Roundtable report



Overcoming Immunogenicity and Safety Assessment Challenges for Biologics and Biosimilars in Oncology

September 16, 2024

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Cerba Research



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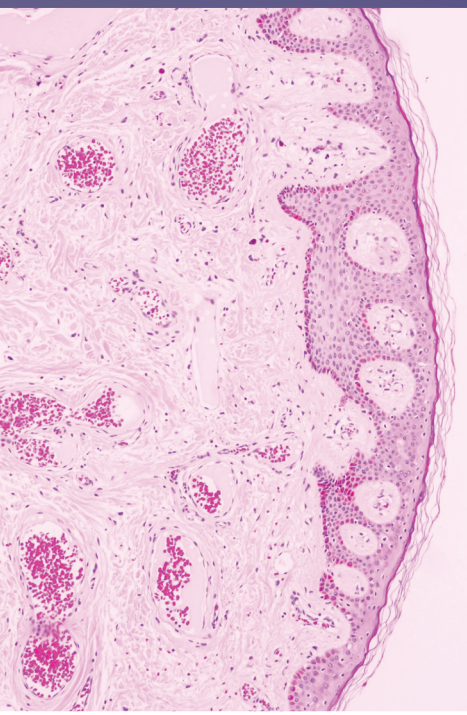
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Key takeaways

There is no 'one-size-fits-all' solution.

Barriers to developing alternative testing methods include differences between different types of drug products, whether they are biologics or biosimilars

Regulators are up to speed with developments in the field.

A flexible approach to neutralizing antibody assay selection can improve results.

Technology will continue to evolve and AI can potentially have a big part to play.

Overview

Biologics and biosimilars continue to be a major source of innovation in the oncology landscape. From advancements in antibody-drug conjugates (ADCs) to tailored approaches using monoclonal antibodies (mAbs), safe and effective treatments across even the most complex cancers are becoming more of a reality.

However, these modalities also present unique operational challenges, especially when it comes to assessing a drug's immunogenicity and safety profile. It is imperative that organizations stay ahead of operational barriers by anticipating and addressing them early in the drug development process.

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Context

In a virtual roundtable held on 16 September 2024, panelists discussed the importance of bridging immunogenicity assays from the pre-clinical to clinical setting and beyond; and the challenges and opportunities of a multi-tier assay approach to immunogenicity testing. They also looked at the key regulatory considerations and ways in which an agile approach to neutralizing antibody assay selection could improve outcomes in the future.

> "Discussions are going on to challenge the current paradigm because, operationally, it costs money to run extra plates, and also justifying what fits best for your program. The key challenge is thinking scientifically about a program rather than following guidance on what you're supposed to do every time."
>
> **Samuel Pine,** Sanofi

Key takeaways

The traditional tests: How to deal with pre-existing antibodies and in-study cut points

The typical cascade of assays performed during immunogenicity testing comprises the screening, confirmatory, and titer tiers. In practice, a high percentage of clinical samples are positive when pre-existing antibodies are present, so Cerba Research has sometimes removed the screening and/ or the confirmatory parts of the tiered testing approach, noted Martin Roberge, Associate Director of the R&D Unit at Cerba Research – Canada.

Some sponsors ask for in-study cut-points "all the time, no matter what". As an alternative, the company proposes to invest in method development to try to minimize the chance of requiring in-study cut-points during clinical sample analysis, which can then be confirmed by verifying the adequacy of the validation cut-point during clinical sample analysis.

Going straight to the titer is already the standard approach in vaccine development, according to Samuel Pine, Global Head of Bioanalysis & Immunogenicity – DMPK at Sanofi. Biotherapeutics first went to a three-tier strategy "because they are so far down in the noise. You need statistically valid cut-points, and it didn't make sense operationally to test eight wells or eight titer points for every sample."

However, the screening and confirmatory tiers are so well correlated that many people argue that there is little point in doing both. What really matters most, Pine and Weifeng Xu, Senior Scientific Director at Merck agreed, is the clinical impact in terms of pharmacokinetics (PK), efficacy, and safety.

