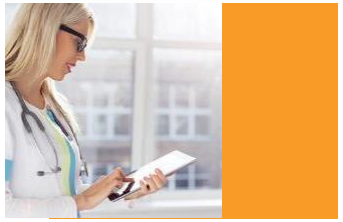
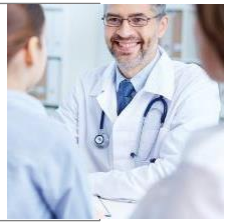
European Federation of Pharmaceutical Industries and Associations

Subcutaneous Biologics: Advancing High-Concentration Technologies and *Justifying CQA Specification Limits Through Translational Immunogenicity Models – Status Update*

Karoline Bechtold-Peters on behalf of the MQEG Biomanuf. WG on "IV to SC Conversion WS“ and on „Use of animal and other models to evaluate product immunogenicity justifying CQA specification limits WS”




CASSS EU CMC Strat Forum
20.10.2025

Use of animal and other models to evaluate product immunogenicity justifying CQA specification limits

Regulatory Feedback at CASSS EU CMC Meeting 2023:

- The support of specifications describing CQAs with influence on immunogenicity by in vitro and in vivo models is welcomed by the authorities, progress in the use of these tests is expected (Mats Welin, Swedish authority/EMA)
- **Reliance on nonclinical models was mentioned as a key element to support specifications, provided that they are demonstrated to be fit for purpose** - i.e., connection with clinical response, appropriate performance **(no "formal" validation needed as for analytical QC methods**, but fit for purpose validation, as discussed during the session dedicated to models). For instance, It is important to combine physchem, nonclinical and (if necessary) clinical verification to support credibility of the nonclinical testing to support use for quality attributes severity confirmation and ranges
- The practical meaning of "fit for purpose" would require further discussion; coherently, reflection will be needed on what to submit in a file on such models. **In this context, attending regulators were supportive about the establishment of the EFPIA working group on nonclinical testing / models to support product understanding/ specifications, or they even expect progress in the use of these models (Mats Welin)**. Position from this group could be the basis for further structured dialogue with regulators. It was also confirmed the opportunity to have vaccines in scope of this group (post-conference proposal: follow-up also with Vaccines Europe to identify potential experts to join this discussion). **The regulators were also encouraging sponsors to report studies using non-clinical models in filings as this would familiarize reviewers with these approaches**.

ISSUE SHEET

Outline of planned IHI Project

Problem statement: The immunogenicity of biological products is evaluated in the context of clinical trials (ADA, NAB, other adverse effects). See USP chapter 1106. There are a number of in vitro and in vivo models that can provide mechanistic information on the immunogenicity potential of certain quality characteristics of a product, but the IVIV correlation is in debate (which also holds true for the vaccines field).

Examples include

- MAPPs (MHCII-associated peptide proteomics) (ref1, ref 2).

- T cell/PBMC assay

- moDC maturation assay (human monocyte-derived DC) (ref2, ref 3).

- Humanized mouse model (ref4, ref5).

The attribute ranges identified in the clinical studies generally do not include preparations with intentionally higher levels of CQAs such as aggregates, chemical modifications, and other CQAs. This raises problems in justifying a specification range that is broader than the historical clinical exposure of the product.

The increased reliance on animal / in vitro assays, is postulated to help setting scientifically sound specs (not grounded on clinical qualification only) and should also be considered for vaccines, essentially when a correlate of protection can be established. It is only sometimes possible to clinically test acceptance ranges from dose-escalation studies or by using batches intentionally designed to have higher attribute levels close to the expiration date.

In many cases, moreover, an accelerated CMC development program at multiple clinical sites limits statistically robust assessment of the effect of product aging data.

IHI PROJECT

Outline of planned IHI Project, Cont.

Participants:

- 10 - 15 pharma companies with expertise in in vitro and in vivo immunogenicity assays/models prepared to invest lab resources
- Proposal to also include CROs
- Include academia, i.e. hospitals and animal labs into program to test in vivo models (various species, humanized versus wild type) and in human (?)
- Include EMA

Questions to be addressed:

- How sensitive are the various models?
- Advance standardized protocols
- Include various protein particle species and amounts, include typical degradation products (mAbs, free fatty acids from PS degradation, silicone) to compare and challenge the models
- Verify in relevant species (humanized minipig? humans?)

