

# IMI1 Final Project Report Public Summary

**Project Acronym:** ABIRISK

**Project Title:** Anti-Biopharmaceutical  
Immunization: Prediction and analysis  
of clinical relevance to minimize the  
risk

**Grant Agreement:** 115303

**Project Duration:** 01/03/2012 - 28/02/2018

## **1. Executive summary**

### **1.1. Project rationale and overall objectives of the project**

Clinical development of new biopharmaceutical products (BPs) offers the opportunity to biologically treat entirely new classes of diseases than ever before. As advances in the design and development of these complex biologic entities evolve, the ability to assess, or even predict, the immunogenicity potential of each new product must be considered to avoid LOR (loss of clinical response) or adverse effects. The overall objective of ABIRISK is to provide an integrated approach to anti-drug (AD) immunization in four major diseases in which BPs provide significant clinical amelioration: multiple sclerosis (MS), rheumatoid arthritis (RA), intestinal bowel diseases (IBD) and haemophilia (HA). The concept of the project is organized around the following driving forces and objectives: Access to and large cohorts of patients treated with BPs, complementary expertise for ADA assays, standardization and characterization of ADA, novel integrated approaches to characterize AD lymphocyte responses, development and validation of innovative prediction tools and collection and integration of immunogenicity-related data and clinical relevance of ADA.

### **1.2. Overall deliverables of the project**

The overall deliverables of the project are the following:

- Build a unique database collecting data both retrospectively from patients suffering from MS, RA, IBD and HA treated with various BPs and prospectively from cohorts of patients in dedicated studies during the 5 years of the ABIRISK program.
- Standardization of ADA assays for the BPs assessed in ABIRISK
- Provide novel integrated approaches to characterize AD lymphocyte responses and biomarkers of immunogenicity from treated patients.
- Development and validation of innovative prediction tools for BP immunogenicity (in silico, in vitro, and in vivo)
- Integration of immunogenicity-related data and clinical relevance of ADA using a single immunogenicity databank. Information will be selected on the basis of its potential value for immunogenicity prediction and safety gathered during the program. This will include the integration of various preclinical, clinical, and immune monitoring factors.

### **1.3. Summary of progress versus plan since last period**

#### **1.3.1. WP1**

Deliverables D1.5, D1.7, D1.11, D1.13, D1.14, D1.15 and D1.16 were still to be accomplished or reports were pending in the last report. During this past year, incomplete deliverables have been finished by now. D1.5 is now written after last results obtained for characterization. For D1.7 predictive cut-off titres for interferon-beta antibodies have been determined and a paper has been drafted and will be submitted soon. In this context, we could determine relevant cutpoints for anti-interferon- $\beta$  and anti-natalizumab antibodies including timing of testing with reference to therapy start. Deliverable D1.11 has also been completed by now, i.e. all audit and monitoring reports of the prospective cohorts have been collected and all queries have been resolved. With that, all data of prospective cohorts have been entered to the database and analysis by WP4 became feasible. However, D1.13 "Analysis of prospective cohorts - understanding of the clinical relevance of ADA

(pre-existing, non-neutralizing, low titer and transient antibodies” and D1.14 “Integrative analysis of cohorts with respect to experimentally generated data” have not been reached yet; analysis are in progress and results should be available in September 2018. These reports have not been uploaded in SOFIA. Also, a new deliverable (D1.17) has been acquired by WP1 which was shifted from WP5 regarding a document summarizing assay development, validation and recommendation for clinical implementation. The report will be available as soon as analyses of clinical and immunological outcomes have been done by WP4. An interim report has been loaded to SOFIA.

### **1.3.2. WP2**

A large number of deliverables were incomplete in 2017 and have been successfully finalized by a final report. This includes D2.4, D2.6, D2.7, D2.8, D2.10, D2.14, D2.17, D2.19, D2.21; D2.22, D2.23, D2.26 and D2.27. For instance, experiments associated with the characterization of the clonal T- and B-cell signatures against the germline sequences in RA patients treated with rituximab have been successfully performed and were extended to TNF $\alpha$  treated RA patients, MS patients (IFN $\beta$ ) (D2.22, D2.23). Deliverable D2.19 was discontinued due to a lack of cells from patients and cell viability issues. Deliverable D2.24 associated with the characterization of responding T- and B-Cell clones during treatment with BP could be not feasible as samples were received too late. D2.28 could not happen due the absence of samples (a report has been provided) and D2.29 could not happen as Astra Zeneca pulled out from the project at its onset. Deliverable D2.2 which deals with the genetics components of the AD response was previously described within D2.2A-D2.2F, D2.12 and D2.13. The respective report is now available in SOFIA.

