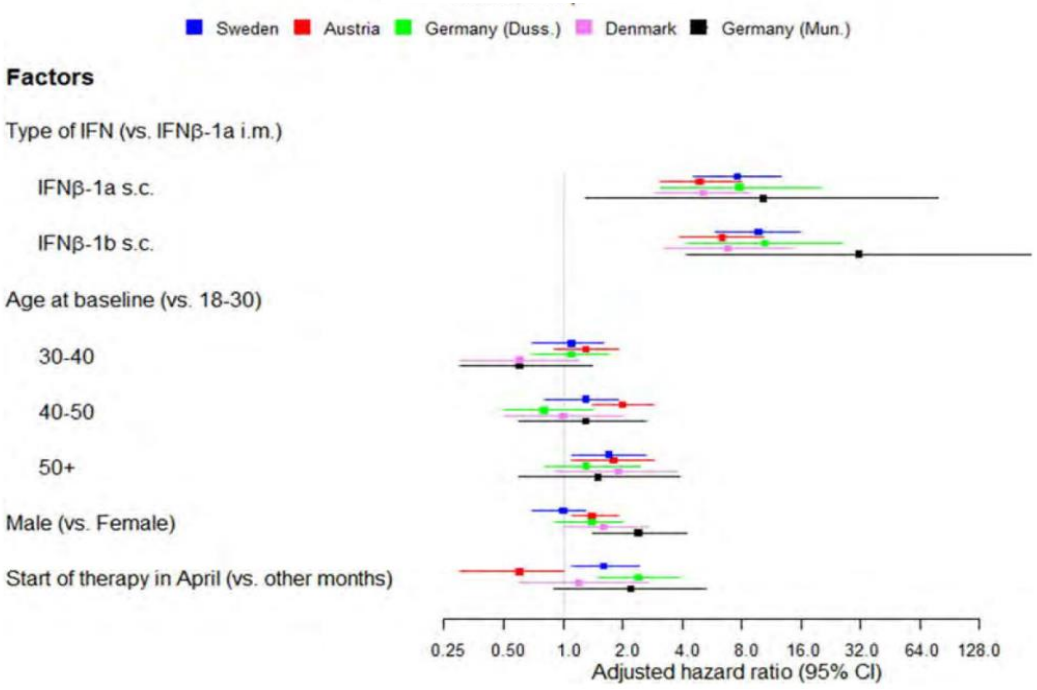


*Link et al. 2014*

# Patient/treatment associated risk factors – Multiple sclerosis

## Product, sex, age at start of treatment are associated with increased risk of ADA development in MS patients

**Interferon β**

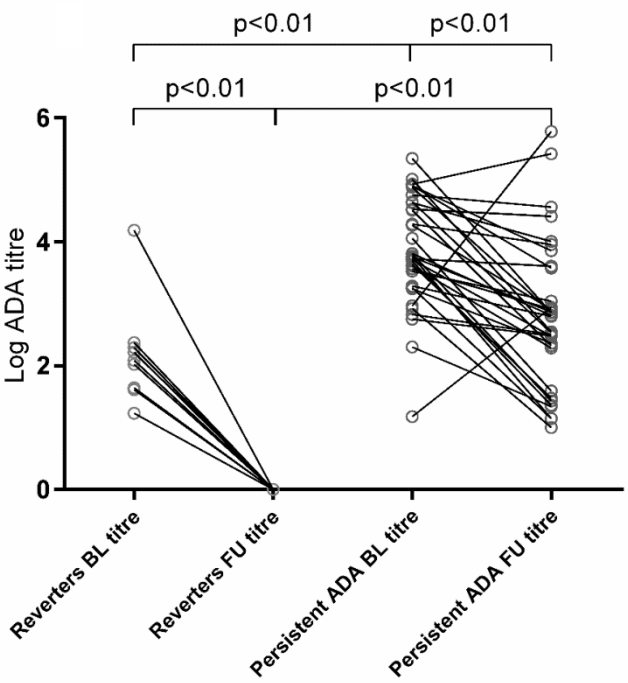


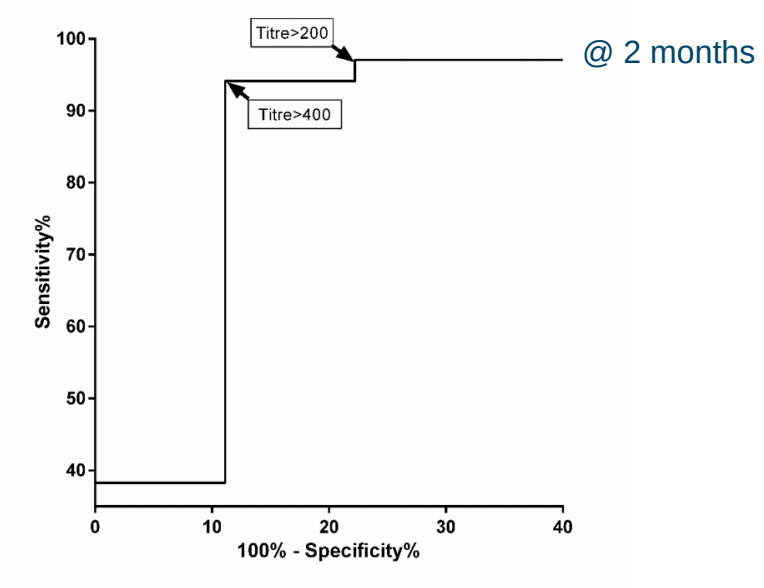

- Higher risk of NAb development associated with INFβ-1a and -1b s.c.
- Higher risk of NAb development associated with being Male and aged over 50 at start of treatment

*Bachelet et al. 2016*

# Predictive biomarkers - NAbs titers

## High NAbs titers at 2 months predict persistence of response to Natalizumab in MS patients




Current recommendation : 2 consecutive low titer > discontinue

**ABIRISK recommendation** : 1 high titer before 3 months > discontinue

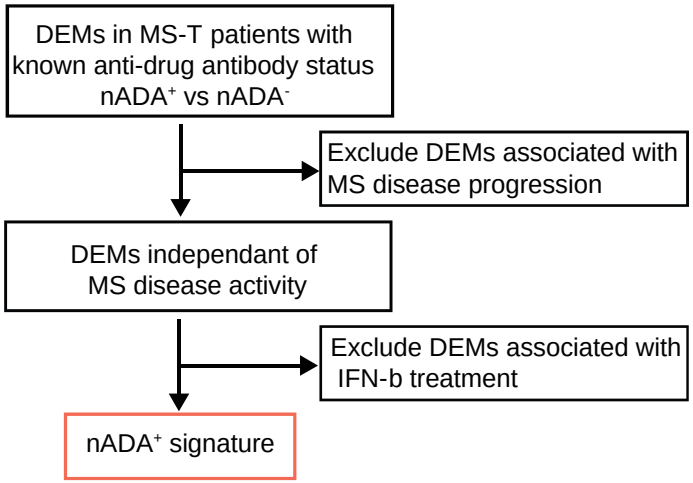


