MABS
2025, VOL. 17, NO. 1, 2487604
https://doi.org/10.1080/19420862.2025.2487604

Taylor & Francis
Taylor & Francis Group

MEETING REPORT

Proceedings of the 15th European immunogenicity platform open symposium on immunogenicity of biopharmaceuticals

Sophie Tourdot[a], Karien Bloem[b], Lysie Champion[c], Anne S. De Groot[d], Axel Ducret[e], Patrick Garidel[f], Joanna Grudzinska-Goebel[g], Michael Gutknecht[h], Timothy Hickling[i], Frank Horling[j], Marina Ichetovkin[k], Alison Johnson[l], Issa Jyamubandi[m], Anette Karle[h], Arno Kromminga[n], Ebru Aydin Kurtulmus[o], Floris Loeff[p], Bernard Maillere[q], Lydia Michaut[r], Francesca Minelli[s,t], Morten Nielsen[u], Vivek Nayak[v], Robert Nelson[w], Marc Pallardy[x], Sofie Pattyn[y], Joao Pedras-Vasconelos[z], Elise Pepermans[aa], Alain Poyau[bb], Matthias Reichel[w], Amy Rosenberg[d], Zuben Sauna[cc], Manisha Saxena[dd], Noel Smith[ee], Veerle Snoeck[v], Lester Thoo[ff], Michael Tovey[gg], Daniela Verthelyi[hh], Rene Wuttke[ii], Daniel Yerly[ff], and Daniel Kramer[jj]

[a]Pharmacokinetics, Dynamics and Metabolism, Pfizer Inc., Andover, MA, USA; [b]Sanquin Diagnostic Services, Amsterdam, The Netherlands; [c]Bioanalytical Services, Celerion Switzerland AG, Fehraltorf, Switzerland; [d]EpiVax, Inc., Providence, RI, USA; [e]Roche Pharma Research and Early Development (pRED), Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Basel, Switzerland; [f]IU-TIP, Boehringer Ingelheim Pharma GmbH & Co KG, Biberach/Riss, Germany; [g]Preclinical Development, Pharmaceuticals R&D, Bayer AG, Berlin, Germany; [h]Immunogenicity and Mechanistic Immunology, Biomedical Research, Novartis Pharma AG, Basel, Switzerland; [i]Pharma Research and Early Development, Roche Innovation Centre Welwyn, Roche, Welwyn Garden City, UK; [j]BioAgilytix Europe, Hamburg, Germany; [k]Pharmacokinetics, Dynamics, Metabolism, and Bioanalytics, Regulated Bioanalytics, Merck & Co., Inc., Rahway, NJ, USA; [l]Drug Metabolism and Pharmacokinetics, Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT, USA; [m]Resolian Bioanalytics, treat as city, UK; [n]BioNTech SE, Mainz, Germany; [o]Research & Development Department, PEPperPRINT GmbH, Heidelberg, Germany; [p]R&D, Sanquin Diagnostic Services, Amsterdam, The Netherlands; [q]Département Médicaments et Technologies pour la Santé, Université de Paris-Saclay, CEA, SIMoS, Gif-sur-Yvette, France; [r]PK Sciences, Novartis Biomedical Research, Basel, Switzerland; [s]NBE-DMPK Innovative BioAnalytics, RBM Merck S.p.A., An Affiliate of Merck KGaA, Darmstadt, Germany; [t]Department of Molecular Biotechnology and Health Sciences, University of Turin, Torino, Italy; [u]Department of Health Technology, Technical University of Denmark, Lyngby, Denmark; [v]Precision Medicine, UCB, Braine-l'Alleud, Belgium; [w]BioAgilitix Europe GmbH, Hamburg, Germany; [x]INSERM, Inflammation, Microbiome and Immunosurveillance, University Paris-Saclay, Orsay, France; [y]In Vitro Immunology, Rlqvia Laboratories, Gosselies, Belgium; [z]Center for Drug Evaluation and Research, Office of Pharmaceutical Quality, Office of Product Quality Assessment III, US Food and Drug Administration, Silver Spring, MD, USA; [aa]ImmuneSpec, Niel, Belgium; [bb]LBA Method Development, KCAS Bio, Lyon, France; [cc]Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD, USA; [dd]PK Sciences - Translational Medicine, Novartis Pharma AG, Basel, Switzerland; [ee]Lonza, Saffron Walden, UK; [ff]Adverse Drug Reaction – Analysis & Consulting, ADR-AC GmbH, Bern, Switzerland; [gg]Chief Scientific Advisor Svar Life Science AB, Villejuif, France; [hh]Division IV, Office of Pharmaceutical Quality Research, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, MD, USA; [ii]Translational Medicine, Bioanalysis, Debiopharm International SA, Lausanne, Switzerland; [jj]Translational Medicine Unit, Sanofi, Frankfurt am Main, Germany

ABSTRACT

The European Immunogenicity Platform (EIP) celebrated the 15th edition of its Open Symposium on Immunogenicity of Biopharmaceuticals and its associated one-day workshop on 22–24 February 2024 in Lisbon. The meeting attracted experts and newcomers across industry, regulatory agencies, and academia, who actively participated in 3 days of discussion on risk assessment, monitoring, and mitigation of unwanted immunogenicity of biologics. Besides oral presentations, poster sessions were held to maximize scientific exchange and networking opportunities. Therapeutic proteins and emerging gene and cell-based therapies present promising therapeutic options for addressing unmet medical needs or when conventional treatment approaches have failed. Nonetheless, the development of an immune response against these therapeutic agents is a significant concern, as it occurs in a considerable number of cases across various products and indications. The specific anti-drug antibodies that develop can lead to adverse safety events, inhibition of drug activity, or accelerated clearance, all of which result in a loss of treatment efficacy. The EIP serves as a forum for experts and newcomers in the immunogenicity field, fostering discussion among scientists from industry and academia, encouraging interactions with regulatory agencies, and disseminating knowledge and advancements in immunogenicity sciences to the broader scientific community. This report covers the main topics discussed during the EIP 15th Open Symposium on Immunogenicity of Biopharmaceuticals, and the one-day workshop on practical aspects of immunogenicity held prior to the conference. Key topics included immunogenicity testing, clinical relevance of immunogenicity, immunogenicity risk assessment and mitigation, and current regulatory considerations.

ARTICLE HISTORY
Received 31 December 2024
Revised 26 March 2025
Accepted 27 March 2025

KEYWORDS
immunogenicity; anti-drug antibody; ADA; risk assessment; clinical relevance

Introduction

Therapeutic proteins have substantially improved the quality of life for many patients with complex, progressive, and/or life-threatening diseases. However, the broad use of these therapeutic agents, as well as new treatments like gene and cell-based therapies, may be hindered by their immunogenicity,

CONTACT Sophie Tourdot sophie.tourdot@pfizer.com Pharmacokinetics, Dynamics and Metabolism, Pfizer Inc., Andover, MA 01810-5901, USA

i.e., their ability to induce an immune response in some patients. Unwanted immune responses can present as development of specific anti-drug antibodies (ADA) and activation of immune cells including helper and cytotoxic T cell. These responses can lead to the loss of treatment efficacy due to inhibition of the agent's activity, accelerated clearance, or reduced exposure and can pose safety issues, sometimes resulting in severe consequences.

Regulatory agencies require comprehensive exploration and characterization of immunogenicity risk and provide guidelines on ADA assays and immunogenicity risk assessment (IRA) for various biologics. Scientists and clinicians developing biologics are faced with the challenge of conducting reliable IRAs, accurately measuring ADA levels, estimating their clinical relevance and impact on safety and efficacy, and correctly reporting immunogenicity data in regulatory dossiers. After marketing authorization is granted, managing unwanted immunogenicity and evaluating its effects on safety and treatment efficacy are essential to ensure high-quality patient care. Establishing a link between ADA development and the loss of efficacy or adverse events depends on accurate and timely measurement of ADA, along with reliable assays to measure serum trough drug levels and approaches to estimate their interconnections.

ADA assays and clinical immunogenicity testing strategies must continuously evolve to adapt to new formats for protein drugs, such as multi-domain monoclonal antibodies (mAbs), and new treatment approaches like gene and cell-based therapies. At an early stage of product development, efforts focus on designing biologics with low immunogenicity risk using in silico and in vitro tools to identify product-related risks and guide the removal of liabilities. Preclinical IRA also includes listing and estimating treatment and patient-related risk factors to provide an overall estimated immunogenicity risk prior to clinical development. New types of biologics, such as multidomain, multispecific and conjugated antibodies, novel scaffolds, cell and gene therapy products, including gene editing, have made IRA more complex. In this context, developers are willing to share their immunogenicity risk and mitigation strategies and practices to accelerate drug development.

The EIP held its 15th Open Symposium on Immunogenicity of Biopharmaceuticals, a longstanding meeting[1–4] that shows how unwanted immune responses are still an issue for biologic-based treatments and need solutions. This Symposium gathered experts in different areas of IRA, such as assay development for clinical or non-clinical immunogenicity evaluation or mitigation by design, with regulatory input on current and future approaches in these areas. The pre-conference workshop featured presentations on various IRA approaches and the use of non-clinical in silico and in vitro tools for product de-risking. It also included "Bring your own problems" sessions for participants to discuss specific issues. This report is organized in 5 main sections: workshop presentations, spotlight talks, non-clinical IRA, immunogenicity monitoring (assay development, novel modalities, clinical relevance) and regulatory aspects. For clarity, the workshop round table discussions' summaries have been included in the relevant non-clinical IRA and immunogenicity measurement sections.

The meeting demonstrated again the collaborative spirit of the Immunogenicity community.

Pre-conference immunogenicity risk assessment workshop (22 February)

Since 2017 and 2019, the European Medicines Agency (EMA) and US Food and Drug Administration Agency (FDA), respectively, have required a stand-alone document named Integrated Summary of Immunogenicity (ISI) be included in submission dossiers.[5,6] To comply with this regulation, companies have established their own processes to develop the ISI. The aim of the workshop was to reveal insights into how companies established their own processes to develop the ISI document and, beyond the generation of the document itself, to provide a forum for discussion of its potential internal value for the drug development process.

Joanna Grudzinska-Goebel (Bayer AG) and Veerle Snoeck (UCB) on behalf of the EIP IRA working group

The IRA group aims to provide a harmonized framework for biotherapeutics IRA throughout product development based on the experiences from different pharmaceutical and biotech companies and examples available from literature. The workshop covered an introduction to immunogenicity of biotherapeutics and a focused discussion on IRA, driven by product, process, and patient- or treatment-related risk factors. The group emphasized how across companies the outcome of the IRA guides preclinical and clinical immunogenicity testing, monitoring, and mitigation strategies. The workshop contained an interactive session with the attendees on how the identified risk factors and potential clinical consequences are translated into an overall low-, medium-, or high-risk level, which will influence the clinical trial design. A manuscript recapitulating the EIP IRA working group's position and recommendations has been submitted for publication.

