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
Anti-Biopharmaceutical Immunization: Prediction and analysis of clinical relevance to minimize the Risk
A European IMI-funded project

Public Private Partnership

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

1EFPIA= European Federation of Pharmaceutical Industries and Associations
ABIRISK - Assets and driving force

- Expertise in Anti-Drug Antibody and PK Assay development
- Expertise in Cellular Immunology and Genetics
- Expertise in biostatistics and mathematical modeling
- Large multi-centric retrospective & prospective cohorts of patients treated with various Biopharmaceuticals
- Integrative database for Immunogenicity Prediction

- **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

Cohort management
ADA assay development and validation

ADA+/- results to identify patients developing ADAs

Define lab data format and select patients

Cellular characterization and mechanisms of the anti-drug immune response

Evaluation and development of technologies for predicting immunogenicity

Patients Immune monitoring Immune responses

Predictive tools and data for integration

Establishment of data base, data analyses and integration
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

<table>
<thead>
<tr>
<th>Biological Product</th>
<th>ADA Assay</th>
<th>PK Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adalimumab</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Infliximab</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Etanercept</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Rituximab</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Natalizumab</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>IFNβ 1-a, 1-b</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>FVIII</td>
<td>✓</td>
<td>-</td>
</tr>
</tbody>
</table>

### Human Positive Controls

<table>
<thead>
<tr>
<th>Targeted Biopharmaceutical</th>
<th>Monoclonal Antibody</th>
<th>Type (Bab/Nab*)</th>
<th>Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rituximab</strong></td>
<td>RXA1 RXA3 RXA10</td>
<td>Nab</td>
<td>IgG1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nab</td>
<td>IgG2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nab</td>
<td>IgG1</td>
</tr>
<tr>
<td><strong>Natalizumab</strong></td>
<td>NAA32 NAA80 NAA84 NAA96</td>
<td>Non-Nab Nab Non-Nab</td>
<td>IgG1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>κ</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>κ</td>
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<td></td>
<td></td>
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<td>λ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>λ</td>
</tr>
<tr>
<td><strong>Infliximab</strong></td>
<td>INA29 INA62 INA79 INA85</td>
<td>Nab</td>
<td>IgG1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nab</td>
<td>IgG4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nab</td>
<td>κ</td>
</tr>
<tr>
<td><strong>Adalimumab</strong></td>
<td>ADA19 ADA23 ADA44 ADA39</td>
<td>Nab</td>
<td>IgG1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nab</td>
<td>IgG1</td>
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<td></td>
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<td>Nab</td>
<td>κ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>λ</td>
</tr>
<tr>
<td><strong>Interferon-β</strong></td>
<td>sa01.53 sa01.54 sa01.71</td>
<td>Nab</td>
<td>IgG2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nab</td>
<td>IgG4</td>
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<tr>
<td></td>
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<td>Nab</td>
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</tr>
<tr>
<td></td>
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<td>λ</td>
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</tbody>
</table>

*Note: Bab/Nab refers to the type of antibody binding, with Bab indicating a bi-specific antibody and Nab indicating a monospecific antibody.*
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Develop mathematical models to predict:
- the occurrence of ADA
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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

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

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

- 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

* > 150 tenfold reduction unit

Link et al. 2014
Product, sex, age at start of treatment are associated with increased risk of ADA development in MS patients

- 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
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*
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

DEMs in MS-T patients with known anti-drug antibody status nADA+ vs nADA-

Exclude DEMs associated with MS disease progression

DEMs independant of MS disease activity

Exclude DEMs associated with IFN-β treatment

nADA+ signature

DEM : differentially expressed markers

Adriani et al. 2017
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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
(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 x10^9 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

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)

<table>
<thead>
<tr>
<th></th>
<th>Infliximab</th>
<th>Rituximab</th>
<th>Adalimumab</th>
<th>Natalizumab</th>
<th>Betaferon</th>
<th>Rebif</th>
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<tbody>
<tr>
<td>Assay A</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Assay B</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Assay C</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Assay D</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

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 in vitro assays for identification of immunogenic sequences

- 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

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

HA, MS, RA Retrospective cohorts
RA, MS and IBD Prospective cohorts
JIA Prospective cohort