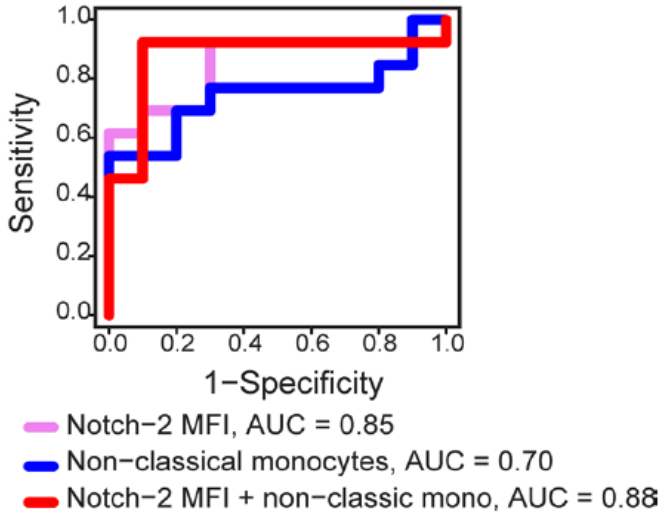
*Deisenhammer et al. 2018*

# Predictive biomarkers – PBMC immunosignature in MS

## Decreased macrophage NOTCH2 expression and increased frequency of pro-inflammatory macrophages predict NAbs development to INFβ in MS patients


LEGENDScreen ™
High through-put immunophenotyping platform
cell surface markers




DEM : differentially expressed markers



*Adriani et al. 2017*

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# Cellular responses associated with ADA$^+$ status - 1

**IL-10–Producing Infliximab-Specific T Cells Regulate the Antidrug T Cell Response in Exposed Patients**

Alessandra Vultaggio,* Francesca Nencini,† Sara Pratesi,† Daniele Cammelli,* Maria Totaro,* Sergio Romagnani,† Enrico Maggi,† and Andrea Matucci* on behalf of the ABIRISK Consortium

*The Journal of Immunology*, 2017, 199: 1283–1289.