Immunogenicity potential assessment at Novartis – Anette Karle (Novartis pharma AG)

Evaluating the immunogenicity potential of biotherapeutics is becoming increasingly important and challenging due to novel emerging formats and modalities. Dr Karle provided an overview of how Novartis conducts immunogenicity potential assessment from the design space to clinical development. Novartis developed and implemented the use of an IRA questionnaire in which the various categories and elements reported include the mode of action of the drug, similarity to endogenous proteins, drug critical quality attributes, or the immune status of the patient. In the design space, different types of in vitro assays can be applied to support the selection of biotherapeutic candidates with the most favorable risk profile. When in the clinic, similar tools can be used to understand mechanistic root causes of immunogenicity.

Immunogenicity mitigation by design – Sophie Tourdot (Pfizer)

Mitigation of unwanted immunogenicity of biologics is crucial for developing safe and effective drugs. In clinical settings, protocols like co-administration of immunosuppressants or desensitization (currently used only for Factor VIII (FVIII) in hemophilia A) help reduce immunogenicity. Ideally, however, the immunogenicity risk of a drug would be minimized before clinical development. Developing an IRA in the early stages of a program helps identify opportunities to mitigate the risk as the program progresses. In most cases, product-related risk factors offer opportunities for reducing the risk early on, with control of critical quality attributes and removal of sequence liabilities as main leverages. Sequence liabilities include post-translational modification sites and foreign CD4+ T-cell epitopes that can trigger immune responses leading to ADA formation. Pfizer's approach to de-risking protein drug sequences consists of organizing several in silico tools and in vitro assays to form a screening funnel that will filter out candidates with higher immunogenicity risks. Tailored screening funnels are used for each program, and a Quantitative Systems Pharmacology model that can predict clinical immunogenicity rates and impact on pharmacokinetics (PK) serves as the final selection step where feasible. The characterization of the sequence risk and the model predictions are then incorporated in the IRA and will contribute to the overall risk level attributed to a program. A growing number of companies apply in silico tools and in vitro assays to de-risking drugs by design. In this context, the importance of standardizing in vitro immunogenicity assays across companies to enhance confidence in de-risking strategies was also emphasized and illustrated by the ongoing efforts by the EIP Non-Clinical IRA working group (NCIRA) to establish Best Practices (see update from the group in the NCIRA section).

Spotlight presentations

MHC class I and its importance for the immunogenicity of novel modalities

Gene therapies, cell therapies such as Chimeric Antigen Receptor T-cell (CAR-T) and gene editing therapies such as the Clustered Regularly Interspaced Palindromic Repeats/Cas (CRISPR/Cas9)-based approaches hold immense promise in treating previously intractable diseases. Understanding the immune response to these modalities is critical for improving safety and therapeutic efficacy. Pre-existing and induced immune responses to components of these novel modalities could either limit the applications of these technologies or be unsuitable for many patients. In addition to CD4+*T-cell activation that leads to ADA development, unwanted immune responses to such modalities include the development of cytotoxic responses mediated by CD8+ T-cells, which recognize epitopes presented by Human Leukocyte Antigen Class I molecules (HLA I).*

In this context, **Morten Nielsen**, **Technical University of Denmark and Zuben Sauna, US FDA**, discussed the growing interest in predicting and mitigating HLA I epitopes content of CAR-T and gene editing therapies.

To date most bio-analytics for assessing the immune response to protein therapies have largely relied on the accurate determination of ADA and determining whether these antibodies are neutralizing. While it is likely that preexisting ADA can be detected for many novel modalities, ADA often do not affect clinical outcomes. The mechanism of action of gene and cell therapies necessitates endogenous expression of the encoded protein of interest. An undesired consequence of which is the possibility that the transcript will be processed and presented by HLA I and induce CD8+ cytotoxic T-cells (CTL) responses. Killing transgene-expressing cells poses a risk of exposure decrease, which can lead to loss of efficacy. Hence, bio-analytics for assessing the immune response to novel modalities cannot rely on the identification and characterization of ADA alone, but must include fit-for-purpose assays to investigate the risk of cellular unwanted responses, carefully designed for each application. In many current applications of novel modalities there are two main components that could be immunogenic (1): the viral vector and (2) the transgene or other payload carried by the vector (e.g., gene editors like Cas). Regarding the vector, adeno-associated virus (AAV) particles undergo proteasomal degradation, and capsid-derived peptides presented by HLA I can trigger CTL activation. In several early liver-directed gene therapy trials, a loss of transgene expression was correlated with a CTL response against the viral capsid. Moreover, the transgene product has often been associated with CTL responses rather than antibody responses. For instance, CTL responses to the α-1-antitrypsin transgene product developed and were associated with a gene expression polymorphism present in the subject. Similarly, gene therapy for Duchenne muscular dystrophy using three products with different transgenes, under different promoters and packaged in different AAV serotypes, all showed CTL immune responses against dystrophin, recognized as foreign antigen by the patient immune system. In some studies, the presence of CAR-specific CTL after infusion has been associated with treatment failure.

The CRISPER/Cas9 gene editors constitute a valuable case study for immune responses to therapeutic proteins that are generated intracellularly. Pre-existing *T*- and B-cell responses to Cas9 have been reported.[7,8] Genome editing in mouse liver was accompanied by an increase in CTL cell responses, hepatocyte apoptosis, and the complete elimination of genome-edited cells. Similarly, efficient AAV CRISPR-mediated dystrophin restoration was demonstrated in canine Duchenne muscular dystrophy models. However, Cas9-specific immune responses were a serious barrier to successful AAV CRISPR therapy.

Thus, there is an urgent unmet need for in silico algorithms, experimental workflows, and analytical methods that can detect the engagement of peptides with HLA I and subsequent CTL responses triggering. This is an emerging field, and further research is needed to determine the clinical relevance of such adaptive immune responses to novel modalities. Similarly, there is a need for new assays, reagents, and even statistical methods for cut-point determination to evaluate immunogenicity in the clinic. However, existing in silico tools for estimating HLA I peptide presentation are very reliable.[9] These tools have a long history of being applied to epitope prediction for rational design of vaccines and can readily be deployed in early IRAs. In this context, it is critical for assays to be biologically and clinically meaningful and reflect the mode of delivery of the target protein.

Non-clinical immunogenicity risk assessment

The development of ADA in response to protein drugs and cell-based therapy products is expected to follow the classical antibody formation pathway, as observed in the context of vaccines targeting pathogens. Immunogenicity risk involves several factors, primarily associated with product characteristics and patient/treatment specificities. To address these risks, non-clinical methods have been devised to evaluate product-related risks of biologics in inducing ADA formation, focusing on the immune cascade stages that lead to antibody secretion and/or cellular responses. It has been well established that CD4+ T-cell epitopes contained in the drug primary structure play a major role in this cascade and de-risking efforts mostly focus on the identification and removal of these epitopes. Innovative approaches are being explored to also predict patient-related risks linked to genetic background, with some efforts directed toward integrating both sources of risk factors for a comprehensive prediction of clinical immunogenicity.

EIP non-clinical IRA working group update

Sophie Tourdot, on behalf of the group

The NCIRA working group focuses on discussing the use and relevance of the many non-clinical IRA in silico, ex vivo and in vitro evaluation tools. The aim of the NCIRA working group is to share knowledge and increase understanding of the product-related drivers of immunogenicity risk, including innate responses, antigen processing and presentation, *T*- and B-cell epitope content, and drug-induced immune regulation in preclinical settings. In 2022, the group recognized the need to standardize and harmonize these tools[10] to further improve the quality of the data and ease their interpretation. Regulators are highly in favor of this initiative, as the numerous assay formats and inconsistency in protocols among assays with similar formats present considerable difficulties in assessing the validity of an assay and ensuring the reliability of their results. As it is unrealistic to impose a common assay/protocol with the community, the group proposed to establish best practices for the application of in vitro and in silico tools for IRA. These include fit-for-purpose validation, control of critical assay parameters and inclusion of appropriate controls. The group aims to publish papers covering best practices for the use of existing in silico and in vitro tools. In parallel, the group participates in the Immunogenicity Database Collaborative (IDC), a global cross-industry and academia consortium. The IDC's purpose is the creation of an open access curated and comprehensive database of clinical immunogenicity records. This database will facilitate the community's efforts to advance understanding and mitigation of the multiple factors associated with the risk of immunogenicity development. The IDC is a grass-roots initiative led by volunteer members and contributors and holds no formal association to any single organization or industry working group. The database is under construction and hosted by the State University of New York at Buffalo (Buffalo, New York, USA) under the supervision of Dr Sathy Balu-Iyer.

As mentioned earlier, a strong effort is taking place in the community to standardize NCIRA in silico and in vitro tools. In this context, Sofie Pattyn, Iqvia Laboratories, provided an update on the joint initiative by the Health and Environmental Sciences Institute (HESI) Immuno-Safety Technical Committee and the American Association of Pharmaceutical Scientists' IRA Mitigation group (AAPS-IRAMP) to contribute to the standardization effort. This initiative aims to develop a novel reference antibody panel for preclinical IRA. Dr Pattyn outlined the rationale and background behind the panel's development, along with key practical observations, limitations, and lessons learned. She then shifted to the preliminary results from the pilot study, offering an interim analysis of data from 5 of the 11 participating laboratories. The raw data was uniformly re-analyzed to enable direct comparison between laboratories. Data from both the positive control keyhole limpet hemocyanin (KLH) and test samples were presented, showing the percentage of donors with a stimulation index above 2. While the dataset remains incomplete, early findings suggest that lyophilized material, the primary focus of the pilot, delivers consistent results in most test cases. Once the dataset is complete, further analysis will be conducted and shared with the Immunogenicity community.

NCIRA in silico tools and in vitro assays can be applied to de-risk molecules by removing sequence liabilities, but also investigate product risk factors linked to the molecule biophysical properties that could enhance the T cell responses. **Marc Pallardy, INSERM**, discussed the role of aggregation in the development of unwanted T lymphocyte responses. The aim of the study discussed was to better understand the role of antibody aggregates in the initiation of specific T-cell responses to therapeutic mAbs. Small, well-characterized infliximab aggregates were generated by exposing the native antibody to ultraviolet light. In a monocyte-derived dendritic cell (moDC) and CD4+ T-cell co-culture assay (DC-T assay), aggregates were found to induce a higher frequency of CD4+ T-cells compared to the native form. Even though infliximab aggregates did not induce healthy donors moDC maturation, increased internalization as compared to native infliximab was observed, with endocytosis being the main pathway. The receptors and mechanisms at play are currently under investigation. Overall, the data indicated that small aggregates have a significant role in immune system activation, emphasizing the importance of assessing the cellular mechanisms that drive the immune response to aggregated proteins to anticipate and prevent mAbs immunogenicity.