ABIRISK processing
ABIRISK eCRF

Transcript-omics
Gen-omics

tranSMART database
eTRIKS

Data export for Predictive analyses

Immuo-phenotyping
ADA PK

✓ 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

BMC Bioinformatics

Bagging survival tree procedure for variable selection and prediction in the presence of nonsusceptible patients
Cyprien Mboving1,2* and Philippe Broet1,2,4

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

ABIRISK legacy as of today

– ADA Human Positive Controls
– Harmonized ADA assays
– Confidence in the use of healthy donors for \textit{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
In particular

<table>
<thead>
<tr>
<th>WP1</th>
<th>WP2</th>
<th>WP3</th>
<th>WP4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Florian Deisenhammer</strong></td>
<td><strong>Tim Hickling</strong></td>
<td><strong>Bernard Maillère</strong></td>
<td><strong>Agnès Hincelin-Méry</strong></td>
</tr>
<tr>
<td>(IMU, AT)</td>
<td>(Pfizer, USA)</td>
<td>(CEA, FR)</td>
<td>(Sanofi, FR)</td>
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<tr>
<td><strong>Mary Birchler</strong></td>
<td><strong>Claudia Mauri</strong></td>
<td><strong>Sebastian Spindeldreher</strong></td>
<td><strong>Philippe Broët</strong></td>
</tr>
<tr>
<td>(GSK, USA)</td>
<td>(UCL, GB)</td>
<td>(Novartis, CH)</td>
<td>(Inserm, FR)</td>
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<tr>
<td><strong>Louis Christodoulou</strong></td>
<td><strong>Vincent Mikol</strong></td>
<td><strong>Christian Ross Pedersen</strong></td>
<td><strong>Pierre Dönnes</strong></td>
</tr>
<tr>
<td>(UCB, GB)</td>
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<td>(NovoNordisk, DK)</td>
<td>(SciCross, SE)</td>
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<table>
<thead>
<tr>
<th>WP5</th>
<th>Cohort leaders</th>
<th>Scientific Advisory Board</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sebastian Spindeldreher</strong></td>
<td><strong>RA : Xavier Mariette</strong></td>
<td><strong>Amy Rosenberg</strong></td>
</tr>
<tr>
<td>(Novartis, CH); <strong>Dan Sikkema</strong></td>
<td>(AP-HP, FR)</td>
<td>(FDA, USA)</td>
</tr>
<tr>
<td>(GSK, USA)</td>
<td><strong>MS : Anna Fogdell-Hahn</strong></td>
<td><strong>Alessandro Sette</strong></td>
</tr>
<tr>
<td><strong>Marc Pallardy</strong></td>
<td>(KI, SE)</td>
<td>(LJI, USA)</td>
</tr>
<tr>
<td>(Inserm, FR)</td>
<td><strong>IBD : Matthieu Allez</strong></td>
<td><strong>Robin Thorpe</strong></td>
</tr>
<tr>
<td><strong>Riccardo Bertini</strong></td>
<td>(GETAID, FR)</td>
<td>(NIBSC, GB)</td>
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<tr>
<td>(ALTA, IT)</td>
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</table>
The BIOPIA initiative

**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.

Contact: Anna Fogdell-Hahn - Anna.Fogdell-Hahn@ki.se

https://ki.se/en/cns/biopia
ADA incidence confounders: Assays and populations

**Adalimumab**

![Graph showing the incidence of ADA for Adalimumab across different years, with population ranges and study sizes indicated.]

**Infliximab**

![Graph showing the incidence of ADA for Infliximab across different years, with population ranges and study sizes indicated.]

**Crohn’s disease/ulcerative colitis**

![Graph showing the incidence of ADA for Crohn’s disease/ulcerative colitis across different years, with population ranges and study sizes indicated.]

Pooled patient population across studies: 
- Adalimumab: n = 3965; sample size range: 3–394 patients.
- Infliximab: n = 4495; sample size range: 5–340 patients.
- Crohn’s disease/ulcerative colitis: n = 1305; sample size range: 23–240 patients.

Gorovits et al 2018 Clin Exp Immunol