Clinical & Experimental Immunology
The Journal of Translational Immunology
Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/cei.12858

**Circulating T cells to infliximab are detectable mainly in treated patients developing anti-drug antibodies and hypersensitivity reactions**

***Vultaggio et al., 2016***

Journal of Autoimmunity 88 (2018) 83–90
Contents lists available at ScienceDirect
Journal of Autoimmunity
journal homepage: www.elsevier.com/locate/jautimm

**Interferon-beta specific T cells are associated with the development of neutralizing antibodies in interferon-beta treated multiple sclerosis patients**

Sudhakar Reddy Kalluri [a], Verena Grummel [a], Zsuzsanna Hracsko [a], Viola Pongratz [a], Verena Pernpeintner [a], Christiane Gasperi [a], Dorothea Buck [a], Bernhard Hemmer [a, b, *], on behalf of the ABIRISK Consortium

frontiers 2017 in Immunology

**Characterization of CD4 T Cell Epitopes of Infliximab and Rituximab Identified from Healthy Donors**

*Moustafa Hamze[1], Sylvain Meunier[1], Anette Karle[2], Abdelaziz Gdoura[1], Amélie Goudet[1], Natacha Szely[3], Marc Pallardy[3], Franck Carbonnel[4], Sebastian Spindeldreher[2], Xavier Mariette[5], Corinne Miceli-Richard[5] and Bernard Maillère[1]*

- ❑ Antigen-specific CD4 T cells are associated with ADA development
- ❑ CD4 T cell cytokine profiles are diverse
- ❑ No difference in Tregs numbers observed so far

# Cellular responses associated with ADA$^{+}$ status - 2

## (Some) On-going/Unpublished work

- Identification of a peripheral B cell immune signature predictive of ADA development in RA patients

- Development of a highly reproducible, sensitive method for early detection and characterization of antidrug T and B cell responses using RNA-seq :
  - NGS-based BCR analysis allows detection of BCR clonal repertoires in samples with undetectable B cells (<0.01 x109 cell/L, flow cytometry)
  - Peak levels of drug-specific T-cells are detected in blood of patients before the detection of anti-drug antibodies
  - The technology can be combined with T cell assay to identify epitope- specific T cells

- Pilot study in untreated, ADA+, ADA- and healthy controls reveals different T follicular helper cell populations in the 4 groups

- Pilot study in RA ADA+, ADA- patients identifies a subpopulation of $CD24^{hi}CD38^{hi}$ IL-10 producing B cells

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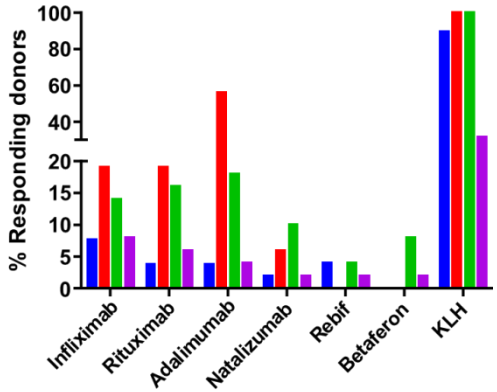
# Evaluation of existing prediction tools - 1

## Evaluation of *in vitro* T cell assays for immunogenicity risk assessment

**Assays - not in same order as in graph**

- EpiScreen™ (Abzena)
- EpiBase™ (Lonza)
- Immuno'line™ (Platine)
- REVEAL® (ProImmune)




| | Infliximab | Rituximab | Adalimumab | Natalizumab | Betaferon | Rebif |
|---|---|---|---|---|---|---|
| **Assay A** | 1 | 3 | 2 | 4 | 2 | 1 |
| **Assay B** | 3 | 2 | 1 | 4 | N/A | N/A |
| **Assay C** | 3 | 1 | 1 | 4 | 1 | 2 |
| **Assay D** | 1 | 2 | 3 | 4 | 1 | 1 |

