Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Adjuvants (for vaccines)

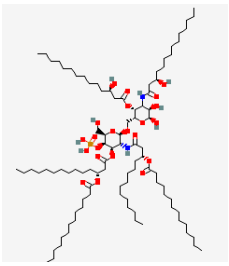
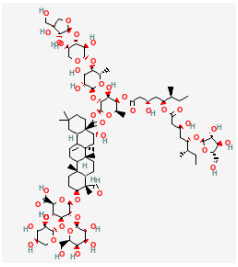
Evidence Summary

Adjuvants can train the immune system in a manner to enhance pathogen control and may impact the progression of chronic conditions associated with immune aging. Adjuvanted vaccines show good safety.

Neuroprotective Benefit: Adjuvanted vaccines have been associated with lower dementia risk. There is suggestive but incomplete evidence that the adjuvant itself plays a role in mediating this risk by modulating the immune system.

Aging and related health concerns: Adjuvants can induce trained innate immunity and enhance protection against pathogen-related illness even in the immunocompromised. Whether they can offer similar protection for age-related conditions is unclear**.**

Safety: Adjuvanted vaccines are well-tolerated and have a good safety profile across global populations. They are associated with increased reactogenicity, such as local injection site reactions, and transient post-injection fatigue, headache, muscle pain, and fever.

Availability: Rx	**Dose**: Adjuvanted vaccines are administered via intramuscular injection with a dosing schedule dependent upon the formulation of the particular vaccine. A dosing regimen for the use of adjuvants as an inducer for trained immunity has not yet been established.	**Monophosphoryl Lipid A (in AS01)**  **Source:** PubChem **QS-21 (in AS01)**  Source: PubChem
Half life: Varies	**BBB**: Not penetrant	
Clinical trials: AS01, AS03, AS04, and CpG 1018 - adjuvanted vaccines have undergone clinical testing in thousands of participants. These vaccines have been administered to millions of people worldwide.	**Observational studies**: Vaccination, particularly with adjuvanted vaccines, has been associated with lower dementia risk.	

What is it?

Adjuvants are used to boost immune responses to vaccines [1]. Historically, aluminum salts: aluminum hydroxide, aluminum phosphate, and potassium aluminum sulfate, commonly referred to as alum, have been used as vaccine adjuvants (FDA). Starting in the 1990s, additional adjuvants were approved for vaccines including the oil-in-water emulsion adjuvant MF59, the Adjuvant System adjuvants AS01, AS03, and AS04, as well as the cytosine phosphoguanosine (CpG)-based adjuvant, CpG 1018 [1]. In addition to boosting the efficacy and durability of immune responses to vaccines, several of these next generation adjuvants have been found to impact general innate immune responses in a manner consistent with the concept of trained immunity, suggesting that these adjuvants may be able to re-wire immune responses more globally [2]. That this immune rewiring may have a beneficial effect on health outcomes, such as mitigating inflammatory processes associated with chronic age-related diseases, comes from observational studies indicating an association between vaccination and risk reduction [2]. It is not yet clear whether these adjuvants alone, independent of their use in vaccines, can be used to meaningfully

impact immune aging and disease trajectories. Some early studies are starting to test these adjuvants as stand-alone therapies.

Next generation vaccine adjuvants used in approved vaccines:

MF-59 is an oil-in-water emulsion adjuvant that contains the oil squalene (4.3%) in citric acid buffer with stabilizing nonionic plant-derived surfactants polysorbate 80 (Tween 80) (0.5%) and sorbitan trioleate (Span 85) (0.5%) [3]. It was first included as an adjuvant in the Fluad® seasonal influenza vaccine for older adults (CSL Seqirus), and has subsequently been included in some pandemic influenza vaccines and other seasonal influenza vaccines. This adjuvant appears to have weaker trained immunity potential relative to the newer adjuvants and will not be a major focus of this report.

CpG 1018 is a cytosine phosphoguanosine oligodeoxynucleotide (CpG ODN) adjuvant comprised of the synthetic immunostimulatory sequence 5'-TGACTGTGAACGTTCGAGATGA-3', from Dynavax Technologies. It is used in the HEPLISAV-B® hepatitis B vaccine.

Adjuvant Systems (AS) adjuvants are a set of adjuvants developed by GlaxoSmithKline.
AS01 contains MPL (3-O-desacyl-4'-monophosphoryl lipid A), produced by GSK, which is a detoxified lipopolysaccharide obtained by hydrolysis of lipopolysaccharide (LPS) from *Salmonella Minnesota* and QS-21 (*Quillaja saponaria* Molina, fraction 21 licensed by GSK from Antigenics LLC, a wholly owned subsidiary of Agenus Inc) in a liposomal formulation [4]. It is used in two doses: 50 µg of MPL and 50 µg QS-21 referred to as AS01B, and half dose of 25 µg of MPL and 25 µg of QS-21, referred to as AS01E. The AS01B formulation is used in the Shingrix® shingles vaccine. The AS01E formulation is used in the Arexvy® respiratory syncytial virus (RSV) vaccine, and the Mosquirix™ malaria vaccine [4].

AS03 is an oil-in-water nano-emulsion adjuvant that contains the oil squalene, the plant-derived surfactant polysorbate 80, and vitamin E (alpha-tocopherol) [5]. High dose (AS03A) formulations contain 11.86 mg alpha-tocopherol, 10.69 mg squalene, and 4.86 mg polysorbate-80 per 0.5 ml dose. AS03B contains a half dose of each of these components [6]. It has been used in pandemic (H1N1 and H5N1) influenza vaccines.

AS04 contains MPL (3-O-desacyl-4'-monophosphoryl lipid A) absorbed onto aluminum salt [7]. It is used in the Fendrix™ hepatitis B vaccine approved by the European Medicines Agency (EMA) for use in individuals with renal insufficiency (EMA). The Ceravix® bivalent HPV (Types 16 and 18) vaccine was used

in the US between 2006 and 2016 (FDA). Its use was discontinued in the US due to the advent of multivalent HPV vaccines, but is still widely used globally.

Neuroprotective Benefit: Adjuvanted vaccines have been associated with lower dementia risk. There is suggestive but incomplete evidence that the adjuvant itself plays a role in mediating this risk by modulating the immune system.

Types of evidence:

- 2 propensity-score matched cohort studies of AS01-adjuvanted vaccines and dementia risk
- 1 post-hoc analysis of a clinical trial for adjuvanted therapy
- Several laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function:

Vaccination has been associated with decreased risk for dementia [8]. While these associations have been observed with vaccines targeting a variety of different pathogens, the robustness of the effect associated with the shingles (varicella zoster virus) vaccine has drawn special attention (see Shingles vaccine report) [9]. This has triggered questions about whether this association stems from the varicella zoster virus (VZV) posing outsized risk due to its neurotropism, or from unique features, such as adjuvants, in the formulation of these vaccines that influence the immune response, or a combination of both.

Evidence that contributions from both virus-specific and formulation factors may play a role comes from propensity-score matched cohort studies, using the federated TriNetX US collaborative electronic health records (EHR) network [10; 11]. There have been two approved shingles vaccines in the US, the live attenuated virus vaccine, Zostavax® (Merck), and the AS01-adjuvanted recombinant vaccine, Shingrix® (GSK). Use of Zostavax® was eventually phased out due to the superior performance of Shingrix® for preventing shingles and associated complications. This transition provided the framework for a natural history experiment that allows for a comparison between the efficacy of the Zostavax® and Shingrix® vaccines on a variety of outcomes. Both vaccines have been associated with a reduction in dementia risk. However, in a propensity-matched cohort of 207,674 individuals (n=103,837 per vaccine), vaccination with the Shingrix vaccine was associated with a lower risk of developing dementia over six

years, equivalent to a 17% increase in time lived without a dementia diagnosis [10]. It is unclear if this is attributable to the higher efficacy of Shingrix® in preventing shingles, or whether differences in the overall immune response elicited by the two vaccines are also involved.