### **1.3.3. WP3**

Multiple deliverables were incomplete in 2017 and have been finalized by a final report. It was the case for deliverables D3.11, D3.13, D3.16, D3.17 and D3.22. In the meantime, two papers have been published in “Frontiers in Immunology” and “Blood advances”. The experiments using the new model of FVIII deficient mice were completed (D3.12). *In vitro* T cell response to aggregated antibodies in comparison to the native antibody were evaluated and showed a differences between the two investigated antibodies (D3.15). Finally, all the data required to generate an integrated view of the WP3 data have been released (D3.19) and a comparison of the WP3 internal data and with all the other WPs has been provided (D3.21). However, D3.18 could not be reached for scientific reasons; a report was uploaded to SOFIA.

### **1.3.4. WP4**

Since the last report, the collection of prospective clinical and assay data was finalized in order to complete the database and perform the planned analysis (D4.3 and D4.5). The results from these analyses are currently being evaluated and manuscripts are in preparation. Merged data has been distributed to other WPs in order for them to complete analyses.

### **1.3.5. WP5**

Missing reports have been uploaded to SOFIA. The publications numbers have been updates accordingly.

## 1.4. Significant achievements since last report

### WP1

- Positive control monoclonal antibodies that were scaled up and provided to authorities (NIBSC)
- New assays for haemophilia (HA) have been implemented for routine analysis in patients using a combination of Lumitope, BAB-assay and the chromogenic FVIII NAB-assay. This is expected to reduce false positive ADA-results and an overall improved detection limit of ADA which considerably improve an early and reliable diagnosis of ADA in patients with HA.
- Results providing insight into immunogenicity of various biological drugs. Translation into clinical used depends on several other factors, such as persistency of ADA, titres, neutralizing capacity, and affinity. Results showing incidence of ADA in the first year of treatment.
- Finalized procedures for methods used to measure binding and neutralizing ADA against IFN $\beta$ -1a, Adalimumab, Infliximab, Etanercept, Rituximab, Tocilizumab, and Factor VIII. Methods for pharmacokinetic analysis of etanercept, adalimumab, infliximab, tocilizumab and rituximab are also provided.
- Development of an innovative method for assessing ADA in patients treated with adalimumab using PK analysis and antibody clearance.

### WP2

- Immunophenotyping of MS patient reveals a signature allowing to characterize ADA positive and ADA negative patients.
- Identification of novel marker for Bregs and whether Bregs are altered in ADA+ versus ADA- patients.
- Characterization of the clonal T- and B-cell signatures against the germline sequences in RA patients treated with rituximab and extension to TNF $\alpha$  treated RA patients and MS patients (IFN $\beta$ )
- ADA response appears to be negatively correlated with clinical responses in IBD patients (first results)
- Crystal structure determination of the natalizumab-ADA complex
- Kinetic development of binding antibodies against IFN $\beta$  for the prediction of neutralizing antibodies

### WP3

- Data from T cell induction experiments with peptides identified in MAPPs, *in silico* and *in vitro* binding assays submitted to ABIRISK database
- Generation of an integrated view of WP3 data
- Data from MAPPs test sets three, four and five submitted to ABIRISK database

### WP4

- Analysis tools for extracting de identified relevant information in pharmacovigilance
- All the results from prospective cohorts have been uploaded to the ABIRISK database

### WP5

- Sustainability plan implemented
- Financial reports finalized

## 1.5. Scientific and technical results/foregrounds of the project

### 1.5.1. WP1

The foreground produced by WP1 was monoclonal antibodies used as positive controls (PC) for various anti-drug antibody assays. These were generated at the Institute for Research in Biomedicine (IRB) from B-cells isolated from patients and scaled-up at Sanofi. For future use the material will be managed by the National Institute for Biological Standards and Control (NIBSC) where the lyophilisation and further characterizations will be conducted. Endorsement by the World Health Organization (WHO) will be sought to act as custodians of these PCs which will be made available to laboratories worldwide. Characteristics of the various antibodies are shown in the tables below.

