

MEETING REPORT



10th European immunogenicity platform open symposium on immunogenicity of biopharmaceuticals

S. Tourdot^a, A. Abdolzade-Bavil^b, J. Bessa^c, P. Broët^d, A. Fogdell-Hahn^e, M. Giorgi^f, V. Jawa^g, K. Kuranda^h, N. Legrandⁱ, S. Pattijn^j, J. A. Pedras-Vasconcelos^k, A. Rudy^l, P. Salmikangas^m, D. W. Scottⁿ, V. Snoeck^o, N. Smith^p, S. Spindeldreher^q, and D. Kramer^r

^aBioMedicine Design, Pfizer Inc, Andover, MA, USA; ^bLarge Molecule Bioanalytical Sciences, Pharma Research and Early Development (pRED), Roche Innovation Center Munich, Hoffmann-La Roche Ltd, Germany; ^cPharmaceutical Sciences, Pharma Research and Early Development (pRED), Roche Innovation Center Basel, Hoffmann-La Roche Ltd, Basel, Switzerland; ^dFaculty of Medicine Paris-Saclay, Orsay, France; ^eDepartment of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden; ^fCertara QSP, Certara UK Limited, UK; ^gPredictive and Clinical Immunogenicity, PPD, Merck & Co, Kenilworth, NJ, USA; ^hTranslational Department, Sparks Therapeutics, Philadelphia, PA, USA; ⁱGenOway, Lyon, France; ^jImmunXperts SA, Gosselies, Belgium; ^kOffice of Biotechnology Products, CDER, Food and Drug Administration, USA; ^lHEXAL AG, Holzkirchen, Germany; ^mNDA Advisory Services Ltd, Surrey, UK; ⁿDepartment of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, USA; ^oTranslational Biomarkers and Bioanalysis, UCB Biopharma SRL, Braine-l'Alleud, Belgium; ^pLonza, Slough, UK; ^qIntegrated Biologix GmbH, Basel, Switzerland; ^rSanofi R&D, Translational Medicine & Early Development, Sanofi, Frankfurt am Main, Germany

ABSTRACT

Therapeutic proteins and emerging gene and cell-based therapies are attractive therapeutic tools for addressing unmet medical needs or when earlier conventional treatment approaches failed. However, the development of an immune response directed against therapeutic agents is a significant concern as it occurs in a substantial number of cases across products and indications. The specific anti-drug antibodies that develop can lead to safety adverse events as well as inhibition of drug activity or accelerated clearance, both phenomena resulting in loss of treatment efficacy. The European Immunogenicity Platform (EIP) is a meeting place for experts and newcomers to the immunogenicity field, designed to stimulate discussion amongst scientists across industry and academia, encourage interactions with regulatory agencies and share knowledge and the state-of-the-art of immunogenicity sciences with the broader scientific community. Here we report on the main topics covered during the EIP 10th Open Symposium on Immunogenicity of Biopharmaceuticals held in Lisbon, 26–27 February 2019, and the 1-d training course on practical and regulatory aspects of immunogenicity held ahead of the conference. These main topics included immunogenicity testing, clinical relevance of immunogenicity, immunogenicity prediction, regulatory aspects, tolerance induction as a mean to mitigate immunogenicity and immunogenicity in the context of gene therapy.

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Introduction

Therapeutic proteins have radically changed the quality of life of a considerable number of patients suffering from diverse complex progressive and/or life-threatening diseases. However, the desired wide use of these therapeutic agents and that of emerging ones such as gene and cell-based therapies, may be impeded by their immunogenicity, i.e., their capacity to induce an immune response in a proportion of treated patients. This immune response, characterized by the development of specific anti-drug antibodies (ADA), can ultimately lead to loss of treatment efficacy through inhibition of the agent activity or accelerated clearance and safety issues, some of them provoking patient death.^{1–4} In this context, regulatory agencies in charge of granting market authorization require the immunogenicity risk to be thoroughly explored and characterized, and have provided sponsors with specific guidelines on ADA assays and immunogenicity risk assessment for biologics of various types.^{5–9} Consequently, scientists and clinicians developing biologics are faced with the challenge of conducting trustworthy

immunogenicity risk assessments, accurately measuring ADA levels, estimating their clinical relevance and impact on safety and efficacy, and correctly reporting immunogenicity data in regulatory dossiers. Once marketing authorization is granted, additional challenges include the efficient management of unwanted immunogenicity should it occur, and evaluation of its consequences on safety and treatment efficacy to ensure that patients receive the highest quality of care. The establishment of a relationship between ADA development and loss of efficacy or the appearance of adverse events heavily relies on accurate and timely measurement of ADA. It also implies that reliable assays to measure serum trough drug levels and approaches to estimate their interconnection are available.^{10,11} In this setting, ADA assays and clinical immunogenicity testing strategies need to continuously evolve to adapt to the emergence of new formats for protein drugs, such as multi-domain monoclonal antibodies, and new approaches to treatment such as gene and cell-based therapies.¹²

In parallel, at a very early stage of product development, efforts will focus on the design of biologics that exhibit a low

immunogenicity risk. *In silico* and *in vitro* tools have been developed to identify the risks inherent to the product itself, and, where possible, guide the removal of liabilities, e.g. T cell epitopes, de-amidation sites, tendency to aggregate. This evaluation can be used to select one candidate over any others to undergo clinical development. Frequently referred to as immunogenicity prediction, pre-clinical immunogenicity risk assessment also includes a comprehensive listing and estimation of the risk factors inherent to the treatment, e.g., dose, frequency of administration, co-medication and to the patient profile e.g. disease, immune status, genetic background. The challenge resides in the ability to integrate and weigh the contribution of product, treatment and patient-related risk factors to provide an overall estimated immunogenicity risk prior to clinical development.^{13 14} By the time, the program is ready for the submission of a Marketing Authorization Application (MAA) in Europe or Biologics License Application (BLA) in the US, clinical immunogenicity data will have been acquired and will be included in the dossier. As the field progressed, regulators increased their prerequisite in terms of ADA assay characteristics and performance, such as sensitivity and drug tolerance, hence the necessity to refer to the latest version of the immunogenicity-related guidelines when embarking upon biologic drug development. The presentation of immunogenicity risk assessment and measurement in regulatory dossiers can be a daunting process, as many pieces of information are reported in various separate sections of the dossier. Recently, however, the European Medicines Agency (EMA), shortly followed by the US Food and Drug Administration (FDA) introduced an Integrated Summary of Immunogenicity to the MAA and BLA dossiers, facilitating regulatory review of the immunogenicity risk assessment of the new biological entities, with the view of reducing the time for a product to reach patients, while ensuring its safety.¹⁵

