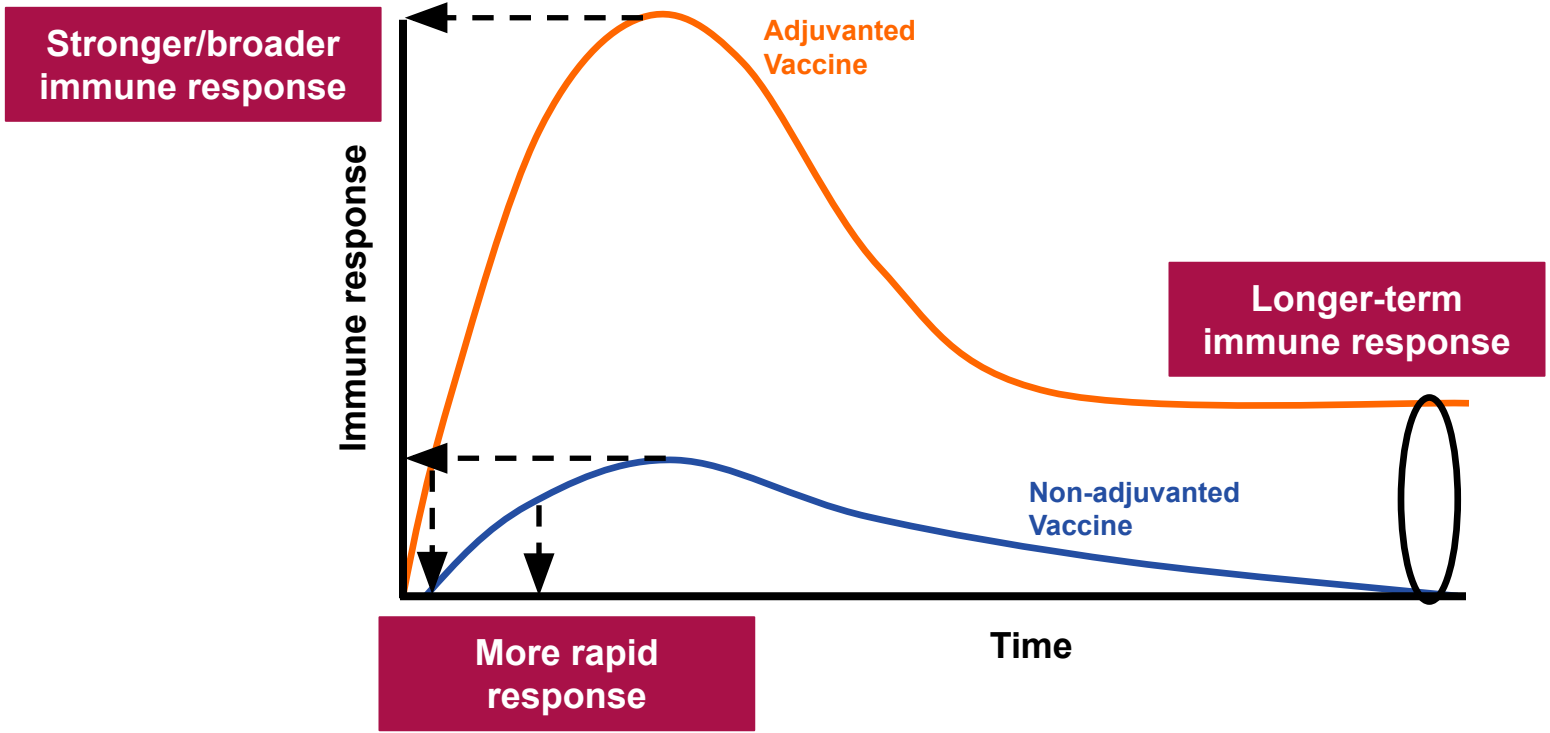
The Science of Vaccine Adjuvants

Derek O’Hagan, Senior Advisor R&D, Senior Fellow GSK

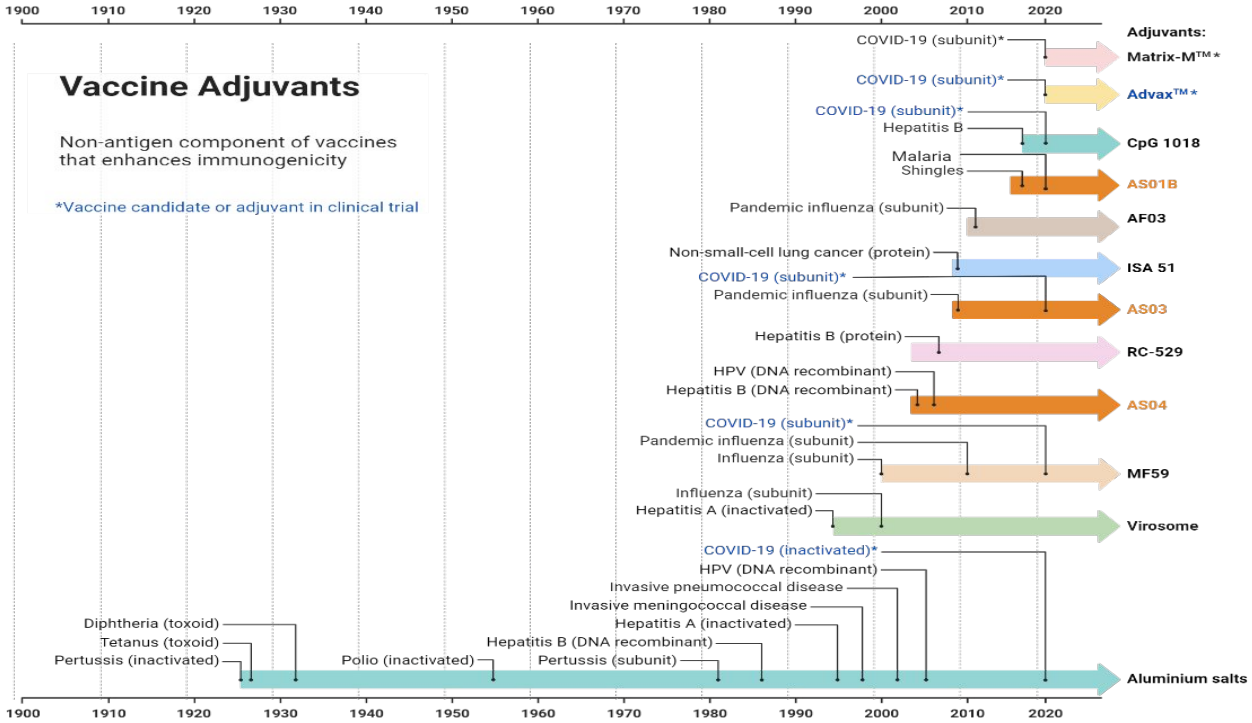
This work was sponsored by GlaxoSmithKline Biologicals SA

Vaccine Adjuvants Enhance immune responses to co-administered antigens.



Adjuvants have been used in vaccines for ~100 years

Newer approaches have finally been included in licensed vaccines in the last two decades



Rational Approach to Adjuvant R&D

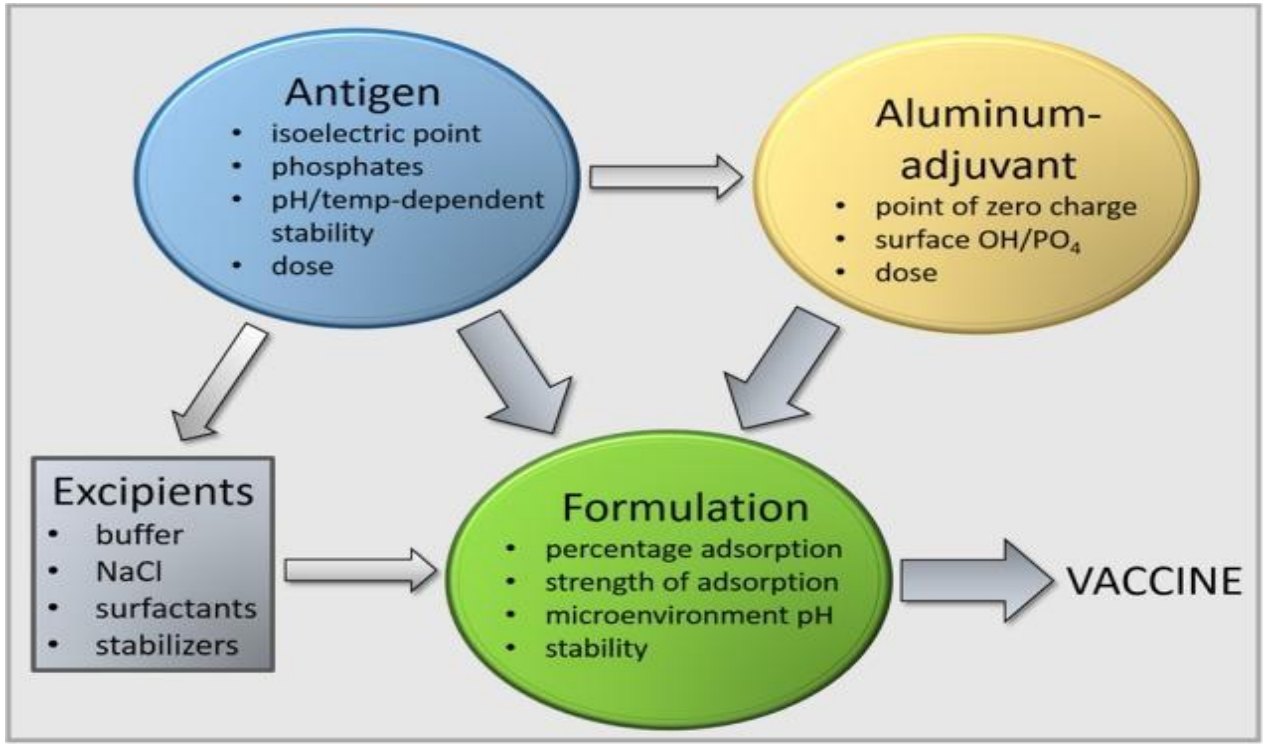
- Dont use them unless you need them – optimize antigen design
- Thoroughly evaluate Aluminum if you need an adjuvant
  (Optimizing the utilization of aluminum adjuvants in vaccines: you might just get what you want. Hogen Esch H, O'Hagan DT, Fox CB. NPJ Vaccines. 2018 Oct 10;3:51.)
- Evaluate if o/w emulsions are a better option, likely more potent
- Use more novel approaches only if necessary and fully justified
  - Need MoA, more data, longer timelines, more Regulatory scrutiny etc
  - Alum+ (TLR) approaches are a good option
- Injectable adjuvant formulations will not be effective for alternative routes