The same group then assessed the potential role of vaccination including the AS01 adjuvant system, irrespective of the specific virus targeted, on dementia risk using 436,788 individuals from the same EHR network, with an 18-month follow-up [11]. This analysis included 35,938 individuals who received only the AS01-adjuvanted respiratory syncytial virus (RSV) vaccine, 103,798 individuals who received only the AS01-adjuvanted Shingrix® shingles vaccine, 78,658 who received both RSV and shingles vaccines, and 218,394 matched individuals who received only the flu vaccine. This study found that the risk of a dementia diagnosis was lower in individuals receiving the AS01 adjuvanted vaccines (shingles or RSV) relative to those receiving the flu vaccine, with a ratio of restricted mean time lost (RMTL) of 0.82 (95% CI 0.74 to 0.91) for the shingles vaccine, ratio of RTML of 0.71 (95% CI 0.61 to 0.83) for the RSV vaccine, and ratio of RTML of 0.63 (95% CI 0.55 to 0.72) for both vaccines, respectively [11]. While this suggests that the inclusion of the AS01 adjuvant system itself may contribute to dementia risk reduction, there are several caveats to this study [12]. Differences in viral control, both in terms of the virus itself and the efficacy of the vaccine, could still account for (some of) this difference. Like VZV, RSV also has the capacity to infect neurons, whereas most common flu strains do not. The efficacy of the VZV and RSV vaccines is typically higher than annual flu vaccines. With respect to RSV, participants receiving either AS01 adjuvanted (Arexvy®) or non-adjuvanted (Abrysvo®) vaccines were included, which complicates the interpretation [12]. Caveats aside, this study provides further support for the notion that vaccines with strong pathogen control can meaningfully mitigate dementia risk. The capacity of the AS01 adjuvant system to enhance to vaccine efficacy may be an important factor in mediating these associations with dementia risk.

Human research to suggest benefits to patients with dementia:

In addition to observational studies indicating that vaccination is associated with lower dementia risk, there is recent evidence to suggest that at least some vaccines, such as the shingles vaccine, may also impact disease course in individuals with dementia [13]. Whether immune modulation from the adjuvant plays a mechanistic role has not yet been established. There are ongoing clinical trials testing vaccine adjuvants in Alzheimer's disease (AD) patients, which may provide additional insight.

AD04 is a water-based aluminum hydroxide gel (i.e. aluminum salt) adjuvant in clinical development by ADvantage Therapeutics. Although this specific formulation is not used in any currently approved vaccines, aluminum hydroxide is the most widely used vaccine adjuvant worldwide. AD04 has been tested in vaccine clinical trials, most notably in the AFF006 trial (NCT01117818) testing AFFITOPE® AD02, an Aβ vaccine, in patients with early AD [14]. AD02 was tested in high (75 µg) and low (25 µg) doses. The 25-µg dose was tested in combination with low (1 mg) and high (2 mg) doses of the AD04 adjuvant. The AD04 adjuvant alone at the 1 mg and 2 mg doses was used as the control comparator. While the AD02 vaccine did not show efficacy relative to the adjuvant control, a beneficial effect was unexpectedly observed by groups receiving the high (2 mg) dose of the AD04 adjuvant relative to those receiving the lower dose, on post-hoc analysis [14]. The benefits were most apparent in those at mild stages (Mini-Mental State Exam [MMSE]≥23). Forty-eight percent of mild and moderate AD patients receiving 2 mg AD04 did not decline on the composite outcome of the Alzheimer's Disease Assessment Scale Cognitive (aADAS-Cog) and the Alzheimer's Disease Cooperative Study Activities of Daily Living (aADCS-ADL) over the course of 18 months, while only 17% to 31% of patients in the other groups did not decline on these measures [14]. While a lack of correction for multiplicity can produce spurious findings in post-hoc analyses, the superiority of 2 mg AD04 over other groups on primary and secondary efficacy outcomes was validated using a more statistically rigorous permutation test [15]. These findings led ADvantage Therapeutics to initiate a clinical trial testing the adjuvant AD04 in AD patients.

AD04 is currently being tested in a multicenter, randomized, double-blind, placebo-controlled, Phase 2b trial in patients with early AD (NCT07107074). Participants are randomized to receive six monthly subcutaneous injections of AD04 or placebo over a five-month treatment period. The primary outcome is a composite score assessing cognition (aADAS-cog), daily function (aADCS-ADL), and a global dementia severity score (CDR-sum of boxes) over six months measured in overall time saved with treatment. The trial has an expected completion date in 2027.

CpG 1018 is a cytosine phosphoguanosine oligodeoxynucleotide (CpG ODN)-based adjuvant containing a synthetic sequence mimicking unmethylated bacterial DNA designed to activate Toll-like receptor 9 (TLR9) and downstream anti-pathogen signaling [16]. It is used as an adjuvant for the approved HEPLISAV-B® hepatitis B vaccine from Dynavax Technologies.

CpG 1018 is currently being tested in a single-center, double-blind, placebo-controlled Phase 1 trial in patients with mild cognitive impairment (MCI) due to AD or mild AD (NCT05606341). The trial is testing three dose levels of CpG 1018 (0.1 mg/kg, 0.25 mg/kg, 0.5 mg/kg administered via subcutaneous injection) for eight weeks, in a staged manner, with a primary outcome of safety and tolerability.

Secondary outcomes include changes to cognition and fluid AD biomarkers. The trial has an expected completion date in late 2026.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

Trained immunity: The primary mechanism through which vaccine adjuvants are thought to impact dementia risk and disease course is through the modulation of the immune system. Immunosenescence refers to an inability to mount the type of robust immune responses needed to effectively clear pathogens and pathological proteins, which then leads to a chronic state of non-productive low-grade inflammation [17]. Immunosenescence can be considered a type of maladaptive trained immunity, such that the immune system gets locked into this non-productive state. It is a common feature of aging and associated with a variety of age-related conditions, including neurodegenerative disease, like AD [17]. In the context of a healthy immune system, signals released from pathogens trigger a stress response that activates the innate immune system and primes it to respond more efficiently to a subsequent pathogen/insult. This is referred to as adaptive trained immunity. It has been demonstrated that certain vaccine components, including some live attenuated vaccines and vaccine adjuvants which resemble pathogen-derived molecules, can induce a form of adaptive trained immunity. This shift in immune system responses may not only protect against pathogens, but also modulate disease course for a variety of other conditions driven by maladaptive chronic low-grade inflammation.
A key question is whether these vaccine components can reset the immune system from a state of maladaptive trained immunity to a state of adaptative trained immunity. There is evidence from preclinical studies suggesting that vaccine adjuvants (and other inducers of adaptive trained immunity) can modify immune responses in a manner that alters neurodegenerative disease trajectories in a protective manner. However, similar evidence from human patients still needs to be established.

<u>Preclinical studies:</u>

AD04: A preclinical study assessed the impact of AD04, administered via subcutaneous injection, on the brains of aged (24 months old) wild-type (C57bl/6) mice [18]. The treated mice were found to exhibit improved performance on a fear conditioning test. This was coupled with a modulation of the phenotype in the brains of the mice, toward a more phagocytic type associated with enhanced debris clearance.

CpG: The immune modulating effects of TLR9 agonism through CpG ODNs has been associated with reductions in AD-related pathology and improved cognition in a variety of animal models [19]. One study tested the impact of CpG ODNs in aged (17–19 years of age) female squirrel monkeys (*Saimiri boliviensis boliviensis*) as a model of sporadic AD, since these animals develop amyloid pathology with age, though it should be noted that deposition typically occurs in the vasculature rather than in the brain parenchyma [19]. This study used the CpG ODN 2006 (also known as CpG ODN 7909) which is in the same class (type B) as CpG 1018, injected subcutaneously at a dose of 2 mg/kg CpG ODN monthly for 24 months. Treated animals exhibited better performance on several cognitive tests, and fibrillar amyloid species were reduced in particular brain regions. Plasma cytokine profiles were consistent with TLR9 activation, including the induction of IL12p40, IFN-γ, and TNFα. The kinetics of the cytokine responses showed considerable inter-subject variability, which is consistent with the variability observed in human studies with innate immune training agents. The repeated injections appear to have been necessary for sustaining the response, as levels of these cytokines waned over time between treatments. Notably, the administration of CpG ODNs did not induce what might be characterized as a pathological neuroinflammatory profile in the brain, based on the lack of effect on markers for microgliosis, astrogliosis, and T cell activation, likely stemming from the lack of BBB penetration.

APOE4 interactions: Vaccination has been associated with reductions in dementia risk, irrespective of ApoE4 status [8]. ApoE4 has a complex relationship with infection risk and outcomes overall, with carriers showing increased protection for some pathogens, but increased susceptibility to adverse outcomes with others [20]. Due to the association of ApoE4 with a maladaptive neuroinflammatory profile [21], there is mechanistic rationale for a strong benefit for ApoE4 carriers.

Aging and related health concerns: Adjuvants can induce trained innate immunity and enhance protection against pathogen-related illness even in the immunocompromised. Whether they can offer similar protection for age-related conditions is unclear.

Types of evidence:

- 2 reviews of TLR-based adjuvants
- 1 review of AS01 adjuvants
- 1 review of AS03 adjuvants
- 2 reviews of CpG 1018-adjuvanted HEPLISAV-B®
- 8 cross vaccine/adjuvant comparison studies

- 2 reviews of CpG ODNs for cancer immunotherapy
- Numerous laboratory studies for trained immunity mechanisms

Infectious disease: BENEFIT

Historically, adjuvants have been used to boost immune responses to inactivated/recombinant vaccines in immunocompromised populations, such as the elderly [2]. The most widely used are aluminum-based salts, such as aluminum hydroxide and aluminum phosphate, often broadly referred to as 'alum' [21]. The addition of the aluminum salt leads to the formation of an antigen depot at the injection site, allowing for greater interaction between the vaccine antigen and antigen presenting cells, resulting in a more efficient immune response. One issue with alum is that it results in a Th2-like immune response, which helps induce a long-lasting antibody (humoral) response, but does not reliably induce a strong Th1-like response, which is needed for cytotoxic T cell-mediated immunity [22]. While the Th2 immune response can effectively combat extracellular pathogens, the effective clearance of intracellular pathogens also requires a strong Th1 response.