Colour coding indicates ranking, from high to low *in vitro* immunogenicity

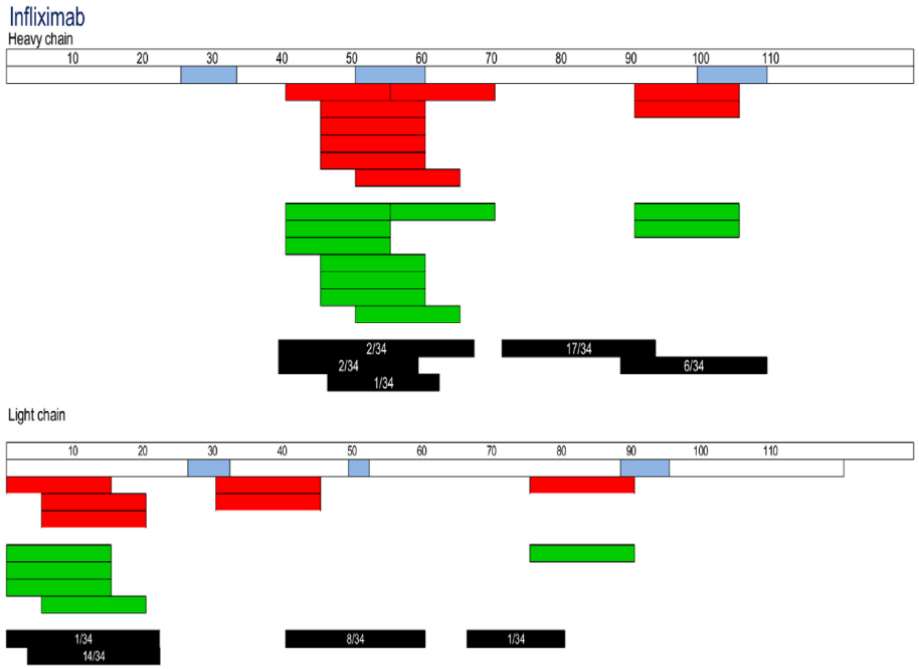
- Discrepancy between assays in their ranking of molecules
- Knowledge on the biology and mechanism of action of the drug is essential as they can interfere with assay

**- One isolated assay cannot predict ADA incidence -**

# Evaluation of existing prediction tools - 2

## Evaluation of *in vitro* assays for identification of immunogenic sequences

Clusters identified with healthy donors T cells
Clusters identified with **infliximab**-treated patients T cells
Clusters identified with MAPPs using healthy donor derived dendritic cells




- **An integrated use of *in silico* prediction, *in vitro* HLA binding, MAPPs and T cell assays identified** 9 infliximab and 8 rituximab CD4 T cell epitopes
- 2/3 of peptides identified in healthy donors **recall a T cell response in ADA+ patients**



*Hamze et al. 2017*

# Development of novel prediction tools - 1

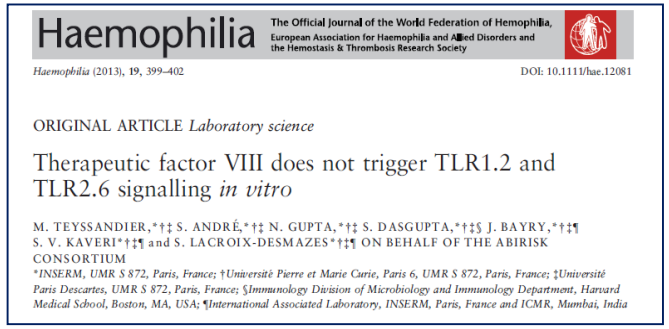
## Comparison of DC activation readouts for danger signal evaluation

Coagulation and Fibrinolysis

Danger signal-dependent activation of human dendritic cells by plasma-derived factor VIII products

Lilija Miller[1]; Sabrina Weissmüller[1]; Eva Ringler[1]; Peter Crauwels[2]; Ger van Zandbergen[2]; Rainer Seitz[3]; Zoe Waibler[1]; on behalf of the ABIRISK consortium