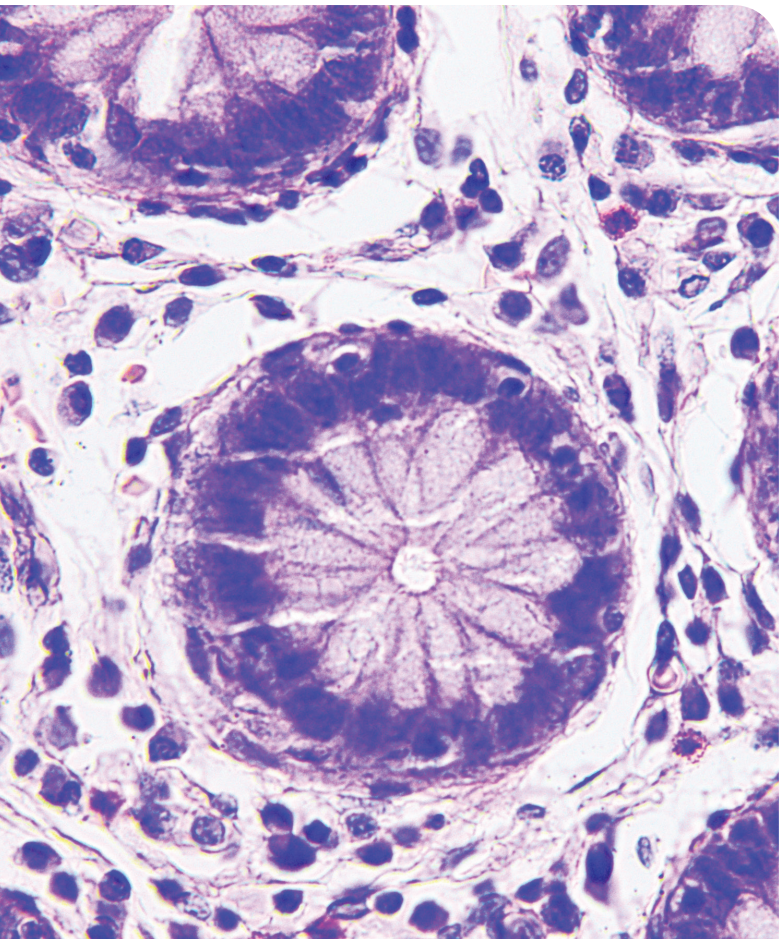


As Jack Ragheb, Senior Vice President - Translational Sciences & Medicine at NexImmune, pointed out, the significance of pre-existing antibodies and their titers depend on what the antibody is related to. In-study cut-points are applicable for biologicals, where sponsors are paying for a statistical basis on which to establish the cut-off during product or assay development. Establishing a cut-point in-study has been proven valuable and they are often different to those established with healthy donors during assay development.

Cerba Research tailors method development based on the drug product (DP), added Roberge. If there are pre-existing antibodies, it would want to characterise them before moving on to method validation, to know if they are specific to the DP.

"When Cerba develops a new method, we work to optimise the assay conditions to make sure the different populations tested within the assay, react differently, and generate similar signals. This will void the need for in-study cut-points later."

Martin Roberge, Cerba Research Canada



Preclinical testing is rarely predictive of clinical testing

Immunogenicity methods have become fairly standardized across industry and many people are using the same methodology to measure their antibodies in terms of relevance. Thus, assay formats in pre-clinical and clinical studies may be similar, if not the same. Immunogenicity seen pre-clinically, while not indicative of what will happen clinically, can be indicative of a worst-case scenario.

During his time at the U.S. Food and Drug Administration (FDA), Ragheb worked on humanized mice with human immune system-specific responses. Those systems were quite good for predicting T-cell responses, which might potentially be useful in assessing the immunogenicity of gene therapy products, since the cellular response can have an important role in the persistence of viral vectors or in the susceptibility of cells transduced to the immune response.

The systems are commercially available but very expensive and they are not complete models because they are restricted by the mouse's major histocompatibility complex (MHC). The most relevant models are those that also have human leukocyte antigen (HLA) molecules in them, in order to obtain an MHC- and HLA-restricted response. "Even if you're measuring antibodies, if the helper cells are restricted to mouse MHC rather than human MHC you don't know that the antibody response is relevant because it may may involve different CD4 helper epitopes," Ragheb said. Meanwhile, others are working on various ex vivo approaches like-organ-on-a-chip.