Next generation adjuvants have been developed which in addition to boosting the efficiency of immune responses, also induce Th1 responses [2]. This has allowed for the development of effective vaccines to pathogens for which the induction of robust immune responses was not possible using alum adjuvants alone. In many cases, vaccines with these newer adjuvant systems exist alongside non-adjuvanted (or alum adjuvanted) counterparts, allowing for a comparison. In general, these new adjuvants are associated with more durable immune responses and greater efficacy in immunocompromised individuals. A key feature of these new adjuvants is that they tend to induce a broader antibody response, such that they may offer at least partial protection against closely related strains/types to the specific virus/pathogen targeted by the vaccine. Another important feature of these next generation adjuvants is the capacity to induce trained innate immunity [2]. This appears to be a critical mechanism through which these adjuvants can promote such robust, and in some cases, long-lasting, immune responses to the antigens contained in the vaccine.

Mechanism of trained immunity

Trained innate immunity refers to the functional modification of innate immune cells, which involves epigenetic and metabolic reprogramming [2]. Due to the changes in the accessibility of the chromatin (i.e. epigenetic marks), the expression of immune response-related genes, such as cytokines, are altered in a manner that typically results in a stronger early anti-pathogen response, followed by a stronger resolving response to prevent the type of excessive inflammation which could cause damage to the self

[23; 24]. Metabolically, this is characterized by an increase in glycolysis, particularly aerobic glycolysis, as well as changes in lipid metabolism.

These changes are typically triggered by a stimulus that induces a strong type 1 interferon (IFN) response [24]. This pro-inflammatory immune response involving the release of interferons and other cytokines, is most robustly induced in response to pathogens and their pathogen-associated molecular patterns (PAMPs). It can also be induced in response to cellular stress because Damage-Associated Molecular Patterns (DAMPs), produced by stressed cells, can engage the same cell receptors as PAMPs. These receptors are referred to as Patterned Recognition Receptors (PRRs). Notable classes of PRRs include Toll-Like Receptors (TLRs), C-Type Lectin Receptors (CLRs), NOD-Like Receptors (NLRs), RIG-I-Like Receptors (RLRs), and AIM2-Like Receptors (ALRs) [24]. The different classes recognize different types of PAMPs/DAMPs.

While this type of anti-pathogen type 1 interferon response is a common downstream response to any type of infection or vaccination, the nature/robustness of the response is highly variable across the population [25]. Some individuals readily develop robust, lasting immunity, while others fail to induce a productive immune response. Immune profiling of participants in vaccine clinical trials has allowed investigators to assess the differences in the baseline immune characteristics of high responders relative to average or low responders [25; 26]. The high responders show evidence of a trained innate immune profile, such that they are poised to induce a robust response to stimulation. There is evidence that some types of live-virus vaccines, such as the tuberculosis vaccine, BCG, can also induce a state of trained innate immunity, which can promote resistance against a variety of pathogens, at least temporarily (see Bacillus Calmette-Guerin Vaccine report) [23]. Recently, it has been recognized that some of the next-generation vaccine adjuvants can also induce features of trained innate immunity, which in turn, enhances the adaptive immunity toward the pathogen targeted by the vaccine [2]. In multi-dose vaccines, the first dose primes the immune system by activating the innate immune system, essentially transforming it into a 'high responder' state, such that the second dose elicits a very robust response [25]. In line with the current molecular understanding of trained innate immunity, the most potent adjuvants tend to be the ones that elicit the strongest early interferon response [27].

Numerous different vaccine adjuvants have been developed that are designed to activate different PRRs [2]. Many of these contain components that activate TLRs, which can be located extracellularly or intracellularly [28]. Upon activation, the TLRs dimerize and recruit adaptor proteins that promote downstream signaling cascades [29]. Most TLRs recruit the adaptor MyD88, leading to the activation of the transcription factors NF-kB and AP-1, and release of pro-inflammatory mediators, such as cytokines [29]. Some of TLRs can instead recruit the adaptor protein TRIF, leading to the activation of a type 1

interferon response [29]. Notably, TLR4 can activate both pathways, by recruiting MyD88 at the plasma membrane, and recruiting TRIF when localized to the endosome [29]. Different types of PAMPs (and DAMPs) will preferentially activate different types of TLRs, thus from an adjuvant development perspective, the components can be tailored to optimize immune responses for different pathogens (vaccine antigens).

Here, I will focus on adjuvants in approved vaccines, including information about their clinical performance in terms of inducing robust adaptive immune responses, as well as evidence that these adjuvants have the capacity to induce trained innate immunity. The evidence that these adjuvants can induce changes in innate immune cells that make them more effective at producing strong adaptive immune responses to the vaccine-associated antigens, is quite strong. However, the evidence indicating that receiving one of these adjuvanted vaccines confers protection toward a variety of other infectious and chronic conditions due to the induction of trained innate immunity is very limited. The best evidence to date comes from the AS01-adjuvanted shingles vaccine, however, those associations are tenuous because they cannot be clearly tied to the presence of the AS01 adjuvant.

While different adjuvants may be more amenable for use with different antigens for complex reasons, such as the type of immune response needed for a particular pathogen, the evidence to date suggests that AS01 is the most potent inducer of trained innate immunity, of the currently available adjuvants [27], such that vaccines containing this adjuvant may confer additional benefits to the immune system. The degree of benefit, as related to the boost to immune responses, is likely to be a function of baseline immune status, such that those already in a 'high responder' state are unlikely to see further gains, while those with low/impaired immune function may experience the most benefit. These adjuvants clearly enhance antigen-specific adaptive immunity. The durability is likely related to the particular antigen. Whether or not the type of antigen impacts the strength and durability of the innate immune responses has not been established. An important outstanding question is the durability of the non-(antigen)specific innate immune training response induced by these adjuvants, and the impact of repeated booster doses.

TAKE HOME MESSAGE: Several of the vaccine adjuvants that induce robust immune responses, especially the AS01 and AS03 adjuvants, also show the capacity to induce trained innate immunity. However, we are currently lacking data regarding whether and how these adjuvants can be used to attenuate processes of immune aging and associated age-related chronic diseases.

AS01

Approved AS01-adjuvanted vaccines: Shingrix® (GlaxoSmithKline); Arexvy® (GlaxoSmithKline); Mosquirix™ (GlaxoSmithKline)

Adjuvant System AS01 is the most potent of the Adjuvant System adjuvants from GSK in potentiating innate and adaptive immune responses [4]. It is currently available in full dose and half dose formulations, referred to as AS01B and AS01E, respectively. AS01 contains a combination of MPL (3-O-desacyl-4'-monophosphoryl lipid A), a detoxified lipopolysaccharide produced through the hydrolysis of lipopolysaccharide (LPS) from *Salmonella Minnes*ota, and QS-21 (*Quillaja saponaria* Molina, fraction 21), a triterpene glycoside purified from the bark of *Quillaja saponaria* Molina, contained in cholesterol-containing liposomes [4]. In addition to serving as a delivery vehicle, the inclusion of cholesterol in the liposomes is important for quenching the hemolytic activity of QS-21. MPL and QS-21 activate the innate immune system in different ways, such that the integration of the different pathways has a synergistic effect on innate immune responses [4]. MPL has the characteristics of a PAMP, leading to its recognition by the patterned response receptor (PRR), TLR4. The activation of TLR4 involves the adaptor proteins MYD88 and TRIF, ultimately resulting in the release of cytokines and type 1 interferon. QS-21 is taken up into cells (endocytosed) and triggers the release of the DAMP, HMGB1 [4]. This activates the ASC-NLRP3 inflammasome and caspase-1, resulting in the production of mature, active IL-1β and IL-18. Additionally, the released HMGB1 also binds and activates TLR4. Studies have found that MPL is a weaker stimulator of interferon responses in humans relative to animal models, but the addition of QS-21 allows for a more potent response [4]. The combination of MPL and QS-21 results in the induction of an emergent innate immune signature, not induced by either component alone. This signature is characterized by IFN-γ and associated cytokines, such as CXCL10 and CXCL9 [4]. Mechanistic studies indicate that this interferon response, a hallmark of trained innate immunity, is a critical component of AS01-mediated potentiation of immune responses, and underlies the robust vaccine responses induced by AS01-adjuvanted vaccines in clinical use [4]. Cell culture studies in human peripheral blood mononuclear cells (PBMCs) found that stimulation with AS01 induces TNF-α and IL-1β production, as well as the upregulation of co-stimulation markers in CD14+ monocytes, which promotes antigen presentation to T cells [30; 31]. With respect to vaccination, AS01 and the antigen drain from the injection site in muscle to the draining lymph nodes resulting in a rapid cytokine and chemokine response, inducing innate immune cell infiltration [4]. This leads to increased interactions between antigen presenting cells and T cells. The presence of AS01 results in a higher degree of diversity and activation of the antigen presenting cells (such as monocytes and dendritic cells), ultimately leading to more efficient T cell priming and a broader humoral immune response.