[1]Junior Research Group "Novel Vaccination Strategies and Early Immune Responses", Paul-Ehrlich-Institut, Langen, Germany; [2]Division of Immunology, Paul-Ehrlich-Institut, Langen, Germany; [3]Division of Haematology / Transfusion Medicine, Paul-Ehrlich-Institut, Langen, Germany



Haemophilia The Official Journal of the World Federation of Hemophilia, European Association for Haemophilia and Allied Disorders and the Hemostasis & Thrombosis Research Society

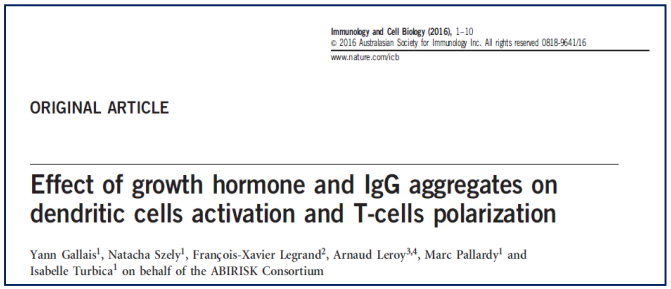
*Haemophilia* (2013), 19, 399–402 DOI: 10.1111/hae.12081

ORIGINAL ARTICLE *Laboratory science*

Therapeutic factor VIII does not trigger TLR1.2 and TLR2.6 signalling *in vitro*

M. TEYSSANDIER,*†‡ S. ANDRÉ,*†‡ N. GUPTA,*†‡ S. DASGUPTA,*†‡§ J. BAYRY,*†‡¶ S. V. KAVERI*†‡¶ and S. LACROIX-DESMAZES*†‡¶ ON BEHALF OF THE ABIRISK CONSORTIUM

*INSERM, UMR S 872, Paris, France; †Université Pierre et Marie Curie, Paris 6, UMR S 872, Paris, France; ‡Université Paris Descartes, UMR S 872, Paris, France; §Immunology Division of Microbiology and Immunology Department, Harvard Medical School, Boston, MA, USA; ¶International Associated Laboratory, INSERM, Paris, France and ICMR, Mumbai, India



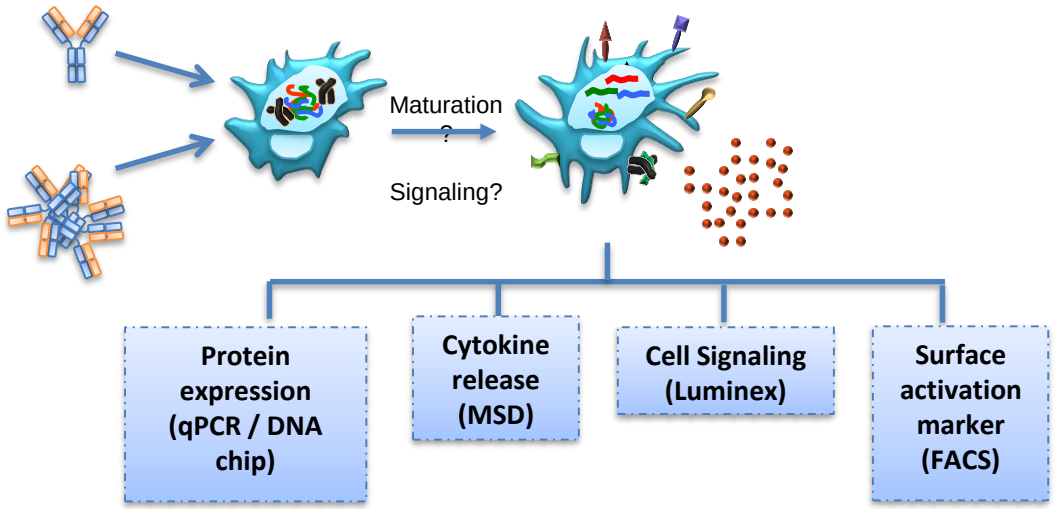
Immunology and Cell Biology (2016), 1–10

www.nature.com/icb

ORIGINAL ARTICLE

Effect of growth hormone and IgG aggregates on dendritic cells activation and T-cells polarization

Yann Gallais[1], Natacha Szely[1], François-Xavier Legrand[2], Arnaud Leroy[3,4], Marc Pallardy[1] and Isabelle Turbica[1] on behalf of the ABIRISK Consortium