"A day may come where we will have predictive preclinical immunogenicity but that day's not here yet."

Jack Ragheb, NexImmune

The humanized mouse model and other preclinical animal models are constrained by the difference in the VDG sequences of humans and animals, and because the anti-drug antibodies (ADAs) from animals for mAbs, including humanized mAbs, "would be mostly against the fragment, crystallizable (Fc) portion," Weifeng Xu said. "When you use ELISA to detect the level of ADA, human serum is added. That pretty much blocked a lot of ADA against the Fc portion so that you only see ADA against the complementarity-determining region (CDR)."

Pine noted that the predictiveness of non-clinical models depends on what sponsors are looking for. Translatable and non-translatable findings can both be seen non-clinically in vivo and sometimes in vitro. The former usually relate to the molecule's mechanism of action (MoA). With immunomodulatory or immunostimulatory molecules, the high ADA incidences found in a nonhuman primate could be translatable to a human, as could other extrinsic factors like impurities.

"There are some pieces of information that we can gather from in vitro models. You really have to pick apart the black box when you go from a drug substance to ADA," Pine said. For example in peptide presentation there are models that do that very well in silico and in vitro in modelling and predicting the presentation of peptides from an MHC Class 1 or 2 molecule, but it is not yet possible to reliably translate the next steps to T-cell response and then later to B-cells.

Cerba Research concentrates on preclinical assays and most of the DP testing has been for correlation with the toxicokinetic (TK) and PK data. Some sponsors also want a neutralizing antibody (NAb) assay to see if these antibodies have the capacity to neutralize the drug activity as well.



There are differences between applying immunogenicity assays to newly developed biologics versus biosimilars.

It is established that newly developed biologics and biosimilars have different responses to immunogenicity testing, particularly in terms of PK and ADAs. Deciding how to carry out the testing can be something of a minefield. The key question is whether to use the same assay for both or carry out two assays and compare the positive controls– and, Weifeng Xu added, measuring not just the ADA positive rate, but also the magnitude of the ADA response.

It also depends on the therapeutic category, according to Sophia Xu, Director - Bioanalysis at Daiichi Sankyo. "Immunogenicity itself is a qualitative assay and if you're trying to do the biosimilars for two drug substances with different processes, there are a lot of factors involved," she said.

For example, Daiichi Sankyo was trying to bridge an old formulation and a new one with a different process. It tested the two drug substances, then the samples. The first objective was to demonstrate the comparability of the in vitro test for those drugs, the processes, and the reagents. The next step was to move into ex vivo sample testing. In one of the two, there was a different biosimilarity between the reference drug and the comparator drug, which was an immunology compound.

"There were no safety concerns in the data, so we attribute that difference to the process change, the variability, and all the factors that we're trying to defend in front of the FDA," she said. "It was eventually approved, but PK in biosimilars is definitely important. A difference in immunogenicity is acceptable between the biosimilar and the originator. As long as you can demonstrate the controls and the processes, you can defend your data."said. Meanwhile, others are working on various ex vivo approaches like-organ-on-a-chip.