Targeted Biopharmaceutical	Monoclonal Antibody	Batch Name	Type Bab/Nab	Activity EC80 (ng/mL)	Affinity EC50 (ng/ml)	Binding-KD (M) SPR	Isotype	
<b><i>Infliximab</i></b>	<i>INA29</i>	VA2-17-221-1	Nab	66	9	3 E-10	IgG1	κ
	<i>INA79</i>	VA2-17-198-1	Nab	489	12	1.7 E-10	IgG4	
<b><i>Adalimumab</i></b>	<i>ADA44</i>	VA2-17-199-1	Nab	155	14	2.5 E-10	IgG1	κ
	<i>ADA39</i>	VA2-17-200-1	Nab	169	12	4.9 E-11		
<b><i>Natalizumab</i></b>	<i>NAA80</i>	VA2-17-218-1	Nab	-	(46.02)	-	IgG1	λ
	<i>NAA96</i>	VA2-17-222-1	Bab	4946	9	1.5 E10		
<b><i>Rituximab</i></b>	<i>RXA3</i>	VA2-17-196-1	Nab	105	11	6.1 E-12	IgG1	κ
<b><i>Interferon-β</i></b>	<i>sa01.54</i>	VA2-17-216-1	Nab	69	31	-	IgG4	K
	<i>sa01.71</i>	VA2-17-217-1	Nab	86	420	-	IgG1	λ

**Table 1. Positive control monoclonal antibodies that were scaled up and provided to authorities (NIBSC)**

BAb Assay	Format	Sensitivity	Cutpoint	Drug Tolerance	nAb Assay
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IFN $\beta$	Bridging ELISA	26 ng/mL	0.078 OD (SCRN) 37.766% (CONF)	0.6 $\mu$ g/mL (@HPC, 1 $\mu$ g/mL) 0.06 $\mu$ g/mL (@LPC, 26ng/mL)	Yes
Anti-TNF: Adalimumab	Bridging ELISA	RA: 5.2 ng/mL IBD: 4.9 ng/mL	RA: 0.015+NC (SCRN), 64% (CONF); IBD: 0.012+NC (SCRN), 55% (CONF)	0.2 $\mu$ g/mL (@LPC) 0.7 $\mu$ g/mL (@HPC)	Yes
Anti-TNF: Infliximab	Bridging ELISA	7.5 ng/mL (RA); 5.1 ng/mL (IBD)	RA: 0.023+NC (SCRN), 55% (CONF); IBD: 0.007+NC (SCRN), 45% (CONF)	0.2 $\mu$ g/mL (@LPC) 0.7 $\mu$ g/mL (@HPC)	Yes
Anti-TNF: Etanercept	Bridging ELISA	6 ng/mL (RA)	RA: 0.027+NC (SCRN), 52% (CONF);	0.05 $\mu$ g/mL (@LPC) 0.4 $\mu$ g/mL (@HPC)	Yes
Anti-TNF: Adalimumab	Bridging ECL	18 ng/ml	RA matrix: 2.08 IBD matrix: 1.17	1.6-6.4 $\mu$ g/ml (LPC-HPC)	
Anti-TNF: Infliximab	Bridging ECL	10 ng/ml	RA matrix: 1.32 IBD matrix: 1.35	0.2 – 1.5 $\mu$ g/ml (LPC-HPC)	
Anti-CD20: Rituximab	Bridging ECL	0.03 ng/mL	Healthy: 1.18 RECL Chronic Lymphocytic Leukemia: 1.12 RECL Non-Hodgkin's Lymphoma: 0.96 RECL 39.1% (CONF)	100 $\mu$ g/mL drug at 500 ng/mL PC	No
Factor VIII	Solid phase ELISA	74.23 ng/mL	0.107+NC (SCRN) 35.32% (CONF)	0.15 U/mL FVIII	Yes
Tocilizumab	Bridging ECLIA	7.5 ng/mL	RA: 1.3 RECL 24% (CONF)	>50 $\mu$ g/mL at 500 ng/mL PC	No
New Natalizumab	Bridging ECLIA	4 ng/mL	Healthy: 1.2 RECL	>50 $\mu$ g/mL at 500 ng/mL PC	No

