Potency Testing for Adjuvanted Vaccines: Progress, Roadblocks and a Vision for Non-Animal testing

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Disclosure

Badiaa Bouzya is an employee of the GSK group of companies.

This work was sponsored by GlaxoSmithKline Biologicals SA.

GSK is committed to the replacement, reduction and refinement of animal studies (3Rs). Non-animal models and alternative technologies are part of our strategy and employed where possible. When animals are required, application of robust study design principles and peer review minimises animal use, reduces harm and improves benefit in studies.

Agenda

1 Introduction

2 Adjuvant overview

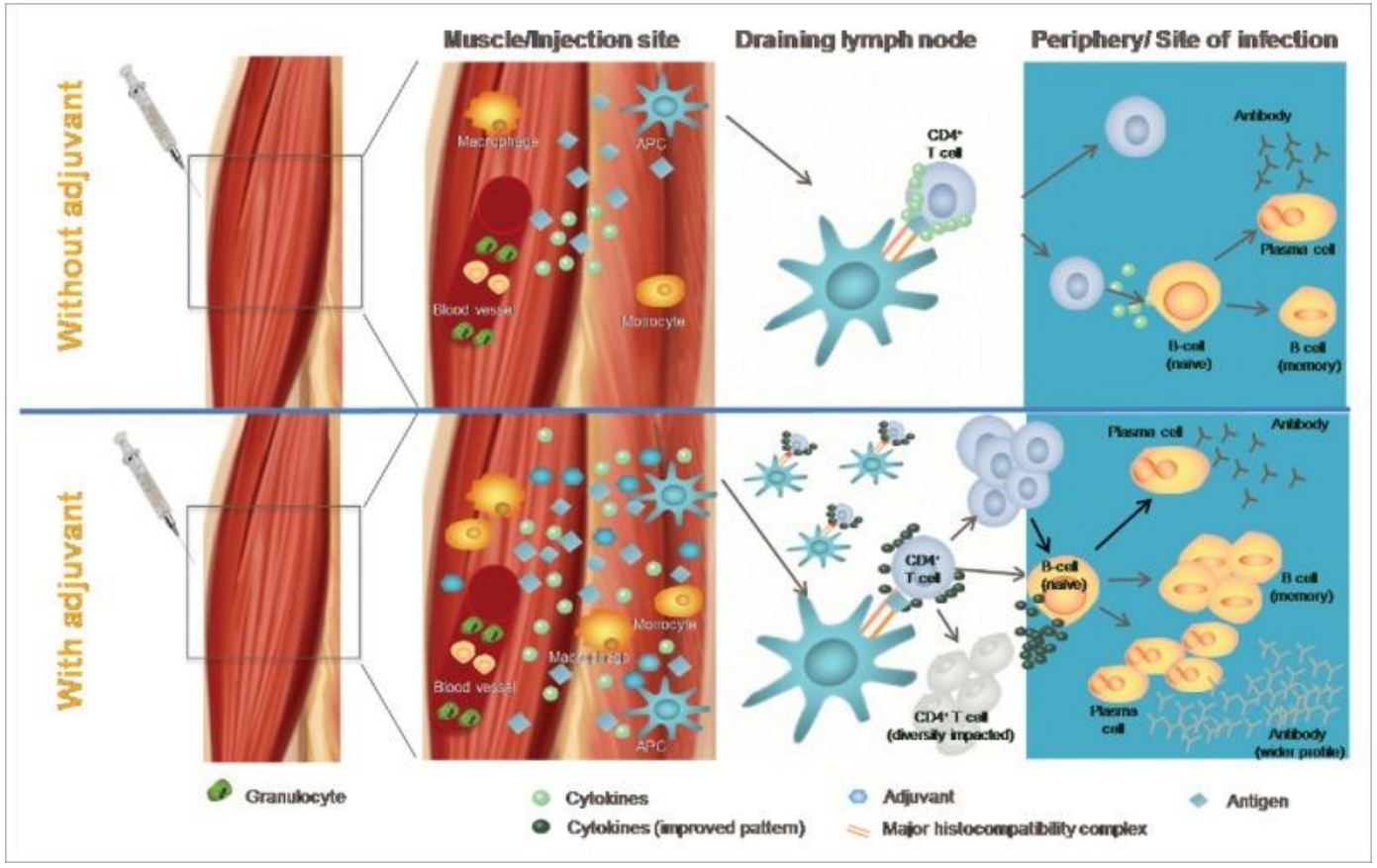
3 Non animal testing strategies

4 Limitations & Roadblocks

5 Take Home Message

Adjuvants play a critical role in enhancing vaccine immune response

- Boost antigen presentation
- Shape the innate and adaptative immune response
- Allow dose sparing and improved response in vulnerable populations