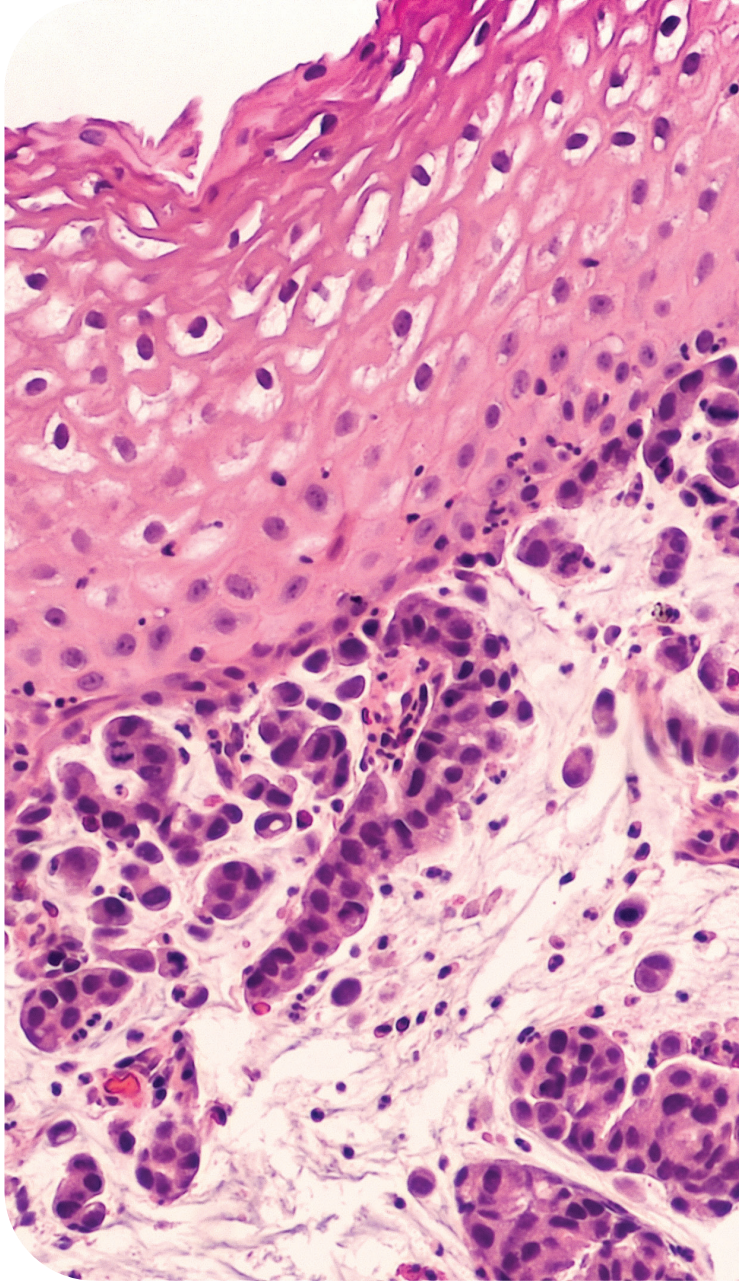
Ragheb, however, regards the differences in the assays between newly developed biologics and biosimilars as a red herring because biosimilars emerged onto the market in the modern era of immunogenicity testing. Many of the reference products (e.g. Humira) were developed many years ago using much less sensitive assays and using new assays finds that the biosimilar and the biologics have very similar immunogenicity.

"Biosimilar immunogenicity is often reduced relative to the reference because there have been improvements in manufacturing processes that reduce the impurities that might contribute to immunogenicity."

Jack Ragheb, NexImmune

The interesting challenge for him now is the implication of reference products having higher rates of immunogenicity in their labels.

Thus far, no agencies have asked a reference sponsor to update their labels, although there are significant differences in the sensitivity of immunogenicity assays for many reference molecules and biosimilars.





Regulators are generally up to speed with developments in the field

For Roberge, the FDA guidance and the latest White Papers show that everyone is "mostly on the same page on what is expected in terms of assay development, assay validation, etc." When companies are subjected to an FDA audit, they will usually have to train the auditors to ensure that they are up to speed with the assay and how and why the company is using it, but Cerba Research's experience here has been good.

FDA experts in cross-functional teams continue to discuss immunogenicity, assays, and clinical impact, Ragheb said. The challenges regulators have posed have not changed much over the years in his view, with the focus on the perennial issues of in-study cut-points, titer, and cell-based neutralizing assays. The agency shows flexibility and acts on a case-by-case basis. As new therapeutic fields come into play, like gene therapy, applying existing immunogenicity tools will have individual challenges.

As new therapeutic fields come into play, like gene therapy, applying existing immunogenicity tools will have individual challenges.

Weifeng Xu agreed that overall the FDA is quite open-minded. For example, its 2019 guidance, mentioned for the first time that a PPP and a biomarker can replace a NAb assay on the regulatory side. There is less certainty on such hot topics as using signal-to-noise to replace titers. Does it really matter, he asked rhetorically, that signal-to-noise saturates at a high titer? Similarly, in vaccines, the FDA still always wants to see the total antibody and the functional antibody.