The generation of safer products in terms of immunogenicity risk may not always involve the removal of sequence liabilities. This is the case, for instance, for recombinant proteins with enzymatic activity, which lose activity if the catalytic site is modified or the conformational structure altered. An alternative approach to de-immunization to mitigate clinical immunogenicity in the case of life-saving replacement therapies is the use of immune tolerance induction regimens. Indeed, immunomodulatory agents, including combinations of low-dose methotrexate, rituximab and intravenous immunoglobulin, are currently used in the clinic.¹⁶ Numerous novel approaches to induce antigen-specific tolerance induction are emerging, albeit still at a pre-clinical stage of development, such as infusion of antigen-specific T regulatory or CAR-T cells, the use of antigen-transduced erythrocytes, or proteasome inhibitors.¹⁷ An approach to immune tolerance induction currently evaluated in clinical trial involves the use of rapamycin synthetic virus particles in the context of gene therapy.¹⁸ Gene therapy has potential to cure a life-threatening disease via a single-dose administration. However, innate and adaptive immune responses to gene therapy vectors remain a major obstacle to achieving efficacy. Application of treatment is further complicated by the high incidence of preexisting immunity to adeno-associated viruses

(AAV), which are the most common gene therapy vectors. Hence, deciphering the mechanisms pertaining to AAV immunogenicity is fundamental to designing immune tolerance induction regimens, which will allow successful expression of the transgene and re-dosing if necessary.¹⁹

At the occasion of the EIP 10th Open Symposium on Immunogenicity of Biopharmaceuticals, 30 experts from academia and industry came together to report on our current knowledge and handling of immunogenicity challenges and what lies ahead. Here, we summarize most of the presentations and discussions that took place on the topics of immunogenicity testing, clinical relevance, immunogenicity prediction, regulatory aspects, tolerance induction to mitigate clinical immunogenicity and immunogenicity consideration for gene therapy.

Immunogenicity testing

Immunogenicity testing is critical to safer drugs development, be it new biological entities or biosimilars to a reference product. Establishing assays that accurately measure ADA, determine their neutralizing or non-neutralizing nature, and identify their isotype in relation to potential safety events is therefore of utmost importance. In this context, assays need to evolve and be tailored to new complex protein drugs, gene and cell-based therapy vectors, as well as alternate matrix to serum.

Dr. Afsaneh Abdolzade-Bavil, from Roche, Germany, discussed the challenges of immunogenicity testing of therapeutic antibodies in ocular fluids after intravitreal injection. Immunogenicity assessment comprises an essential part of the development program for therapeutic antibodies, which supports the correct interpretation of pharmacokinetic (PK), pharmacodynamic and safety data. High drug concentrations in ocular fluids after intravitreal (IVT) administration preclude the use of drug-sensitive immunoassays. A drug-tolerant immunoassay is therefore desirable for immunogenicity testing in ophthalmology. An immune complex (IC) ADA assay was established for aqueous humor (AH), vitreous humor (VH) and plasma samples. The assays were compared to the bridging ADA assay. This sophisticated solution has been implemented for a wide range of studies in different ophthalmological projects with IVT administration including different animal species. This sensitive and drug-tolerant ADA assay enabled the detection of ADAs in ocular samples for the first time. Good correlation of AH and VH ADA data allows immunogenicity monitoring without termination of the animals. Strong correlation of systemic immunogenicity data with histopathological findings in retina and inflammatory response in the eye was observed. The IC assay allows a reliable ADA detection in matrices with high drug concentrations, such as ocular fluids after IVT injection. Dr. Abdolzade-Bavil concluded that systemic ADA analysis might be sufficient for the evaluation of immunogenicity in non-clinical and clinical ophthalmological studies.

Dr. Vibha Jawa, from MSD, USA reported on the American Association of Pharmaceutical Scientists' ADA validation reporting initiative. The initiative is a year-long effort led by biopharmaceuticals biotechnology companies and

regulatory agencies. The talk summarized the recommendations for the harmonization of validation testing and data reporting and alignment with the regulatory agencies through their input and insight. The aim was to increase clarity around communications to health authority queries received during filling. The effort also partnered with the European bioanalytical forum to gain alignment with EU industry members as well as European regulatory agencies. The final outcome would be a collaborative industry manuscript ensuring streamlining of communications to regulatory agencies.

The key parameters during assay validation, such as assay cut point, assay sensitivity, drug tolerance, sample stability, selectivity, as well as considerations for multi-domain biologics were summarized. There are several interpretations to the guidance on assay validation. Hence, the need to harmonize and streamline across industry, and align with the regulatory agencies. For instance, correctly establishing the assay's cut point is critical to suitable clinical performance, henceforth the following recommendations were provided around assay cut point: 1) The screening assay cut point should provide a 5% false positive rate (FPR) to reduce the risk of false negative results; 2) Lower 90% confidence limit of the 95th percentile helps ensure a 5% FPR 90% of the time; and 3) FPR < 2% or > 11% post-outlier exclusion can trigger the need for in-study cut point. Regarding assay sensitivity, it was recommended that sensitivity be calculated by using data points that flank the cut point to calculate the concentration of positive control (PC) that correlates to the raw response of the assay cut point. If screening and confirmatory sensitivities are similar, the low positive control (LPC) that meets criteria for both tiers should be selected. The sensitivity can then be defined as the lowest ADA level that is consistently (>99%) screened and confirmed positive.

It was recommended that drug tolerance be reported separately for screening and confirmatory assays. The drug tolerance is defined as the highest drug concentration that can still enable a reactive result to be detected in a screening assay and a positive result in a confirmatory assay. Multiple validation runs should be performed and median tolerated drug concentration at each ADA level assessed. The drug tolerance in other populations should be recommended only if population-specific cut points are implemented. Regulatory considerations expect a sufficient drug tolerance to enable detection of ADA in the presence of serum drug at the time of sample collection. However, if no clinical consequences are observed in patients with low-level ADA responses, assay drug tolerance may be acceptable even if the expected drug level is tolerated only at ADA concentrations higher than 100 ng/mL. For selectivity, both screening and confirmatory tiers should be tested to ensure that matrix does not interfere with the confirmatory state. The use of one LPC set at a level that robustly screens and confirms should be considered. Other factors to be considered during selectivity assessments include hemolytic/lipemia samples, diseased state and preexisting antibodies. There is no recommendation for assessing selectivity during co-mediations.

The assays for next-generation biologics, such as fusion proteins, antibody-drug conjugates, bispecific antibodies and

pegylated proteins, may need additional considerations. The critical reagents would need a careful design of positive controls against the multiple binding domains and labeling the drug. Some anticipated challenges would include the generation of different domains leading to differences in 3D structures vs the entire molecule. Some risks with novel structural formats would be the creation of neo-antigens or exposure of cryptic epitopes. Hence, there is a regulatory expectation that the immune response will be characterized, and appropriate controls against the multiple epitopes will be used. Specifically, the competitive inhibition strategy can be applied to confirm the specificity of the ADA reactivity to each domain.