N Garçon, A Di Pasquale , "From discovery to licensure, the Adjuvant System story", Human Vaccines & Immunotherapeutics 2017

Adjuvant overview

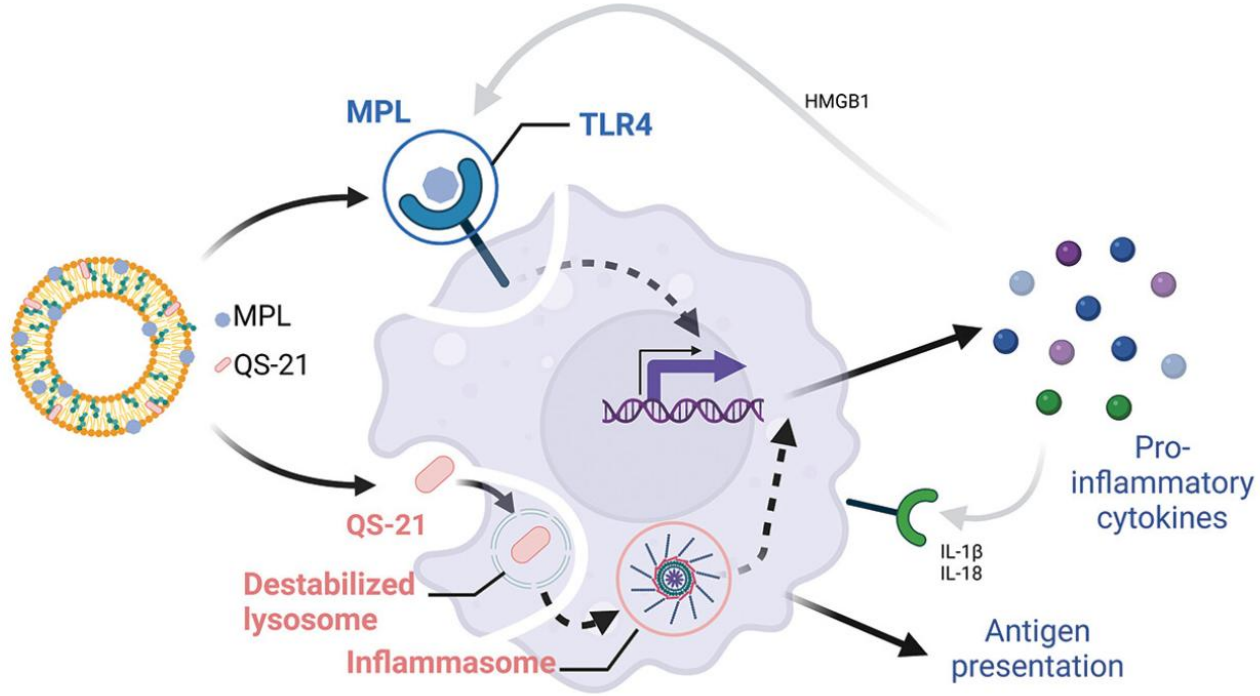
Common classes of adjuvants :

- Aluminium salts (Alum)
- Oil-in-water emulsions (MF59, AS03)
- AS04, AS01 :
  - Toll-like receptor (TLR) agonists (CpG, MPL)
  - Saponin-based adjuvants (e.g., QS-21) : Liposomes, nanoparticles

Mechanisms :

- Activation of dendritic cells (DCs), cytokine production, and T-cell polarization

Cellular mechanism signaling pathways triggered by MPL and QS-21



F.Roman et al, " Adjuvant system AS01: from mode of action to effective vaccines" , EXPERT REVIEW OF VACCINES ,2024

Why Potency testing matters ?