*Adapted from S. Spindeldreher, Coral Gables 2016*

- Native biologics alone do not induce detectable DC maturation
- Some aggregated forms give a danger signal
- Cytokine profiles are diverse

# Development of novel prediction tools - 2

## Whole systems analysis of risk

No surrogate readout of immunogenicity : ADA induced by the protein drugs are measured

- ABIRISK new hemophilic mice
- State of the art humanized mice ( Axenis BRGSF™ )
- Human Artificial Lymph nodes (ProBiogen)

Results obtained with KLH as a model antigen as pilot experiment or with therapeutic drugs were inconclusive

Further exploration is required to assess the value of these models for immunogenicity prediction of therapeutic proteins

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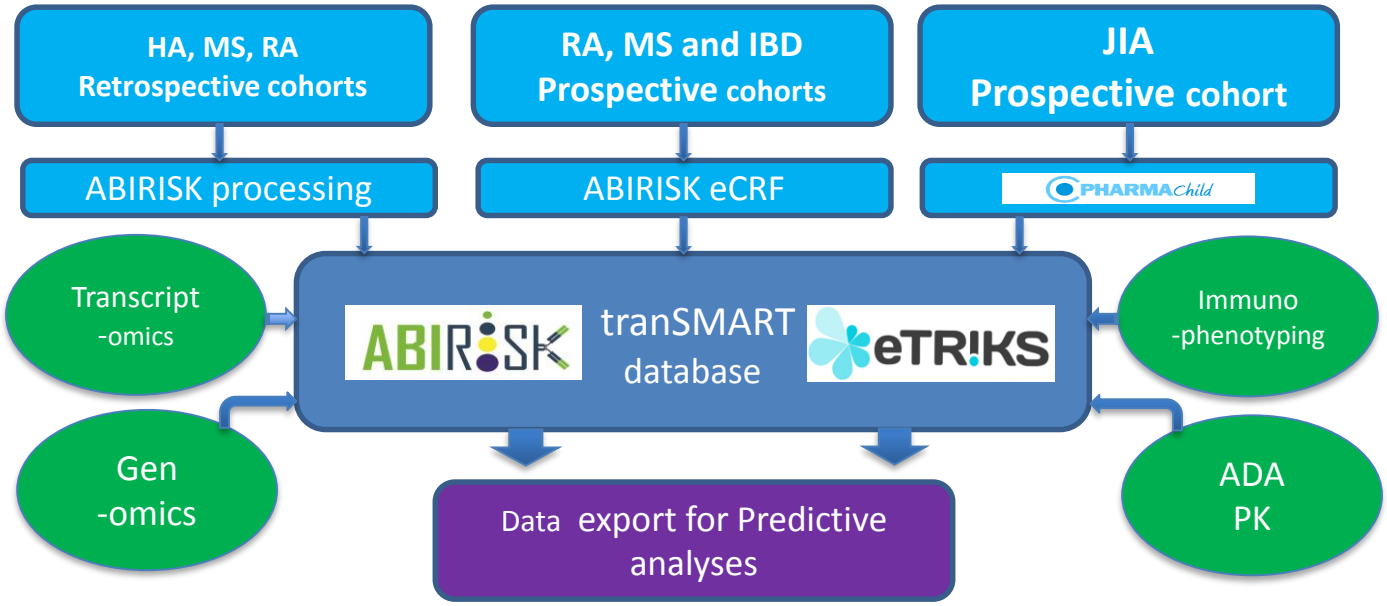
# Database & statistical model




Mbogning and Broët *BMC Bioinformatics* (2016) 17:230
DOI 10.1186/s12859-016-1090-x

BMC Bioinformatics

METHODOLOGY ARTICLE Open Access

## Bagging survival tree procedure for variable selection and prediction in the presence of nonsusceptible patients

Cyprien Mbogning[1,2*] and Philippe Broët[1,2,3,4]

https://cran.r-project.org/web/packages
Comprehensive R Archive Network Project

- ✓ Take into account high-order interactions and high dimensional data
- ✓ Cope with time-to-event data and a mixed population of immune/tolerant patients
- ✓ Provide biomarker selection for prediction
- ✓ Provide stable and accurate individual prediction