By contrast, Pine has seen "quite a variation in expertise and the types of questions and concerns we get from different health agencies and different regulators." There are plenty of knowledgeable people at health agencies, but some "ask questions that reflect less than complete understanding of the risks involved in the types of assays being run." Sponsors thus sometimes have to educate regulators in the nuances of immunogenicity when defending their dossier and their approach, and they should be prepared to push back on demands for tests they know to be unnecessary.

Sophia Xu added that Daiichi Sankyo, which focuses on ADCs, has found regulatory agencies to be very interested in domain-specificity in immunogenicity. They are not yet very concerned about domain-specificity in NAbs, but companies with multiple domain portfolios must plan a domainspecific assay ahead of time. The company also receives many questions about drug tolerance from both FDA and the European Medicines Agency (EMA).

"In your Phase III trials on drug tolerance, you really have to meet those criteria. Sometimes you have to revisit the drug tolerance you initially validated…later on, if you improve your reagent, you probably can achieve drug tolerance, but I'd suggest assessing that early instead of waiting until Phase III trials."

Sophia Xu, Daiichi Sankyo

Lorella DiDonato, Senior Vice President at Cerba Research – Canada, hopes for more information exchange among regulatory and the scientific people within the sponsors and the contract research organizations (CROs) on the risks and how the data correlates with real clinical outcome. Cerba often sees sponsors being wary of taking new approaches because of how regulatory investigators might respond, so having sessions to keep abreast of new information gathered from testing, could help "keep the regulators at the same momentum as the scientific community within the sponsor and the CROs."

Conversations like this are already happening at industry events, Pine observed, notably on signal-tonoise at the last WRIB (Workshops on Recent Issues in Bioanalysis). The FDA is in some ways ahead of the sponsors in thinking about "what goes in the label and is read by the physicians." Right now, that means titer. To avoid unnecessary testing, sponsors should think ahead about the data going to those making treatment decisions, and work with CROs to define it and explain it in their dossier and then in the label. That has to be balanced against the desire to avoid unnecessary testing.

"Having those discussions upfront with the regulators and trying to define how you're going to present your results to a clinician who wants to use your product to make a treatment decision is where those discussions should ultimately lead."

Samuel Pine, Sanofi

Part of the problem with immunogenicity is that every product is unique, and it is very difficult to make generalized rules other than for biosimilars, in Ragheb's view. The FDA encourages sponsors to ask questions prior to submission but will hardly ever tell them what they can or cannot do or what will be accepted. This is always a review matter. "You have to take your best shot at asking the questions that you want them to address and then interpret those responses in developing your ASK."

There has been progress, however, in the form of a recent White Paper that has substantially reduced the amount of immunogenicity assays needed at the preclinical stage. The sponsors all agreed that this has had an effect. Sanofi no longer uses the three-tiered approach for non-clinical studies and directs its partners not to either. From the other side of the fence, Cerba Research has revised its standard operating procedures (SOPs) to drop three-tier resting and go straight to titer in support of PK or TKR data.

"We propose a minimum approach, and we have optional assessments to do during method validation," said Roberge. "That's the discussion we have with every sponsor before we start working on these projects, because we need to align with the sponsor's needs and what they want to do with these immunogenicity data in the preclinical stage."

"We will tailor assay development and assay validation based on what sponsors expect"

Martin Roberge, Cerba Research



A flexible approach to neutralizing antibody assay selection can improve results Whilst Cerba Research has developed many cell-based neutralizing antibodies, it is aware that many sponsors would prefer a simpler, less costly assay. The FDA has been open to the use of competitive ligand-binding assays here, and cell-based neutralizing antibodies are often still required in practice.

In one project Daiichi Sankyo worked on, it was unable to achieve NAb drug tolerance in cell-based assays, so it requested a Type C meeting with FDA for permission to use competitive ligand-binding. This was permitted but the agency asked the company to generate some other peripheral data to support the assay because the cell-based assay reflected the MoA much better. This assay is yet to be validated.