Potency testing ensures that a vaccine **induces the intended Immune response**.

For adjuvanted vaccines, this becomes more complex because:

- The **antigen** and **adjuvant** work synergistically
- Small changes in antigen content, adjuvant composition or dose, or excipient levels can strongly affect immunogenicity
- Traditional in vivo assays were designed to capture this combined effect

Limitations of Traditional Animal-Based Potency Tests

Operational and Ethical considerations :

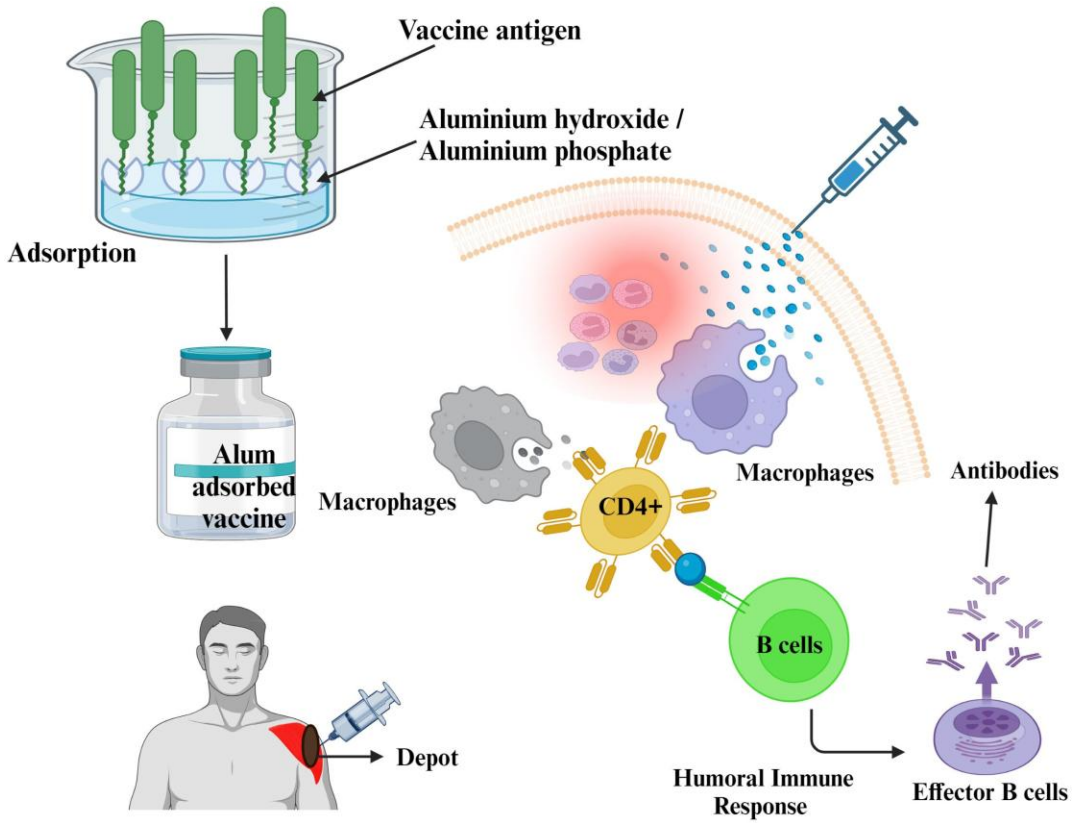
- Embracing 3Rs principles strengthens ethical standards
- High costs and long study durations remain a challenge
- Logistical constraints continue to impact implementation

Scientific Limitations :

- High variability due to biological and environmental factors
- Stress and welfare conditions impacting immune responses
- Limited mechanistic information. They measure outcome rather than pathway. It is well suited to detect non-potent products but is less sensitive for identifying sub-potent decreases in activity
- Slow turnaround and poor scalability for modern manufacturing

Challenges in Potency Testing for adjuvanted vaccines

- Antigen-adjuvant interactions can alter stability and immunogenicity
- Potency often depends on **both** components functioning correctly
- Replacing in vivo potency assays requires a validated package of analytical, functional, and immunochemical methods that together reflect the vaccine's critical quality attributes (CQAs)
- New in vitro potency assays for adjuvanted vaccines must measure the adjuvant's contribution to immune activation, not just antigen activity
- The whole analytical portfolio of the antigen and adjuvant need to be considered in the consistency-approach framework