The risk factors identified at each stage of drug development are scored to develop a bioanalytical strategy for clinic as discussed below. The risk assessment and bioanalytical strategy based on the activities performed during pre-clinical development can be provided as part of the Investigational New Drug (IND) application. The main components would include a brief background on the therapeutic protein with respect to its modality (e.g., monoclonal antibody, multi-domain, cell/viral/nucleic acid) and the target and disease indication. The structure/sequence-based risk, any posttranslational-related attributes, as well as disease state risk can all be summarized as part of this risk assessment. If there is prior experience in the clinic, a summary of the results related to immunogenicity and its impact on exposure, efficacy and safety can be provided. This initial assessment can then support the development of the sampling strategy in clinic with a high probability of risk driving the frequent monitoring vs low level of risk factors leading to reduced monitoring and a collect and hold strategy. The bioanalytical assays to support such a strategy can also be streamlined with minimal assay development for molecules with a low probability of risk vs additional characterization (e.g., titer, domain specificity) for molecules with a high probability of risk.

Immunogenicity is a potential concern for all biopharmaceuticals. For biosimilars, the focus is on confirming that there are no clinically meaningful differences between the reference product and the biosimilar. Immunogenicity is analyzed in head-to-head clinical trials (reference vs. biosimilar) using state-of-the-art bioanalytical assays. **Dr. Anita Rudy** from Sandoz, Germany reported on immunogenicity testing for GP2017, a biosimilar to reference adalimumab, indicated for use in rheumatoid arthritis, psoriatic arthritis, plaque psoriasis, ankylosing spondylitis, juvenile idiopathic arthritis, hidradenitis suppurativa, Crohn disease, ulcerative colitis and uveitis. Similar PK bioequivalence between GP2017 and adalimumab was demonstrated in healthy volunteers and similar efficacy, safety and immunogenicity were demonstrated in confirmatory clinical studies. GP2017 is now approved in the US and Europe. Immunogenicity of GP2017 was assessed in a multi-tiered approach: serum samples were first analyzed in a screening assay and, if positive, the specificity for anti-adalimumab antibodies was evaluated in a confirmatory assay. Confirmed positive samples were further characterized for their potential neutralizing capacity. A proper cut-point determination and set-up of quality controls (i.e., low positive control determination) are of specific importance for

biosimilar immunogenicity assessment. Sandoz used highly sensitive and highly drug tolerant state-of-the-art assays to determine the immunogenicity of GP2017, which was shown to be similar to the reference adalimumab.

Dr. Veerle Snoeck, from UCB Biopharma SRL, Belgium gave the EIP Assay Working Group update, in which she presented the two major topics the working group will focus during 2019 on: 1) Collecting general feedback on guidelines and identify major point of challenges. Based on the combined feedback, topics that require further discussion with agencies will be identified; and 2) Discussion of a risk-based bioanalytical testing strategy for multi-domain drugs. The objective is to work out and find alignment on the best practice considering the nature of the biologic and immunogenicity risk. The Working Group intends to share the output from these activities at the EIP Open symposium and at other meetings.

Clinical relevance

ADA formation has been linked to lower serum drug levels and loss of clinical response. However, in a significant number of cases, ADA development has also been found not to affect patient treatment efficacy or safety. Hence, assessing the temporal evolution of both ADA and drug levels is essential to determine the biological consequences of unwanted immunogenicity, in terms of loss of clinical response and occurrence of adverse events. This includes accurate assessment of pre-existing immunity stands, as a prerequisite to establishing reliable immunogenicity mitigating strategies.

Dr. Anna Fogdell-Hahn, from the Karolinska Institute, Sweden discussed the evaluation of the clinical impact in heterogeneous populations and additional monitoring of ADA and PK parameters using appropriately sensitive and specific bioanalytical methods. ADA and PK data are only partially introduced in clinical routine, even though it could be beneficial to aid personalized medicine by tailoring the right treatment regime to the right patients, and thereby optimize health-care quality and resources allocations. Lack of guidance on when to test, with what method and how to interpret the results have left clinicians skeptical of the benefits of using PK and ADA data as integrated in the treatment decisions. Furthermore, as ADA methods are becoming more sensitive, there is a need to determine a clinical threshold titer value and distinguish this from being ADA positive.

A post-marketing observational study in a real-life setting of rheumatoid arthritis (RA) patients treated with infliximab was presented. They compared results of PK and ADA from a randomized clinical trial (RCT) with: 1) a cross-sectional cohort of patients that been treated from over 2 y to up to 18 y of treatment; and 2) a prospective cohort of patients followed from treatment initiation up to 28 weeks with samples taken in trough. The proportion of RA patients with low, medium and high drug levels were different in the RCT compared to the real-life studies, showing that results from an RCT cannot be directly translated to the clinical situation. Optimal trough level of drug was found to be between 0.85 and 6.35 $\mu\text{g/ml}$, meaning that both a too low and a too high level of drug gave unfavorable treatment responses. Even in

the cross-sectional cohort of patients that had been on the treatment for several years, only 58% had the optimal level, whereas 34% had too low and 8% had too high level. Regarding patients with very low drug level, in 99% of the cases, this could be explained by the development of ADA. The patients with too high level had already been subjected to a dose escalation, probably because they had more severe disease. However, these patients might benefit from switching to a drug with another mode of action.

The number of additional patients positive for ADA when using the drug-tolerant precipitation and dissociation method (Panda) was relatively low, only 18% in the group with a drug level between 0.2 and 3 $\mu\text{g/ml}$. Thus, the formation of immune complexes does not seem to explain a large proportion of the failure to detect ADA, at least not in samples taken in trough. However, using the drug-tolerant Panda method resolved many of the undetermined test results from the RCT cohort and allowed detection of patients earlier than when the drug-sensitive ELISA was used. In summary, ~60% of the RA patients treated with infliximab have a presumed optimal effect of the treatment, and the others would benefit from an informed decision of treatment switching or dose regulation with the aid of test results for drug level and ADA. They suggest a treatment algorithm for how to best integrate this in clinical routine and propose that similar treatment algorithms would be beneficial to generate for other biologics when implementing ADA testing in clinical routine. To this end, they have set up a web page of laboratories in Europe providing tests for PK and ADA, BIOPIA (<https://ki.se/en/cns/biopia>), were facilities offering services for testing can be searched.

Prediction of immunogenicity

The development of ADA to protein drugs and cell-based therapy products is likely to follow the classical path to antibody formation described, for instance, for pathogens in the vaccine field. Multiple factors pertain to immunogenicity risk, mainly related to product and patient/treatment specificities. In this context, non-clinical methods have been developed to assess the product-related risks of biologics to provoke ADA formation, based on the various steps of the immune cascade leading to antibody secretion. Novel approaches are also being investigated for prediction of the patient-related risks, some aiming for an integration of both sources of risk factors to predict clinical immunogenicity.