***Poster*: Patrick Garidel**, representing the EIP Particle Characterization Working Group

After a pause of several years, the EIP Particle Characterization Working group (PCS) is resuming its activities. The mission of the PCS is to share and discuss experiences on protein characterization and particle formation in the context of immunogenicity and safety to increase fundamental understanding of product-related immunogenicity risk factors. The PCS working group aims are the following: share experiences on suitability of the various technologies; evaluate and assess current and upcoming particle detection technologies, including standards, define common strategies and methodologies for particle detection and characterization. EIP members who are interested in these

subjects are invited to contact Patrick Garidel to join the group and engage in their discussions.

Removing sequence liabilities necessitates their identification. If a region of a drug is to become a CD4+ T-cell epitope and activate CD4+ T-cells, that region must be generated during internal protein degradation and bind HLA II and then that peptide-HLA II complex must be expressed at the surface of antigen-presenting cells (APCs). This can be assessed using the MHC-Associated Peptide Proteomics assay (MAPPs). **Elise Pepermans, ImmuneSpec**, discussed how high-sensitive MAPPs analysis enables the precise identification of all the regions of a protein that may evoke an immune response. This information is key to an accurate risk assessment and modulation of the immunogenicity risk of biotherapeutics. ImmuneSpec's high-sensitivity MAPPs workflow leads to higher numbers of identified peptides (self and non-self peptides) and provides the depth of analysis required for confident immunogenicity derisking. The importance of detecting peptides presented by HLA-DP and HLA-DQ isotype (next to the standard HLA-DR isotype-presented peptides) was also highlighted. Most importantly, a pioneer comparison of peptides presented across APCs was reported. The investigation revealed that HLA II complexes in moDC, true dendritic cells (myeloid DCs, myDCs), and primary B cells from the same donor show similar presentation patterns for the same proteins, albeit increasing numbers of HLA II-associated peptides are observed from primary B cells to myDCs to moDCs. This study demonstrated that moDCs serve as an excellent in vitro proxy for studying HLA II presentation of biotherapeutic-derived peptides by APCs.

To date, the MAPPs assay has been restricted to studying HLA-DR epitope presentation due to the lack of well-characterized HLA-DP and HLA-DQ specific and pan antibodies availability. **Axel Ducret, Roche**, discussed the necessity of expanding the MAPPs Assay to HLA-DR, -DP and -DQ Receptors. Recent data demonstrate that the "minor" HLA-DP and HLA-DQ alleles also contribute to triggering a significant CD4+ T-cell response. For example, the presence of the HLA-DQA1 × 05 allele, which is carried by approximately 40% of Europeans, has been shown to significantly increase the rate of immunogenicity in patients treated with adalimumab. Hence, an accurate preclinical drug IRA must integrate comprehensive in silico- and in vitro-based tools considering the HLA II diversity encountered in the general population. In this context, a more general application of the MAPPs assay may in the future provide supportive information in the context of biotherapeutics approval by regulatory authorities. Roche's strategy is to survey HLA-DP, -DQ and -DR using MAPPs. Commercially available antibodies, such as the gold-standard HLA-DR (L243), the HLA-DQ (SPV-L3 and 1a3), the HLA-DP (B7/21), and the HLA II pan (Tü39, CR3/43, and WR18) antibodies, were included in the study, characterized, and their specificity were assessed using the epitope prediction algorithm NetMHCIIpan.[11] Although the use of these antibodies in the assay collectively increased the number of identified compound-specific cluster profiles, no individual antibody clone was able to recover the whole experimental HLA II peptide repertoire. Such findings revealed that a mixed immunoprecipitation strategy using a minimum of three antibody clones with differing specificities (HLA-DR-specific clone L243, HLA II pan-specific clone WR18, and HLA-DQ-specific clone SPVL3) leads to more robust compound-specific peptide detection in one single analysis. This strategy was applied to determine an experimental format compatible with screening biotherapeutic candidates during preclinical IRA. Several illustrative examples were presented to demonstrate the strength and added value of screening all HLA II peptide complexes. Expanding the MAPPs assay to leverage HLA-DP and HLA-DQ alleles may vastly improve the predictability of immunogenicity through the identification of a greater number of potential CD4+ T-cell epitopes during preclinical drug development.

Immunotherapy with biologics encompasses replacement therapy, i.e., correct the lack or insufficient expression of endogenous proteins that are the root cause of a disease by providing patients with the protein (administration of recombinant protein or delivery by gene therapy are common approaches). This is the case for FVIII for treatment of hemophilia A. Replacement therapy comes with the risk of development of unwanted immune responses to the drug, as the drug could be recognized as foreign by the patient's immune system. Immunogenicity development will depend on the level of tolerance to the drug each patient presents. In this context, **Bernard Maillère, CEA**, discussed how naive and memory FVIII-specific regulatory and conventional CD4+ T-cells share common epitopes in healthy individuals and influence FVIII immunogenicity. Multiple observations suggest that immune tolerance to the hemorrhagic factor FVIII is weak, even in healthy individuals. Auto-reactive CD4+ T-cells specific to FVIII circulate in the blood of healthy individuals at a frequency close to that of CD4 + T-cells specific to foreign protein Ovalbumin and comprise memory T-cells, which have therefore been activated in the periphery. To shed light on T-cell tolerance to FVIII in heathy donors, a comprehensive analysis of the FVIII-specific conventional CD4+ T-cell and regulatory CD4+ T-cell (Treg) repertoire was conducted. The first focus was on conventional CD4+ T-cells. Sequencing of the complementarity-determining region (CDR) CDR3β of T-cell receptor from isolated FVIII-specific CD4+ T-cells revealed limited usage and pairing of TRBV and TRBJ genes, with only 13 clonotypes accounting for half of the anti-FVIII response. Through large-scale epitope mapping of the full-length FVIII sequence, 18 immunodominant epitopes located in the A1, A3, C1, and C2 domains were identified, which encompassed half of the conventional CD4+ T-cell response. T-cell priming with this set of epitopes revealed that highly expanded clonotypes specific to these epitopes individually constituted up to 35% of the total FVIII repertoire.

The same set of 18 immunodominant epitopes stimulated CD4+ CD127- CD25+ Foxp3+ CD4+ Tregs collected from healthy donors. FVIII-specific Tregs were found at a lower frequency than conventional CD4+ T-cells and divided into naive and memory Tregs. Tregs exhibited antigen-specific suppressive capacity in vitro. Using FVIII-dextramers staining, the shared epitope specificity within the regulatory and conventional CD4+ T-cell compartment was confirmed. This work strongly suggests that tolerance to FVIII does not result from large deletion of autoreactive CD4+ T-cell in the

thymus, but from a balance between conventional CD4+ T-cell that escape thymic selection and committed Tregs cells that mitigate expansion of memory conventional FVIII-specific CD4+ T-cells.

As previously mentioned, binding to HLA II is a perquisite for a drug sequence to constitute a CD4+ T cell epitope. HLA II allele expression greatly varies between individuals and binding of a given epitope is restricted to specific alleles. Hence, this epitope will not be presented by individuals lacking the specific alleles, thereby considerably reducing the risk of that an individual will develop ADA. **Anne S. De Groot, EpiVax**, reported how differences in ADA incidence to the same drug might be due to differences in HLA alleles prevalence in regional populations. In the realm of therapeutic mAbs, pre-clinical immunogenicity evaluation has traditionally relied on a one-size-fits -all approach that overlooks the genetic diversity influencing individual immune responses. This traditional approach may be ill-suited for diverse populations that are marked by different frequencies of HLA-DR alleles, a critical variable in antigen presentation and subsequent immunogenicity. She described groundbreaking methodology for personalizing immunogenicity analysis using in silico tools designed to fine-tune HLA-DR frequencies in alignment with their prevalence within regional populations.[12] Results from a case study of mAbs used for the treatment of rheumatoid arthritis demonstrated a more nuanced landscape of potential immunogenicity that is profoundly influenced by regional HLA-DR variations.[13] For instance, mAbs considered low risk in one population (for example, Asian populations, where HLA-DRB1 × 0901 is more common) were found to pose elevated risks in others (for example, American or European populations, where HLA-DRB1 × 0301 is more common). Thus, differences in ADA that are observed in different clinical trials may be due to differences in the prevalence of HLA alleles in the regional populations.

Furthermore, within each population, higher risk and lower risk combinations of HLA-DR alleles could be defined. This was illustrated in a retrospective case study of Pompe disease subjects who developed anti-enzyme replacement antibodies. More specifically, the odds of developing high ADA were 52 times higher in subjects that had HLA-DRB1 that presented more epitopes, as compared to those that presented fewer T cell epitopes and more putative Treg epitopes.[14] Dr De Groot advocated for a more personalized risk assessment in clinical practice, while also anticipating the impact of regional differences in immunogenicity risk on clinical trial outcomes. This approach may provide important guidance to developers and clinicians for the development and deployment of mAbs. Thus, individualized risk assessment has the potential to become a clinical tool for management of individual patients' therapy, in the future. The era of personalized medicine calls for innovations that accommodate human diversity at every level, including immunogenicity. Platforms that assess regional, and even personal, immunogenicity risk represent a significant leap toward that ideal, promising to redefine how we evaluate and employ mAbs therapies across diverse populations.

Poster: **Michael Gutknecht, Novartis**. In Silico Immunogenicity Profiling Based on Drug/Pathogen Analogy

Immunogenicity can result in the discontinuation of development of a biotherapeutic owing to adverse events and their impact on clinical outcomes or withdrawal of an approved product due to an inferior immunogenicity profile in a highly competitive market. Understanding and mitigating immunogenicity of biotherapeutics is paramount for drug efficacy and patient safety and is a clear competitive advantage. The immunogenicity potential assessment should be started as early as possible in the biotherapeutic development process to inform necessary de-immunization approaches early on and to avoid spending resources on candidates with a high inherent immunogenicity potential in later stages. Oftentimes, this is only possible using in silico tools, since in early drug development, high-quality candidate material is not available in the quantities necessary for most in vitro assays. Additionally, high cost and long timelines of in vitro assays are also factors that can be hurdles for pharma and biotech companies alike. Most in silico tools used in biotherapeutic development predict peptide binding to HLA II molecules (e.g., NetMHCIIpan – 4.0[15]), frequently with the option to apply a weighting matrix, based on the hypothesis that self-peptides and germline sequences have a lower immunogenicity potential. Based on recent experience of root cause analysis of adverse events, exploration of additional options to improve this weighting matrix were conducted. The study showed biotherapeutic sequences can bear analogues to pathogen sequences, which theoretically may result in a high number of memory CD4+ T-cells that are cross-specific to the biotherapeutic, as well as a high prevalence of preexisting ADA. So, for a comprehensive in silico immunogenicity analysis, not only germline white-listing and self-peptide filtering should be applied, but also a pathogen database lookup to identify analogue peptides that could lead to the development of a strong immunogenicity response in a larger proportion of the patient population.