S Moni et al ," Advancements in Vaccine Adjuvants: The Journey from Alum to Nano Formulations " Vaccines 2023
J-F Dierick et al , " The consistency approach for the substitution of in vivo testing for the quality control of established vaccines: practical considerations and progressive vision" , Open Research Europe,, 2002

Scientific Progress Toward Non-Animal Potency methods but not yet GMP-release ready

Mechanism-Based Analytical Assays

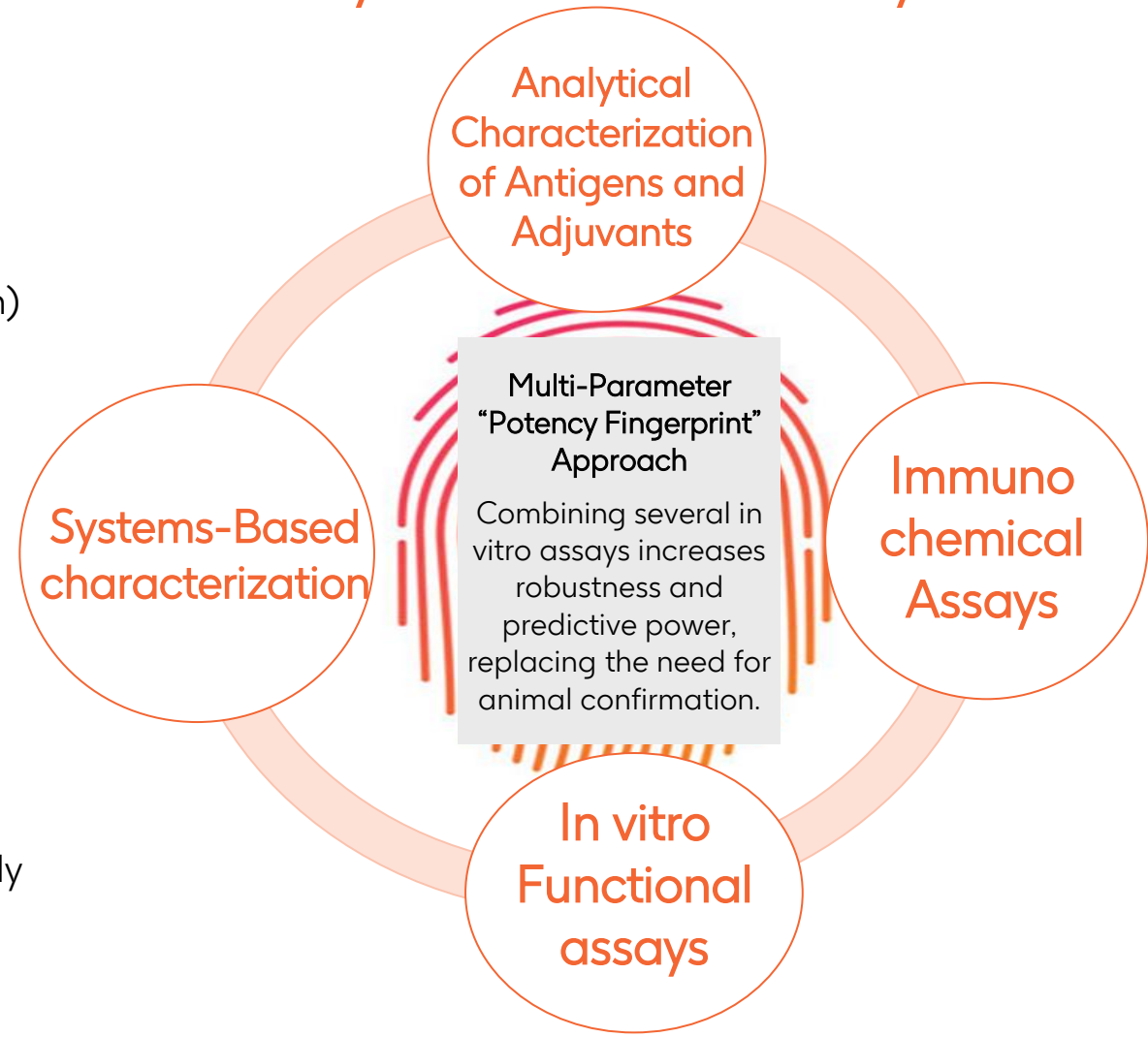
- Antigen structural integrity assays (HPLC, mass spectrometry, DLS)
- Epitope-specific binding assays
- Adjuvant characterization (particle size, charge, interaction strength)