Dr. Sofie Pattijn from ImmunXperts, Belgium, and an EIP Team member covered the basic concepts of the current understanding of unwanted immunogenicity. Immunogenicity or “the ability of a particular substance, such as an antigen or epitope, to induce an immune response,” can be desirable (in the context of vaccines) or unwanted (in the context of using biologics and cell-based therapy to treat diseases). In the latter case, the body mounts an immune response toward these treatments, which can lead to neutralization or rejection of the therapy vectors. Most antibodies are formed via the T-cell dependent B cell activation pathway. Both B cells and T cells are lymphocytes that are derived from specific types of stem cells, called multipotent hematopoietic stem cells, in the bone

marrow. On leaving the bone marrow, B and T-cells remain naïve until they encounter their cognate antigen. When a B cell recognizes its antigen, it becomes activated. Upon an antigen-specific interaction with an activated T cell, it undergoes different modifications and the process of clonal expansion, affinity maturation, isotype switching take place. This eventually results in the production of high-affinity antibodies and the differentiation of B cells to a memory phenotype, ready for a recall response.

The immunological memory is a product of B and T lymphocytes activation, and antibodies can be considered the end products of a cascade of evolutionary learning processes. ADA development is thought to follow this universal pathway. Factors increasing the risk of the development of unwanted immunogenicity are commonly classified as product and treatment/patient-related. The former comprises the biopharmaceutical sequence, structure, presence of novel epitopes, post-translational modifications such as glycosylation, deamidation, oxidation, presence of impurities (host cell proteins), aggregation and degradation products, formulation and storage conditions. The mode of action of the product also might represent a risk factor: expression of the target on immune cells, immune-checkpoint inhibitors, which release the brakes of the immune system, combination therapies. The latter refers to patients' characteristics, such as age, gender, disease status, genetic makeup (human leukocyte antigen (HLA) type), immune status, and the presence of preexisting or cross-reacting antibodies, and therapy regimens such as dose, length, route of administration and co-treatment. Finally, the most important factor for the type of unwanted immunogenicity seen today is probably the interplay between the abovementioned factors. The same is seen in the field of vaccines, where when a group of people is vaccinated, not everyone develops a protective response. Immunogenicity is a complex interplay between the innate and adaptive immune system, and different drug-related, therapy-related and patient-related factors contribute to the final outcome. Consequently, collaborative efforts currently focus on predicting the likelihood that unwanted immunogenicity will occur by weighting each risk factor at each step of the immune cascade leading to ADA development.

Product-related risk factors

Aggregates

The presence of aggregates in protein pharmaceuticals is an important risk factor for unwanted immunogenicity, but relatively little is known about the effect of aggregate size on their immunogenic potential. **Dr. Wim Jiskoot**, of Leiden University, The Netherlands reported on a pre-clinical model that allows the assessment of aggregate size impact on ADA development. A murine monoclonal IgG1 was subjected to a set of stress conditions (a combination of pH, heat and stir stress) to generate heterogeneously sized aggregates. The stressed sample was fractionated in four size classes: monomers, oligomers, nano-sized aggregates and micron-sized aggregates. In a separate experiment, dimers were generated by either heat, pH, or light stress; for all three cases, the dimers were separated from the (stressed) monomers. The

fractions were analytically characterized and tested for immunogenicity in a Balb/c mouse model. Nano-sized aggregates were found to be more immunogenic than oligomers and micron-sized aggregates. Unstressed and stressed monomers as well as dimers were not immunogenic in the mouse model used. This suggests that, from an immunological perspective, it is important to control the levels of nano-sized aggregates in protein biopharmaceuticals.

Dr. Juliana Bessa, from Roche, Switzerland, further discussed protein impurities, including antibody aggregates, and in particular the formation of neo-epitopes as potential risk factors related to the immunogenicity of biologics. The immunogenic properties of soluble antibody aggregates as well as sub-visible particles have been studied in the Roche human IgG1 transgenic (Tg) mouse immunogenicity model. The immunogenicity mouse model consists of a transgenic mouse expressing a mini-repertoire of soluble human IgG1 antibodies. Elimination of membrane Ig-H chain expression was intended to avoid allelic exclusion of endogenous mouse Ig genes. The expressed transgenic repertoire represents the most commonly used antibodies (V genes) in humans and therapeutic antibodies. Immunogenicity of antibody aggregates of different sizes generated under different stress conditions (low pH, process-related and harsh UV-light) was assessed in the current study. Moreover, sub-visible particles of 15 µm size, generated with different oxidizing agents were also tested. Collectively, their findings demonstrated that both soluble aggregates as well as sub-visible particles carrying neo-epitopes derived from extensive covalent modifications in the primary structure of the protein are immunogenic and break immune tolerance in the transgenic mouse model. In contrast, aggregates and/or sub-visible particles of same size but devoid of chemical modifications were not immunogenic in the same model. The role of aggregate size in tolerance breakdown was addressed using IgG1 monomers and preparations carrying increasing amounts of high molecular weight aggregates. As both modified monomers and preparations carrying low contents of large aggregates failed to elicit an immunogenic response, the role of size in tolerance breakdown was also established. The results presented showed that T-cell neo-epitopes are required for immunogenicity of antibody aggregates in a pre-clinical immunogenicity mouse model. As the Ig repertoire covered by the transgenic mice is broad and comprises V families commonly used in therapeutic antibodies, the model can be consistently applied as a pre-clinical tool to assess antibody format- and mechanism-related immunogenicity of therapeutic antibodies across the Roche portfolio.

Practical advice for antigenicity profiling and de-immunization

Dr. Noel Smith from Lonza, UK and an EIP Team member provided an overview of the various assays currently available to assess the risk of biopharmaceutical with regards to the steps of the immune cascade leading to ADA development. Immunogenicity is a challenge for the majority of biopharmaceuticals and contributes to the high attrition rate associated with their development. Despite this, immunogenicity risk is

often not assessed until clinical studies by which time it can be too late to sufficiently address the problem. Pre-clinical animal models are routinely used to study immunogenicity but are ultimately a poor indicator of immunogenicity risk in humans. Alternative fully human systems can be used in pre-clinical development to significantly reduce the immunogenicity risk of biopharmaceuticals prior to first-in-human trials. *In silico* and *in vitro* HLA-binding assessment tools were discussed along with a host of human primary cell assays to assess immunogenicity risk. The primary cell assays are based on human peripheral blood mononuclear cells (PBMC) and can assess both the innate and adaptive immune response to biopharmaceuticals. These *in silico* and *in vitro* platforms have been shown to be a rapid, cost effective way to assess immunogenicity risk during the development of biopharmaceuticals. The *in vitro* assays in particular can be used to assess the risk of inducing a CD4 + T cell response that could lead to the generation of ADA, as well as the risk of product- and process-related impurities inducing an unwanted immune response in the target patient population. *In silico* and *in vitro* immunogenicity risk assessment tools are now widely used during the discovery and development of biopharmaceuticals, in particular, to support lead selection and for assessing the impact of altered product- or process-related impurity profiles. These tools are currently not a regulatory requirement, but are now commonly included in the pre-clinical immunogenicity risk assessment IND package.