***Poster*: Aydin Kurtulmus, PEPperPRINT**. A Chip-Based Tool for Epitope-Specific IRA

Given the growing numbers of therapeutic protein products being developed, the generation of tools to identify immune responses mounted against them has become critical. While it was previously assumed that fully humanized proteins would have negligible immunogenicity, many of them still fail to eliminate ADA formation associated with a wide spectrum of immune-related adverse events. The success of current IRA efforts is constrained by challenges, including limitations in labor-intensive processes and requirement for substantial amounts of patient material in addition to dependence on predictions and complex computational methodologies. PEPperPRINT's poster presented a novel chip-based methodology that effectively surmounts these limitations, allowing the detection of cellular and humoral anti-drug responses at epitope level. To pinpoint immunogenic sites recognized by ADA, they analyzed two well-known PDL-1 inhibitors (a group of immune checkpoint blockers) with differing rates of ADA formation. They generated a high-density peptide microarray by converting the drug sequences into 15 amino acid peptides with a peptide- peptide overlap of 14 amino acids for high-resolution epitope data. The peptide microarrays were tested with the serum samples of PD-L1- treated patient and control groups to identify IgG and IgM antibody responses at

the epitope level. Since the root cause of ADA generation is typically T-cell dependent, they developed a novel, chip-based MAPPS technique to identify potential HLA II peptide epitopes of the TPP that is presented to T-cells. Briefly, the same arrays were screened for binding of recombinant human HLA II constructs instead of serum samples. Analysis of the PDL-1-treated patient serum samples using the arrays revealed a class-switched, high affinity immune response with several linear epitopes being recognized by ADA. For root cause analysis, incubating the same content arrays with 5 allotypes of human recombinant HLA II constructs led to the identification of potential epitopes for T-cell dependent immunogenicity. Overall, the study highlights the sensitivity and potential of this novel approach for defining appropriate risk mitigation strategies and identifying patient populations at greater risk for ADA formation

Round table discussion

Summary by Tim Hickling and Sophie Tourdot

Attendees interested in NCIRA joined a round table discussion led by experts from the EIP NCIRA working group. Here, we provide an overview of the main topics and the ideas and comments that were shared for each. The aim of this summary is not to provide answers, but rather offer food for thought on topics of interest in this area.

In silico tools

A key role of immunogenicity scientists is to influence the design of molecules to optimize for low immunogenicity risk in parallel to other developability criteria and not to rely on de-immunizing once issues are found. Ensuring protein engineers understand and access in silico tools is an effective way to embed this quality-by-design approach. In silico tools, are increasingly used for their cost-effectiveness and high throughput. For example, the use of EpiVax's ISPRI system[12] by toolkit users more than quadrupled, from 1 M per year to more than 4 M sequences in 2024. While it has been accepted that HLA-DR is the focus of risk assessment using HLA supertypes[16] there is still a need to better understand the roles of HLA-DR 3/4/5 and HLA-DP and HLA-DQ, including the impact of HLA allele expression level, and regional differences in HLA. Some have argued that preclinical assays should include additional HLA types/haplotypes to cover subjects of non-European heritage and/or disease-associated HLA alleles for therapeutics targeting a specific disease indication.

Assay critical parameters

Sensitivity is currently established using 'benchmark molecules' of known immunogenicity incidences, with investigators setting 'reasonable' levels of immunogenicity, usually below 20% incidence of ADA. More recently, emphasis has been placed on the use of assay controls from comparable molecules, i.e., similar length (peptides/proteins). Assay critical parameters, including sensitivity and controls are being reviewed for T cell, MAPPs, innate immune response assays, and silico in vitro tools as part of manuscripts from the EIP NCIRA workstream. At present, each lab establishes their own assay based on trial and error from published studies. A framework for harmonizing assays was published by the EIP NCIRA in 2022.[10] Dr Sauna and Dr Verthelyi underlined that "an absence of signal does not equal an absence of risk".

Application of the MAPPs assay

The assay was at the center of the round table discussion. The MAPPs assay use is increasing and now extends to identification of CD8+ T-cell epitopes of novel modalities. Several EIP members are running MAPPs with a common pan-Class I immunoprecipitation step. A recent report from Eli Lilly[17] described a method and showed AAV capsid peptide presentation. Other applications include gene editing and messenger Ribonucleic Acid/Lipid Nanoparticle (mRNA/LNP) therapeutics. A few tips emerged from the conversation:

HLA I MAPPs:

- All types of cells are amenable to HLA I assessment.
- Use mRNA transfected DCs to have HLA I presentation pathway.
- Remove the b2-microglobulin to improve sensitivity.
- Use cell lines that express only 1 allele to identify specific allele risk.
- Avoid CD8+ T-cells as they kill the cells that express the construct.
- It is possible to do HLA I and HLA II from the same APC lysates. Precipitate HLA I first.
- As HLA I peptides are shorter than HLA II, the sensitivity of the assay may be lower. Care should be taken if analysts are used to HLA II before starting HLA I work.

HLA II MAPPs:

- MAPPs for B cells can be performed. It is sometimes used to compare with the DC MAPPs outcome.
- Multiple compounds can be run at the same time.
- Try a MAPPs run with no lipopolysaccharide (LPS). This could increase sensitivity, as LPS contributes to cell death.
- Align donor set with DC-T assay so that what is presented in MAPPs is what is presented in the DC-T assay.
- Most protocols use whole cell lysates and therefore include cell membrane and internal HLA molecules for the immunoprecipitation stage.
- Unprocessed peptides will be longer. This may indicate a technical problem.
- Different time-points might be needed for viral gene therapy products to identify more of the capsid peptides.
- Some of the most eluted IgG peptides are tolerogenic epitopes (also known as Tregitopes. Do not assume that all eluted T-cell epitopes are bad actors in terms of immunogenicity risk.

Matching MAPPs to DC uptake: DC uptake assays require protein labeling. No one present had tried running labeled proteins in the MAPPs assay with the same donors for a direct comparison. Internalization controls the labeling. There is a risk of following fluorescence and not the protein

Generic peptides impurities

One question that frequently arises in response to the FDA guidance[18] is related to the concentration of the impurities to be tested in T-cell assays. Given that the lower limit of 0.5% (concentration of impurity as compared to the active pharmaceutical ingredient (API)) defines which peptide impurities have to be tested, a question often asked is: Why is it necessary to test impurities at the same concentration as whole product? The answer to this question is that the FDA generally would like to see an 'apples-to-apples' comparison of immune responses to impurities and to the API. Considering that generic drugs are usually used over long periods of time, dosed weekly or daily, this approach provides the best proxy for longer-term immunogenicity risk, even though the impurity may be present only at low concentrations. The first essential step for IRA of impurities is to determine a sufficient concentration to get a signal, then to compare that signal to an appropriate (peptide-sized) control that gives a similar signal, allowing sponsors and FDA to assess if the presence of the impurity might add to the overall immunogenicity of the drug. Currently, in vivo models would likely not be accepted by the agencies as a tool to examine how different levels of impurities might affect risk level. The orthogonal approach to IRA for peptide impurities has been reported for several FDA-funded research programs.[19,20]

Biosimilars

Understanding chemical differences can be important due to the impact of post-translational modifications on uptake and presentation. Assay preparations should be of high quality, considering aggregates and impurities, and controls should be comparable molecules with known immunogenicity. The MAPPs assay can be applied. However, since post-translational modifications affect uptake and possibly presentation, a good understanding of the chemical differences might be needed to interpret the data. Difference in production processes can also contribute to different host cell protein profiles (HCP) in biosimilars; the FDA is ramping up requirements for assessing the HCP content of biosimilar drugs and may request immunogenicity profiling in the future.

To what degree can we trust and need the assays?

These remain in vitro assays with translational risks, which is why EIP prefers not to use the term 'predictive', but rather 'risk assessment', when referring to these tools. FDA emphasizes the need for comprehensive data aggregation. For smaller companies it can be difficult to translate data from in vitro assays into new developments, so it helps to have an expert in the team, or access to outside expertise. Educating colleagues on immunogenicity and the limited predictiveness of assays is a broad challenge. Most of the assays we discuss across the NCIRA are related to assessing the risk of ADA incidence, not the clinical impact of ADA. Therefore, questions arise on the value of running the assays. A counterpoint to this is that the clinical outcome of zero ADA is zero. The presence of ADA themselves is a risk factor for consequences. Assays help mitigation at the design stage. Companies must balance the risk of immunogenicity against development timelines and budgets, often optimizing a molecule only if time and risk levels permit.

Patient-centric approaches

IRA approaches should, in theory, consider how products affect individual patients or patient groups. Furthermore, it may be important to use samples from populations affected by the disease of interest (consider, for example, using peripheral blood mononuclear cells (PBMC) from patients who have rheumatoid arthritis (RA), or at least ensure that the alleles associated with RA are represented in the population of subjects used for preclinical assays). Current IRA assays that are performed with healthy donor samples provide a broad assessment of risk, however access to patient samples and industry-wide data sharing may be necessary to gather sufficient information to deploy individualized assessments. It may also be important to develop assays that have higher sensitivity to detect small differences between patients. Statistics should be used to help understand differences between groups, when the results of preclinical assays do not provide a strong enough signal or show clear differences between comparator molecules.

Finally, FDA scientists made three important points:

(1) It is not enough to demonstrate that we have done everything we can to select the most favorable candidate to call a molecule 'low risk'
(2) It is essential to aggregate the data. Although separately two low signals in assays might appear acceptable, it is possible that additional assessment of combined risk could show a higher risk level.
(3) Sharing data from the NCIRA assays with the agencies helps move the field forward, as they are uniquely positioned to see data across many products.

Immunogenicity monitoring: measurement

Immunogenicity testing is essential for the development of safer drugs, whether they are new biological entities or biosimilars to a reference product. It is crucial to establish assays that accurately measure ADA, determine their neutralizing or non-neutralizing nature, and identify their isotype in relation to potential safety events. Consequently, these assays must evolve and be tailored to address the complexity of new protein drugs, gene and cell-based therapy, as well as alternative matrices to serum.

Assay development

Cut points

***Poster*: Lysie Champion, Celerion**. Low Cut Points: Where Has Our Biological Variability Gone?

Advancements in technology have considerably mitigated nonspecific binding in ADA assays, resulting in decreased assay background and increased sensitivity. Consequently, newly developed ADA assays frequently exhibit very low assay cut points (CP) and sensitivities. Celerion's poster presented the development of a bridging electrochemiluminescent immunoassay to detect ADA against a bispecific therapeutic antibody ranked with moderate immunogenicity risk. The initial assay exhibited exceptional

performance, with minimal matrix interference observed, resulting in a preliminary screening CP factor < 1.05 and < 10% confirmatory CP. The background noise of the assay was remarkably low, raising concerns about the assay's ability to detect biological variability as sample response of drug naïve samples approached the lower detection limit of the instrument. Throughout method development, the suitability and robustness of the assay was confirmed by testing different conditions, including matrix type (healthy versus diseased population) and dilution, capture and detection reagent concentration, assay buffers, and washing steps to assess their impact on overall variability. As a result, the assay parameters were confidently confirmed despite the low established CP.