GSK

Brito LA, Malyala P, O'Hagan DT. Sem. Immunol. 2013, 25, 130.

Optimizing the use of Aluminium Adjuvants in Vaccines

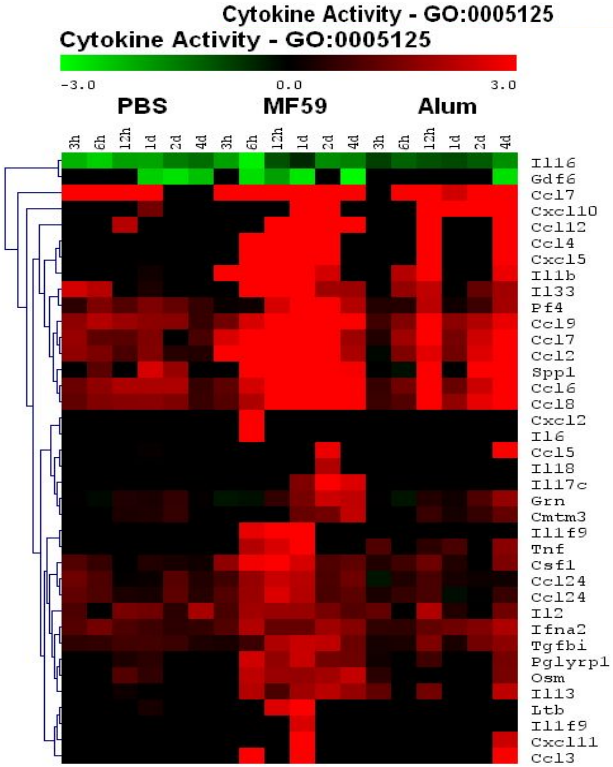
The importance of formulation science in the optimal use of Aluminium adjuvants

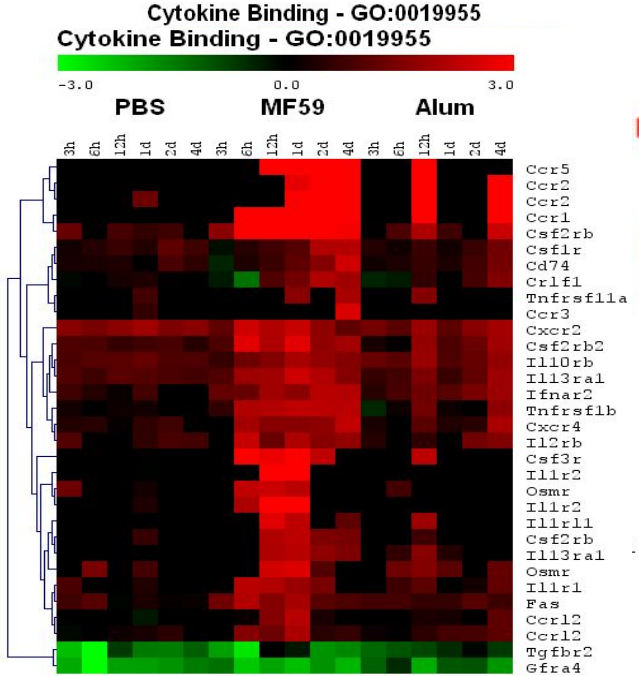


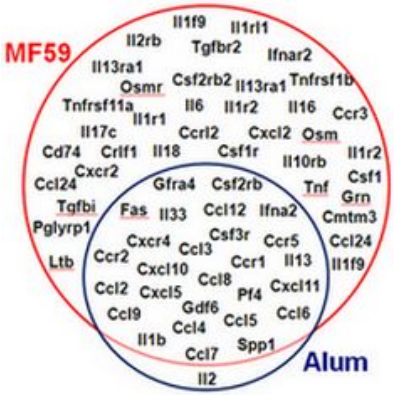
Hogen Esch H., et al., NPJ Vaccines. 2018 Oct 10;3:51.

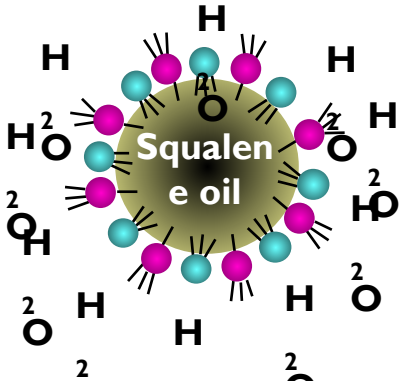
MF59 Acts Locally to Activate Innate Immunity

Defining the MoA of vaccine adjuvants is key to their successful development





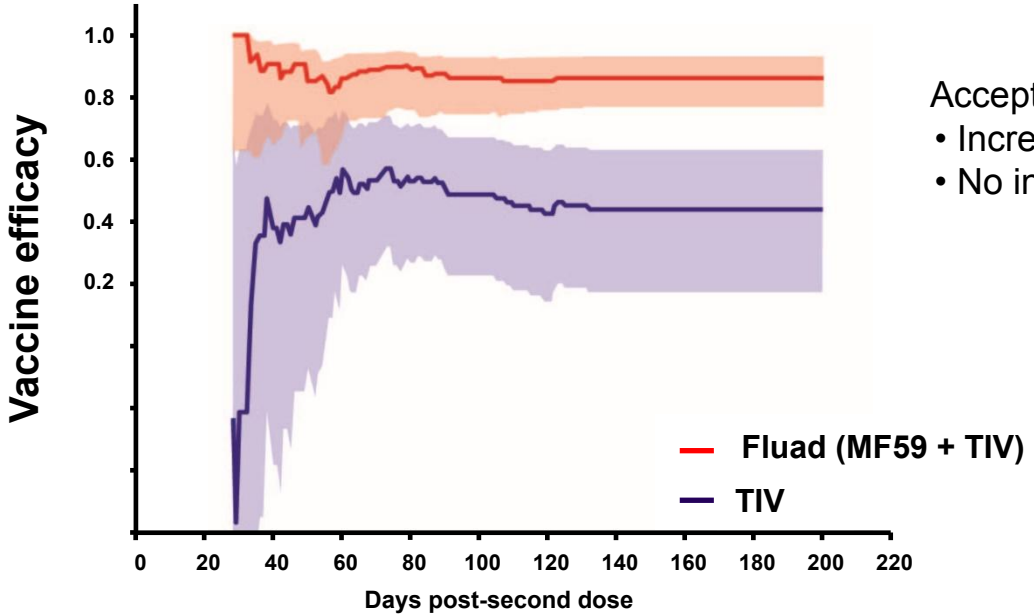




GSK

Mosca, F. et al PNAS, 105, 10501, 2008.