Dr. Sophie Tourdot from Pfizer, USA, and **Dr. EIP Team** member, discussed the deployment of the immunogenicity risk assessment assay suite for protein design, de-immunization, risk assessment and retrospective analysis of high immunogenicity biopharmaceuticals. The predictive tools described above can be applied early in development to guide the design and screen leads to select a clinical candidate that exhibits the lowest immunogenicity risk. In this context, a selection funnel strategy was proposed. If sequence liabilities such as the presence of CD4 T epitopes, de-amidation sites are identified in otherwise promising leads, the molecule can be re-designed to engineer out the undesirable amino-acid sequences and undergo another cycle of assay screening. This process is generally referred to as de-immunization. The tools can also be used as part of the immunogenicity risk assessment of a designated clinical candidate prior to an investigator-initiated IND application. The results will be incorporated into the Immunogenicity Risk Assessment and Mitigation Plan (IRAMP). The IRAMP will report on the assessment of many other risk factors, such as mechanism of action, intended study/patient population, co-medication, and route of administration, and estimate the overall immunogenicity risk of the clinical candidate. What assay(s) to perform will depend on the desired level of information, budget, timelines, hence might vary for each program. Last, the assay suite can be of value in deciphering the mechanisms underlying the high immunogenicity of a clinical asset. These studies are mostly conducted for programs that were terminated due to the clinical consequences of the observed high immunogenicity, such as safety issues or decrease of efficacy. For instance, a combination of assays was applied to bococizumab, a highly immunogenic humanized monoclonal antibody designed to inhibit the pro-protein convertase subtilisin-kexin type 9

(PCSK9) and indicated for the treatment of primary hyperlipidemia and mixed dyslipidemia. The combination of *in silico*, PBMC peptide proliferation, dendritic cell (DC) activation and DC-CD4 T cell proliferation assays retrospectively identified sequence risks that might play a role in the high immunogenicity observed in the Phase 3 clinical trials. In summary, a suite of *in silico* and *in vitro* assays is available to assess the risk at each step of the immune cascade thought to lead to ADA development, but timing is key to have an opportunity to influence design or de-immunize a candidate. It is worth emphasizing here that not a single assay nor the overall combined risks will predict ADA clinical incidence, but will rather provide an estimate the likelihood of ADA development. Applied retrospectively, the assays are of value to advance understanding of the mechanisms of ADA development and increase confidence in risk assessment.

The EIP Non-Clinical Immunogenicity Risk Assessment (NCIRA) Working Group update was given by one of its co-leads, **Dr. Sebastian Spindeldreher**, Integrated Biologix GmbH, Switzerland on behalf of the NCIRA working group. Unwanted cellular and humoral immune responses to therapeutics can have major safety, efficacy, as well as commercial implications. Various non-clinical evaluation tools, such as *in silico* algorithms or ex vivo and in vivo experimental setups, are commonly used to assess the immunogenicity risk, e.g., ADA. However, the pharmacology of the drug candidates can influence the results of these assessments and lead to false positives or negatives. In addition, the diversity of HLA and polymorphisms in other components of the immune system, as well as assay specifics such as sensitivity and robustness, may render assay results unreliable or inconsistent. Therefore, robust and consistent and, where feasible, standardized approaches and methods are required to better inform and mitigate the immunogenicity risk. The scope of the NCIRA working group is to provide an evaluated position on the limits of ex vivo and in vivo assays, to suggest best assay combinations to more robustly inform drug design, development, lead selection and risk assessment. The group also works on increasing understanding of the drivers of immunogenicity, including the innate response, antigen processing and presentation, T- and B-cell epitopes as well as immune regulation. In the group, the utility of pre-clinical/non-clinical assays to inform critical quality attributes such as aggregation, glycosylation, deamidation and others are discussed and evaluated. The short-term goal is publishing a position paper that covers the current diversity in ex vivo and in vivo assay methods such as DC maturation, MHC-associated peptide proteomics, T-cell assays, preexisting antibodies and B-cell precursors assays. It will provide a description of drawbacks and difficulties in comparing various methods addressing the same elements of the immune response and provide proposals for strategies that allow cross-comparison between different methods, as well as same methods conducted in different laboratories.

Patient-related risk factors

Innovative methods for predicting clinical immunogenicity with high-dimensional data

Dr. Philippe Broët, from Paris-Saclay University, France discussed innovative methods for predicting clinical immunogenicity

with high-dimensional data in the light of what is expected from a predictive model, i.e., capacity to: 1) predict clinical immunogenicity of a drug based on patient-related high-dimensional data (genomic/patient's genetic makeup); and 2) build a rule based on observations from clinical studies for future risk prediction. To achieve such objective, statistical predictive models need to be considered. First, prediction should not be confused with explanation, as the aim of a predictive model is to accurately predict an outcome, or that an event will or will not occur. The explanation and interpretation of why an event will or will not happen is a second step. The hypotheses tested in the context of explanatory studies relate to the disease process, most commonly interrogating a relationship between a phenotype and a biochemical factor.

The main challenge posed by prediction in the context of high-dimensional data, is to cope with so-called 'Fat matrices,' where the number of predictors is colossal compared to the number of observations (number of patients). Time-to-event analysis was found to fit the specific requirements of clinical immunogenicity prediction. Compound predictor and regularized methods both exhibit advantages and drawbacks. Machine learning (ML), Artificial Neural Networks (ANN) and Random Forests (RF) approaches might also be considered. Overall, when high-dimensional data with complex interactions are expected, ML approaches can be applied. Prediction for clinical use should strike an acceptable balance between accuracy and interpretability. In this context, RF offers an appreciable trade-off. ANN are still relatively new and not fully transparent. In conclusion, there is no one-size-fits-all predictive tool. Methods should be chosen and tailored to specific questions and problems.

Integrative approaches

Construction of humanized mouse models for pre-clinical risk assessment

Dr. Nicolas Legrand, of GenOway, France provided an update on the state of the art of ultra-humanized mice as a putative new whole system prediction model for immunogenicity of biopharmaceuticals.

The lack of relevant pre-clinical models hinders the proper evaluation of human immune cell reactivity against a variety of therapeutics. Over the past decade, new generations of mouse models humanized for cellular and molecular components of the immune system (also known as Human Immune System [HIS] mice) have been generated, but several challenges remain before optimal human immune responses in such models can be achieved. Dr. Legrand's team focused their interest on HIS mice based on the transplantation of cord blood-derived CD34+ hematopoietic stem cells into pre-conditioned (sub-lethal irradiation) BRGS (Balb/c Rag2-/- Il2rgc-/- SirpaNOD) newborn mice. The BRGS mice lack mouse T, B and natural killer cells and contain mouse phagocytes that are tolerant toward human cells. Consequently, these immune-deficient BRGS mice are highly permissive to human hematopoietic cell transplantation, and they show multi-tissue, multi-lineage, long-term human hematopoiesis. The BRGSF mouse strain includes a supplementary deficiency for Flk2, the receptor for the FLT3L cytokine that is critical