In Vitro Functional Assays

- APC/dendritic cell activation assays
- Reporter gene assays for TLR or other PRR
- Multiplex cytokine/chemokine assays for secreted mediator profiles

Immunochemical Assays

- ELISA-based potency assays for antigen quantification
- Competitive inhibition assays reflecting epitope recognition
- Monoclonal antibody–based assays that simulate protective antibody binding

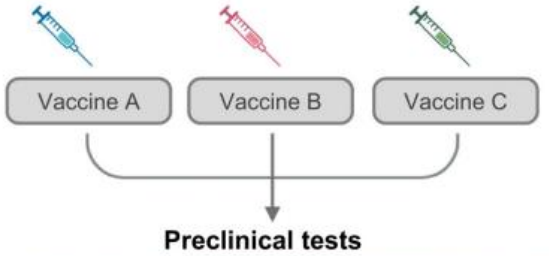


Although these mechanism-based, functional and immunochemical assays are available, several are not yet suitable for GMP release.

Morrocchi et al , " Modeling human immune responses to vaccination in vitro" , Trend Immunol. 2024

Alternatives to in vivo methods

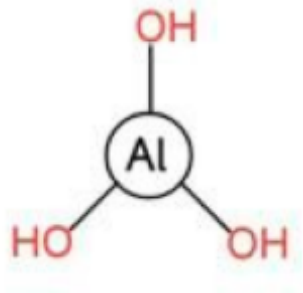
- Limitations of animal models: Species-specific differences, ethical concerns, and low throughput.
- In vitro models:
  - **Human Dendritic Cells (DCs)**:
    - Single-cell assay
    - Assess activation and cytokine profiles.
  - **PBMC Assays**:
    - Multi-cellular component assay
    - Evaluate immune responses in human blood samples.
  - **Organs-on-a-Chip**: Mimic human tissue microenvironments for precise immune modeling.
- Benefits: Enhanced translational accuracy, reduced costs, and 3R compliance.



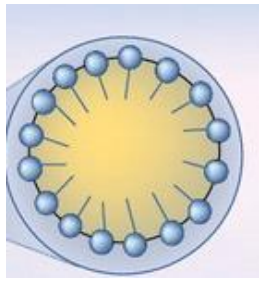
	2D models	3D models	Animal models	Human clinical trials
Production method	Cells grown on a rigid and flat surface	3D scaffold that resembles ECM	In vivo natural structure	In vivo natural structure
Cell type	Depends on model type	Multitype	Highly diverse cell types	Highly diverse cell types
Natural cell morphology	No	Yes	Yes	Yes
Tissue architecture	Absent	Complexity based on design	Naturally present	Naturally present
Vascularization/perfusion	Absent	Present according to model type	Present	Present
High-throughout screening	Medium to high	Low to medium	No	No
Biobanking	Yes	Yes	Yes, only at the cellular level	Yes, only at the cellular level
Fidelity to human processes	Oversimplified, non physiological conditions	More physiological conditions	Species-specific differences	High fidelity
Costs	Low	Moderate	Moderate	High
Bioethical concerns	No	No*	Moderate	High

Morrocchi et al , " Modeling human immune responses to vaccination in vitro" , Trend Immunol. 2024

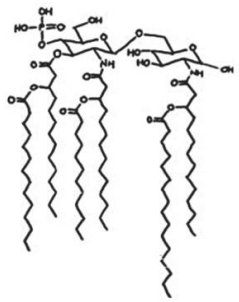
Integrated functional assays : Cell-based assays



Aluminum Adjuvants Cell-based assays capturing inflammasome activation or phagocytosis



Emulsion Adjuvants (MF59, AS03) Innate immune activation assays (cytokine responses from dendritic cells)