**Table 2. Newly developed or validated assays**

### 1.5.2. WP2

WP2 has taken advantage of assays validated in WP1 to identify patients who develop ADA. The immunological and genetic profiles of ADA+ and ADA- patients will be correlated and presented by the WP4 database. Predictive markers identified by WP2 analysis will be cross-verified with results generated in WP3. We have established different approaches cellular, antibody and genetic characteristics of patients treated with a range of different BP in different diseases. To gain insight into the mechanisms by which BPs drive immune cell activation. We have utilised the samples collected both retrospectively (i.e. SLE, RA, IBD, and HA-patients in the first phase) and prospectively (MS, RA, IBD, SLE and HA) from a range of cohorts across Europe (WP1).

The activation, maturation and differentiation of T and B lymphocytes in patients before and after BP treatment and the mechanisms underlying the breakdown in cellular immune tolerance leading to generation of ADA responses has been studied and several findings have been reported. The nature, avidity, persistency, glycosylation and other biochemical characteristics of ADA have also been established.

The following scientific results have been also achieved in WP2:

- Identification of an immune signature that predicts the development of ADA has been identified in RA patients treated with adalimumab, and in MS patients treated with IFN $\beta$ .
- Identification of novel marker for Bregs and whether Bregs are altered in ADA+ versus ADA- patients.
- Functional assessment of infliximab (IFX)-specific T cells in patients with inflammatory diseases with or without ADA demonstrated that IFX develops memory IL-10-producing T cells in exposed individuals that may contribute to the anti-inflammatory activity of the drug and regulate its immunogenicity.
- Epitope mapping in healthy donors and drug-treated patients identified responses to several adalimumab and rituximab epitopes using a peptide-based T-cell stimulation assay followed by three-color fluorospot assay.
- Next-generation sequencing (NGS)-based repertoire analysis was applied to identify, fingerprint and quantify clonal T- and B-cell receptor responses longitudinally and in different tissues from prospectively collected peripheral blood samples of patients starting a biologic therapy. Treatment with adalimumab is likely to influence the phenotypic composition of the BCR repertoire in peripheral blood.
- Development of a protocol for detection of immunogenic antigen-specific T-cells clones by comparing samples before and after *in vitro* antigen stimulation. Taking advantage of this technique, we were able to show that anti-drug T-cell responses can be detected in blood before anti-drug antibodies are detectable.
- Crystals could be grown successfully for five BP-ADA complexes.
- Polymorphisms and fragment analysis data from 586 patients with Haemophilia A (HA) were uploaded to the common database platform (tranSMART) for analyzing the risk of ADA development together with other potential risk factors of ADA development recorded in that database.
- Genetic analyses were performed by whole genome sequencing using the Illumina platform. Data was compiled in the tranSMART database for analysis with clinical outcomes in WP4.

### 1.5.3. WP3

The following scientific accomplishments have been achieved in WP3:

- Comparative analysis of T-Cell assays outcomes.
- A complete characterization of T cell epitopes of therapeutic proteins including *in silico* data, HLA binding activity and MAPPs data.
- Comprehensive analysis of impact of aggregation of therapeutic antibodies on peptide presentation by dendritic cells (DC) or DC maturation.
- Comparison of methods to assess DC maturation.
- New FVIII deficient mouse models were developed.

- Assessment of new potential models of immunogenicity prediction including artificial lymph nodes and immunodeficient mice grafted with human hematopoietic stem cells.

#### **1.5.4. WP4**

WP4 has generated foreground in terms of merged datasets from the studies (both retrospective and prospective) conducted in ABIRISK and by development of novel algorithms for statistical analysis. The technical setup for data processing and QC heavily relies on the tranSMART processing scripts. Here WP4 has interacted with the eTRIKS/tranSMART community and the technology for data processing and loading are in the public domain.