Shingles (Herpes Zoster; Varicella-Zoster Virus; VZV):

Approved AS01-adjuvanted vaccine - Shingrix®

There is currently one FDA approved shingles vaccine available in the US for adults 50 years and older, which is the AS01B-adjuvanted recombinant zoster vaccine (RZV), Shingrix (GSK) [4].

Clinical Performance: Shingrix® replaced a prior live attenuated virus vaccine, Zostavax®, due to its superior efficacy and durability. The difference in efficacy is largely attributed to differences in the magnitude of the VZV T cell responses. VZV (antigen specific) T cell responses are significantly boosted following the second dose with the AS01-adjuvanted (Shingrix®) vaccine, resulting in 90% vaccine efficacy after two doses, compared with 48% efficacy for Zostavax® [32]. While a subset of participants generate adequate T cells to the first dose, a single dose is insufficient to generate sufficient VZV T cells, in the majority of older adults [32].

Mechanisms of trained immunity: One study found that the first dose of the Shingrix® vaccine altered the gene expression in immune cells in a manner that mimicked an expression profile identified in the subset of individuals with a robust response to the first dose [32]. This priming effect likely then drove the robust response to the second dose. The Shingrix® vaccine induces robust innate immune cell memory-like responses, which were coupled with transcriptional and epigenetic modifications in innate immune subsets, particularly monocytes and dendritic cells [33]. The induction of robust antigen-specific T cell activation/adaptive immunity following vaccination is at least partially mediated by the early induction of innate immune responses triggered by the AS01 adjuvant.

This suggests that AS01 induces a trained immunity phenotype, which allows for the induction of strong immunity following vaccination in a much larger subset of the population that would otherwise be possible.

The shingles vaccine has been linked with positive health outcomes in addition to protection from shingles. Details about the shingles vaccine and its association with various health outcomes can be found in the Shingles vaccine report. It remains to be determined whether at least some of these benefits can be attributed to the trained immunity induced by the AS01 adjuvant.

Respiratory Syncytial Virus (RSV):

Approved AS01-adjuvanted vaccine - Arexvy® (GlaxoSmithKline)

There are currently three RSV vaccines approved for use in adults aged 60 and older. These include the monovalent (RSV-A) **AS01E-adjuvanted** RSV prefusion F protein-based (adjuvanted RSVPreF3) vaccine

(Arexvy®, GSK), the non-adjuvanted bivalent (RSV-A and RSV-B) RSVpreF vaccine (Abrysvo®, Pfizer), and the mRNA-1345, encoding the RSV F glycoprotein, vaccine (mResvia®, Moderna) [34].

Clinical performance: The adjuvanted RSVPreF3 and RSVpreF vaccines were approved a year prior to the mRNA vaccine, so there is more cumulative data on them, and most real-world studies focus on these two. To date, real-world observational data indicates that these vaccines have high vaccine efficacy, around 75%, with similar protection from severe disease as observed in clinical trials [34; 35]. Encouragingly, while the protection is slightly less in immunocompromised individuals, it is still very robust, indicating that both vaccines have the capacity to elicit robust immune responses to RSV, even when baseline immune function is diminished [35]. Preclinical animal testing indicated that the addition of AS01 led to more robust polyfunctional T cell responses and titers of RSV-A/B-specific neutralizing antibodies broadly across RSV strains, relative to non-adjuvanted or alum-adjuvanted formulations of the RSVPreF3 vaccine [36]. Additionally, an analysis of serum from participants in a Phase 1/2 trial testing the RSVPreF3 vaccine in older adults found that the inclusion of the AS01 adjuvant induced a greater breath of RSV neutralizing antibodies [37]. Thus, without the AS01 adjuvant, the monovalent RSVPreF3 vaccine likely would not have been able to elicit such robust RSV immunity in older adults.

Malaria:

Approved AS01-adjuvanted vaccine - Mosquirix™, GSK)

Malaria is caused by the parasite, *Plasmodium falciparum*. RTS,S/AS01E (Mosquirix™, GSK) is the world's first approved malaria vaccine, which was developed as part of a public–private partnership between GSK and PATH's Malaria Vaccine Initiative [38]. In 2015, this became the first AS01-containing vaccine to be approved for widespread clinical use. The World Health Organization (WHO) recommends it be administered on a four-dose schedule starting at 5 months of age, in children living in regions with moderate to high malaria transmission [38]. Efficacy is relatively modest, reducing severe malaria by around 30%, and wanes over time, such that its use is not recommended in areas without endemic malaria [38; 39].

AS03

Adjuvant System AS03 from GSK is an oil-in-water emulsion adjuvant containing squalene, polysorbate-80, and α-tocopherol (Vitamin E) [5]. Preclinical studies indicate that following intramuscular injection, AS03 is rapidly drained into the draining lymph node, where it promoted the activation of resident innate immune cells. When taken up into macrophages, AS03 modulated their gene expression, including the down regulation of genes involved in fatty acid and cholesterol metabolism, and upregulation of genes for cytokines and chemokines [40]. Due to the lipid nature of AS03, its uptake into cells alters their lipid content, including decreases in cholesterol coupled with increases in triacylglycerol and phosphatidylcholine, and triggers the formation of lipid droplets [40]. The endoplasmic reticulum (ER) is a major site of lipid biogenesis, and is sensitive to lipotoxic stress [40]. The intake of excess lipids contained in AS03 induces an ER stress response involving the unfolded protein response (UPR), including the activation of IRE1α and JNK-associated inflammatory pathways [40]. In mice, induction of this ER stress response was found to contribute to the enhanced quality of the antibody response, such as the increased avidity and breadth of antigen-specific antibodies, in AS03 adjuvanted vaccines [40]. Uptake of squalene-based emulsion adjuvants has also been shown to trigger the release of DAMPs, which also enhances the innate immune response, such as by promoting the maturation, activation, and antigen-presenting efficiency of dendritic cells [41].

Influenza:

The AS03 adjuvant has been tested in a variety of influenza vaccines. Its addition has been shown to induce around five to ten-fold higher antibody levels, and generate a greater diversity of anti-influenza antibodies, even when the vaccines contain low doses of antigen [5; 25].

H5N1 is a subtype of Influenza A which is responsible for avian influenza (i.e. bird flu). In the absence of an adjuvant, immune responses to H5N1 antigens tend to be weak, resulting in poor vaccine efficacy [42].

Clinical performance: AS03-adjuvanted H5N1 vaccines were found to elicit higher neutralizing antibody responses, with robust cross-reactive anamnestic (memory) antibody responses, and evidence suggesting that a two-dose regimen may provide protection for up to three years [43]. For example, vaccination with an AS03 adjuvanted H5N1 A/Indonesia/05/2005 monovalent inactivated vaccine resulted in the generation of higher affinity antigen-specific antibodies as well as significant cross-clade neutralization of other clade 2 H5N1 strains [42].

Stemming from the global H5N1 outbreaks in the early 2000s, the AS03-adjuvanted Q-Pan H5N1 monovalent influenza vaccine (AREPANRIX™, GSK) was approved in 2013 and added to national stockpiles [44]. Over time, strain changes have been approved from the A/Indonesia/05/2005 H1N5 strain, to the A/American wigeon/South Carolina/22-000345-001/2021-like H5N1 strain, and most recently the A/Astrakhan/3212/2020-like H5N8 strain (FDA). The MF59-adjuvanted, inactivated, cell-based monovalent H5N1 vaccine (Audenz™, Seqirus) was approved in 2020.

Trained immunity mechanisms: An analysis of immune-related gene networks from whole blood transcriptomic data from clinical trials testing AS03-adjuvanted vs non-adjuvanted H5N1 vaccines identified NR4A1, SDC1, and ID3 as hub genes involved in immune response enhancement by AS03 [43]. Suppression of NR4A1 promotes the activity of the transcription factor, AP-1, a master regulator of immune activation, leading to more robust T and B cell responses. SDC1 is important for dendritic cell maturation and migration. ID3 plays an important role in the generation of memory T cells. The most enriched network was the NOD-like receptor signaling pathway, which is an important driver of innate immune responses, particularly in the context of trained immunity, suggesting that the enhanced immune responses induced by AS03 may be largely attributable to its capacity to induce trained innate immune responses.