# Summary

## ABIRISK legacy as of today

- ADA Human Positive Controls
- Harmonized ADA assays
- Confidence in the use of healthy donors for *in vitro* risk assessment T cell assays
- Identification of markers associated with ADA development, including predictive markers
- Position paper on Terms and Definitions
- Database
- Biobank

## Looking forward : on-going analyses

- Validation of the statistical model of ADA occurrence prediction
- Clinical relevance of pre-existing, transient, low titer non-neutralizing antibodies
- Immune mechanisms pertaining to ADA development
- Disease/Product-specific predictive markers of ADA development
- …

# Acknowledgment : ABIRISK consortium participants

## EFPIA MEMBER COMPANIES

GlaxoSmithKline Research & Development Limited
United Kingdom
www.gsk.com

Bayer Pharma AG
Germany
www.bayer.com

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Sanofi-Aventis Research and Development
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en.sanofi.com

UCB Pharma S.A.
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www.ucb.com

## SMEs

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www.altaweb.eu

Biomonitor A/S
Denmark
www.biomonitor.dk

SciCross AB
Sweden
www.scicross.com

## ACADEMIC INSTITUTES

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www.inserm.fr

Academisch Medisch Centrum
the Netherlands
www.amc.nl

Leids Universitair Medisch Centrum
the Netherlands
www.lumc.nl

Centre National de la Recherche Scientifique
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www.cnrs.fr

Commissariat a L'Energie Atomique et aux Energies Alternatives- France
www.cea.fr

DRK-Blutspendedienst Baden-Württemberg – Hessen gemeinnützige GmbH - Germany
www.blutspende.de

Fondazione per l'Istituto di Ricerca in Biomedicina
Switzerland
www.blutspende.de

Fundació Institut de Recerca de L'hospital Universitari Vall D'hebron - Spain
www.vhir.org

Groupe d'Etudes Therapeutiques des Affections Inflammatoires du Tube Digestif - France
www.getaid.org

Istituto Giannina Gaslini
Italy
www.gaslini.org

Johann Wolfgang Goethe Universität - Klinikum und Fachbereich Medizin - Germany
www.kgu.de

Karolinska Institutet
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ki.se

Klinikum rechts der Isar der Technischen Universitaet Muenchen - Germany
www.med.tum.de

Medizinische Universität Innsbruck
Austria
www.i-med.ac.at

Paul-Ehrlich-Institut, Bundesinstitut für Impfstoffe und biomedizinische Arzneimittel - Germany
www.pei.de

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www.unifi.it

Universitaetsklinikum Bonn
Germany
www.ukb.uni-bonn.de

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University Hospital Basel
Switzerland
www.unispital-basel.ch

Univerzita Karlova v Praze
Czech Republic
www.cuni.cz

Assistance Publique-Hôpitaux de Paris
France
www.aphp.fr

# In particular

## WP1

- **Florian Deisenhammer** (IMU, AT)
- **Mary Birchler** (GSK, USA)
- **Louis Christodoulou** (UCB, GB)

## WP2

- **Tim Hickling** (Pfizer, USA)
- **Claudia Mauri** (UCL, GB)
- **Vincent Mikol** (Sanofi, FR)

## WP3

- **Bernard Maillère** (CEA, FR)
- **Sebastian Spindeldreher** (Novartis, CH)
- **Christian Ross Pedersen** (NovoNordisk, DK)

## WP4

- **Agnès Hincelin-Méry** (Sanofi, FR)
- **Philippe Broët** (Inserm, FR)
- **Pierre Dönnes** (SciCross, SE)

## WP5

- **Sebastian Spindeldreher** (Novartis, CH); **Dan Sikkema** (GSK, USA)
- **Marc Pallardy** (Inserm, FR)
- **Riccardo Bertini** (ALTA, IT)



## Cohort leaders

- **RA : Xavier Mariette** (AP-HP, FR)
- **MS : Anna Fogdell-Hahn** (KI, SE)
- **IBD : Matthieu Allez** (GETAID, FR)

## Scientific Advisory Board

- **Amy Rosenberg** (FDA, USA)
- **Alessandro Sette** (LJI,USA)
- **Robin Thorpe** (NIBSC, GB)