"From the FDA's guidance and also through our communication we still prefer a cell-based assay for neutralizing antibodies in general, unless we can't achieve the desired drug tolerance," she said. "We have to write a very long method development report to demonstrate the experiment.

Many assays have been exhausted to convince the reviewer to allow us to move to the plate-based assay."

Merck recently published a paper on its use of a non-cell-based assay, added Weifeng Xu. Cell-based assays cannot monitor any of the MoAs of an ADC other than the receptor involved. Cell-based assays were historically preferred but there is increasing interest in alternatives. FDA guidance now encourages the use of a PK, pharmacodynamic (PD), and biomarker package. He believes that the trend here is in the right direction.

"When I joined Merck, we developed a competitive ligand-based assay for mAbs.

For one particular ADC, we still have to do the cell-based carrier assay first because it's a new modality, but we believe it will probably follow the same path as the mAbs."

Weifeng Xu, Merck



There has been a long tension between cell-based assays and binding assays, Ragheb said.

The FDA recognizes that a cell-based assay cannot be developed for every programme, but they usually want to see some justification for this. "Clearly a binding assay by itself is not going to fully reflect the MoA of an ADC, but the purpose of the assay is to ask if there are ADAs present that prevent the therapeutic from being efficacious. If it can't bind its ligand, it's not going to be very efficacious," he said.

Conceivably, Ragheb and Pine agreed, if a confirmatory domain-specificity assay showed no incidence of payload-specific ADA, a ligand-binding method for the NAb for the targeted moiety might be sufficient. Whether that will happen in practice remains to be seen and the science is not yet clear, as Merck's experience has shown.

Cerba Research has used a highly sensitive cell-killing assay for an ADC, Roberge noted. It saw many antibodies that were directed against the payload, as well as many pre-existing ones and many drug-boosted antibodies against that were actually neutralizing.

"So when we put that in a cell-based cell killing assay, they would prevent cell killing."

Technology will evolve and AI has a big role to play

New technology will definitely make ADAs more sensitive, Weifeng Xu said, but a very sensitive array is not always necessarily the best.

The industry might "shoot its own feet" by using very sensitive arrays with very high positive rates. Safety, sensitivity, and clinical relevance must be balanced against each other.

Pine agreed that the industry is "throwing the whole kitchen" at multi-domain and complex therapeutics. It must learn to take leaner approaches and find out what is really applicable as it gains more experience, and more compounds reach the market.

For multi-domain bispecifics, we have enough information to start confirmatory testing for domain specificity, but for the next generation of complex therapeutics, "extending the conjugations and seeing what other combinations of lipid nanoparticles (LNPs) and other types of targeted viral vectors we might have, there are very big immunogenicity questions that need to be answered."

"My take-home message is 'Do not be scared about high immunogenicity or worry about your array sensitivity when you have ultra-low immunogenicity rates,'" said Sophia Xu. Daiichi Sankyo has experienced both, for example, genetic positivity of up to 40-50% in a single dose in an immunology indication and an extremely low immunogenicity rate in an ADC.

"You just need to monitor the in-study cut-point and the false-positive rate, ensure it's all science-based, and communicate with regulatory agencies ahead of time."

Companies are already selecting candidates based on in silico predictions of immunogenicity risks, Ragheb said, and it will be interesting to see whether AI has an impact on these tools. Probably the largest obstacle will be sponsors' unwillingness to share enough immunogenicity data for an AI programme to be developed. The field will also evolve as new therapeutic modalities come online.

In this context, said Sophia Xu, the challenge might be how to ask the right questions of tools like ChatGPT 406. Daiichi Sankyo has already used this in other fields like stop data review and data comparison. The potential for time saving is immense. "Nobody's prepared for that," Roberge agreed. "I think it's a bit scary, a bit encouraging, and it certainly is something exciting. We'll see where it goes."

Biographies



Dr. Lorella Di Donato

Senior Vice President,
Co-CEO Cerba Research-Canada

Dr. Lorella Di Donato has over 29 years of experience in the development and validation of specialized assays to measure cellular and protein biomarkers, as well as drug products for both large and small molecules using different technology platforms. She received her PhD in Biochemistry from the University of Montreal and held significant positions within the scientific operations of Cerba Research as Chief Operating Officer and Chief Scientific Officer.