A novel predictive tool was implemented to analyse the time-to-event outcome data generated in ABIRISK. This method is called improper Bagging Survival Tree (iBST) and has been made freely available in an R package. Furthermore, the GPLTR for binary outcomes procedures developed by WP4 has also been made available as a free R package.

### **1.6. Potential impact and main dissemination activities and exploitation of results**

Biopharmaceutical products (BPs) offer a great opportunity to treat entirely new classes of diseases than ever before. However, due to the fact that BPs are proteins, the body can mount an immune response against them in the form of anti-drug antibodies (ADAs). Some ADAs can increase BP clearance or neutralise the function and thus decrease the BPs efficacy, resulting in patient secondary unresponsiveness. Also, adverse events such as hypersensitivity reactions can result from an ADA response. In situations, where a BP replaces an endogenous protein (replacement therapies) with a unique biological function, development of neutralizing ADA can even lead to life-threatening conditions. Thus, besides the huge benefits that come with the use of BPs, development of ADA is one of the major limitation. ABIRISK was world-wide the first orchestrated large-scale endeavour to understand underlying factors that influence development of ADA and to increase the knowledge on predicting development of ADA on a patient as well on a drug product level.

Despite immunogenicity being one of the limitation for the treatment of patients with BPs, it has not been a strong focus of academic research in Europe before ABIRISK started. Some industry-driven research activities had been ongoing but only relatively few academic and clinical groups in Europe focussed their basic research on understanding the immunological events that drive anti-BP immunisation.

ABIRISK brought industry and academia in Europe closer together and paved the way for a joint understanding of the need to investigate immunogenicity of BPs further and to speak a common language in Europe. The alignment of the language resulted in an important publication on the standardization of terms, definitions and concepts for describing unwanted immunogenicity to BPs that is referenced in the current EMA Guideline on Immunogenicity assessment of therapeutic proteins (EMA/ CHMP/ BMWP/ 14327/ 2006 Rev 1). Not only language needed a standardization but also the assays that were established to test for immunogenicity. It is a known caveat of immunogenicity assays that results may differ between different assay types and therefore ABIRISK developed reference standards that were derived from patients that were immunized during the



treatment with the studied BPs. For the first time, such human-derived reference standards will be made available publicly through the National Institute for Biological Standards and Control (NIBSC) in the United Kingdom.

The six years of close collaboration between industry, basic research and clinical institutes within the ABIRISK consortium was for many of the consortium participants just the beginning. Already during the course of ABIRISK, additional projects between industry and academia but also between different academic institutes flourished, nourishing the ground for further biopharmaceutical research in Europe.

ABIRISK's results increased the understanding of the immunological events that drive or contribute to anti-BP immunisation. Similarly, they support the concept of applying *in vitro* assays, such as antigen presentation or T cell response assays, for selection of drug candidates that bear a lower risk for immunogenicity. Thus, future BP generations will benefit from potentially reduced immunogenicity incidence rates. Consequently, less patients may be facing a loss of efficacy due to anti-BP immunisation.

In addition, potential biomarkers that may predict development of anti-BP immunisation have been discovered. In case where several different BPs are available as treatment options, for example the TNF- $\alpha$  blockers, the most appropriate treatment for a patient may be identified by testing for the respective immunogenicity-related biomarkers. This means that patients could receive cost-effective treatment without risking the development efficacy-limiting anti-BP immunisation.

Based on ABIRISK's published assays to test for immunogenicity response as well as for BP concentrations (pharmacokinetics, PK) in patient samples, treatment guidelines will be influenced. These will guide clinicians on treatment decisions for the BPs that were studied in ABIRISK. Based on the pharmacokinetics, the immunogenicity status and efficacy information, the clinicians would then be able to decide to the benefit of the patient to stay on the BP, to switch to another BP within the same class, i.e. with the same target, or to switch to a different class of BP targeting a different part of the pathway that is associated with the respective disease. In this context, the ABIRISK laboratories that developed assays to determine BP serum levels and anti-BP immunogenicity are providing this service to interested clinicians.