MF59 Emulsion Adjuvant Enhanced Flu Vaccine Efficacy in Children from 6 Months to <72 Months of Age (Fluad)

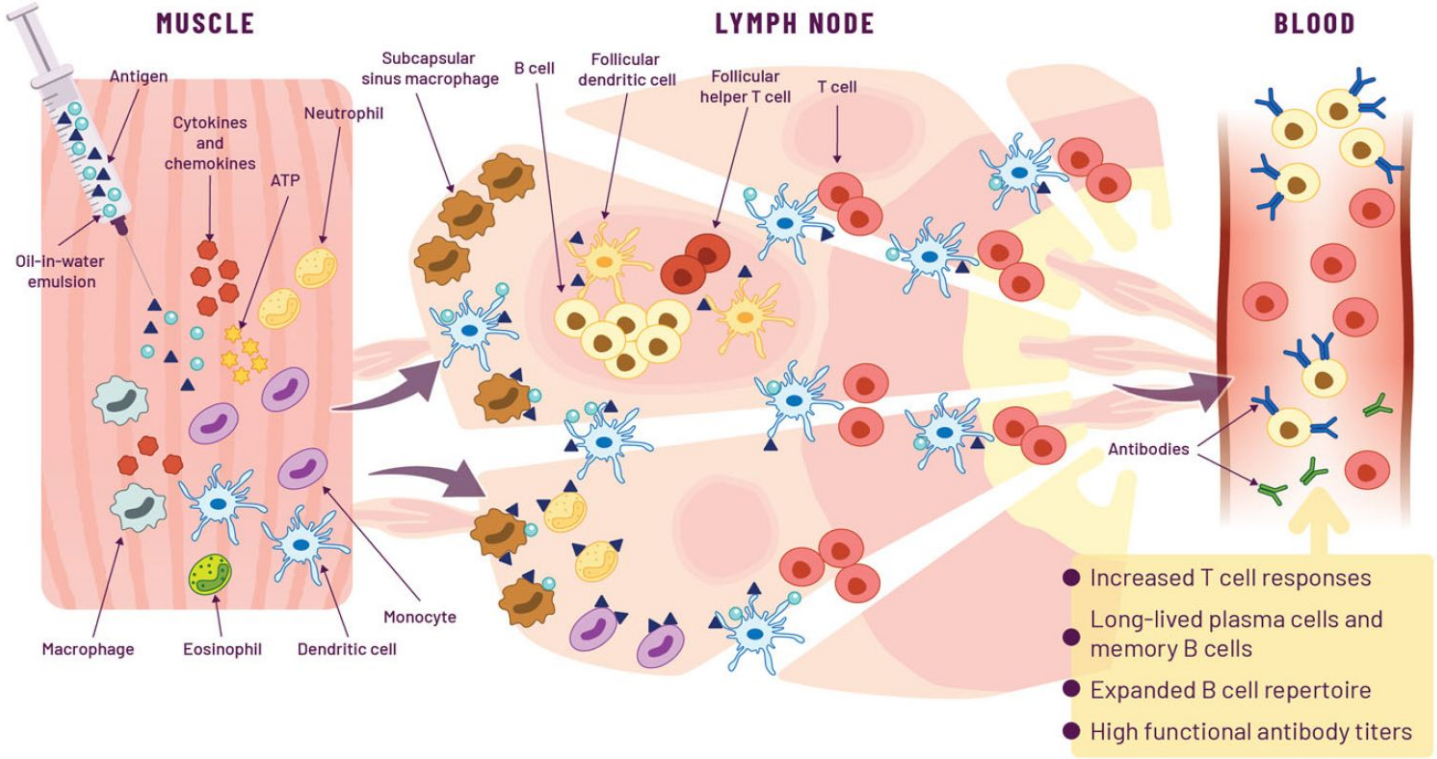


Acceptable safety profile:
- Increased local reactogenicity
- No increase in serious adverse effects

MF59 composition
0.5% Polysorbate 80
0.5% Sorbitan Triolate
4.3 % Squalene
Water for injection
10mM Na-citrate

Vesikari T, *et al.* NEJM. 365, 1406, 2011

O/W Emulsion adjuvants enhance B-cell repertoire and enhance functional antibody titers and T cell responses



O'Hagan, D. et al., NPJ Vaccines. 2021 Dec 21;6(1):158

Composition of alternative emulsion adjuvants

Table 1. Composition of emulsion adjuvants.

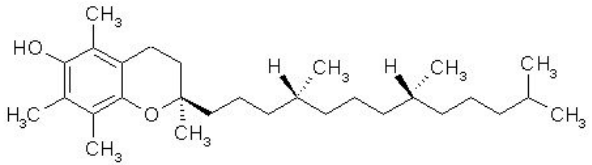
Emulsion adjuvant*	Clinical development stage	Composition in a dose administered to adult humans
MF59**	Licensed for seasonal and pandemic Flu vaccine	Squalene oil (9.75 mgs); Span85 (1.18 mgs) and Polysorbate 80 (1.18 mgs) as surfactants
AS03**	Licensed for pandemic Flu vaccine, Ph III for COVID-19	Squalene oil (10.75 mgs) and α-tocopherol (11.86 mgs); Polysorbate 80 (4.83 mgs) as surfactant
Squalene-in-water emulsion (SWE)	Preclinical, Ph I for COVID-19	Squalene oil (9.75 mgs); Span85 (1.18mgs) and Polysorbate 80 (1.18mgs) as surfactants
Stable emulsion (SE) and GLA-SE or SLA-SE***	SE in Ph II for pandemic Flu; GLA-SE/SLA-SE in Ph I - Ph III for diseases such as TB, Schistosomiasis, Leishmaniasis	Squalene oil (8.6 mgs); Poloxamer188 (0.125 mgs) and synthetic phosphatidylcholines (2.73 mgs)
AF03	Was licensed for pandemic Flu	Squalene oil (12.5 mgs); Span80 (1.85 mgs) and Eumulgin B1 (2.38 mgs); also contains mannitol
CoVaccine	Ph III for COVID-19	Squalane (40 mgs); Polysorbate 80 (10 mgs) and sucrose fatty acid sulfate esters (10 mgs)

GLA-SE glucopyranosyl lipid adjuvant–stable emulsion, *Ph* phase, *SE* stable emulsion, *SLA-SE* synthetic lipid A stable emulsion, *SWE* squalene-in-water emulsion, *TB* tuberculosis.
*All emulsion adjuvants are either similar or lower than AS03/MF59 in droplet size.
**Both AS03 and MF59 can be and have been investigated at lower doses in clinical trials in pediatric populations[63].
***SE composition in GLA-SE and SLA-SE is the same as stable emulsion.

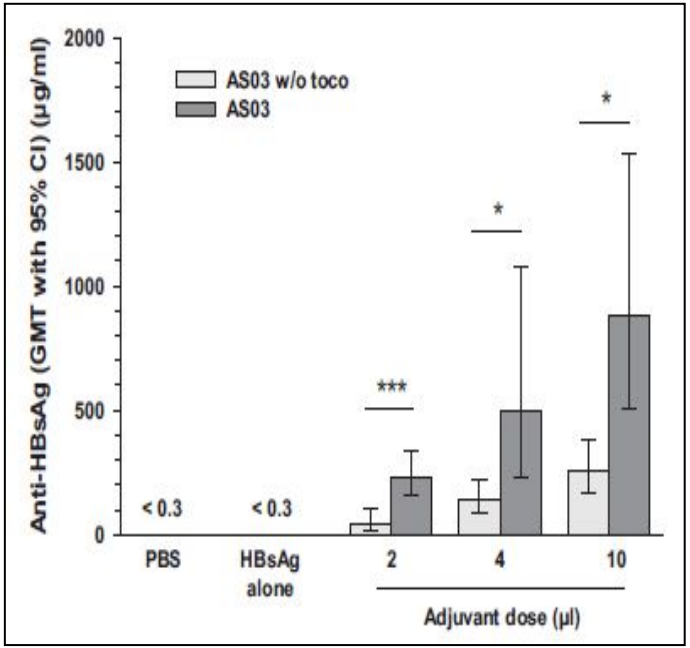
O'Hagan et al., NPJ Vaccines. 2021 Dec 21;6(1):158

Alpha tocopherol is an immune potentiator

- Immune enhancing component in AS03
- Vitamin E, anti oxidant, viscous oil
- Included in veterinary vaccines
- Enhanced antibody titers in mice in comparison to squalene alone emulsion (eg. MF59)
- Higher antibody response in humans for AS03 compared to MF59 for pandemic preparedness (H7N9 and H5N8)



Alpha tocopherol



Morel, S., et al. Vaccine *29 (2011) 2461*

AS03 vs MF59 – H2H clinical trial with influenza H7N9

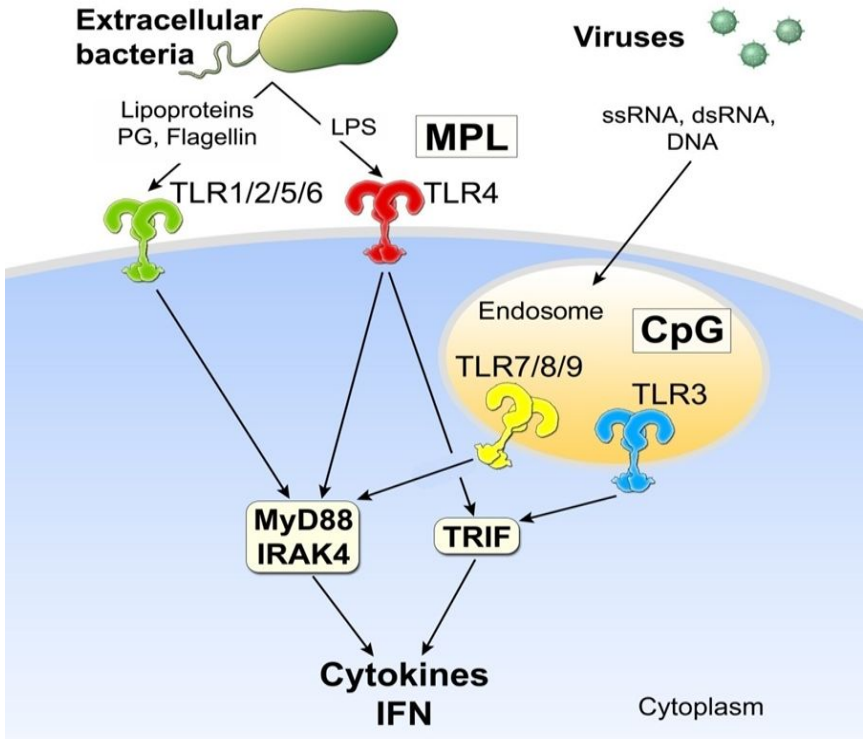
AS03 out-performed MF59 in a head-to-head trial with H7N9 PanBlok influenza vaccine