# Back-up

# The BIOPIA initiative

https://ki.se/en/cns/biopia

## *Objectives*

Raise awareness about biopharmaceuticals and their immunogenicity, with the aim of integrating testing of these factors in order to improve the care and overall health of patients


## *Means*

- Provide easy, accessible information about ADA and drug level testing
- Create a site for clinicians to help them assess biologic responses in their patients and choose the correct treatment for each person
- Connect European labs together, with the goal of implementing routine, clinical testing for immunogenicity and drug levels

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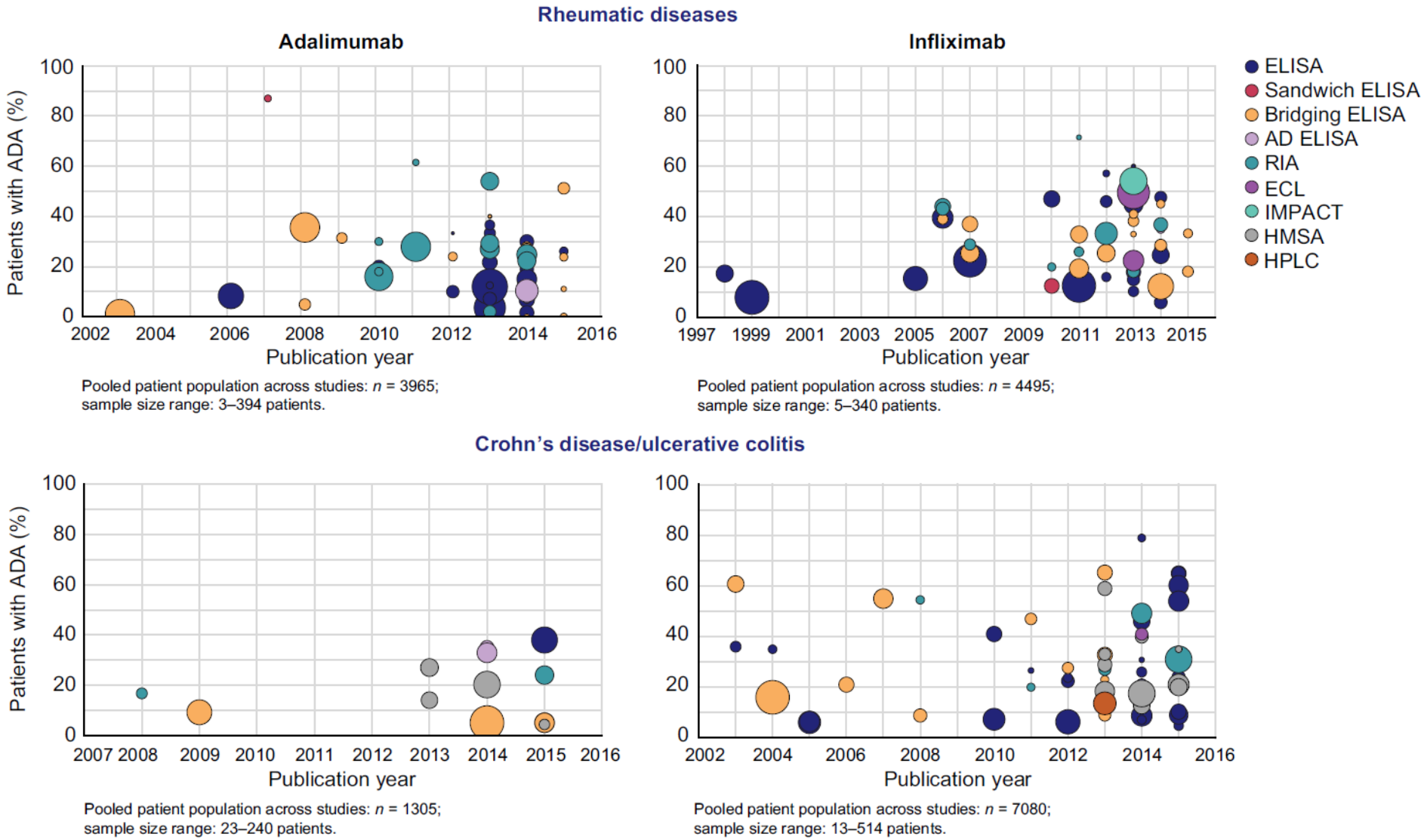
# ADA incidence confounders: Assays and populations



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