Generic assays

Poster: **Francesca Minelli, Merck KGaA/University of Turin**. Generic ADA Assay: A Universal Approach to Enhance Early Phase and Preclinical Immunogenicity Testing

Detection of ADA plays a crucial role in biopharmaceutical drug development to mitigate potential adverse effects such as safety concerns and reduced drug efficacy due to immunogenicity. The immunogenicity assessment is based on risk evaluation and the likelihood of immune response occurrence. When a drug candidate is highly immunogenic, additional resources and time are invested in these evaluations. As a result of this approach, since ADA determination is not predictive and therefore not mandatory during the preclinical phase, companies must balance the risk of not having an ADA assay ready or dedicating excessive resources to develop unnecessary ADA assays. In response to this dilemma, a generic preclinical ADA assay, which could quickly be implemented to provide insight into preclinical study results was. developed. The assay is able to detect ADA against human IgG-like molecules in preliminary murine studies. This generic ADA assay utilizes the same capture and detection reagents across all drug candidates, with rigorous screening to identify optimal conditions for various drug modalities. The method was qualified following current guidelines and adapted for the specific Context of Use. Finally, this method demonstrates good drug tolerance and sensitivity, supporting immunogenicity evaluations during the early stages of drug development in mouse models.

Cross-reactivity

Poster: **Karien Bloem, Sanquin Diagnostic Services**. Cross-Reactivity of ADA Against Anti-CD20 Therapeutic Monoclonal Antibodies with Other Anti-CD20 Antibodies

Rituximab is a B cell-depleting anti-CD20 antibody, used to treat B cell leukemia, as well as autoimmune diseases including rheumatoid arthritis and pemphigus vulgaris. It has proven to be an often highly effective treatment. However, one reason for lack of response to rituximab, as well as infusion-related adverse events, is the development of anti-rituximab antibodies, despite its B cell-depleting activity. Besides rituximab, multiple other therapeutic antibodies targeting CD20 are available that may be considered as alternatives. Before switching to another anti-CD20 drug, it would be good to know that there is no cross-reactivity of ADA between the different drugs, especially in the case of infusion-related adverse events. Sanquin Diagnostic Services' poster provided details of their investigation of the potential cross-reactivity of anti-rituximab antibodies to three other anti-CD20 mAbs: ofatumumab, obinutuzumab, and ocrelizumab. While these all target CD20, they differ in their exact binding mode and precise details of the B cell-depleting mechanisms. Previous data from their lab has shown that the antibodies developed against mAbs are mainly directed to the target binding site. Although the overall sequence of the humanized ocrelizumab and chimeric rituximab is not that similar, the core epitope shows some striking similarities. To investigate if the ADA pool of patients treated with either rituximab or ocrelizumab cross-react, both direct binding assays to the other anti-CD20 drugs and blocking experiments with the different drugs were performed. These results show that for some patients the ADA pool against the drug they are treated with contain antibodies cross-reacting with other anti-CD20 drugs, suggesting that it could be wise to check cross-reactivity before switching to another anti-CD20 drug to circumvent infusion-related adverse events or decreased efficacy of the new therapy.

Cross-validation

Marina Ichetovkin, Merck & Co., Inc., provided an update on the EIP Immunogenicity Assays Working Group activities. The EIP Immunogenicity Assays Working Group conducted a survey to gather information on ADA assay cross-validation assessment practices from member companies. Of 15 respondents, the majority were from pharmaceutical companies (53.3%), biotechnology companies (26.7%), and CROs (20.0%), and most had performed cross-validations (60%). However, only a few (14.3%) had included cross-validation in Health Authority submissions, and none had received feedback from the authorities. The survey revealed diverse conditions under which cross-validation is performed, including different reagent requirements and parameters assessed. Challenges reported included obtaining consent for sample use and limitations when conducting studies in China. Importantly, the survey revealed that respondents used various definitions and approaches for defining successful cross-validation. Given these findings, there is a clear need for harmonized guidance from EIP summarized in a publication on when cross-validation is needed, how it should be conducted and evaluated, and impact on clinical immunogenicity reporting

Multidomain scaffolds

Domain characterization

Administration of bispecific antibody therapeutics, like any other biologic, can induce ADA.[21] Hence, the development of suitable ADA and neutralizing antibodies (NAbs) detection methods is necessary, as unwanted immunogenicity against therapeutic drug remains one of the root causes of clinical program termination. The generation of ADA/NAbs can affect PK exposure, bioavailability and pharmacodynamics (PD) depending on

their distinct characteristics. The bi-functional property of bispecific antibodies requires additional consideration to develop a fit for purpose assay, because ADA/NAbs against each of the domains may have different clinical impact. Therefore, the ADA/NAbs assay developed for bispecifics should be able to assess the presence of ADA/NAbs to each individual domain. **Issa Jyamubandi, Resolian Bioanalytics**, discussed examples of two bispecific therapeutic mAbs targeting different tumors. For one bispecific the domain characterization was carried out in the ADA assay whereas for the other bispecific, domain specificity was not developed in the ADA assay but developed and validated in the Nab assay. The critical nature of the selection of adequate positive controls for both ADA and the NAbs assays, as well as the choice of assay format to achieve the desired assay sensitivity, drug and target tolerance was underlined

Many biotherapeutics currently in development have complex mechanisms of action and contain more than one domain/component, each with a specific role or function. Examples include bispecific and tri-specific antibodies, antibody-drug conjugates, PEGylated proteins/peptides, fusion proteins, and LNP encapsulated RNA or deoxyribonucleic acid (DNA). As with other biotherapeutic molecules, a multi-domain biotherapeutic can elicit immune responses resulting in the production of specific ADA. Understanding the contribution of the different domains/components to the ADA response usually requires specific characterization assays, with several different approaches available. **Matthias Reichel, BioAgilytix**, provided insights on phase-appropriate implementation of a domain specificity strategy, which were shared through discussion of case studies of contrasting analytical approaches, which were taken for different immunogenicity programs. Pros and cons related to sensitivity in domain-specific antibodies detection, investment of time and resources for assay development and validation, and requirements for specific reagents were discussed.

Drug tolerance

ADA assays are essential tools for evaluating and monitoring immunogenicity during drug development. However, the presence of free peptide drugs can challenge the precision of these assays. This is further exacerbated when combined with sustained release formulations. In addition to the complication carried by high drug concentrations, peptide drugs are notoriously difficult to work with due to their small size relative to the cadged functional groups and labels required for the assay chemistry. **René Wuttke, Debiopharm**, presented a strategy for developing an assay with enhanced Free Drug Tolerance. The various stages of the development of a bispecific ADA assay with increased sensitivity and drug tolerance for octreotide were presented. It included the use of bead-based separation techniques to selectively isolate drug-ADA complexes from free peptide drugs. The selection process of different critical reagents generated using different conjugations and purification strategies was discussed.

Manisha Saxena, Novartis, discussed how the drug tolerance of ADA assays represents a significant bioanalytical challenge during the clinical development of biologics in cases of high and frequent dosing

A case study describing clinical ADA assay development and validation for an mAb directed against a soluble cytokine that is known to dimerize was presented. In addition to known target interference, ADA needed to be detected in the context of high drug levels, followed by effective integration and interpretation of the PK/PD/IG and clinical data. Moreover, Dr Saxena highlighted how feedback from regulatory authorities enabled agreement on the best-suited ADA assay to be implemented during clinical development. A standard and robust ADA bridging electrochemiluminescence assay (ECLIA) format was initially validated and implemented in Phase 1 clinical study, but the assay's drug tolerance was deemed too low for its use in clinical Phase 2 and 3 by regulatory authorities. Subsequent efforts to improve the assay using cutting-edge technologies, such as acid dissociation, solid phase extraction with acid dissociation, precipitation and acid dissociation, and competition with antibodies to the target, failed to improve assay drug tolerance, or compromised other key assay parameters such as sensitivity and target interference. To overcome the challenge of drug tolerance and target interference, a magnetic bead-based approach combined with acid dissociation was developed. In this assay, a two-step process was used to first enrich ADA followed by removal of the target. Although this approach provided superior drug tolerance, this assay lacked robustness and significantly increased the amount of time required for processing and analyzing samples. In parallel, additional positive controls, both monoclonal and polyclonal, were produced and characterized at various concentrations in the presence of increasing concentrations of both drug and target in each assay. The investigation showed that the original ECLIA ADA assay performance and its drug tolerance was greatly improved using multiple positive controls. Overall, the original ECLIA ADA assay was considered optimal to meaningfully characterize the ADA response in clinical studies. The results of the investigations, Novartis' position and justification to use the original ECLIA approach were shared with FDA through a Type D meeting. Novartis' position was endorsed by the regulatory agency, exemplifying how technical improvements and regulatory interactions can be efficiently paired during assay life cycle. Additionally, this case study illustrates that drug tolerance is highly dependent on the characteristics of surrogate positive control used in the assay, which may not reflect the real drug tolerance observed in study patients.

Complex modalities

Complex modalities such as AAV-based, cell-based, or nucleic acid-based therapies can induce not only ADA and NAb formation, but also trigger cellular responses with a potential impact on drug exposure, treatment efficacy, and safety. Hence, there is a need to develop adequate assays to measure cellular unwanted immune responses to these drug classes.

mRNA-LNP

mRNA-LNP are among modalities with multiple components and their use requires adjustment of monitoring strategies. **Joanna Grudzinska-Goebel, Bayer AG**, provided an overview

of the importance of evaluating immunogenicity risk during the development of mRNA/LNP therapies, highlighting the regulatory requirements and offered practical guidance on the identification and evaluation of immunogenicity risk. It outlined key immunogenicity risk factors within mRNA-LNP therapies, including mRNA recognition by the innate immune system, the contribution to innate and adaptive immune responses by the LNP delivery system including PEGylated lipids, and the immunogenicity risks associated with transgene protein expression. Examples from marketed products and literature provided practical context for risk identification and evaluation. Various mitigation strategies were presented, focusing on RNA modification, LNP design optimization, and transgene sequence engineering, all aimed at reducing potential immune responses during early development stages, thereby significantly improving the probability of clinical success. Dr Grudzinska-Goebel emphasized the profound impact of the IRA on the fit-for-purpose bioanalytical monitoring strategy, which facilitates the generation of robust data packages required for regulatory submissions while allowing for strategic adaptations based on emerging clinical evidence. In conclusion, the implementation of the IRA is essential for advancing safe and efficacious mRNA/LNP therapies from development through clinical practice, ultimately benefiting patient care.