Seroprotection rates (SPRs) after two doses of adjuvanted vaccine (serum HAI titers)

PanBlok H7N9 + Adjuvant	3.5 µg + AS03	7.5 µg + AS03	15 µg + AS03	3.5 µg + MF59	7.5 µg + MF59	15 µg + MF59
SPR homologous strain A/GD/2016	94.6%	98.1%	100%	81.0%	82.5%	82.8%
SPR antigenically related strain A/HK/2017	85.7%	96.3%	98.2%	63.8%	61.4%	81.0%
SPR antigenically distant strain A/SH/2013	76.8%	79.6%	89.3%	25.9%	38.6%	44.8%

Oshansky et al., npj Vaccines 6, 41, 2021

Toll-like Receptors (TLR) Recognize Microbial Structures* – Many Established Adjuvants are TLR Agonists



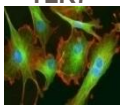
- TLR are Pathogen Recognition Receptors (PRR)
- TLRs on cell membranes recognize bacterial products
- TLRs in the endosome recognize nucleic acids
- TLRs trigger signal transduction pathways to activate innate immunity
- All TLRs are targets for vaccine adjuvants

*Nobel Prize for Medicine 2011 – Beutler, Hoffman and Steinman

The Discovery of Small Molecule Immune Potentiators (SMIPs) – TLR7 agonists

High-throughput Screening

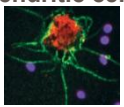
TLR7



Macrophages

B cells

Dendritic cells



Robotic Drug Screening:
Cell-Base Assays
Target-Based Assays

2 Pronged Approach

NOVARTIS VACCINES

NOVARTIS INSTITUTES FOR BIOMEDICAL RESEARCH

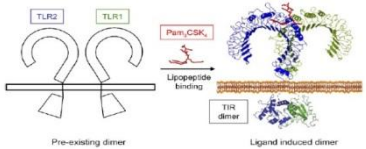
DEFENSE THREAT REDUCTION AGENCY · UNITED STATES OF AMERICA

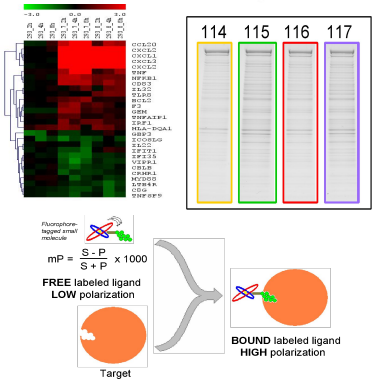
Genomics Institute of the Novartis Research Foundation

DoD: Funding/Biodefense

Chemically & Mechanistically Diverse SMIP Portfolio

Rational Design



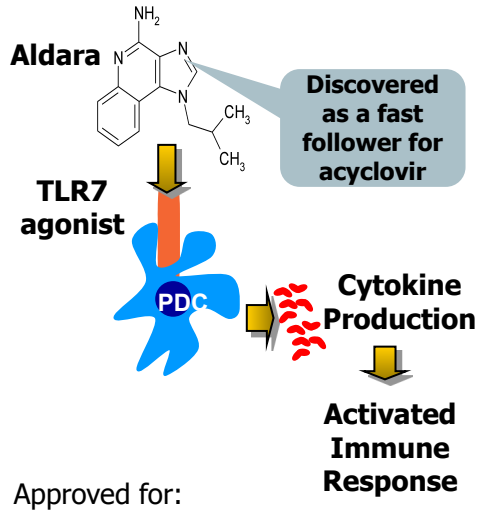


Literature Analysis
Genomic Screen
Target ID

Wu et al., Sci Transl Med. 2014 Nov 19;6(263)

Why pick TLR7 as the first target for Adjuvant Discovery ?

Aldara (Imiquimod) is a topical Immune Response Modifier (IRM)



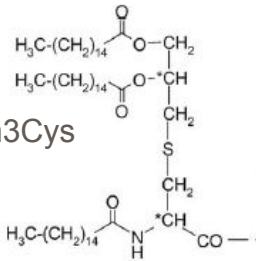
Approved for:

- Actinic Keratosis (AK)
- Basal Cell Carinoma (BCC)
- Genital Warts

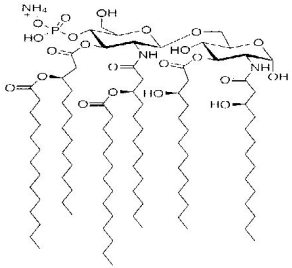
Potential Advantages of Small Molecule Immune Potentiators (SMIPs) as Adjuvants

- Simple synthetic molecules, minimize supply/production challenges
- Discrete chemical identity, lack of heterogeneity
- Pure agonists, structures easily manipulated
- Simplified analytics
- Many years of successful development as ‘drugs’ to build on

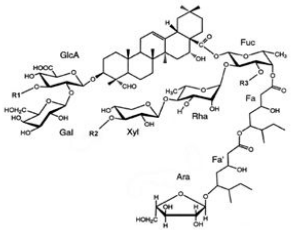
TLR9: CpG



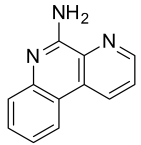
TLR2: Pam3Cys



TLR4: MPL



Saponins (QS21)



TLR7a SMIP

Which is the best way to Deliver SMIPs ?

For initial clinical progression we chose Alum:

- Well established and inexpensive
- Safe and generally well tolerated
- Well known by regulatory agencies
- Allows co-delivery of antigen and SMIP
- Can be used with existing vaccines
- Established platform to deliver TLR agonists (AS04)
- Potentially enables stable single dose vials

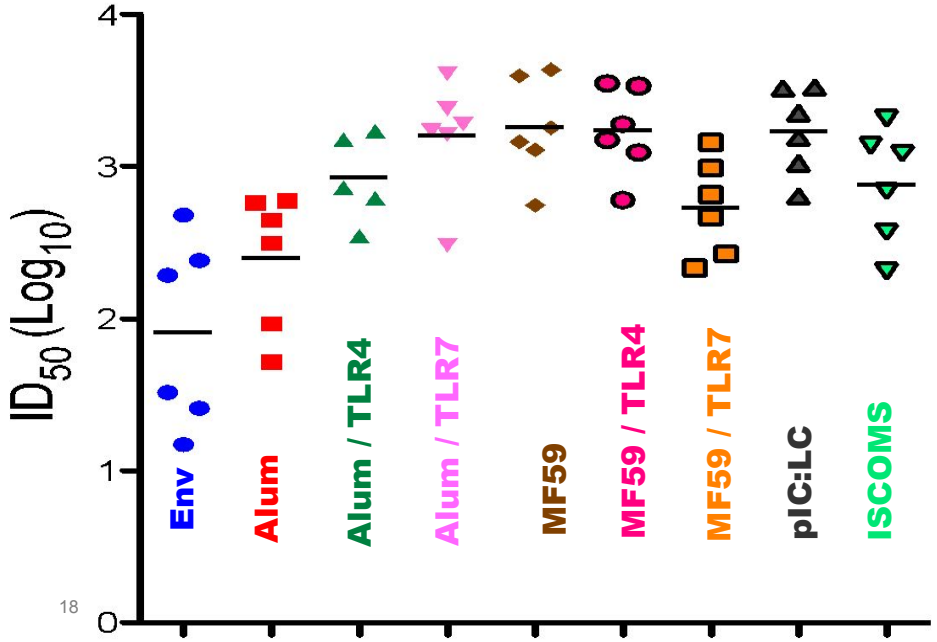
Next Generation of Alum based Adjuvants (Alum+)

Adjuvant	First described	Current clinical Phase (US or Eur)
AS04 (GSK) MPL – TLR4 agonist	2002	Licensed (HPV and HBV)
Alum/CpG (Coley) CpG – TLR9 agonist	1998	Licensed (COVID – Dynavax/Valneva)
AS37 (GSK) SMIP – TLR7 agonist	2014	Phase I/II (HBV)
Alum/Resiquimod (AAHI) TLR7/8 agonist	2016	Phase I (HIV and COVID)

GSK

Laera, D., et al Pharmaceutics, 15, 1884, 2023.