Following ABIRISK, BIOPIA was founded which is a non-profit collaborative effort of multiple European laboratories with expertise in biopharmaceutical pharmacokinetics and immunogenicity. It is an initiative that aims to 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. BIOPIA involves several labs from all across Europe that specialize in biotherapeutic immunogenicity and assessing drug serum levels in many diseases. Through the BIOPIA website (<https://ki.se/en/cns/biopia>), these labs will work together to (1) provide easy, accessible information about ADA and drug level testing, (2) create a site for clinicians to help them assess biologic responses in their patients and choose the correct treatment for each person and (3) connect European labs and hospitals together, with the goal of implementing routine, clinical testing for immunogenicity and drug levels.

ABIRISK organized and co-organized three important open conferences. On 6<sup>th</sup> of September 2015, ABIRISK held a Satellite Symposium on "Immunogenicity of Biopharmaceuticals" at the European

Congress of Immunology in Vienna which was attended by more than 100 researchers, many coming from basic research institutes that were not involved in ABIRISK project. In the following year, on 1<sup>st</sup> of April 2016, ABIRISK organized the “1<sup>st</sup> ABRISK Drug Immunogenicity Conference” in Innsbruck. In its final year, ABIRISK co-organized together with the European Immunogenicity Platform (EIP) the “Joint ABIRISK-EIP Open Symposium – Immunogenicity of Biopharmaceuticals” in Lisbon from 13<sup>th</sup> to 16<sup>th</sup> November 2017 which was attended by about 150 participants with key representatives from both, the FDA and the EMA.

As a consortium, ABIRISK has delivered numerous scientific presentations at international conferences, and already has 38 manuscripts published in peer-reviewed journals such as PLoS One, Clinical & Experimental Immunology, Cellular Immunology, Arthritis & Rheumatology, Haemophilia, Neurology, Multiple Sclerosis, Blood Advances, Bioanalysis and many more. About 40 more manuscripts are still in preparation or under review. Additional dissemination activities such as a training course for PhD students and additional presentations at international conferences and at an FDA workshop are planned for 2018 and beyond.

### **1.7. Lessons learned and further opportunities for research**

**For WP1:** With respect to assay development and validation the cooperation has been extremely fruitful and complementary. On the academic side access not only to patients’ samples but also to full clinical background was an essential input. Also, experience in assay development and application in the clinical setting was already implemented in some academic partners. Very few academic partners were highly specialized, e.g. on selecting and producing monoclonal antibodies directly from patients’ blood samples. On industry’s part experience in regulatory affairs and market access was very welcome and helpful. Further, large scale certified production opportunities provided by industry was essential to make positive controls available to the public.

Regarding cohort management organizational structures and experience in running clinical trials by industrial partners was again essential to warrant high quality of creating study protocols, follow-up, and data completeness.

**For WP2,** we have obtained several immunosignatures related to ADA and it would be important to test these now in larger cohort of patients as well as in other diseases. The analysis of a vast amount of markers has also generated a wealth of results that would be important to test in patients from different cohorts. (e.g. predictive of disease). Many studies were pilot studies with WP2. The conclusions of these studies would need to be evaluated in other diseases, with other biotherapeutics and with more samples from ADA+ patients. This applies to the genetic and structural analysis for instance but also to the characterization of the anti-drug antibody.

**The WP3 deals** with the prediction of immunogenicity, which is a topic with both basic and applied aspects. Interest for the prediction could have been perceived differently by the pharmaceutical companies and academic researchers. We overcame our differences by trying to fulfil both requests of evaluating the relevance of the prediction assays and by revealing fundamental mechanisms of immunogenicity. **Sharing the governance of the WP between academic researchers and industrial partners was a key point to succeed in maintaining a good balance between these two objectives.** From an academic side it has been much appreciated to benefit from the operational organisation of the industry partners. This allowed for instance centralization of the sourcing, supply and distribution

of BPs to the different partners. On the other hand the industry partners benefited from the rich scientific expertise and the innovative spirit of the academic partners. In fact, scientific complementarity of the team was very good and the project took advantage from all the expertise. For instance, aggregates of BPs have been produced and characterized by industry partners and their effects on DC and T cell activation as well as on antigen presentation have been evaluated by multiple teams from both, academia and industry.