AAV

Michael Tovey, SVAR, outlined an orthogonal approach to AAV immunogenicity assessment based on the evaluation of both total and neutralizing antibodies directed against AAV. Although AAV has become the predominant vector for gene therapy, its widespread application is limited by both a humoral and cellular immune response directed primarily against the capsid proteins. A large proportion of the world population has been exposed to a particular AAV serotype, often in early childhood, which, coupled with the high degree of cross-reactivity of antibodies against different serotypes, results in a memory response when exposed to a recombinant AAV vector later in life that can seriously comprise therapy. There is thus a need for the development of specific and sensitive methods for the quantification of both total and neutralizing antibodies against wild type AAV serotypes as the basis for the establishment of inclusion-exclusion criteria prior to treatment of patients with AAV gene therapy. A highly sensitive plate-based assay based on an antigen capture format using whole capsids and a labeled secondary antibody has been developed together with a highly sensitive neutralizing antibody assay using the i*Lite*® reporter-gene technology comprising a packaging cell line expressing a recombinant AAV virus expressing a serotype-specific *cap* gene and containing a tag sequence within the inverted terminal repeat sequences. The tag sequence is recognized specifically by a reporter cell line stably transfected with the firefly luciferase reporter-gene placed under the control of an AAV-responsive chimeric promoter. The reporter cells also contain the Renilla luciferase normalization gene under the control of a constitutive promoter that renders the assay independent of cell number and provides a means to unambiguously distinguish between sera that inhibit virus transduction due to the presence of immunoglobulins and other "matrix factors" that inhibit virus transduction *in vitro* by an unrelated mechanism, but may not be relevant *in vivo*. These assays provide highly sensitive, rapid, and precise methods for quantifying the humoral antibody response to both wild type AAV serotypes and a wide range of recombinant AAV vectors.

Vivek Nayak, UCB, asked which of the total antibody (TAb) and NAb assay is best for AAV ADA monitoring in preclinical studies. Antibody responses against AAV can include both TAb and NAb. As the presence of preexisting anti-AAV ADA can affect transduction efficiency and trigger immunotoxicities, the impact of these preexisting anti-AAV antibodies on vector biodistribution and level of transgene product should be evaluated during preclinical development. To allow this evaluation, a possible approach consists of grouping animals prior to start of the study based on their negative or positive AAV ADA status. An evaluation of a TAb and a cell-based NAb assay was performed to select the best assay format for animal grouping during toxicology studies. UCB developed a bridging MSD-based assay to analyze AAV TAb in cynomolgus monkey serum. The specificity was determined using a confirmatory assay. Similarly, a transduction inhibitory assay was developed to analyze AAV Nab. Both methods were qualified, and characteristics compared. The prevalence of preexisting TAb and NAb was evaluated in the cynomolgus population and pros and cons of the different assays for animal grouping during preclinical studies were discussed. To group animals based on the preexisting anti-AAV status, either TAb, NAb or both assays can be used. As it has been demonstrated that the NAb assay detects neutralizing potential that does not seem to be antibody mediated (TAb negative), using both assays would be a conservative approach to provide the highest level of detail on the nature and preexisting antibody status of the animals.

As for mRNA-LNP products, the AAV end-product protein is produced intracellularly. If the protein contains HLA I epitopes, there is a risk of cytotoxic CD8 T cell activation. Hence, besides measuring ADA, monitoring should include assessment of cellular responses. In this context, **Alison Johnson, Boehringer Ingelheim**, discussed an ELISpot assay for the assessment of AAV peptides to examine immune safety. AAV-based gene therapies have shown promise as novel treatments for rare genetic disorders such as hemophilia A and spinal muscular atrophy. However, cellular immune responses mediated by CTL and CD4+ T-cells may target vector-transduced cells, as well as healthy immune cells, impacting safety and efficacy. Dr Johnson described the optimization and reproducibility of interferon-γ (IFNγ)- and interleukin-2 (IL-2)-based enzyme-linked immunosorbent spot (ELISpot) assays for measuring T-cell responses against AAV peptide antigens. For method optimization, PBMC were isolated from healthy human donors and stimulated with HLA I or HLA II specific peptides as positive controls. Peptide pools were designed from published AAV8 and AAV9 capsid protein sequences and then used to assess the presence of AAV-specific T-cell responses. The results showed a measurable increase in IFNγ and IL-2-producing cells after AAV peptide presentation. Furthermore, there was an observed difference in the

magnitude and specificity of response to peptide pools based on AAV serotype and donor. Finally, using individual peptides, we identified a region of the AAV9 capsid protein that can elicit an immunogenic response. This work shows the applicability of ELISpot in assessing anti-AAV immune responses and provides insight into how novel recombinant AAV vectors could be designed to reduce immunogenic potential.

Poster: **Alain Poyau, KCAS Bio**. Monitoring Immunogenicity of Your Candidate Vaccine By Elispot: How To Reduce Assay Variability?

Unraveling the mechanisms by which an investigational product influences the immune system holds substantial value across a spectrum of therapeutic domains and indications. The ELISpot assay, developed over 10 years ago for detecting antibody-secreting cells, has since emerged as a widely used method for assessing antigen-specific T-cell responses in the context of immunotherapies. T-cell ELISpots can help evaluate different contexts of use, including efficacy for preventive or therapeutic vaccines (both universal and individualized) and oncolytic viral therapies or safety for therapeutic antibodies/immune checkpoint inhibitors, and cell and gene therapies. The method's popularity extends to various fields, including immuno-oncology, infectious diseases, autoimmunity, (neuro)inflammation/neurodegeneration, and allergy. While the ELISpot assay offers indisputable advantages, such as sensitivity, measurement of cell function, and cost-effectiveness, for assessing cell-mediated immunogenicity in large-scale clinical trials, the performance and outcomes of the assay can be significantly impacted by various factors if not carefully controlled. A comprehensive understanding of the context of use is essential, including the indication (acute vs. chronic), the expected magnitude of immune responses, the treatment regimen, and the sampling schedule. Additionally, it is important to take into consideration the way the ELISpot data will be analyzed (statistical analysis) and used, e.g., establishing correlates of protection, identifying surrogate markers, or dose-finding. These factors collectively influence the design, and the minimum level of validation required for the assay. They also dictate the degree of flexibility in optimizing sample preparation procedures to ensure reliable and meaningful results. The impact of pre-analytical and experimental conditions on ELISpot assay sample quality and antigen-specific immune responses was illustrated. Solutions through method standardization and harmonization to generate reliable data for monitoring therapy immunogenicity in clinical development were discussed.

Immunogenicity monitoring: clinical relevance

Floris Loeff, Sanquin Diagnostic Services, described the wonderful world of immunogenicity: scientific understanding, clinical relevance, and regulatory necessity. Treatment with biologics generates polyclonal antibody responses composed of a mixture of affinities and isotypes and of varying titers. More profound scientific understanding of this antibody response, whether unwanted in case of biotherapies or wanted in the case of vaccination, will help develop more effective drugs. Advancement in assay technology and design are a key driving factor, as assay characteristics determine whether this biological immune response is technically detectable. Driven by regulatory guidelines, increasingly sensitive immunogenicity testing using highly drug tolerant assays is performed in market-approval studies, resulting in detection of ever lower ADA levels. Consequently, ADA positivity is poorly correlated with meaningful impact on PK and thus loss of clinical efficacy of the drug. To gain proper insight into clinically relevant ADA development, humoral immune responses in patients in the post-approval clinical setting were analyzed for titers, temporal patterns, isotype (IgM, IgG1–4), anti-idiotype, and a reconciliation with PK and PD data was conducted. This characterization revealed that a simplified immunogenicity testing scheme would provide sufficient information for securing safe and efficient treatment of patients with therapeutic mAbs and its implementation could be beneficial to regulatory bodies and in the clinic. After initial ADA formation, tolerance development can set in, which is characterized by dwindling ADA titers and class switching to IgG4. Sanquin's latest studies on factors, including design of the dosing schedule and concomitant medication, that promote tolerance or impact tolerance in a dose-tapering setting were shared. Tolerance induction in the vaccination setting is detrimental and raises questions regarding the impact of widely used immunosuppressive drugs on the response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination. A substantial increase in the proportion of receptor-binding domain-specific IgG4 antibodies (median 21%) was observed in healthy/untreated controls after the third injection of an mRNA-based vaccine. Interestingly, this IgG4 skewing was absent when an adenoviral vector-based product was used for the first vaccination. The humoral response to the vaccines was profoundly reduced in both dupilumab and tumor necrosis factor inhibitor-treated patients.

Amy Rosenberg, EpiVax, asked whether novel approaches for treatment of autoimmunity could be leveraged as a means to mitigate the immunogenicity of biological therapeutics. Single-agent therapy has been the standard of drug development for treatment of diseases from those with simple etiologies, including microbial infections, in which it succeeded initially, to treatment of complex diseases including autoimmune disease, cancer, and allergy. However, the success of single-agent therapeutics for diseases with more complex etiologies has been dismally low. This fact has spurred greater investigation into the immunologic mechanisms promoting disease and strategic targeting of immune pathways known to contribute to disease genesis and maintenance. The Immune Tolerance Network has outlined three critical immunologic pathways that must be addressed for successful tolerance induction: interrupt immune effector mechanisms; restrain innate immune activation; and boost regulation. While some success has accrued in the treatment of autoimmune diseases such as Type 1 diabetes disease by therapeutics that diminish immune effector mechanisms by inducing T-cell exhaustion with an anti-CD3 mAb (Teplizumab) that does not bind the crystallizable fragment receptor (FcR), this was sufficient to delay, but not prevent, progression to full blown disease as it failed to "boost regulation." Novel approaches to

boosting T-cell regulatory control following effector elimination include administration of antigen specific or polyclonal Tregs populations and administering pro-Treg agents. Similarly, for patients generating ADA to life-saving and highly effective therapeutics, there is a critical need to both prevent ADA, as well as to treat those who have generated ADA that neutralize the activity of the therapeutic and cause adverse events. Current promising strategies involve: 1) diminishing epitope generation and epitope spread and eliminating antibody secreting plasma cells via proteasome inhibitors, 2) inhibiting B cell progression to ADA secreting plasma cells, and 3) boosting T-cell regulation with therapeutics such as rapamycin, Tregitopes, and cytokines.

Round table discussion (Held at the pre-conference workshop on Monday 22nd February)

Similarly to the NCIRA round table discussion, attendees interested in immunogenicity monitoring joined a round table discussion led by experts from the EIP Assay working group. A brief account of the main topics brought up, as well as ideas and comments that were shared for each are given below. As previously mentioned for the NCIRA round table, the aim of this summary is not to provide answers but rather offer food for thoughts on topics of interest in this area.

Clinical relevance of ADA is more important than incidence

Participants discussed how ADA can influence the safety and efficacy of biologics and emphasized that the clinical relevance of ADA is more important than incidence. Especially in the case of high drug trough levels, ADA may have no impact on PK/PD and efficacy. However, the impact on safety may not be related to drug levels. The use of a study-specific CP for Phase 3 studies is rather standard, as the false positive rate (FPR) does not fit in the 2–11% preferred FPR using the validation CPs set on samples from healthy volunteers. A higher false positivity rate does not necessarily require the use of an in-study CP, though FPR of 20% may be considered the higher end and would warrant reevaluation of the CPs (though this would lead to changed sensitivity of the method). New CPs make it more complex to combine data set generation in support of the Integrated Summary of Immunogenicity.