for DC development. In BRGS-HIS mice, human T cell homeostasis is optimal, permitting the accumulation of resting, naïve T cells in the lymphoid tissues of the animals. Furthermore, BRGSF-HIS mice can be specifically boosted on demand for the human DC compartment when treated with exogenous FLT3-L. Without human DC boosting, the extent of human immune responses following vaccination is limited, since only a fraction (20–40%) of HIS mice (referred to as 'responder mice') show antigen-specific B-cell responses (ELISA), mostly limited to close-to-germline IgM, and no human T cell responses (ELISPOT IFN γ). In contrast, major improvements are achieved when applying FLT3L (which boosts human DC density) and a hGM-CSF/hIL-4 cocktail (which boosts human DC maturation status) prior to vaccination. The large majority (around 85%) of HIS mice now show IgM responses, half of the 'responder' mice also show detectable levels of antigen-specific IgG, and most of the responder mice exhibit human T cell responses by ELISPOT. Overall, these results demonstrate that the optimal preparation of the human DC compartment is critical to induce proper human immune cell responses in HIS mice. Of note, HIS mice also show reactivity against tumor cells when triggered with proper stimuli (e.g., immune checkpoint inhibitors or T-cell engager bispecific antibodies). In conclusion, there have been major improvements regarding HIS mouse generation over the years. Standardized production methods, as well as the use of optimized immune-deficient mouse strains, ensure the reproducible generation of homogeneous batches of HIS mice with improved human leucocyte content. Still, the number of human leucocytes remains relatively low in HIS mice, as compared to wild-type mice or normal human individuals. Addressing the specific requirements and limitations of each application field will help achieving the next incremental steps of HIS mouse model optimization.

Mathematical modeling and simulation

Immunogenicity is a significant and common issue specific to the development and clinical use of biologics. Currently, immunogenicity is mostly tackled pre-clinically through T-cell epitope prediction and protein engineering. Even if this approach is showing considerable improvements, it is very unlikely that it will lead to complete eradication of immunogenicity. **Dr. Mario Giorgi**, from Certara, UK presented the work conducted by The Immunogenicity consortium (AbbVie, Astellas, Lilly, Pfizer, Roche/Genentech, Bristol-Myers Squibb; Certara), which aims to develop a quantitative systems pharmacology (QSP) model to predict and manage immunogenicity and guide decision-making in drug development. The consortium mechanistic model is composed of two parts representing immune response and biologics physiologically based pharmacokinetics (PBPK). The PBPK is simulated by Simcyp simulator, while the mechanistic model of immune system is implemented in a biological process map interface. Both models are integrated through common variables, which are compound concentrations in physiological compartments. The QSP model has sufficient mechanistic detail to integrate diverse inputs, including bioinformatics prediction of MHCII binding to antigenic peptides, *in vitro* assay and clinical measurements of compound ADA

concentrations. Preliminary simulation results showed good agreement with the adalimumab clinical trial described by Bartelds and colleagues.²⁰ When the HLA allele frequencies for a European population are used, the simulation can classify the virtual subjects into those who do and do not exhibit immunogenicity using the same criteria used in the clinic. The PK profiles for these two categories well matched the clinical reported PK, as well as the percentages of subjects who showed immunogenicity. To conclude, QSP models can provide the basis for model-informed management of immunogenicity. For example, these models could be used to understand the effect of immunogenicity on different target populations or on disease populations. It can be used to explore the effect of co-therapy or used for phase III/IV extrapolation. It is anticipated that QSP models will play an increasing role in the management of immunogenicity.

Risk assessment and data presentation for regulatory dossiers

Over time, assessment of the immunogenicity risk carried by any new biological entity has become an essential element of submission dossiers. Regulatory authorities have issued guidelines to help the biopharmaceutical industry conduct such assessments in a comprehensive and integrated manner. Guidelines undergo revision cycles as the field progresses and novel modalities and vector emerge. For sponsors, the challenge lies in translating the guidelines into internal procedures and presenting the collected immunogenicity data in a clear, concise and integrated manner to facilitate regulatory review.

Dr. Daniel Kramer from Sanofi, Germany and **Dr. Arno Kromminga**, from BioAgilitix, Germany, both EIP Team members, gave an overview of the new FDA Immunogenicity Guideline and discussed the challenges in the assessment of immunogenicity. The long-expected FDA guideline “Immunogenicity testing of therapeutic protein products – developing and validating assays for anti-drug antibody detection” became effective in January 2019. Overall the guideline is highly appreciated by the biopharmaceutical industry, especially as it now clearly outlines the agency’s expectations that were anyway already requested from Sponsors/Applicants during IND/BLA reviews. With respect to validation parameters, FDA explains that some are preliminarily assessed during assay development and verified during validation such as cut-point, sensitivity, drug tolerance. In contrast, other parameters, such as the minimum required dilution (MRD) or the concentration of a therapeutic protein product concentration used to stimulate cells in a neutralizing antibody (NAb) assay, are only assessed during assay development, but results should be presented in the validation report. Also, the expected 1% failure rates for both the confirmatory assay as well as the NAb assay are clearly described in the guideline. The agency now also points out that the confirmatory assay needs to be fully validated – a comment that was received by many companies in the past years during assay review at the Office of Biotechnology Products.

The audience in general highly appreciated that many comments of consortia such as the EIP during the

consultation phase made it into the final guideline. For example, in respect to sensitivity, FDA now acknowledges this validation parameter is assessed using positive control antibody preparations that may not represent the ADA response in a specific subject, and they also agree that it is important to determine assay sensitivity in the presence of the expected concentration of onboard drug. For years the biopharmaceutical industry argued that “long term stability testing” (LTS) for ADAs does not really add value, as one can only use the positive control (usually derived from animals) to assess this parameter. In addition, the stability of antibodies frozen in matrix is well known (and should be independent of the complementarity-determining regions). It was great to see that the agency finally concurred and deleted the need for LTS from its 2019 guideline.

The new guideline ends with a chapter about the “Integrated Summary of Immunogenicity” (ISI). Although this concept was already introduced in the revision of the 2017 EMA “Guideline on Immunogenicity assessment of therapeutic proteins,” the FDA counterpart does provide much more practical advice, for example, with respect to which subsections should be included in an ISI.

Dr. Paula Salmikangas, from NDA Advisory Services, UK, discussed the challenges posed by conducting immunogenicity risk assessment of Advanced Therapy Medicinal Products (ATMPs) and regulatory expectations for risk mitigation. The number of authorized ATMPs (cell/gene therapy and tissue engineered products) is steadily increasing worldwide and more and more complex products are proceeding to the clinical phase of development, e.g., first ex vivo CRISPR/Cas9 genome-edited cells. At the same time novel technologies are being used, for example, in vector production and large-scale manufacturing of cell-based ATMPs to enhance commercial production of ATMPs. Although there have been many improvements in the field, development paths often suffer from challenges that may be difficult to predict and which may come as surprises during clinical studies. One aspect that has not been much discussed is the unwanted immunogenicity of ATMPs and the regulatory expectations for immunogenicity studies as part of pre-clinical and clinical development. For many of the authorized ATMPs, limited non-clinical and clinical data on immunogenicity has been provided as part of MAAs and in most cases, further safety data to cover missing immunogenicity data is requested post-authorization. Most commonly reported have been the severe immunologically related adverse reactions of the CAR T cells, which have led to severe and life-threatening conditions, and even deaths of study participants due to severe cytokine release syndrome (CRS). CRS is caused by the high activation of the modified T cells, which is related to the efficacy of the product. In addition, simultaneous destruction of large tumor masses (tumor lysis syndrome) affects the severity of the CRS. The toxicity, however, seems to be mediated by recipient macrophages and the cytokines (IL-1, IL-6) secreted by them. Allogeneic mesenchymal stromal/stem cells (MSCs) have been widely used, e.g. for treatment of graft-versus-host disease (GvHD), and the cells have been considered immunomodulatory without having significant alloreactivity in patients. However, the

way the MSCs modulate different immune responses in various tissue environments, and whether all actions are positive, remains still unclear.