HIV Neutralization Titers for Alum/TLR7 in NHP versus Benchmark Adjuvants



Groups:
1. **ENV**
2. **ENV** + Alum
3. **ENV** + Alum + TLR4
4. **ENV** + Alum + TLR7
5. **ENV** + MF59
6. **ENV** + MF59/TLR4
7. **ENV** + MF59/TLR7
8. **ENV** + pIC:LC
9. **ENV** + ISCOM

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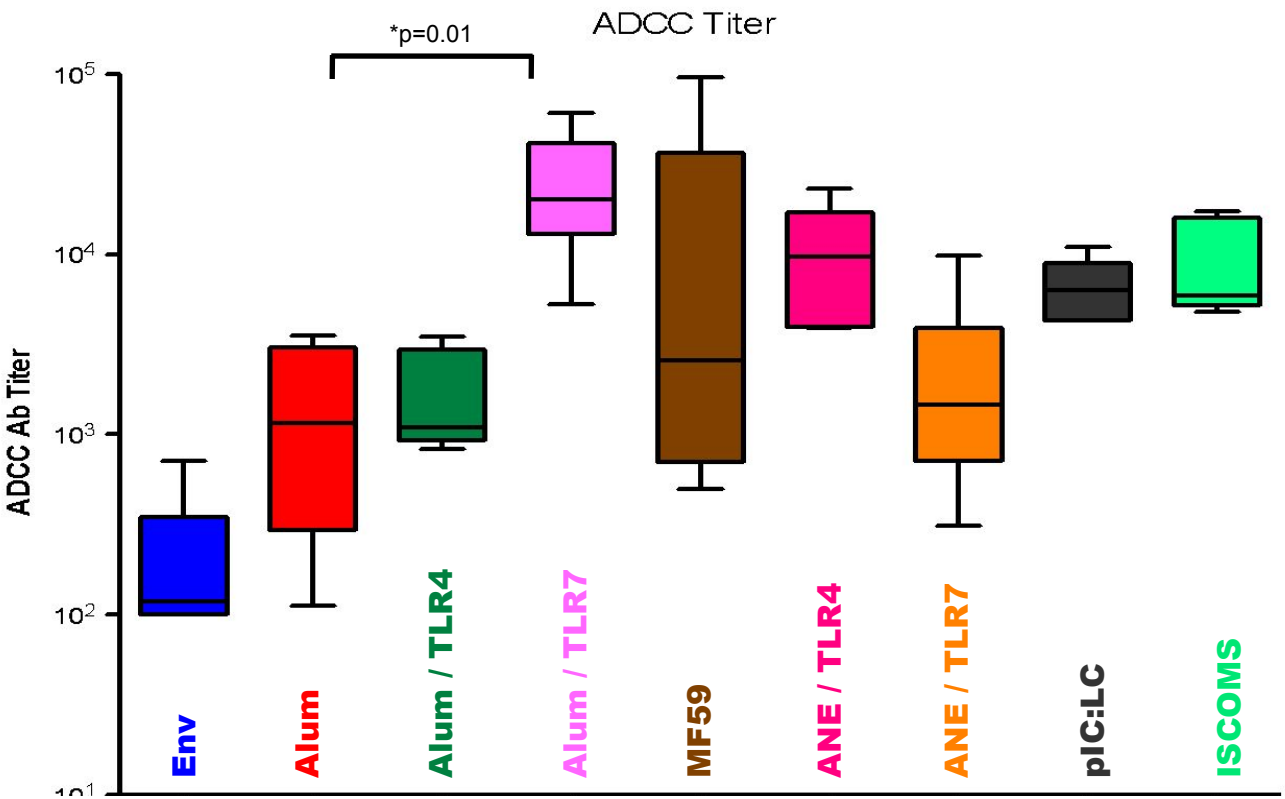
Francica, J., et al., Blood Adv. 2017 Nov 17;1(25):2329

Antibody Dependent Cellular Cytotoxicity (ADCC) titer for Alum/TLR7 vs benchmark adjuvants

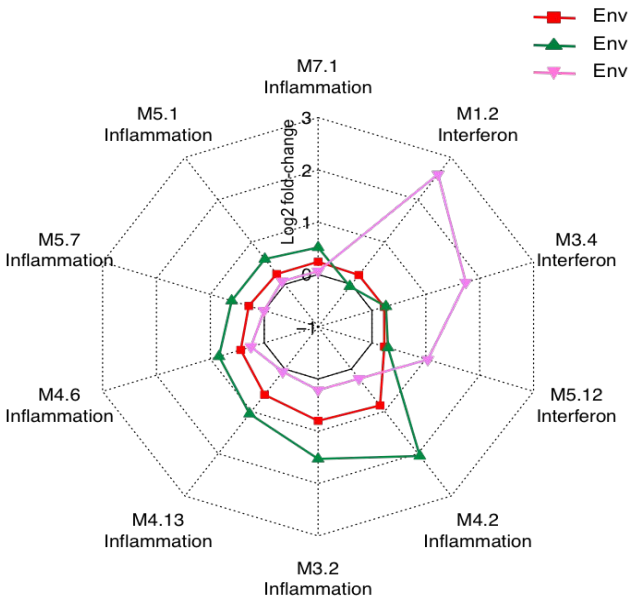


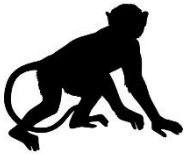
GSK

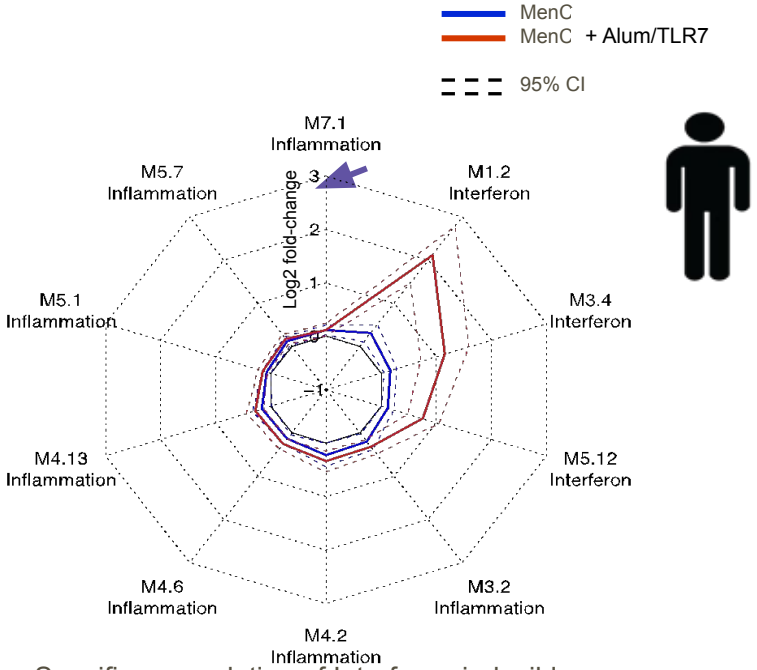
Guido Ferrari

Alum/TLR7 (AS37) induced a specific upregulation of interferon-inducible genes in NHP and man.

Transcriptome response in peripheral blood; 24 hours vs baseline.







TLR7: upregulated interferon inducible genes

TLR4: upregulated pro inflammatory genes

Human interferon response confirmed by cytokines (CXCL10)

Specific upregulation of Interferon-inducible genes (M1.2, M3,4, M5.12).

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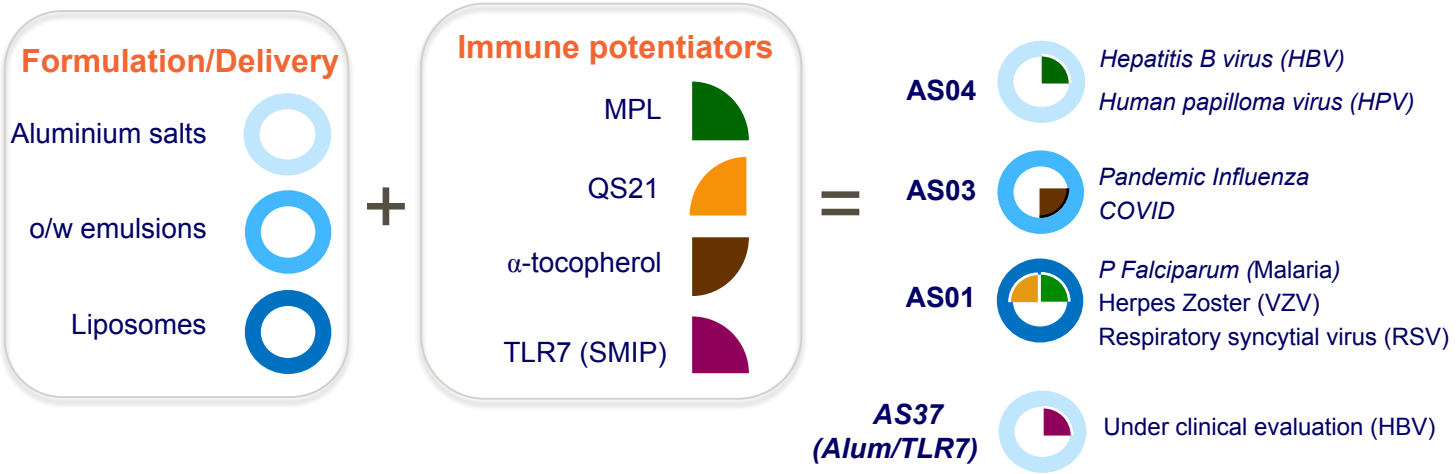
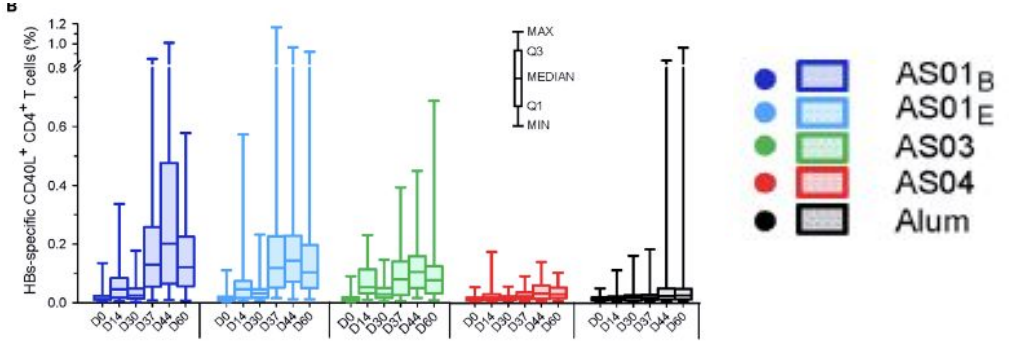
Siena, E. et al., Vaccine 2023, 41, 724.