Biosimilars require adaptation of CPs and reporting

Participants discussed how biosimilars may differ from originators and how this may affect CPs and reporting. They mention that CPs need to be adjusted for different indications or studies, and that clinically relevant incidences and titers should be reported. Again, the overall incidence of ADA needs to be discussed in comparison to the incidence of clinically relevant ADA.

Available approaches to increase drug and/or target tolerance were discussed and included the Panda method, and heat or acid treatment with several stringencies. Information on the anticipated target accumulation upon dosing in clinical studies could come from modeling preclinical data with the anticipated levels to be tested during ADA assay validation.

Calculating drug tolerance could save time and money: Some participants suggest calculating drug tolerance based on thermodynamic, kinetic, and mass action law parameters instead of determining it experimentally. This could help to improve understanding of drug tolerance as a function of ADA characteristics (e.g., affinity), facilitating optimization of the ADA assay pre-treatment protocols. The participants concluded that this topic requires further discussion and experimental proof of modeling drug tolerance. So far, no detailed information on such models is available.

Generic ADA methods in the preclinical phase

Generic assay strategies for the detection of human mAbs in different species based on the Meso Scale Discovery platform and the antigen-binding fragment (Fab) were discussed. The generic ADA assay strategy can be applied throughout preclinical development strategy, including Good Laboratory Practices toxicology studies.

ADA measurement in singlicates

The participants discussed the advantages and disadvantages of using singlicates instead of duplicates for ADA analysis and the impact on plate design, read time and cut-point assessment. There was consensus that when validation is to be done in singlicates, all parameters should be evaluated

Immunologically related adverse events

Delayed type drug allergies are complicated, as they may manifest as heterogeneous diseases like drug reaction with eosinophilia and systemic symptoms, Steven-Johnson syndrome or toxic epidermal necrolysis. T cells mediate these drug hypersensitivity reactions through cytokine secretion and cytotoxicity. **Lester Thoo, ADR-AC**, reviewed the detection of drug-specific T-cells using the cytokine release in vitro assay (Cyto-LTT). Based on previous investigations, the measurements of IFNγ, IL-5, IL-13, Granzyme B and Granulysin was conducted after a 7-day culture of PBMCs in the presence of the drugs. While measurement of IFNγ alone is a common method for assessing T-cell reactivity, this method runs the risk of missing reactivity mediated through other cytokine responses. To the contrary, because the Cyto-LTT interrogate both CD4+ T helper 1 (Th1) and CD4+ T helper (Th2) cytokines, and cytotoxic granules, it enhances the capability of detecting any deleterious responses induced by a drug. Dr Thoo provided examples where the Cyto-LTT aided with the identification of immunogenic large molecule drugs (biologics), as well as small chemical drugs (e.g., beta-lactams) in sensitized patients. The necessity to also evaluate the main metabolite of a drug for its immunogenicity potential (e.g., Oxipurinol and Allopurinol) was emphasized and data regarding drugs cross-reactivity were presented (e.g., Amoxicillin and Cefuroxim). Beyond identifying drug-specific T-cell reactivity in previously exposed patients, preclinical assessment for immunogenicity in unexposed individuals is also possible in vitro when a cofactor is

present and known (e.g., defined HLA allele). Nonetheless, such reactions typically require more intense (addition of IL-2) and longer stimulations (e.g., for Abacavir, Oxipurinol). In conclusion, the Cyto-LTT is an efficient tool to evaluate drug immunogenicity, through assessment of T-cell secretion of drug hypersensitivity relevant cytokines. The method can be applied to both drug allergy testing and preclinical immunogenicity evaluations. Furthermore, cross-reactivity assessments can be performed for new drug derivatives to determine if such reactions are diminished with newer derivatives.

Daniel Yerly, ADR-AC, discussed how immediate type drug allergy and immunostimulation can be evaluated in vitro with the basophil activation test. Basophils express a range of FcRs, including FcεR, FcγR and complement system receptors such as C3aR and C5aR. These circulating blood cells are easily available from patients or blood donors, and various protocols to monitor basophil reactivity in the Basophil Activation Test (BAT) have been established, e.g., flow cytometry evaluation of the degranulation marker CD63 and the activation marker CD203c. Importantly, known assays are those detecting specific IgE, but activation of basophils can also be observed beyond IgE (via IgG, or complement fragments C3a, C5a). Case study examples were provided that highlighted the utility of BAT for drugs of various formats such as mAb (e.g., antibodies including those with modifications in the Fc or Fab), LNPs (e.g., SARS-CoV-2 vaccines and Paclitaxel), small chemical drugs (e.g., Cephalosporins) and disinfectants (Chlorhexidine). BAT enabled evaluation of whether basophil reactivity is caused by the drug excipients (e.g., polysorbate 80 and polyethylene glycol 2000 evaluations in SARS-CoV-2vaccines) and how immunogenicity may be reduced through excipient formulation (Paclitaxel in its liposomal-bound versus albumin-bound formulations). An example of cross-reactivity is the IgE response against Chlorhexidine and other biguanide disinfectants. In such cases, BAT can help determine whether a patient is truly sensitized (specific IgE has formed) to a particular drug after exposure or the reaction to that drug is due to IgE induced by a different drug previously administered. Additionally, when used with blood from healthy donors, BAT can evaluate the stimulatory potential of a drug in the general population through non-IgE mediated mechanisms. Such non-IgE mediated mechanisms include activation via innate receptors like C3aR and C5aR or FcγR. These reactions are exemplified by basophil activation at high test concentrations of the drugs, which reflects the supra-physiologic local concentrations reached with subcutaneous administration. This may explain why some reactions are also observed at first administration, a common adverse event of therapeutic antibody treatments. In conclusion, the talk gave an overview of the BAT's utility in the assessment of a drug's potential to induce immediate reactions in preclinical drug development by evaluating non-IgE mediated responses and in the clinic by diagnosing IgE-mediated allergy in exposed individuals.

Regulatory aspects

The European Union In Vitro Diagnostic Medical Devices Regulation (IVDR) 1 was published on 05 April 2017, becoming applicable on 26 May 2022. **Robert Nelson, BioAgilytix**, discussed its impact on clinical immunogenicity development. Initially, the bioanalytical community had little concern with the regulations, as it was believed that most bioanalytical activities supporting clinical trials would be out of scope of the new legislation. However, upon publication of the Medical Device Coordination Group Document MDCG 2022–10 on the interface between clinical trials and the IVDR2 in May 2022, it became clear that clinical trial assays used for patient selection, allocation and/or monitoring were within scope of, and required compliance to, the IVDR. Thus, the IVDR has a direct impact on clinical assay development by increasing the stringency of method establishment (design control) and validation (performance evaluation), with extensive technical documentation requirements. The IVDR has added increasing regulatory complexity to TAb and/or NAb assays used to screen for preexisting antibodies to viral vector capsid in gene therapy trials for patient selection, to biomarker assays applied for treatment group allocation, and to polymerase chain reaction assays used to monitor for replication competent retroviruses or in shedding evaluation that may lead to discontinuation of post-study monitoring, to name but a few. Dr Nelson described BioAgilytix's approach and progress in bringing such assays into compliance with the IVDR.

Joao A. Pedras-Vasconcelos, US FDA, provided an overview of the FDA Center for Drug Evaluation and Research's (CDER) current integrative immunogenicity review processes for biologics, focusing on the roles played by various regulatory stakeholder offices within CDER. Integrative immunogenicity reviews involve multi-disciplinary interactions between several CDER offices, including the Office of Product Quality (OPQ), which provides quality reviews focusing on the understanding of critical quality attributes of biologics and how they may contribute to product immunogenicity, and provide a review of the clinical immunogenicity assays used to access ADA. Other key stakeholders include the Office of Clinical Pharmacology (OCP), which provides feedback on clinical immunogenicity study design (e.g., ADA sampling plans and testing strategies), PK and biomarker PD assays, and assesses the impact of immunogenicity on PK/PD and efficacy of the product. Another critical stakeholder, the Office of New Drugs (OND) provides clinical reviews focusing on efficacy and safety of the product in the clinical study patient population and collaborate with OCP regarding the impact of immunogenicity on safety. OND also performs pharmacology/toxicology reviews focusing on preclinical immunogenicity and immunotoxicity data to support product safety. Finally, the Office of Study Integrity and Surveillance (OSIS) performs inspections of bioanalytical sites, including ADA testing sites, and audits bioavailability/bioequivalence studies and non-clinical studies conducted under Good Laboratory Practice (GLP). Dr Pedras-Vasconcelos also provided an overview of CDER's immunogenicity outreach efforts, and their importance to ensure that industry sponsors are informed about CDER's expectations regarding immunogenicity-related regulatory filings.

Daniela Verthelyi, US FDA, discussed regulatory considerations focused on emerging strategies and approaches to assessing immunogenicity risk. Dr Verthelyi described the

current FDA thinking regarding the IRA of generic peptides[18] and other drug products, where the assessment relies at least in part on the clinical trial experience that supported the licensing of the innovator-referenced licensed drug (RLD). In that space, several assays generally used to assess the immunogenicity risk of proteins and peptide products are being deployed to interrogate the potential for product- and process-related impurities to modify the immunogenicity risk relative to that of the RLD. The talk focused on the importance of identifying and controlling key assay performance parameters, such as cell platform, provenance and processing of primary cells, cell composition, and viability of specific cell types, before and after the studies to understand the responses and ensure that the assays are fit for purpose, sensitive, and reproducible. Dr Verthelyi stressed the importance of providing a full description and justification of the methods and suitability controls used, including a description of any criteria used to accept or exclude data and data analysis strategy. Lastly, the need for common reference standards for the community to understand the data stemming from the diverse assays, as well as the need to connect in vitro and in silico data with clinical outcomes, was emphasized.

Conclusion and outlook

The biopharmaceutical industry is increasingly moving away from classical biotherapeutics and toward the development of more complex modalities, such as gene therapies, CRISPR/Cas gene editing or multispecific mAbs. The fact that these new scaffolds present novel challenges to assess their immunogenicity was a prominent theme throughout the 15th EIP Open Symposium. In addition to ADA risk assessment, the risk of developing HLA I-restricted cellular immune responses against transduced cells need to be assessed for products such as mRNA/LNP, AAV-gene therapies or CRISPR/Cas gene editing. Similarly, innate immune responses against nucleic acids (e.g., mRNA in mRNA/LNP therapies) or the AAV capsid must be taken into consideration. Assays for those assessments are still much less standardized and robust compared to classical ADA assays. Preexisting ADA to AAV capsids are often an exclusion criterion for clinical trials, which will put the total antibody or transduction inhibition assays used for this purpose under IVDR/IDE regulations. As a result, bioanalytical labs are confronted with new expectations for IVDR-compliant assay validations.