Much recent debate has concerned the immunogenicity risks of viral vectors used for gene therapy, especially those of the AAV widely used to treat monogenic diseases. However, vectors associated with the highest host immune responses appear to be first-generation adenoviruses (AdV), which can provoke strong innate immune responses through multiple host defense recognition systems and complement activation. AAV and AdV vectors are derived from commonly spreading wild-type viruses infecting humans; AdV cause mainly upper respiratory system infections, whereas AAVs are not known to cause any diseases in humans. Nevertheless, neutralizing antibodies against both AAV and AdV are detected in a healthy population, and the preexisting immunity may hamper the use of the vectors for gene delivery. Where replication competent (oncolytic) AdV are used, immunotoxicity is highly dependent on the viral dose and route of administration. Plasmids, used for gene delivery, have also been shown to induce innate immune responses and high T-cell responses. Integrating lenti- and retroviruses are used for indications where persistent expression of the transgene is intended, as in many inherited monogenic diseases. Lentiviruses are developed from human immunodeficiency virus and the current third-generation vectors are heavily modified to prevent toxicities. Although the current vectors have pseudotyped envelopes and the endocytic entry into the cells inactivates lentiviral particles, immune responses against the lentiviruses may occur, especially in the case of repeated administration. The fast-developing genome editing technologies are expected to provide new opportunities for *in vitro* and *in vivo* genetic modification of cells. However, these technologies also bear risks for unwanted immunogenicity, as recently reported for the CRISPR/Cas9 system. The clinical consequences of host immune responses against ATMPs may range from minimal/absent to life-threatening adverse reactions and/or loss of efficacy. Factors affecting innate and adaptive immune responses toward an ATMP, including the immune status of recipient, should be considered early in the development process. For cell-based products, the main factors are related to the starting and raw materials, whereas for GTMPs these include the transgene delivery vehicle, transgene promoter, presence of selection markers or suicide genes, the transgene product and its transfer protocol, preexisting immunity toward the vector and host factors, such as prior exposure to the viral vector and/or transgene product, and immune competence. Furthermore, the use of an ATMPs with immunogenic properties should be carefully considered for patients who may later require other ATMPs or organ, tissue or cell transplants.

Dr. Joao Pedras-Vasconcelos from US FDA's Office of Biotechnology Products, USA provided some practical guidance on Immunogenicity Risk Assessment and data presentation for regulatory dossiers. Currently, the data relevant to the assessment of immunogenicity for therapeutic biologics are dispersed throughout different locations of the eCTD, including 2.7.4 Summary of Clinical Safety, 5.3.1.4 Reports on Biopharmaceutical Studies and 5.3.5 Reports of Efficacy

and Safety Studies. The scattering of immunogenicity information in the regulatory file makes both the applicant's preparation of the immunogenicity information and the subsequent FDA review process quite challenging. To facilitate both the clinical development of therapeutic biologics and the subsequent regulatory review process, FDA recommends a life-cycle management approach to immunogenicity through the creation of an Integrated Summary Immunogenicity report that applicants begin populating early in product development, and update at regular intervals as the individual product clinical program progresses through IND filing to BLA submission and even post-approval stages. This recommendation was formalized in the finalized FDA ADA assay validation guidance Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection published January 2019 and harmonizes well with EMA immunogenicity guideline recommendations.

Tolerance induction and gene therapy

As discussed above, consequent efforts are directed to the design and production of drugs predicted to exhibit low immunogenicity risk in the clinic. However, in some instances alterations of the product to reduce its immunogenicity potential is not achievable because it may lead to a decrease in potency or loss of efficacy. This is particularly true for replacement therapy agents with enzymatic function. An alternative approach to mitigating clinical immunogenicity is the use of tolerance-induction regimen to dampen the unwanted immune response to the drug. Tolerance induction is of particular interest in the context of gene or cell therapy, where cellular agents have multiple antigenicity determinants and patients can exhibit strong preexisting immunity to the vectors.

Dr. David W. Scott, Uniformed Services University, USA, presented the work carried out by his team on the use of engineered antigen-specific regulatory T cells for modulation of immunogenicity. Clinical studies with expanded human regulatory T-cell therapy are already in progress. However, these are polyclonal T cells that include a diverse repertoire of relativities. Dr. Scott's approach focused on the generation and use of specific rather than polyclonal Tregs. Antigen specificity was introduced into polyclonal human Tregs via retroviral transduction of specific "receptors," e.g. T-cell receptor (TCR), CAR (single-chain variable fragment) or BAR (B-cell Antibody Receptor). The specific Tregs ability to reduce immunogenicity was evaluated in a pre-clinical model of hemophilia A. Antigen-specific Tregs specifically suppressed both proliferation and cytokine production by antigen-specific T effectors, and antibody formation *in vitro* and *in vivo* in multiple model systems. Recent data with "BAR" CD8s and Tregs (expressing antigen domains) may allow multiple approaches to regulate adverse immune responses. As such, engineered specific Tregs hold promise for the treatment of inhibitor formation.

A therapeutic area that would benefit from efficacious tolerance induction regimen is gene therapy. **Dr. Klaudia Kuranda**, from Sparks Therapeutic, USA gave an overview of the use of AAV and the challenges and opportunities

encountered in the context of gene therapy. Gene transfer with AAV vectors is one of the most promising approaches, permitting long-term therapy in various diseases. Exciting results from clinical trials targeting spinal muscular atrophy, Duchenne muscular dystrophy, hemophilia or Leber's congenital amaurosis demonstrated the potential of the AAV vector technology in delivering unprecedented results in patients. However, immune responses to both viral capsid and transgene remain an obstacle for long-term efficacy, vector re-administration and, potentially, the safety of patients. The first evidence that AAV vectors can be immunogenic in humans came from the early trials of liver-targeted hemophilia gene therapy, in which preexisting immunity to wild-type AAV was identified for the first time as an obstacle to the efficient transduction. These trials also shown that hepatotoxicity could be accompanied by increased levels of AAV-specific T cells in peripheral blood. Since then, our understanding of the immunological implication of AAV gene transfer has evolved. Today, it is clear that immune responses triggered by AAV vectors are the result of a combination of factors, which include the vector itself in all its components, pre-exposure to wild-type AAV, and host-specific variables. Many of these variables differ from one trial to another, making each trial unique and difficult to compare. Generally, if a gene is not delivered to the "immune privileged" site of the body, some form of immune suppression accompanies the treatment. While it is well established that vector immunogenicity can be a challenge to successful gene transfer, it should be kept in mind that not all immune responses associated with gene transfer are necessarily detrimental. Induction of transgene tolerance is in fact a key factor in transgene engraftment and, therefore, in achieving safe and long-lasting effects. In this context, expressing transgene in liver seems to be particularly beneficial. It has been shown that hepatic expression of antigens can drive the induction of antigen-specific immunological tolerance. For instance, in small and large animal models of hemophilia, the expression of coagulation factors from hepatocytes leads to the eradication of inhibitors. Deep understanding of the vector-host immune system interactions will be key to successful modulation of immune responses in gene transfer, enabling safe and persistent transgene expression and eventually allowing vector re-dosing.