Transcriptomic PBMC analysis from healthy young adult participants in a Phase 1 trial testing a H5N1 vaccine with or without the AS03 adjuvant identified an early innate immune response-related signature triggered by the inclusion of AS03 [45]. This included a stronger induction of IFN-inducible genes, including IP-10, as well as the JAK-STAT, NF-κB, NOD-like, TLR, and TNF pathways. Antigen presentation and processing were also upregulated, including MHC class I and class II pathways and FcγR-mediated phagocytosis [45]. Similarly, an analysis of sorted cell fractions also noted the early upregulation of interferon signaling in immune cell subsets following vaccination with the AS03-adjuvanted vaccine [46]. The interferon-induced gene CXCL10 gene encoding for the IP-10 cytokine was upregulated in monocytes and dendritic cells. The JAK-STAT pathway was upregulated in neutrophils, monocytes, and dendritic cells. NOD-like receptor signaling was enriched in neutrophils, while the complement and coagulation cascade pathway was enriched in dendritic cells. MHC-mediated antigen processing presentation was also upregulated in these cell types. Notably, these early transcriptional changes were correlated with subsequent cytokine and antibody responses induced by the vaccine [45; 46].

An analysis of immune cell responses from healthy participants from a Phase 1/2 trial testing a H5N1 vaccine with or without the AS03 adjuvant found that AS03 induced an early transcriptional response that enhanced the capacity of innate immune (myeloid) cells to sense environmental signals, such as the upregulation of pattern recognition receptors (PRRs) [25]. While vaccines of any type induced IFN-

related pathways, the early induction was much stronger in the presence of AS03. Genes associated with apoptosis (cell death) were also found to be downregulated in naïve B cells, leading to a greater diversity of B cells entering germinal centers, and ultimately a broader antibody response [25].
In conjunction with the transcriptomic effects, epigenetic modulation of innate immune subsets, an important feature of trained immunity, has also been observed following vaccination with the AS03-adjuvanted H5N1 vaccine [47]. This included a significant reduction in the chromatin marks, H3K27ac, H4K5ac, H3K9ac, and PADI4, in classical monocytes, along with reduced chromatin accessibility of the transcription factor AP-1 [47]. In contrast, chromatin accessibility of genes involved in antiviral responses, including interferon response genes and STAT genes was increased. This is consistent with elevated levels of IFN gamma and IP-10 in the plasma of participants vaccinated with the AS03-adjuvanted vaccine [47]. The induction of this antiviral program could potentially confer some degree of protection to heterologous (other) viral pathogens. In cell culture, PBMCs from participants vaccinated with the AS03-adjuvanted H5N1 vaccine had significantly lower titers of Dengue or Zika, following *in vitro* infection with those viruses [47].
Similar to what has been observed with the AS01-adjuvanted shingles vaccine, vaccination with the AS03-adjuvanted H5N1 vaccine shifted the innate immune profile in the majority of participants to a state very similar to the baseline (pre-vaccination) immune profile observed in the subset of high responders [25]. Thus, the inclusion of the AS03 adjuvant allows for the induction of robust immune responses toward vaccination by a much greater proportion of the general population.

H1N1 is a subtype of Influenza A that originated in pigs (i.e. swine flu). A strain of H1N1 was responsible for the 1918 Spanish flu global pandemic [48]. A novel strain emerged in 2009 (H1N1pdm09 virus) with pandemic potential, resulting in the launch of a nationwide vaccination campaign in the US [49]. H1N1 strains were included in annual (seasonal) flu vaccines the following year, such that standalone H1N1 vaccines are typically no longer used. Two AS03-adjuvanted split-virion vaccines were developed against the A/California/7/2009 H1N1 strain by GSK and licensed during the H1N1pdm09 pandemic [44]. These include Pandemrix, used mostly in Europe, and Arepanrix H1N1, which was primarily used in Canada.

Clinical performance: In a multinational study, Arepanrix H1N1 was found to have an efficacy of 76.8% for prevention of H1N1 illness in children aged 6 months to 9 years relative to that of a non-adjuvanted H1N1 vaccine during the 2010-2011 flu season [44].

Cross adjuvant comparison: Various H1N1 vaccines targeting the H1N1pdm09 strain were developed and tested during that time, allowing for cross study comparisons. An indirect comparison meta-analysis

including 10,734 participants from 22 studies found that both AS03-adjuvanted and MF59 adjuvanted vaccines provided better immunogenicity compared with unadjuvanted vaccines [50]. AS03 and MF59 are both squalene-based oil-in-water emulsion adjuvants, however, AS03 also contains the immune potentiator vitamin E (DL-α-tocopherol). Likely due to the additional immune potentiator, AS03 provided robust immune responses at a lower dose of antigen, relative to MF59, and superior performance, based on indirect comparison.

H7N9 is a subtype of Influenza A which primarily circulates in birds (i.e. bird flu). There are currently no approved H7N9 vaccines. Outbreaks emerging in 2013 led to efforts to develop H7N9 vaccines, some of which have been tested in clinical trials.

Cross-adjuvant comparison: A clinical trial assessed the improvement of immunogenicity achieved by including the AS03 or MF59 adjuvants to a monovalent vaccine targeting the A/Shanghai/2/2013(H7N9) strain in a head-to-head manner in adults younger than 65 [51]. The AS03 adjuvant had the best performance, with 84% of vaccine recipients achieving antigen-specific antibody titers ≥ 40, compared with 57% for those receiving MF59-adjuvanted vaccines, and 2% for those receiving unadjuvanted vaccines (administered as two doses using 15 µg of hemagglutinin antigen, 21 days apart).

Clinical performance: The impact of including the AS03 adjuvant was further evaluated in clinical trials testing the Yangtze River Delta (YRD) lineage inactivated A/Hong Kong/125/2017 fifth-wave H7N9 vaccine [52; 53]. One trial assessed the impact of two injections (21 days apart) of three different doses of the inactivated vaccine (3.75, 7.5, and 15 µg of hemagglutinin) containing the **AS03 adjuvant** as well as two doses (15 and 45 µg) without the adjuvant [52]. At 21 days post vaccination with the second injection, the mean proportion of participants achieving (hemagglutinin) antibody titers ≥ 40 ranged from 69% to 76% in adults younger than 65, and from 45% to 51% in adults aged ≥65, in those receiving the AS03 adjuvanted vaccines, whereas it was effectively 0% in those receiving the unadjuvanted vaccines. Cross-reactivity to other H7N9 strains was also observed, and followed a similar trend to the primary strain [52]. A study examining ways to optimize vaccine-induced immunity found that the inclusion of the AS03 adjuvant in the boost (2^{nd}) dose resulted in significantly higher antibody titers against multiple strains, including antigenically drifted strains [53]. Additionally, delaying the timing of the adjuvanted boost dose from 21 days to 4 months further enhanced antibody levels [53].
These studies suggest that the inclusion of robust adjuvants, such as AS03, may be necessary for the development of effective vaccines for antigenically unstable zoonotic influenza subtypes with pandemic potential.

AS04

Approved AS01-adjuvanted vaccine- Ceravix® (GlaxoSmithKline) **No longer available in the US*

Adjuvant System AS04 from GSK is composed of MPL (3-O-desacyl-4'-monophosphoryl lipid A) mixed with an aluminum salt (phosphate or hydroxide) [7]. As described above for the AS01 adjuvant, MPL acts as a PAMP to activate TLR4, and by promoting IFN-γ production, skew immune responses toward a Th1 profile, which promotes protection against intracellular pathogens [4]. Aluminum salts, as an adjuvant, boost vaccine responses by acting as a depot, to enhance interactions between antigens and antigen-presenting cells [7]. In PBMCs, AS04 has been shown to induce CD14+ monocytes to express IL-6 and TNF-α, and to secrete IL-1β [54]. AS04 also promoted the activation of dendritic cells, including the production of IL-6 and TNF-α, and the expression of costimulatory molecules. These effects were all mediated by MPL. AS04 does not directly act on T cells [54]. While not contributing to the innate immune response, the inclusion of the aluminum salt results in the production of a localized immune response at the injection site [54].

Human Papillomavirus (HPV):

Approved AS01-adjuvanted vaccine- Ceravix® (GlaxoSmithKline) **No longer available in the US*

Currently, the only available FDA approved HPV vaccine in the US is the recombinant human papillomavirus (HPV) nonavalent Gardisil-9® (alum-adjuvanted) vaccine (Merck), which protects against the HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58, and has replaced an earlier quadrivalent (6, 11, 16, and 18) version [55]. An **AS04-adjuvanted** bivalent (16 and 18) HPV Cervarix® vaccine (GSK) is no longer available in the US, but is available in some other countries.