GSK Adjuvant Systems (AS)



Adjuvant systems differ in their ability to induce humoral and cellular immune responses

HBs-specific CD40L$^+$ CD4$^+$ T cells



Anti HBS IgG

Reactogenicity

No vaccine related serious adverse events were reported in the study

Leroux-Roel & Marchant et al. Clin Immunol 169, 16, (2016)

AS01: vaccine adjuvant success story

AGE	50–59	60–69	70–79	≥80
	Data from ZOE-50		Pooled data from ZOE-50 and ZOE-70	
% EFFICACY (95% CI)	96.6% (89.6, 99.4)	97.4% (90.1, 99.7)	91.3% (86.0, 94.9)	91.4% (80.2, 97.0)

AREXVY
(RESPIRATORY SYNCYTIAL VIRUS VACCINE, ADJUVANTED)

High and consistent efficacy across spectrum of RSV symptomatic disease in >60YOA adults

Overall vaccine efficacy against RSV-LRTD (Primary endpoint)	Observed efficacy in those with certain comorbidities	Observed efficacy against severe disease
82.6%	94.6%	94.1%

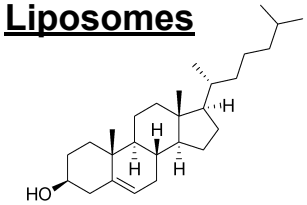
GSK

Adapted from SHINGRIX Product Monograph; Adapted from Papi A *et al, N Engl J Med 2023; 388:595-608*

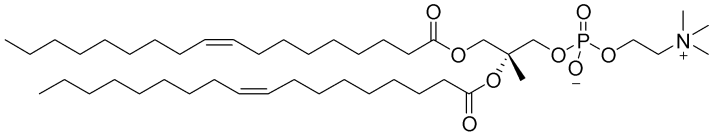
AS01 Composition

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

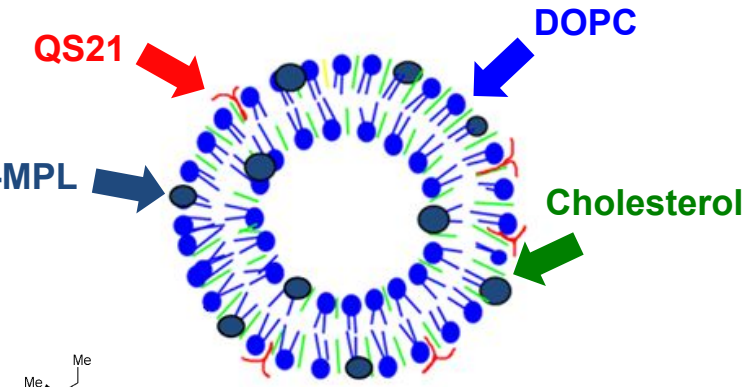
Liposomes



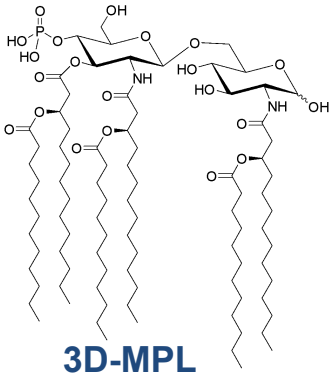
Cholesterol



DOPC

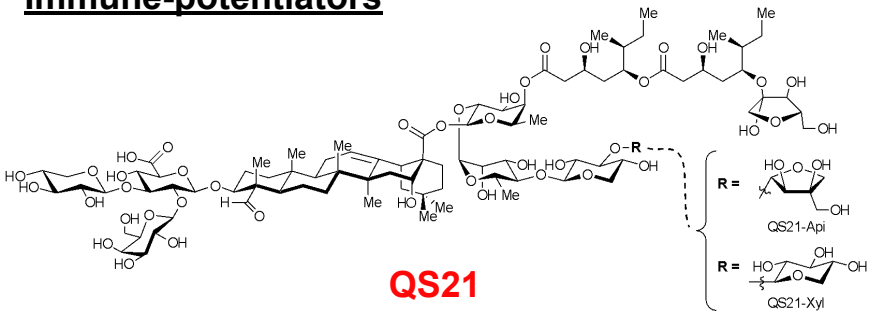


Size : +/- 100 nm



3D-MPL

Immune-potentiators

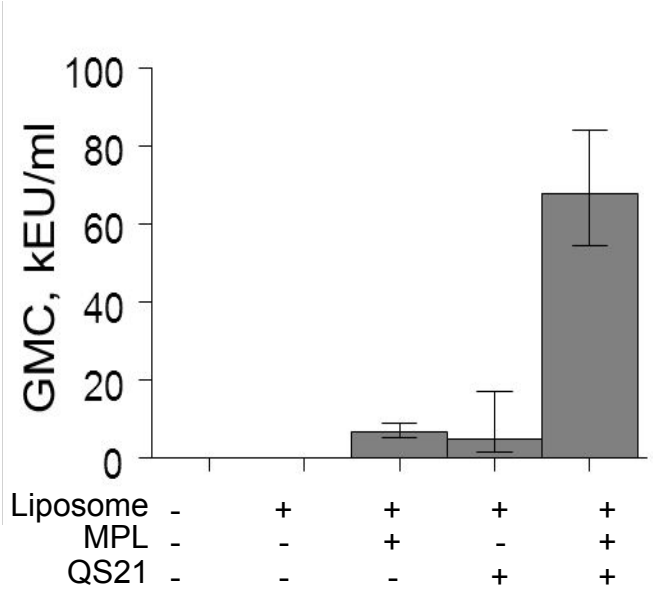


QS21

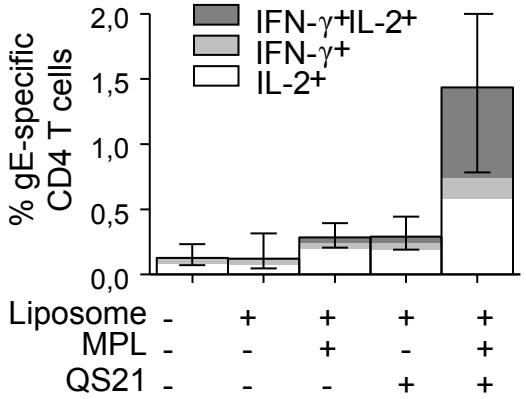
QS-21; licensed by GSK from Antigenics LLC, a wholly owned subsidiary of Agenus Inc., a Delaware USA corporation

Synergy of MPL and QS21 in liposomes is critical for adjuvant effect of AS01 (gE - Herpes Zoster)

Total IgG



CD4+ T cell response

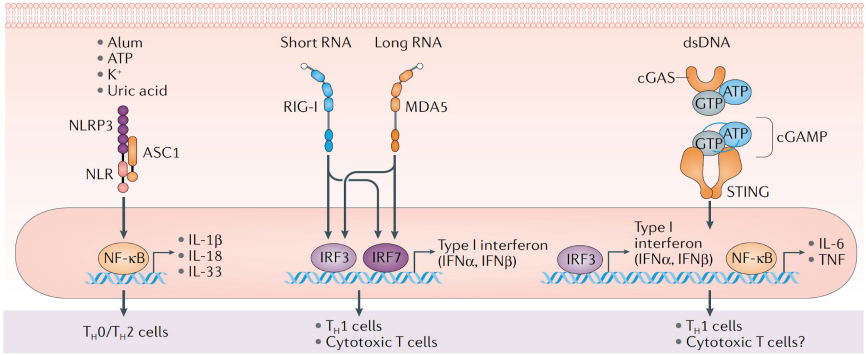


Dendouga, N. et al,

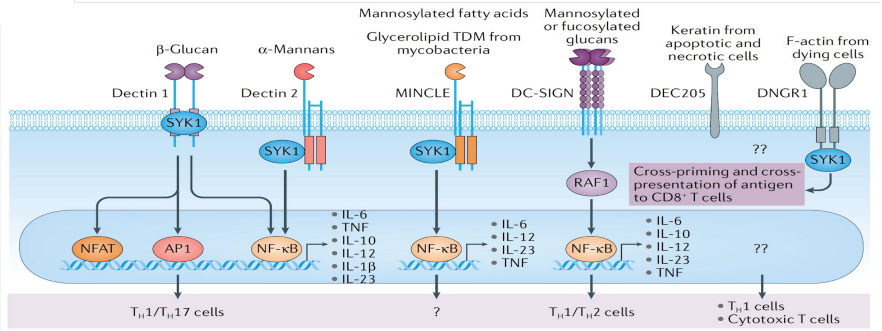
There are Many New Targets for Adjuvant Discovery

Beyond pattern recognition receptors (TLR, RIG-I, NLR, MINCLE etc)

- Cell death
- Tissue damage
- Metabolic adjuvants
- Treg modulators
- Epigenetic adjuvants



Cytosolic targets



CLR targets

Pulendran B, et al. Nat Rev Drug Discov. 2021 Jun;20(6):454.