Dr. Jack Ragheb

SVP, Translational Sciences
& Medicine, NexImmune

Dr. Jack Ragheb received his MD and PhD from Johns Hopkins University and is a diplomate of the American Board of Allergy & Immunology. Formerly, he was the Senior Medical Fellow for Immunology in Global Patient Safety and Co-Chair of the Immunogenicity/Immunosafety Working Group at Eli Lilly. Before joining industry, he was a Senior Clinical Investigator at the National Institute of Health (NIH), where he conducted clinical and basic research on immune tolerance, and a Chief Medical Research Officer in the FDA's Office of Biological Products, where his research group established the BLT humanized mouse model to predict the immunogenicity of biotherapeutics.

He has published in the areas of immunogenicity, immune tolerance, IL-2R signaling, CD28 and CD40L regulation, retrovirology,and gene therapy.



Dr. Samuel Pine

Global Head of Bioanalysis &
Imunogenicity, DMPK, Sanofi

Dr. Samuel Pine is an accomplished drug development expert with a broad background in biotherapeutic, vaccine, and diagnostic R&D and a strong focus in immunogenicity. In his current role, he provides leadership and guidance to scientists developing and implementing PK, ADA, and biomarker methods for Sanofi's R&D portfolio. He regularly contributes to scientific discussions at international symposia and is an active member of industry working groups. Prior to Sanofi, Samuel held roles of increasing responsibility at BioAgilytix, Allergan, the Infectious Disease Research Institute, the Fred Hutchinson Cancer Research Center, and Chiron/Novartis.

He received his bachelor's degree at the University of California, Santa Cruz, and his PhD in Pathobiology from the University of Washington.



Dr. Martin Roberge

Associate Director – Cerba Research BioAnalytical Center of Excellence Cerba Research Canada

Dr. Martin Roberge has over 26 years of experience in the life science industry. Throughout his career in biotech and CROs, he has developed a thorough expertise in protein engineering, bioanalysis, and drug development. At Cerba Research, he provides leadership and scientific expertise for method development and GLP validation projects for large molecule drug products in support of preclinical and clinical studies.

The methods used include immunogenicity assays (ADA, neutralization antibody or NAb), PK assays, biomarkers, quantitative polymerase chain reaction (qPCR), and others.

Biographies



Dr. Weifeng Xu

Senior Scientific Director,
Regulated Bioanalytics Merck & Co.

Dr. Weifeng Xu leads the scientific strategy and liaison for regulated bioanalytics at Merck. He has been active in the field of immunogenicity for more than ten years and co-leads the AAPS NAb working group, which has multiple publications and patents. At Merck, Weifeng established the Cell Assay Group within PCD Regulated Bioanalytics to support NAb assays for biologic therapeutics and cell therapy, as well as infectivity, neutralizing, and bacteriology methods for vaccines.

He was the Bioanalytical Lead for Merck's COVID-19 vaccine and its first cell therapy and is now supporting the 21-valent V116 pneumococcal vaccine, as well as multiple ADCs.



Dr. Andrew Warmington

Manufacturing Editor,
Citeline (Moderator)

Dr. Andrew Warmington has been writing about pharmaceuticals and related industries since becoming editor of Speciality Chemicals Magazine in 2002.

His particular area of expertise is in the C(D) MO drug substance and CRO markets, plus the regulatory side, which was developed during a stint with Chemical Watch. During the last 20 years, he has attended every CPHI Worldwide exhibition, plus other key industry events, and has been a regular moderator of expert panels.

He joined Citeline as Manufacturing Editor in 2022.