Clinical performance: While no longer relevant (in the US) due to the availability of the nonavalent vaccine which covers a wide variety of strains, studies comparing the earlier bivalent (Cervarix®) and quadrivalent (Gardasil-4®) vaccines highlighted the capacity of the AS04 adjuvant to promote cross-reactivity to strains not included in the vaccine itself [56]. An analysis of Phase 3 trial data and real-world observational studies found that while both vaccines performed well at reducing the incidence of HPV-related cancers associated with the included strains, the AS04 adjuvanted vaccine offered superior protection against HPV types not included in the vaccines [56]. A comparison of Phase 3 data from the two vaccines found that the vaccine efficacy against cervical intra-epithelial neoplasia grade 3 or greater related to any HPV subtype was 93% (95% CI 79 to 99) for the AS04-adjuvanted bivalent vaccine, and 46% (95% CI 18 to 64) for the alum-adjuvanted quadrivalent vaccine.

Trained immunity mechanisms: A mechanistic study including six pairs of monozygotic female twins vaccinated with either the AS04-adjuvanted Cervarix® or (alum-adjuvanted) Gardasil-4® HPV vaccines assessed differences in immune cell responses [55]. The AS04-adjuvanted vaccine generated higher cross-neutralizing antibody titers to strains not included in either vaccine. This was coupled with higher frequencies of antigen presenting dendritic cells, which have been shown to be activated by AS04, and to a lesser extent B cells, which had enhanced expression of genes involved in RNA translation, cell metabolism and cell proliferation. AS04 stimulates the follicular helper T cells (Tfh) that interact with memory B cells, allowing them to rapidly produce high-affinity antibodies. Notably, diversity/breath of the neutralizing antibody response was positively associated with this enhanced expression of RNA translation, cell metabolism and development in dendritic cells and B cells. The AS04-adjuvanted vaccine also resulted in greater induction of conventional dendritic cells, whereas the alum-adjuvanted vaccine primarily induced plasmacytoid dendritic cells. One of the key pathways induced in immune subsets response to the AS04-adjuvanted vaccine is NOTCH signaling. This results in increased communication between T cells and memory B cells, as well as greater survival of memory B cells. These effects all favoring memory B cells result in a broader and longer lasting humoral response.
These mechanisms are consistent with the potentiation of innate immunity, through activation of dendritic cells, by the AS04 adjuvant as a key driver of augmented adaptive immune responses.

Hepatitis B (HBV):

EMA Approved AS04-adjuvanted vaccine: Fendrix™ (GlaxoSmithKline)

A specialized HBV vaccine developed for adults and adolescents with reduced kidney function approved by the EMA, Fendrix™ (GSK), uses the AS04 adjuvant system to boost immune responses in this immunocompromised population (EMA).
Clinical studies have found that this vaccine may be useful for boosting HBV immune responses in other populations that are nonresponsive to the traditional approved alum-adjuvanted Engerix-B (GSK) vaccine [57]. Relative to Engerix-B, the AS04-adjuvanted Fendrix vaccine induces seroprotection more rapidly, coupled with a higher overall antibody response [57].

CpG ODNs

Cytosine phosphoguanosine oligodeoxynucleotides (CpG ODNs) are synthetic sequences designed to mimic the type of bacterial DNA sequences that activate TLR9, which is an intracellular TLR [29]. The nature of the downstream signaling response can be tuned based on the intracellular compartment that TLR9 is localized and presence of co-factors, which vary based on cell type and activation status [45]. Different types of CpG ODNs have been developed which differentially act on different cell types and downstream processes [28]. Type A CpG-ODNs contain a central phosphodiester palindrome region with a CpG-hexamer motif in the palindrome and a phosphorothioate backbone attached to the 5' and 3' ends. They primarily activate the maturation of plasmacytoid dendritic cells and induce the production of IFN-α [28]. Type B CpG-ODNs contain a phosphorothiolate backbone throughout their entire sequence and strongly induce B cell proliferation and the production of pro-inflammatory cytokines [28]. Type C CpG ODNs contain a phosphorothioate backbone with one or two CpG-hexamer motifs and a palindromic sequence at the 3' end and induce an immune profile that is a hybrid of the other two types [28]. The CpG ODNs used in clinical studies to date have predominantly been type B, meaning they mainly act on B cells, and also have some effects on the maturation and activation of dendritic cells, monocytes, and NK cells [28].

Preclinical evidence of trained immunity: In PBMCs (from sheep), both type A and type B CpG ODNs have been shown to be able to induce features of the type 1 interferon response, including IFN-α secretion and 2'-5'A synthetase, an enzyme with antiviral properties, though the degree of induction was higher for type A CpG ODNs [58]. Administration of type B CpG ODNs into neonatal chicks altered the serum metabolic profile in a manner consistent with immune cell proliferation and activation [59]. This was coupled with increased resistance to bacterial septicemia. CpG ODNs have also been shown to improve survival following infection with various bacterial and viral pathogens in rodent models, consistent with a boost in non-specific innate immunity [29].

CpG 1018

Approved CpG 1018-adjuvanted vaccine - HEPLISAV-B® (Dynavax)

CpG 1018 is a type B CpG ODN containing an unmethylated CpG motif, known as a synthetic immunostimulatory sequence (ISS), designed to stimulate the innate immune system via TLR-9 [16]. The 1018 ISS is a 22-mer, 7.15 kDa phosphorothioate oligodeoxyribonucleotide with the sequence 5'-TGACTGTGAACGTTCGAGATGA-3', from Dynavax [16]. The activation of TLR9 drives Th1 responses [16]. It recruits the adaptor protein MyD88, leading to the induction of type 1 interferons and cytokines, such as

IL-12, IL-18 and IFN-γ from macrophages, dendritic cells, and NK cells [29]. The only approved CpG 1018-containing vaccine to date is the HEPLISAV-B® hepatitis B vaccine from GSK.

Hepatitis B (HBV):

Approved CpG 1018-adjuvanted vaccine - HEPLISAV-B® (Dynavax)

There are currently three FDA approved vaccines specifically targeting HBV, as well as several multi-antigen vaccines that also include HBV antigens. The earliest approved recombinant HBV vaccines, Recombivax-HB® (Merck) and Engerix-B® (GSK), contain aluminum-based adjuvants. The next generation HEPLISAV-B® (Dynavax) HBV vaccine contains the CpG 1018 adjuvant, providing it superior seroprotection and antigen-specific immune responses in immunocompromised populations, including older adults [60]. And, as described above, the specialized HBV vaccine, Fendrix™ (GSK), approved by the EMA for adults and adolescents with reduced kidney function, uses the AS04 adjuvant system to boost immune responses in this immunocompromised population [57].

Clinical Performance: In Phase 1 studies, the highest dose groups receiving CpG 1018 adjuvanted HBV vaccines achieved 100% protection within four weeks of the second dose, which was maintained 12-15 months later [16; 60]. Meanwhile, the unadjuvanted vaccine was unable to elicit a protective antibody response. Similarly, Phase 2 trials showed that nearly 80% of participants achieved seroprotection following the first dose, and 100% achieved seroprotection within one week of the second dose. This indicated a more rapid and robust response relative to the alum-adjuvanted Engerix-B vaccine, for which only around 11% of participants achieved seroprotecion after the first dose, and full (97.9%) seroprotection wasn't achieved until one month after the third dose [60]. The rapid induction of seroprotection with the CpG 1018 adjuvanted vaccine (HEPLISAV-B®) was also observed in older adults (aged 56–70), with a similar degree of seroprotection achieved with two doses of HEPLISAV-B® relative to three doses of Engerix-B [60]. Notably, the CpG 1018 adjuvanted HBV vaccine has been shown to provide superior seroprotection in patients with chronic conditions that reduce immunocompetency, including diabetes, chronic kidney disease, chronic liver disease, and HIV-infected patients [60].

Potential therapeutic utility: In addition to its use to prevent HBV-related illnesses, a preprint for a preclinical rodent study provides support for the notion that a CpG 1018 adjuvanted vaccine may also facilitate viral clearance in those with chronic HBV [61]. HBV infected mice vaccinated with a CpG 1018-adjuvanted HBV vaccine elicited enhanced IFN-γ and TNF-α responses in $CD4^+$ and $CD8^+$ T cells and higher quality B cell responses, suggesting a restoration of HBV antigen-specific immunity [61]. The

interaction between CD40 on B cells and its ligand CD40L (CD154) on activated CD4 T cells was found to be critical for this process.

Adjuvant Head-to-Head Comparison: AS01, AS03, AS04, Alum (with HBV antigen)
The hepatitis B (HBV) vaccine was used by GSK to perform a head-to-head clinical comparison of immune responses elicited by their different Adjuvant Systems, AS01, AS03, and AS04 (NCT00805389) [62]. The adjuvant systems were compared to one another, as well as to the reference alum-adjuvanted Engerix-B vaccine. AS04 was tested using the Fendix vaccine, while the AS01 (AS01B and AS01E) and AS03 adjuvants were tested in experimental (GSK223192A) vaccines. In the trial, 623 participants received a primary injection (i.m.) followed by a second dose 30 days later [62].