STING is a key Pathogen Recognition Receptor (PRR)

- Natural ligands of STING receptor were shown to boost immune responses against infectious agents, mostly via IFN signaling with limited production of inflammatory cytokines
- GSK designed a new class of highly potent STING agonists: di-amidobenzimidazole (diABZI)

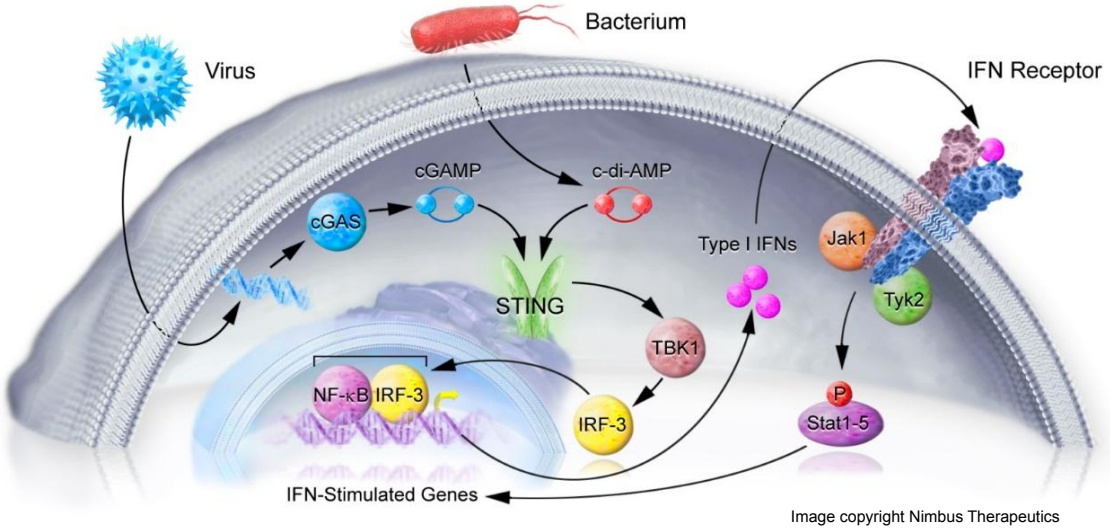
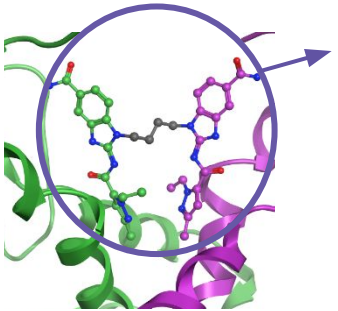
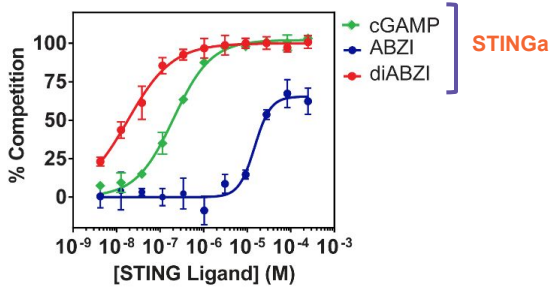


Image copyright Nimbus Therapeutics



diABZI filling STING receptor recognition groove



Ramanjulu J *et al*, Nature 2018 doi: 10.1038/s41586-018-0705-y

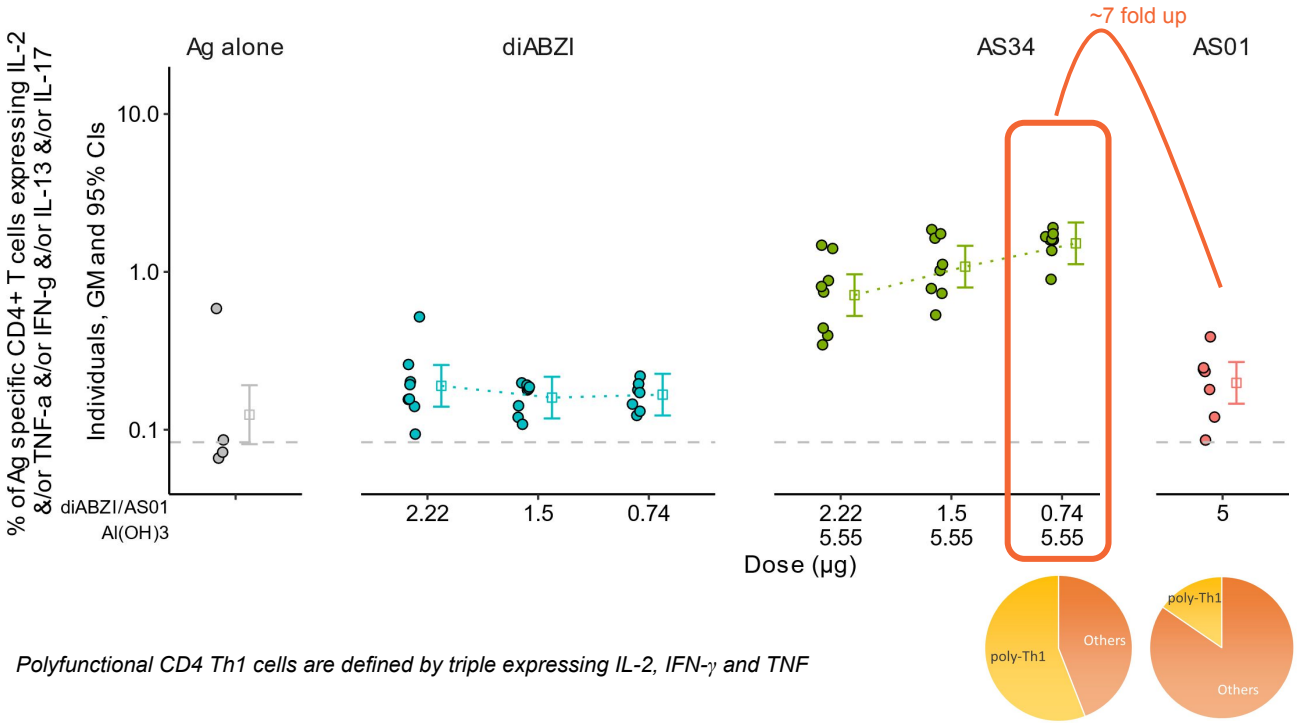
AS34 increases magnitude and polyfunctionality of Th1 cytokine -expressing CD4+ T cells in mice