Dr. Xiaohui (Sophia) Xu

Director, Daiichi Sankyo

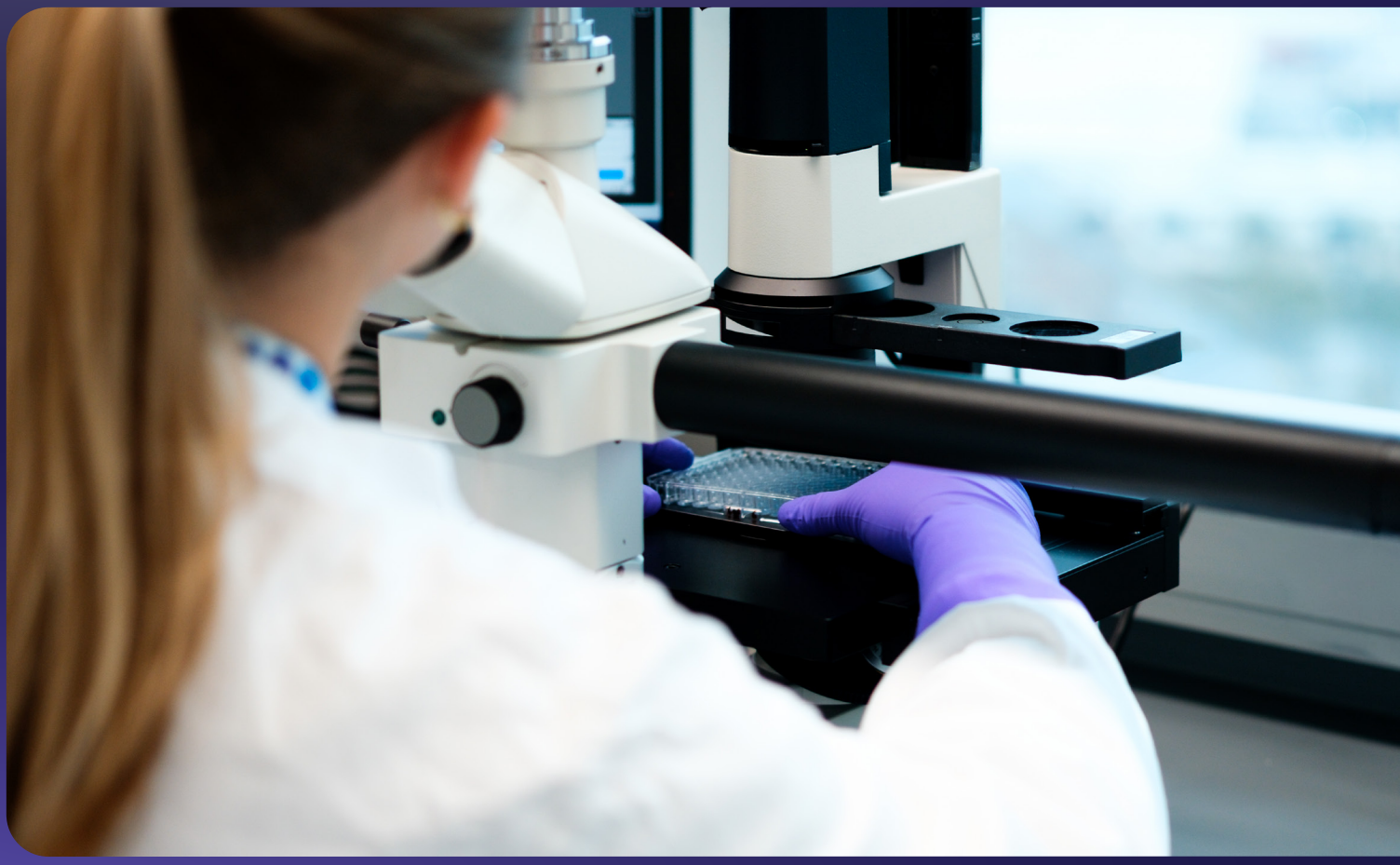
Dr. Xiaohui (Sophia) Xu is currently a director at Daiichi Sankyo. She is serving actively in the American Association of Pharmaceutical Scientists (AAPS) PK Assay Group and also in different groups in the IQ Consortium. Sophia has extensive experience in the regulated bioanalytical field, including global submissions covering small molecules, biologics, and new modalities, and has chaired sessions at various scientific conferences. She has also published extensively including two chapters of 'Bioanalytical Aspects in Biological Therapeutics, which she co-edited. She received her doctorate in Pharmaceutical Analysis from the University of Georgia's College of Pharmacy.



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