- Timing: Submission of project in 2026

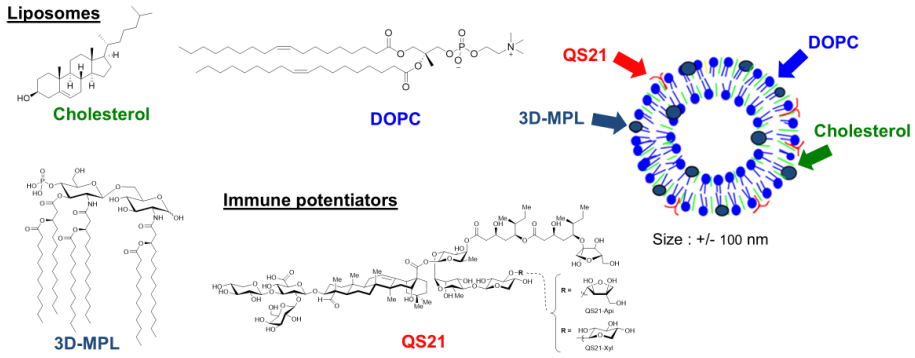
EXAMPLE FOR A SPECIFIC TOPIC DISCUSSED IN REGULAR MEETINGS OF WS

Adjuvant effect of polysorbate degradation products?

- A session with Derek O'Hagan, GSK, covered the science and mechanisms of vaccine adjuvants, including traditional (aluminium salts, oil-in-water emulsions) and emerging types, with emphasis on how formulation and delivery impact immune response.
- Two EFPIA work streams—immunogenicity/patient-centric specifications and polysorbate degradation—collaborated to discuss current practices, challenges, and future strategies for controlling polysorbate degradation and its safety implications.
- The group highlighted concerns about the potential immunogenicity of free fatty acids and particles formed from polysorbate degradation, questioning whether these could act as adjuvants and how best to assess their impact using relevant assays and models.
- Comparative experiences were shared, such as replacing polysorbate 80 with poloxamer to reduce sub-visible particles without affecting immunogenicity, and the need for specialized assays to evaluate these effects.
- **Conclusion from the adjuvant expert: Free fatty acids resulting from polysorbate degradation are not known to act as adjuvants. Their composition is critical, and a complex, optimized system would be required for any adjuvant effect—simple presence of free fatty acids is likely not sufficient to confer adjuvant properties.**

AS01 Composition

AS01 is an Adjuvant System comprising MPL, QS-21 and liposomes



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QS-21; licensed by GSK from Antigenics LLC, a wholly owned subsidiary of Agenus Inc., a Delaware USA corporation

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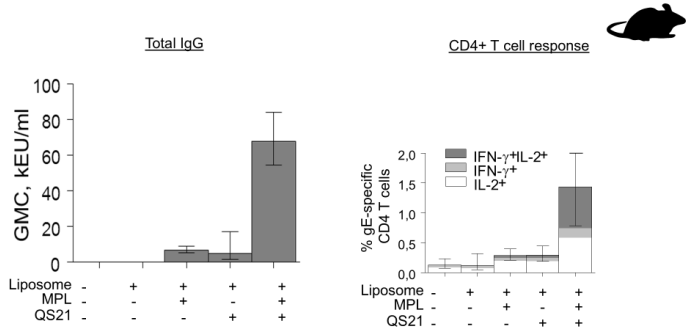
Synergy of MPL and QS-21 in liposomes is critical for adjuvant effect of AS01 (gE - Herpes Zoster)



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Dendouga, N. et al, Vaccine, 30, 3126, 2012