The other dominant theme of the Symposium was the recent advancement of tools to predict the human immunogenicity of biopharmaceuticals. Accuracy of HLA II in silico prediction has significantly improved due to training of algorithms with experimental MAPPs data, identification of tolerated (or even tolerizing) residues and the inclusion of HLA-DP /DQ alleles. State-of-the-art in silico tool predictions of HLA II epitopes are now considered as accurate across all three HLA-II loci as that of HLA I methods. Additionally, the understanding of in vitro assays, such as MAPPs and DC-T assays, and interpretation of their outcomes has significantly increased over the past years. Nevertheless, standardization and harmonization of these assays remain a key challenge, which consortia such as HESI and scientific societies such as the EIP are collaboratively tackling.

The EIP via its annual Open Symposium and active working groups will continue to act as central meeting place for biopharmaceutical companies, academic institutes, contract research organizations and scientific experts to address and discuss the challenges described above to further advance the science of unwanted immunogenicity.

Acknowledgments

The EIP extends heartfelt thanks to all speakers, chairs, poster presenters, and attendees of the 15th Open Symposium for their active participation and valuable contributions. Special thanks to Barbara Vercruyssen for her pivotal help in organizing the meeting and to our sponsors Agro-Bio, ImmunXperts, QPS, Svar, Celerion, BioAgilytix, and PEPperPRINT for their support. Presentations and posters are available on the EIP website.

Disclosure statement

ST is ae full-time employee of Pfizer Inc. and might hold shares of the company.
SD reports no conflict of interest.
VD reports no conflict of interest.
JGG is a full-time employee of Bayer and holds shares of the company.
AK is a full-time employee of BioNTech and might hold shares of the company.
Anette K is a full-time employee of Novartis and might hold shares of the company.
GPL is a full-time employee of Roche Diagnostics GmbH and might hold shares of the company.
Laurent M is a full-time employee and stockholder of Eli Lilly and Company.
Lydia M is a full-time employee of Novartis and might hold shares of the company.
KNW is an employee and a shareholder in Novo Nordisk A/S.
JAP reports no conflict of interest.
LISF reports no conflict of interest.
SS is a full-time employee, partner, and managing director of Integrated Biologix GmbH.
ZS reports no conflict of interest.
VS and NV are full-time employees of UCB and might hold shares of the company.
DV reports no conflict of interest.
DK is a full-time employee of Sanofi Aventis Deutschland GmbH and might hold shares of the company.
MT is a full-time employee of Svar Life Science France.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

Abbreviations

AAPS	American association of pharmaceutical scientists
AAV	Adeno-associated virus
ADA	Anti-drug antibodies
APC	Antigen-presenting cell
API	Active pharmaceutical ingredient
BAT	Basophil activation test
CAR-T	Chimeric antigen receptor T cells
CDR	Complementary-determining region
CP	Cut point
CRISPR/Cas9	Clustered regularly interspaced palindromic repeats
CTL	Cytotoxic CD8+ T-cell

DC	Dendritic cell
DC-T asay	Monocyte-derived dendritic cell and CD4+ T-cell co-culture
DNA	Desoxyribonucleic acid
EIP	European immunogenicity platform
ELISpot	Enzyme-linked immunosorbent spot
EMA	European medicines agency
FcR	Crystallizable fragment receptor
FDA	United-states food and drug administration
FPR	False positive rate
FVIII	Factor VIII
HCP	Host cell protein
HESI	Health and environment sciences institute
HLA I	Human leukocyte antigen class I
HLA II	Human leukocyte antigen class II
IDC	Immunogenicity database collaborative
IRA	Immunogenicity Risk Assessment
IRAMP	Immunogenicity Risk Assessment and Mitigation
IVDR	In vitro diagnostic medical devices regulation
KLH	Keyhole limpet hemocyanin
mAbs	Monoclonal antibodies
MAPPs	MHC-associated peptide proteomics assay
mDC	Myeloid dendritic cells
moDC	Monocyte-derived dendritic cell
mRNA-LNP	mRNA/Lipid Nanoparticle
NAb	Neutralizing antibody
PBMC	Peripheral blood mononuclear cells
PD	Pharmacodynamics
PK	Pharmacokinetics
RLD	Reference licensed drug
RNA	Messenger ribonucleic acid
SARS-CoV-2	Respiratory syndrome coronavirus 2
TAb	Total antibody
Treg	Regulatory CD4+ T-cell

References

1. Tourdot S, Abdolzade-Bavil A, Bessa J, Broet P, Fogdell-Hahn A, Giorgi M, Jawa V, Kuranda K, Legrand N, Pattijn S, et al. 10 th European immunogenicity platform open symposium on immunogenicity of biopharmaceuticals. MAbs. 2020;12(1):1725369. doi: 10.1080/19420862.2020.1725369.
2. Tourdot S, Quaglia CB, Chamberlain P, De Groot AS, Dellas N, Guillemare E, Kromminga A, Lotz GP, Mingozzi F, Piccoli L, et al. European immunogenicity platform 11th open scientific symposium on immunogenicity of biopharmaceuticals. Bioanalysis. 2020;12(15):1043–1048. doi: 10.4155/bio-2020-0150.
3. Tourdot S, Baltrunkonis D, Denies S, Devanarayan V, Grudzinska-Goebel J, Kromminga A, Lotz GP, Malherbe L, Michaut L, Weldingh KN, et al. Proceedings of the 14th European immunogenicity platform open symposium on immunogenicity of biopharmaceuticals. MAbs. 2024;16(1):2324801. doi: 10.1080/19420862.2024.2324801.
4. Reynisson B, Barra C, Kaabinejadian S, Hildebrand WH, Peters B, Nielsen M. Improved prediction of MHC ii antigen presentation through integration and motif deconvolution of mass spectrometry MHC eluted ligand data J Proteome Res. 2020 Jun 5;19 (6):2304–2315. doi: 10.1021/acs.jproteome.9b00874 . Epub 2020 Apr 30. PMID: 32308001.
5. EMA. Guideline on immunogenicity assessment of therapeutic proteins. European Medicines Agency; 2017.
6. FDA. Immunogenicity testing of therapeutic protein products — developing and validating assays for anti-drug antibody detection (Guidance for industry). U.S. Food and Drug Administration; 2019.
7. Simhadri VL, McGill J, McMahon S, Wang J, Jiang H, Sauna ZE. Prevalence of pre-existing antibodies to CRISPR-Associated nuclease Cas9 in the USA population. Mol Ther Methods Clin Dev. 2018;10:105–112. doi: 10.1016/j.omtm.2018.06.006.
8. Simhadri VL, Hopkins L, McGill JR, Duke BR, Mukherjee S, Zhang K, Sauna ZE. Cas9-derived peptides presented by MHC class II that elicit proliferation of CD4(+) T-cells. Nat Commun. 2021;12(1):5090. doi: 10.1038/s41467-021-25414-9.
9. Reynisson B, Alvarez B, Paul S, Peters B, Nielsen M. NetMHCpan-4.1 and NetMHCIIpan-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. Nucleic Acids Res. 2020;48(W1):W449–W454. doi: 10.1093/nar/gkaa379.
10. Ducret A, Ackaert C, Bessa J, Bunce C, Hickling T, Jawa V, Kroenke MA, Lamberth K, Manin A, Penny HL, et al. Assay format diversity in pre-clinical immunogenicity risk assessment: toward a possible harmonization of antigenicity assays. MAbs. 2022;14(1):1993522. doi: 10.1080/19420862.2021.1993522.
11. Nilsson JB, Kaabinejadian S, Yari H, Kester MGD, van Balen P, Hildebrand WH, Nielsen M. Accurate prediction of HLA class II antigen presentation across all loci using tailored data acquisition and refined machine learning. Sci Adv. 2023;9(47):eadj6367. doi: 10.1126/sciadv.adj6367.
12. Mattei AE, Gutierrez AH, Seshadri S, Tivin J, Ardito M, Rosenberg AS, Martin WD, De Groot AS. In silico methods for immunogenicity risk assessment and human homology screening for therapeutic antibodies. MAbs. 2024;16(1):2333729. doi: 10.1080/19420862.2024.2333729.
13. Sugiyama N, Terry FE, Gutierrez AH, Hirano T, Hoshi M, Mizuno Y, Martin W, Yasunaga S, Niiro H, Fujio K, et al. Individual and population-level variability in HLA-DR associated immunogenicity risk of biologics used for the treatment of rheumatoid arthritis. Front Immunol. 2024;15:1377911. doi: 10.3389/fimmu.2024.1377911.
14. De Groot AS, Desai AK, Lelias S, Miah SMS, Terry FE, Khan S, Li C, Yi JS, Ardito M, Martin WD, et al. Immune tolerance-adjusted personalized immunogenicity prediction for pompe disease. Front Immunol. 2021;12:636731. doi: 10.3389/fimmu.2021.636731.
15. Reynisson B, Barra C, Kaabinejadian S, Hildebrand WH, Peters B, Nielsen M. Improved prediction of MHC II antigen presentation through integration and motif deconvolution of mass spectrometry MHC eluted ligand data. J Proteome Res. 2020.
16. Sette A, Sidney J. HLA supertypes and supermotifs: a functional perspective on HLA polymorphism. Curr Opin Immunol. 1998;10 (4):478–482. doi: 10.1016/S0952-7915(98)80124-6.
17. Brito-Sierra CA, Lannan MB, Malherbe LP, Siegel RW. The HLA class I immunopeptidomes of AAV capsid proteins. Front Immunol. 2023;14:1212136. doi: 10.3389/fimmu.2023.1212136.
18. FDA. ANDAs for certain highly purified synthetic peptide drug products that refer to listed drugs of rDNA origin for certain highly purified synthetic peptide drug products that refer to listed drugs of rDNA origin. 2021.
19. De Groot AS, Roberts BJ, Mattei A, Lelias S, Boyle C, Martin WD. Immunogenicity risk assessment of synthetic peptide drugs and their impurities. Drug Discov Today. 2023;28(10):103714. doi: 10.1016/j.drudis.2023.103714.
20. Roberts BJ, Mattei AE, Howard KE, Weaver JL, Liu H, Lelias S, Martin WD, Verthelyi D, Pang E, Edwards KJ, De Groot AS. Assessing the immunogenicity risk of salmon calcitonin peptide impurities using in silico and in vitro methods. Front Pharmacol. 2024;15:1363139. doi: 10.3389/fphar.2024.1363139.
21. Trivedi A, Stienen S, Zhu M, Li H, Yuraszeck T, Gibbs J, Heath T, Loberg R, Kasichayanula S. Clinical pharmacology and translational aspects of bispecific antibodies. Clin Transl Sci. 2017;10 (3):147–162. doi: 10.1111/cts.12459.