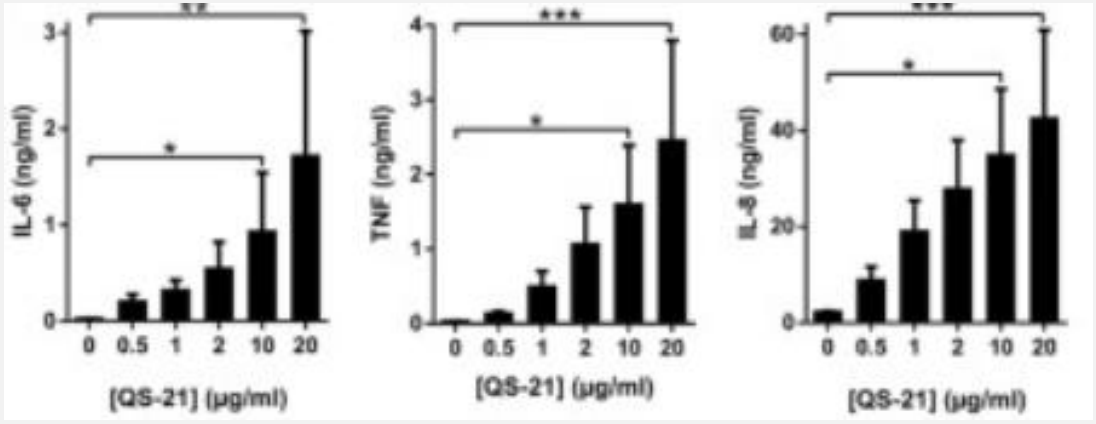
TLR Agonist Adjuvants In vitro Dendritic cell activation TLR-specific reporter assays (e.g., NF-κB activation)

Saponin or Nanoparticle Adjuvants (QS21) Cell-based assays mimicking innate immune activation

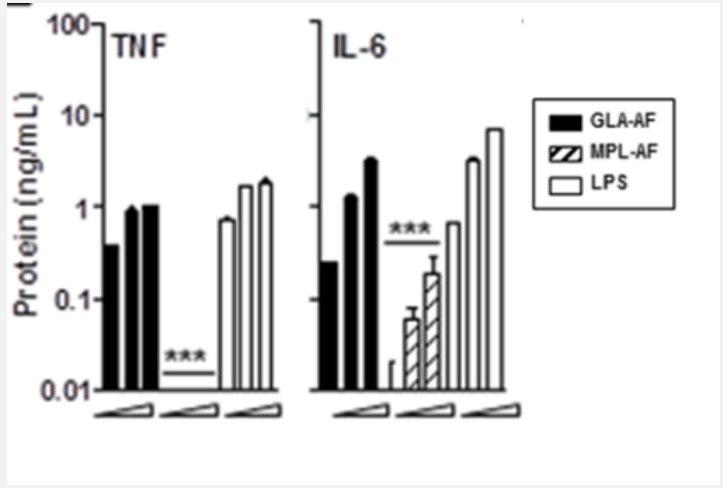
- Cytokine profiling (IL-6, TNF-α, IFN pathway activation)
- Gene expression signatures of innate activation
- High-throughput immunophenotyping platforms
- These assays collectively represent **modern, relevant, and predictive** alternatives to animal potency testing

QS-21 and MPL directly activate human dendritic cells

However the potential as a stability indicating Analytical Target profile is not clarified



- Monocyte-derived Dendritic Cells were stimulated with increasing concentrations of QS-21
- IL-6, TNF, and IL-8 were quantified in the culture supernatant after 24 h