**For WP4:** The PPP collaboration was very important to set up a number of studies distributed in several European countries. The industry partners have well-defined processes for running clinical studies which has been very useful in obtaining high quality data. Using only academic resources it would have been challenging to run so many multi-national studies within the project. The academic groups have on the other hand brought new ways to explore and analyse the data, which means a very good combination between industry and academia.

As the nature of the game, industrial efforts need to follow commercial interests. Academic institutions are probably freer choosing their lines of scientific interest and will not suffer as much from unsuccessful research outcome. Getting public funding onto the interaction between academic/public institutions and industry, there will be interest in commercially less promising research lines.

The ABIRISK project was very ambitious in terms of scope of diseases and types of biotherapeutics which made it scientifically very attractive but also in a way not focused enough. It has opened up many very exciting scientific avenues that would need to be further investigated before it becomes useful for developing new drugs or for changing the medical practice of existing medicine. Many of the deliverables have not translated into practical tools that can be used in a routine fashion by the drug developers and clinicians to provide safer treatments to the patients. **This also highlights that if a PPP such as ABIRISK has promising or unmet goals it should be continued under specific rules or calls.**

**One of the biggest hurdles for the project was the contracting, especially the legal aspects of contracting.** For example, setting up a collaboration with the IMI-eTRIKS consortium took more than 2 years. Of course, we were able to collaborate during that time, without exchange of any relevant data but it took a lot of time and energy of the involved participants. In a similar way we are currently negotiating with ELIXIR to host the ABIRISK data for future access and use, also by the broader scientific community. Ideally, the procedures of sharing data after project end should have been regulated in the original project agreement. Getting legal teams of 39 partners to review documents / contract / collaboration agreements and so on is huge challenge. A clear legal basis provided by the IMI JU for such exchange and for sustainability after the finalization of a project would be of great benefit.

**Comment from the IMI JU managing entity:** One major last item is the necessity to have a continuity of the personnel from EFPIA companies along the project. In ABIRISK we had to face the loss of very strong and committed researchers from several companies and concomitantly a loss in scientific expertise and a delay in the program. Also, a stronger commitment from the management is sometime needed to allow a better reactivity from EFPIA partners.

In the field of ADA assays, development of highly sensitive and specific tests needs to be advanced, particularly addressing drug & target interference. Various methods, particularly mass spectrometry should be explored, the latter looking into specific signature peptides that might evolve during antibody-antibody interaction as compared to unbound proteins.

There are many areas from WP2 that would need to be followed up. Having reliable predictive biomarkers of immunogenicity would accelerate the successful development of new biotherapeutics and support patient stratification. This consortium has focused on protein therapeutics but there is a growing number of new biotherapeutics modalities (multi-specific antibodies, fusion proteins, conjugated proteins, cell therapies, gene therapies, RNA-based therapies) for which similar studies will be also required.

Including a larger variety of markers such metabolomics, epigenetics, microbiome, could give more powerful insight into the preliminary signatures which have been identified. In addition, there is by now capability to run single cell analysis. Single cell analysis could provide a much more comprehensive picture of the immune response and provide some new avenues for biomarker identification and for the understanding of the underlying mechanism of immune response.

The issue of immunogenicity of therapeutic proteins is not entirely resolved. Immunomonitoring of the cellular response in patients treated with therapeutic antibodies might be improved. The relationship between the number of infused products, its biodistribution and lifetime, its mode of injection and its immunogenicity is finally poorly documented and not at all understood. Another issue is to improve the developability of the therapeutic proteins in the early steps of development by increasing the methods of sequence optimisation (immunogenicity, production, stability, homogeneity, aggregation).

From a data and data analysis perspective the challenge is always to get as much data as possible to reach statistical significance. Of course, it would be key that research data on industry owned projects can be shared effectively and publicly between research organizations and industry, while ensuring that competitive information remains protected. There are currently many attempts to bring data together on a large scale, like ELIXIR for example. It would be very beneficial to build on the knowledge obtained in ABIRISK in order to create a strong data repository for this type of data (data related to immunogenicity of biologics). The process to collect such data from multiple sources is a major outcome of ABIRISK and could be of benefit to future projects. Larger datasets will also enable development of new algorithms for data analysis, potentially also identifying low frequency risk groups.