Conclusion and outlook

The field of biopharmaceuticals is experiencing fast and exponential growth by means of the generation of numerous innovative protein-based drug modalities, cell-based and gene therapies. Based on the current success of biologics at treating complex and life-threatening diseases, this rapid expansion offers confidence in finding new approaches to treatment for patients with unmet needs. In this context, the impediments associated with unwanted immunogenicity need to be addressed and lessened. The advancement of immunogenicity sciences tremendously benefits from scientists and clinicians sharing their experience, knowledge and breakthroughs. Moving forward, the EIP aspire to continue, through its annual

open symposium and working groups, to provide a place where experts can participate in this common effort.

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References

- Chirmule N, Jawa V, Meibohm B. Immunogenicity to therapeutic proteins: impact on PK/PD and efficacy. *Aaps J.* 2012;14:296–302. doi:10.1208/s12248-012-9340-y.
- Mok CC, van der Kleij D, Wolbink GJ. Drug levels, anti-drug antibodies, and clinical efficacy of the anti-TNF α biologics in rheumatic diseases. *Clin Rheumatol.* 2013;32:1429–35. doi:10.1007/s10067-013-2336-x.
- Saint-Remy JM, Lacroix-Desmazes S, Oldenburg J. Inhibitors in haemophilia: pathophysiology. *Haemophilia.* 2004;10(Suppl 4):146–51. doi:10.1111/j.1365-2516.2004.01009.x.
- Svenningsson A, Dring AM, Fogdell-Hahn A, Jones I, Engdahl E, Lundkvist M, Brännström T, Gilthorpe JD, et al. Fatal neuroinflammation in a case of multiple sclerosis with anti-natalizumab antibodies. *Neurology.* 2013;80:965–67. doi:10.1212/WNL.0b013e3182840be3.
- Immunogenicity assessment for therapeutic protein products. Guidance for industry. U.S. Food and Drug Administration; 2014. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/immunogenicity-assessment-therapeutic-protein-products>.
- Guideline on Immunogenicity assessment of therapeutic proteins. European Medicines Agency; 2017. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-immunogenicity-assessment-therapeutic-proteins-revision-1_en.pdf.
- Preclinical safety evaluation of biotechnology-derived pharmaceuticals. ICH guideline S6 (R1). European Medicines Agency; 2011. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-s6r1-preclinical-safety-evaluation-biotechnology-derived-pharmaceuticals-step-5_en.pdf.
- Guideline on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use. European Medicines Agency; 2012. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-immunogenicity-assessment-monoclonal-antibodies-intended-vivo-clinical-use_en.pdf.
- Considerations in demonstrating interchangeability with a reference product. Guidance for industry. U.S. Food and Drug Administration; 2019. <https://www.fda.gov/media/124907/download>.
- Shankar G, Arkin S, Cocea L, Devanarayan V, Kirshner S, Kromminga A, Quarmby V, Richards S, Schneider CK, Subramanyam M, et al. Assessment and reporting of the clinical immunogenicity of therapeutic proteins and peptides-harmonized terminology and tactical recommendations. *Aaps J.* 2014;16:658–73.
- Passey C, Suryawanshi S, Sanghavi K, Gupta M. Reporting, visualization, and modeling of immunogenicity data to assess its impact on pharmacokinetics, efficacy, and safety of monoclonal antibodies. *Aaps J.* 2018;20:35. doi:10.1208/s12248-018-0194-9.
- Gorovits B, Peng K, Kromminga A. Current considerations on characterization of immune response to multi-domain biotherapeutics. *BioDrugs.* 2019;34(1):39–54. doi:10.1007/s40259-019-00389-8.
- Sauna ZE, Lagasse D, Pedras-Vasconcelos J, Golding B, Rosenberg AS. Evaluating and mitigating the immunogenicity of

- therapeutic proteins. *Trends Biotechnol.* 2018;36:1068–84. doi:10.1016/j.tibtech.2018.05.008.
14. Tourdot S, Hickling TP. Nonclinical immunogenicity risk assessment of therapeutic proteins. *Bioanalysis.* 2019;11:1631–43. doi:10.4155/bio-2018-0246.
 15. Chamberlain P. Effective presentation of immunogenicity risk assessments and related data in regulatory dossiers. *Bioanalysis.* 2019;11:1581–92. doi:10.4155/bio-2018-0209.
 16. Kazi ZB, Desai AK, Troxler RB, Kronn D, Packman S, Sabbadini M, Rizzo WB, Scherer K, Abdul-Rahman O, Tanpaiboon P, et al. An immune tolerance approach using transient low-dose methotrexate in the ERT-naive setting of patients treated with a therapeutic protein: experience in infantile-onset Pompe disease. *Genet Med.* 2019;21:887–95. doi:10.1038/s41436-018-0270-7.
 17. Salazar-Fontana LI, Desai DD, Khan TA, Pillutla RC, Prior S, Ramakrishnan R, Schneider J, Joseph A. Approaches to mitigate the unwanted immunogenicity of therapeutic proteins during drug development. *Aaps J.* 2017;19:377–85. doi:10.1208/s12248-016-0030-z.
 18. Meliani A, Boisgerault F, Hardet R, Marmier S, Collaud F, Ronzitti G, Leborgne C, Costa Verdera H, Simon Sola M, Charles S, et al. Antigen-selective modulation of AAV immunogenicity with tolerogenic rapamycin nanoparticles enables successful vector re-administration. *Nat Commun.* 2018;9:4098. doi:10.1038/s41467-018-06621-3.
 19. Mingozi F, High KA. Therapeutic in vivo gene transfer for genetic disease using AAV: progress and challenges. *Nat Rev Genet.* 2011;12:341–55. doi:10.1038/nrg2988.
 20. Bartelds GM, Kriekaert CL, Nurmohamed MT, van Schouwenburg PA, Lems WF, Twisk JWR, Dijkmans BAC, Aarden L, Jan Wolbink G. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. *JAMA.* 2011;305:1460–68. doi:10.1001/jama.2011.406.