Adaptive Responses: Immunological profiling was conducted from blood collected on days 0, 14, 30, 37, 44 and 60. Adaptive immunogenicity was assessed in 599 participants, while antibody and memory B cell responses were assessed in 293 participants [62]. Antigen (hepatitis B)-specific CD4+ T cells increased with the AS01 and AS03 adjuvanted vaccines. The AS01 adjuvanted vaccines led to the highest frequencies of antigen-specific CD4+ T cells [62]. The polyfunctionality of the CD4+ T cells was similar across adjuvant groups, and no antigen-specific CD8+ T cell responses were detected. Hepatitis B antibody titers were highest for the AS01 and AS03 adjuvanted vaccines, followed by AS04, which were all significantly higher than alum [62]. Antigen-specific memory B cells were also highest for the AS01 and AS03 adjuvanted vaccines. Additionally, B cell responses peaked faster for all the Adjuvant Systems (AS01, AS03, and AS04) relative to alum.

Innate Responses: The impact to innate immune responses was assessed in 291 participants. Innate immune response signatures were transiently elevated following vaccination for all the Adjuvant Systems (AS01, AS03, and AS04), including C-reactive protein (CRP) and IL-6 [63]. Increases in IP-10 and IFN-γ were only observed after the second injection, predominantly with AS01-adjuvanted vaccines. Notably, **the magnitude of the early induction of innate immune responses was positively associated with the magnitude of the adaptive immune response.** More specifically, the AS01, and to a lesser extent, the AS03 adjuvants led to the induction of an interferon-related signature, including MX1 and STAT1, and was associated with the stronger adaptive immune responses, suggesting it may be a key driver [63].

Long-term responses: The durability of the adaptive immune responses, up to one-year post-vaccination was assessed in a cohort of 265 participants [64]. Antigen-specific antibody, memory B cell, and CD4+ T

cell responses were maintained for all groups. However, quantitatively, the magnitude of these long-term immune responses followed the same pattern described for the early responses [64]. All of the Adjuvant Systems induced higher proportions of high-avidity antibodies and a stronger promotion of avidity maturity to an antigen recall challenge, relative to alum. This is likely attributable to differences in the memory B cells induced by the Adjuvant Systems [64]. Amongst the Adjuvant Systems, AS04 produced a lower degree of high-avidity antibodies, relative to AS01 and AS03. This may stem from the higher degree of inter-individual variability in memory B cell responses with the AS04 adjuvanted vaccine [64]. Further analysis identified differences in the antibody profiles induced by AS01 and AS03, relative to AS04 and alum [65]. The AS01 and AS03 adjuvants produced more robust and durable FcR-engaging and IgA-biased responses [65]. Additional post-hoc analysis using data from 98 participants provides further evidence that the early innate response, characterized by the IFN gene signature, is the key driver of the unique functional antibody response induced by AS01 and AS03 [27].

Overall assessment: These studies found that, at least with respect to the hepatitis B antigen, the ranking of adjuvants for both the innate and immune responses was
(AS01B ≥ AS01E > AS03 > AS04 > Alum) [63].

Adjuvant Cross-Comparison: AS01B, MF59, and Alum (with HIV antigen)
A retrospective cross-protocol, cross-sectional analysis compared four RCTs (HVTN 100, 107, 120, and 702) including adults without HIV who received the ALVAC-HIV (vCP2438) vaccine and a bivalent gp120 protein booster containing either MF59, alum, or AS01B as an adjuvant [66]. The magnitude of the antigen-specific CD4+ T cell response was significantly higher for AS01B relative to MF59 or alum. AS01B also was associated with superior antibody binding for some of the HIV antigens.

Cancer: POTENTIAL BENEFIT IN COMBINATION WITH IMMUNOTHERAPY

The addition of potent adjuvants has been proposed as a mechanism to potentiate immune responses in the context of cancer immunotherapy approaches [67]. Numerous different adjuvants have been clinically tested in cancer vaccines under development [67].
CpG 1018 was tested at four dose levels in combination with the anti-CD20 monoclonal antibody rituximab in a Phase 1 trial in patients with relapsed non-Hodgkin lymphoma [68]. The addition of CpG 1018 was shown to promote the induction of several IFN-α/β–inducible genes in a dose-related manner. The addition of the CpG 1018 adjuvant to neoantigen mRNA vaccine was found to potentiate anti-tumor immunity by increasing the level of tumor-infiltrating CD8+ T cells in rodents [69].

The type C CpG ODN nelitolimod (SD-101) from TriSalus Life Sciences has also been tested in several clinical trials in cancer patients in combination with anti-PD-1 (e.g. pembrolizumab) immunotherapy, but has not yet been approved [70].

Safety: Adjuvanted vaccines are well-tolerated and have a good safety profile across global populations. They are associated with increased reactogenicity, such as local injection site reactions, and transient post-injection fatigue, headache, muscle pain, and fever.

Types of evidence:

- 2 meta-analyses of clinical trials testing adjuvanted vaccines
- 1 review of clinical studies testing AS01-adjuvanted vaccines
- 1 review of clinical studies testing AS03-adjuvanted vaccines
- 1 analysis of adverse events from RCTs testing CpG 1018-adjuvanted HEPLISAV-B®
- 1 review of immune-related adverse events from studies testing CpG ODNs
- 2 post-marketing surveillance analyses from AS04-adjuvanted Ceravix®
- 1 phase III/IV trial assessing long-term safety of AS04-ajuvanted Ceravix®

Due to the role of adjuvants in potentiating the immune system, the primary theoretical safety risk associated with their use is the development of autoimmune conditions [71]. Clinical data from adjuvanted vaccines to date has not shown supportive evidence for this potential risk.

A meta-analysis including 47,602 participants from 7 clinical trials testing AS01/AS02 adjuvants, 7 clinical trials testing AS03 adjuvants, and 12 clinical trials testing MF59 adjuvants, assessed safety outcomes [72]. Rates of serious adverse events, deaths, and immune-mediated diseases were similar for vaccines using these adjuvants relative to control (placebo or alum-adjuvanted) vaccines. Reactogenicity was higher with these newer adjuvants, especially AS01 and AS03. In line with the increased reactogenicity, rates of fatigue (Relative Risk [RR]: 2.48, 95% CI 1.69 to 3.64), headache (RR: 2.94, 95% CI 1.24 to 6.95), and myalgia (RR: 2.68, 95% CI 1 86 to 3.80) were higher with the AS01/AS02, AS03, and MF59 adjuvanted vaccines [72]. Similarly, a meta-analysis of 29 trials including 25,056 children (age≤10) who received at least one dose of a vaccine containing one of these adjuvants also found an increase in reactogenicity, but no other consistent pattern of adverse events [73].

AS01

Of the GSK Adjuvant Systems, AS01 has undergone the most extensive clinical testing and use, as it has been used in vaccines received by millions of people, ranging from infants to the elderly [4]. The main effect of AS01 is an increase in the reactogenicity, or the physical inflammatory response of the vaccine, such as pain, redness, and swelling at the injection site, and systemic responses, like fever and fatigue [4]. The side effect profile with AS01-adjuvanted vaccines has been found to be comparable between immunocompetent and immunocompromised populations [4].

AS03

Based on data from over 55,000 clinical trial participants receiving at least one dose of an AS03-adjuvanted influenza vaccine, these AS03-contaning vaccines were generally well tolerated [44]. Relative to non-adjuvanted vaccines or placebo, the administration of AS03-adjuvanted H1N1 or H5N1 influenza vaccines was associated with increased reactogenicity. This included increased incidences of injection site pain, as well as systemic effects, including irritability and drowsiness in young children, and muscle pain (myalgia), headache, and fatigue in older children and adults [44]. Fever was more common following the second dose of the AS03-adjuvanted vaccine in children under age 3 [44]. A pooled assessment of adverse events of special interest (anaphylaxis, Bell's palsy, convulsion, demyelination, encephalitis, Guillain-Barré syndrome (GBS), neuritis and vasculitis) as defined by the Committee for Medicinal Products for Human Use of the European Medicines Agency (EMA), including 22,521 adults from 28 clinical trials who received AS03-adjuvanted H5N1 or H1N1 vaccines, found that the inclusion of the AS03 adjuvant was not associated with a significant increase in the incidence of these adverse events [44].