2 IM inj.
14-d
apart

Ag/
diABZI
AS34
AS01

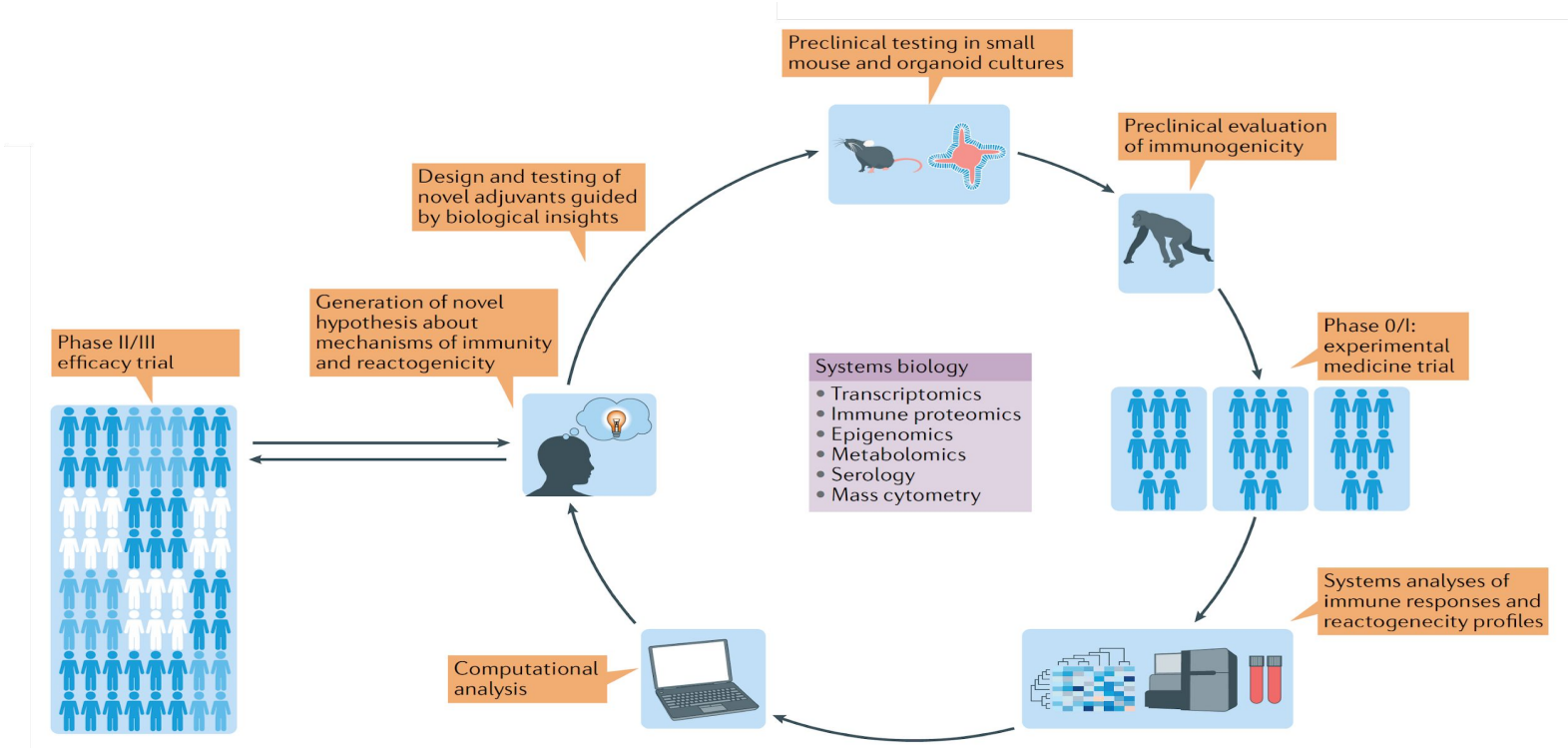
Spleen Th1 CD4+ T cells by ICS

Created with BioRender.com



Polyfunctional CD4 Th1 cells are defined by triple expressing IL-2, IFN-γ and TNF

Accelerating Adjuvant Development



Pulendran B, S Arunachalam P, O'Hagan DT. Nat Rev Drug Discov. 2021 Jun;20(6):454.

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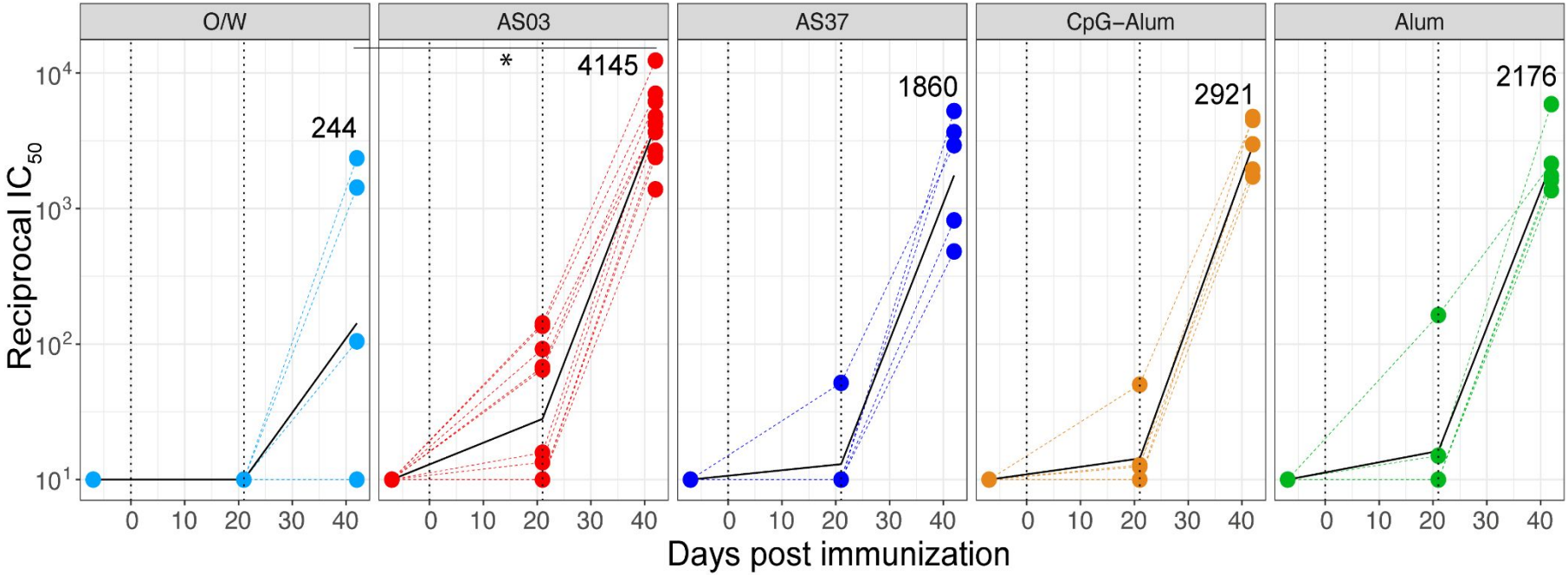
Adjuvants – what are the high level trends ?

- Additional adjuvanted vaccines will be approved in the coming years
- Responsible agencies will continue to ask detailed scientific questions, including the requirement for MoA studies
- New adjuvant molecules will be discovered, more of the same or different ?
- Ideally new generation adjuvants will be pure, synthetic, potent agonists, rather than heterogeneous ‘natural products’
- Formulation/delivery will continue to be key to success
- There is continued scope to seek synergy between different adjuvant cpds (AS01 etc)

Thank you for your attention

SARS-CoV-2 neutralization titers in NHP following immunization with a recombinant spike NP and alternative adjuvants

O/W = SWE (squalene emulsion)

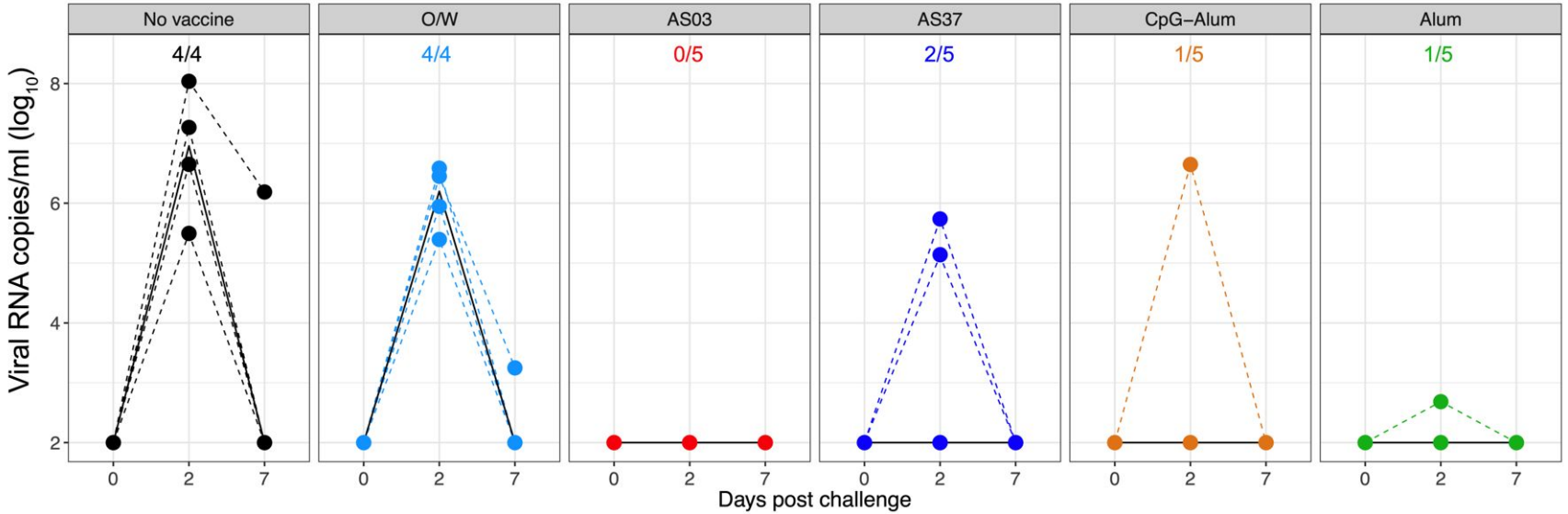


GSK

Arunachalam P. et al, Nature, 594, 253, Jun 10, 2021

Protection against challenge with SARS-CoV-2 after immunization with spike protein and alternative adjuvants

O/W = SWE (squalene emulsion)



Arunachalam P. et al, Nature, 594, 253, Jun 10, 2021