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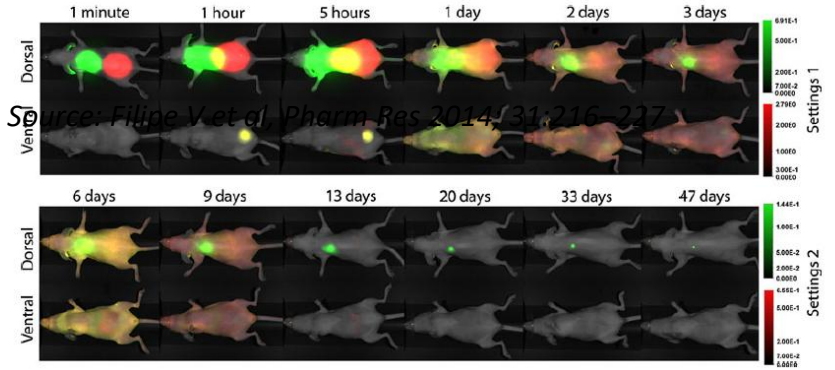
CONNECTING
WORKSTREAM GOALS:

IV to SubQ Workstream

Still needed: more flexibility on specification setting for SC DP

- In the concentrated to ultra-highly concentrated SC preparations, there are more protein molecules per unit volume than traditionally. The previous specification of particles per volume or container creates an imbalance. The specification should consider the total applied amount.
- Adjusted specifications may be justified for the parameters
  - Subvisible Particles
  - Visible Particles
  - Aggregates
- Pharmacopoeial specifications may not apply
- Need to include aged material in clinical studies
- **Follow the approach of "patient-centric specifications" rather than follow pharmacopoeial standards**

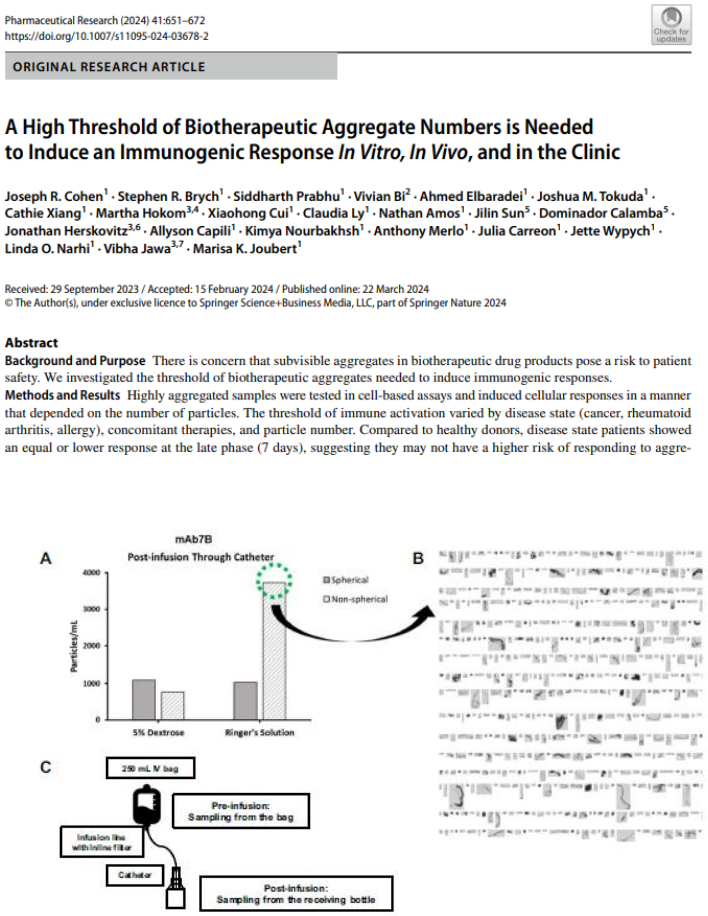


Source: Filipe V et al, Pharm Res 2014; 31:216–217

EXAMPLE FOR A SPECIFIC TOPIC DISCUSSED IN REGULAR MEETINGS OF WS

Summary of Paper A High Threshold of Biotherapeutic Aggregate Numbers is Needed to Induce an Immunogenic Response *In Vitro, In Vivo*, and in the Clinic