In addition to those receiving AS03-adjuvanted vaccines in clinical trials, it has been estimated that approximately 31 million doses of *Pandemrix* and 59 million doses of *Arepanrix* H1N1, the A/H1N1pdm09 influenza vaccines, were administered, including at least 9.5 million doses to children and 300,000 doses to pregnant women [44]. Real-world post-licensure experience similarly found that the AS03-adjuvanted vaccines had an acceptable safety profile and were generally well-tolerated [44]. While some case series suggested possible risk for some neurological conditions, a Swedish cohort study including 3.3 million vaccinated and 2.5 million unvaccinated individuals found, there was no evidence of a role for the Pandemrix vaccine in triggering any of more than 30 neurological and immune-related diseases, with the exception of narcolepsy [44]. Studies in Europe found that vaccination with *Pandermix* was associated with an increased risk for narcolepsy, with a pooled relative risk ranging from 5.0 to 14.3 in children and from 2.9 to 7.0 in adults [44]. However, a similar safety signal was not

identified with the *Arepanrix* H1N1 vaccine, which was primarily used in Canada. This association is not well understood, but possible explanations for this effect include confounding, an interaction between viral exposure and vaccination, as well as possible cross-reactive antibodies between the influenza virus nucleoprotein and human hypocretin receptor 2 [44]. Administration of these vaccines was not associated with adverse pregnancy outcomes, and they were generally well tolerated in immunocompromised populations [44].

AS04

As of 2018, more than 71 million prophylactic doses of the AS04-adjuvanted Ceravix® human papillomavirus (HPV-16/18) vaccine had been administered worldwide [74]. Global post-licensure passive surveillance from 2007 to 2011 found that the most common adverse events associated with vaccination with the AS04-adjuvanted HPV-16/18 vaccine were consistent with those identified during clinical trials and including in the prescribing information, with no new safety concerns [74]. A separate post-marketing surveillance cohort study in China also did not identify new safety concerns [74]. A phase III/IV controlled, randomized study (NCT00534638) assessed the long-term safety, up to 6.5 years, of vaccination with the AS04-adjuvanted HPV-16/18 vaccine based on a cohort of 32,175 Finnish adolescents aged 12–15 years at the time of vaccination [74]. Rates of adverse events in participants vaccinated with the AS04-adjuvanted HPV-16/18 vaccine (n= 14,837) were compared to those in a control cohort who received the aluminum salt-adjuvanted Engerix B® hepatitis B vaccine (n=17,338), which has a long history of a favorable safety record. Rates of serious adverse events considered as possibly related to vaccination were similar, as were rates of new-onset autoimmune disease, and pregnancy outcomes. Other studies have also found that vaccination with Ceravix® does not impact rates of miscarriage or other adverse pregnancy outcomes [74; 75].

CpG 1018

A post-hoc analysis of safety outcomes from three Phase 3 clinical trials including a total of 9,365 vaccinated with the CpG 1018 adjuvanted hepatitis B (HBV) (HEPLISAV-B®) vaccine and 3,867 participants vaccinated with an alum-adjuvanted HBV vaccine (Engerix-B®) found that the vaccines had similar safety profiles [76]. Rates of local and systemic reactions, serious adverse events, new-onset immune-mediated adverse events and autoantibodies were similar between the vaccine groups. The initial FDA review for HEPLISAV-B® was rejected due to potential safety concerns about rare autoimmune events, but this concern has not been supported by clinical evidence to date, and it was approved upon later review [16; 60].

A review of immune-related adverse events including over 14,000 participants from 11 clinical trials using the CpG 1018-adjuvanted hepatitis B (HBV) vaccine found that rates of immune-related adverse events were similar between the CpG 1018 and the alum adjuvanted HBV vaccines [70]. Similarly, rates of new-onset immune-mediated adverse events were similar between adults receiving CpG 1018 and alum adjuvanted HBV vaccines in a postmarketing electronic health record study (HBV-26) including nearly 70,000 adults.
The CpG 1018 adjuvant was used in six covid-19 vaccines developed outside of the US. The inclusion of the CpG 1018 adjuvant was not associated with an increased risk for autoimmune disease with these vaccines [70].

Drug interactions: Vaccines containing these adjuvants potentiate the immune system, thus they may have interactions with immunosuppressants.

Sources and dosing:
All of the Adjuvant Systems (AS0) adjuvants and associated vaccines are from GlaxoSmithKline.

AS0-adjuvanted approved vaccines
Shingrix® is a recombinant Zoster vaccine containing the AS01B adjuvant (50 µg of MPL and 50 µg of QS-21). It is approved for the prevention of Herpes Zoster (shingles) in adults 50 years and older as well as in adults 18 years and older with immunodeficiency. It is administered via intramuscular injection as a two-dose regimen, with the second dose administered two to six months after the first dose (FDA Prescribing Information).
Arexvy® is a recombinant Respiratory Syncytial Virus Vaccine containing the AS01E adjuvant (25 µg of MPL and 25 µg of QS-21). It is approved for the prevention of lower respiratory tract disease caused by respiratory syncytial virus in adults 60 years and older as well as adults 18 years and older at increased risk for RSV. It is administered as a single dose via intramuscular injection (FDA Prescribing Information).
Mosquirix™ (RTS,S/AS01) is a recombinant protein malaria vaccine containing 25 micrograms of RTS,S and the AS01E adjuvant (25 µg of MPL and 25 µg of QS-21). It is indicated for children aged 6 weeks up to 17 months living in regions with high malaria burden. It is administered via intramuscular injection in three doses at a monthly interval, with a fourth dose recommended from 12 to 18 months after the third dose (EMA Prescribing Information).

AS03-adjuvanted vaccines

There are currently no approved vaccines in regular use that utilize the AS03 adjuvant. However, several AS03-adjuvanted vaccines were developed and used during previous influenza outbreaks.
Pandemrix is an inactivated split-virion H1N1 pandemic influenza vaccine targeting the A/California/7/2009 (H1N1)v-like strain. It contains the AS03 adjuvant. It was approved by the EMA for use during the 2009 H1N1 pandemic (EMA).
Arepanrix H1N1 is an inactivated split influenza vaccine containing the antigen A/California/7/2009 (H1N1)v-like strain (X-179A) (3.75µg haemagglutinin per 0.5mL dose). It contains the AS03 adjuvant. It was approved by Health Canada for use during the 2009 H1N1 pandemic (Health Canada).
Q-Pan H5N1 monovalent influenza vaccine (AREPANRIX™) was approved for use in individuals 6 months of age and older at increased risk of exposure to the influenza A virus H5 subtype contained in the vaccine (FDA Prescribing Information). It contains the AS03 adjuvant. It is included in the National Pre-Pandemic Influenza Stockpile.

AS04-adjuvanted vaccines

Ceravix® is a recombinant Human Papillomavirus bivalent (Types 16 and 18) vaccine containing the AS04 adjuvant. It was approved for the prevention of the diseases caused by oncogenic human papillomavirus (HPV) types 16 and 18 in females aged 9 through 25. It is administered via intramuscular injection in three doses, with the second and third doses administered one and six months after the first dose, respectively (FDA Prescribing Information). It is still used globally, but is no longer used in the US due to the development of newer HPV vaccines.

Fendrix™ is a recombinant Hepatitis B vaccine containing the AS04 adjuvant. It is approved by the EMA for use in adults and adolescents (aged ≥15) with renal insufficiency. It is administered via intramuscular injection in four doses, with subsequent doses occurring at 1 month, 2 months and 6 months from the date of the first dose (EMA Prescribing Information).

CpG 1018

The CpG 1018 adjuvant is available through Dynavax Technologies.
HEPLISAV-B® is a recombinant Hepatitis B vaccine containing the CpG 1018 adjuvant. It is approved for the prevention of infection caused by all known subtypes of hepatitis B virus in adults 18 years and older. It is administered via intramuscular injection in two doses with the second dose 1 month after the first (FDA Prescribing Information).

Research underway:

There are clinical trials underway testing several of these adjuvants in novel vaccines, and as a therapy to induced trained immunity.

AS01 is being tested in a single-center, randomized, single-blind, placebo-controlled in older adults for its ability to induce trained immunity (NCT07527247), which has an expected completion date in 2029. The AS01-adjuvanted Shingrix® vaccine is being tested in combination with a GLP-1R agonist for preservation of residual beta-cell function in adults with recent-onset type 1 diabetes (NCT07614412).

CpG 1018 has been tested in clinical trials of novel vaccines for HIV and Herpes Zoster. It is also being tested in as an adjuvant in combination with cellular immunotherapy in recurrent epithelial ovarian cancer (NCT06366490), and as a standalone therapy in patients with MCI and mild AD (NCT05606341). The AD study is being sponsored by NYU Langone Health, with the CpG 1018 provided by Dynavax.

Search terms:

Pubmed, Google: Vaccine Adjuvants

- Alzheimer's disease, neuroprotection, aging, trained immunity, clinical trials, cancer, safety

Websites visited for Adjuvants:

- Clinicaltrials.gov (AS01, AS03, AS04, MF59, CpG 1018)
- Drugs.com (Shingrix; Arexvy; Heplisav-B)

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