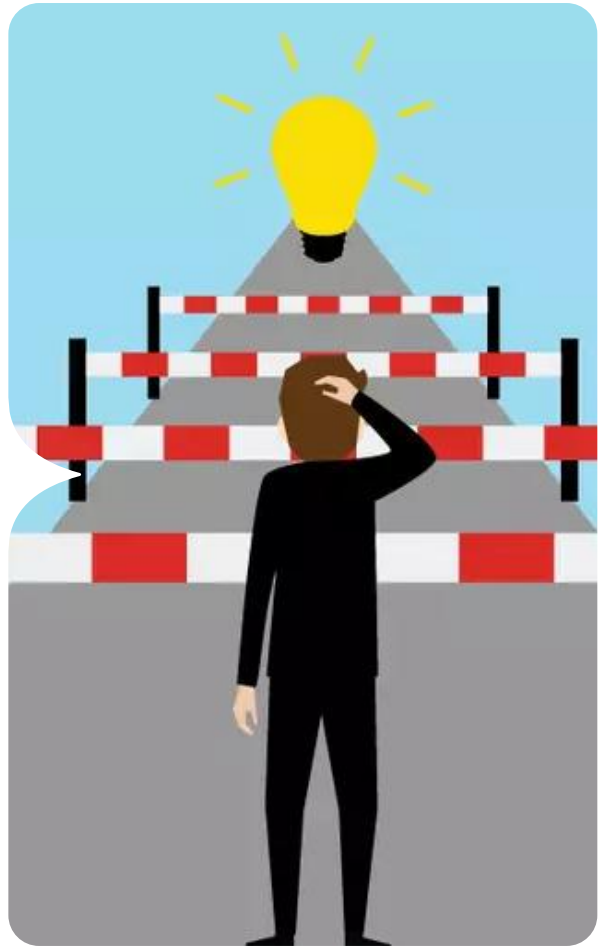
- Dendritic Cells were stimulated with 1 mg/mL of GLA-AF, MPL-AF, or LPS
- Protein levels were determined after 4, 8, and 24 h stimulation for a subset of molecules in culture supernatants by ELISA

I Welsby et al , " Lysosome-Dependent Activation of Human Dendritic Cells by the Vaccine Adjuvant QS-21" Frontiers in Immunology, 2017
N. Coler et al, " Development and Characterization of Synthetic Glucopyranosyl Lipid Adjuvant System as a Vaccine Adjuvant" , Plos One , 2011

Roadblocks Still Facing Non-Animal Potency Testing

1. **Scientific** Complex adjuvant–antigen interactions not fully captured by single analytic assays
   - Limited mechanistic understanding of some adjuvants
   - Lack of standardized correlates of protection for certain diseases
2. **Regulatory**
   - Some pharmacopeias still require in vivo potency tests
   - Regulatory acceptance differs across regions
   - Reluctance to change validated legacy methods
   - Need for cross-industry harmonization
3. **Manufacturing**
   - Legacy production platforms not designed with mechanism-based assay in mind
   - Need for method transfer, cross-validation, training, equipment, and documentation

The science exists, but the **ecosystem** must adapt.



A Vision for a Fully Non-Animal Potency Testing Framework

1. **Mechanism-informed Design of New Vaccines**
   - Defined **CQAs** connected to potency
   - Clear understanding of how adjuvant enhances immune response
   - Built-in **analytical endpoints** for potency from day one
2. **Multi-Assay Potency Strategy**
   - Structural integrity assay (physico-chemical)
   - Antigen-specific assay (immunochemical)
   - Functional assay mimicking innate activation by the adjuvant
   - Stability-indicating assays
3. **Increased International Collaboration**
   - Regulatory convergence is essential
   - Harmonized acceptance criteria
   - Shared validation data
   - Collaborative studies across regulatory bodies and manufacturers
4. **Digital and AI-driven Prediction Models**
   - Advanced analytics
   - Predictive models correlating CQAs with potency

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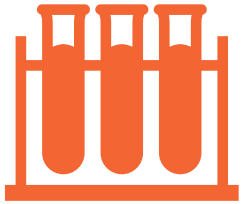
Take Home Message

Adjuvanted vaccines pose unique potency-testing challenges, but modern science allows precise, mechanism-based in vitro alternatives

Non-animal strategies combine analytical, immunochemical, and functional assays into integrated potency platforms



Adjuvants are characterized, **the combination is assessed in the clinical study**, and quality is ensured through a **consistency approach** and GMP-compliant processes.

Adjuvant–antigen combinations are controlled to protect **patients** and to ensure consistent product efficacy.

The path forward includes **harmonization, predictive modeling, and designing QC into vaccines**, paving the way for 100% non-animal potency testing independent if they are adjuvanted or not.

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Thank you

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