- **Background and Purpose**
- The paper investigates the threshold of biotherapeutic aggregates needed to induce immunogenic responses. There is concern that aggregates in biotherapeutic drug products pose a risk to patient safety.
- **Methods and Results**
- **In Vitro Studies**: Highly aggregated samples were tested in cell-based assays. The immune activation threshold varied by disease state (cancer, rheumatoid arthritis, allergy), concomitant therapies, and particle number. Disease state patients showed an equal or lower response at the late phase (7 days) compared to healthy donors.
- **In Vivo Studies**: Xeno-het mice were used to assess the threshold of immune activation. Highly aggregated samples (1,600,000 particles/mL) induced a weak and transient immunogenic response in mice, while a 100-fold dilution of this sample (16,000 particles/mL) did not induce immunogenicity.
- **Clinical Data**: Subvisible particles (up to ~18,000 particles/mL) produced under representative administration practices did not induce a response in cell-based assays or increase the rate of adverse events or immunogenicity during phase 3 clinical trials.



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ORIGINAL RESEARCH ARTICLE

A High Threshold of Biotherapeutic Aggregate Numbers is Needed to Induce an Immunogenic Response *In Vitro, In Vivo*, and in the Clinic

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Abstract

Background and Purpose There is concern that subvisible aggregates in biotherapeutic drug products pose a risk to patient safety. We investigated the threshold of biotherapeutic aggregates needed to induce immunogenic responses.

Methods and Results Highly aggregated samples were tested in cell-based assays and induced cellular responses in a manner that depended on the number of particles. The threshold of immune activation varied by disease state (cancer, rheumatoid arthritis, allergy), concomitant therapies, and particle number. Compared to healthy donors, disease state patients showed an equal or lower response at the late phase (7 days), suggesting they may not have a higher risk of responding to aggre-

Other topic discussed in 2025

- Bridging of attributes between IV and SC
- Excipients enabling SC administration and requirements for new excipients
- Effect of hyaluronidase
- Higher SC volumes without enzyme
- PBMK/PK models
- Goal in 2026 (tentative):
  - Summarize combined knowledge of group (primary authors of many scientific papers) and publish position paper („the knowns and the unknowns about SC application“)

CASSS

Bridging Strategies During Clinical Development (Dosage Forms, Specifications, Devices) – WS is supporting this session

The development and approval of combination drug products benefit significantly from risk-based approaches, which utilize predictive models to assess the impact of changes on pharmacokinetics (PK), stability, compatibility, and real-world usability. The acceptability of these models by regulatory bodies is crucial, as it ensures that the predicted outcomes are reliable and can be used to streamline the approval process. Effective communication with regulatory bodies is essential for identifying and resolving potential issues before they become critical, while ongoing dialogue ensures that any changes or updates are promptly addressed. This collaborative approach fosters a transparent and efficient approval process, reducing the likelihood of delays and facilitating the timely introduction of new drug products to the market.

Clinical bridging strategies, whether conservative or smart, provide a structured approach to transitioning between different stages of drug development. Conservative strategies focus on maintaining safety and efficacy by adhering to established protocols and guidelines, while smart strategies leverage innovative methods and technologies to optimize the development process. It is the aim of the session to present more advanced and novel tools to support smart strategies.

Bridging between dosage forms is another critical aspect, ensuring that changes in the form of the drug (e.g., vial to 2 x PFS to 1 x PFS) do not compromise its efficacy or safety. This involves rigorous testing and validation to confirm that the new dosage form delivers the drug consistently and effectively. Similarly, bridging between specifications involves ensuring that any changes in the drug's specifications, such as its chemical composition or manufacturing process or subvisible particles content, do not affect its quality, safety, or efficacy. How can in-silico simulation and novel characterization means help here?

By implementing robust risk-based approaches, maintaining continuous communication with regulatory bodies, and employing effective clinical bridging strategies, including bridging between dosage forms, devices and specifications, developers can navigate the complexities of combination drug product development with confidence and precision. These practices collectively contribute to the successful development and approval of combination drug products, ensuring that they meet all necessary safety and efficacy standards.

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Thank You