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Review

Adjuvant Systems for vaccines: 13 years of post-licensure experience in diverse populations have progressed the way adjuvanted vaccine safety is investigated and understood

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ABSTRACT

Adjuvant Systems (AS) are combinations of immune stimulants that enhance the immune response to vaccine antigens. The first vaccine containing an AS (AS04) was licensed in 2005. As of 2018, several vaccines containing AS04, AS03 or AS01 have been licensed or approved by regulatory authorities in some countries, and included in vaccination programs. These vaccines target diverse viral and parasitic diseases (hepatitis B, human papillomavirus, malaria, herpes zoster, and (pre)pandemic influenza), and were developed for widely different target populations (e.g. individuals with renal impairment, girls and young women, infants and children living in Africa, adults 50 years of age and older, and the general population). Clearly, the safety profile of one vaccine in one target population cannot be extrapolated to another vaccine or to another target population, even for vaccines containing the same adjuvant. Therefore, the assessment of adjuvant safety poses specific challenges. In this review we provide a historical perspective on how AS were developed from the angle of the challenges encountered on safety evaluation during clinical development and after licensure, and illustrate how these challenges have been met to date. Methods to evaluate safety of adjuvants have evolved based on the availability of new technologies allowing a better understanding of their mode of action, and new ways of collecting and assessing safety information. Since 2005, safety experience with AS has accumulated with their use in diverse vaccines and in markedly different populations, in national immunization programs, and in a pandemic setting. Thirteen years of experience using antigens combined with AS attest to their acceptable safety profile. Methods developed to assess the safety of vaccines containing AS have progressed the way we understand and investigate vaccine safety, and have helped set new standards that will guide and support new candidate vaccine development, particularly those using new adjuvants.

Focus on the patient: What is the context? Adjuvants are immunostimulants used to modulate and enhance the immune response induced by vaccination. Since the 1990s, adjuvantation has moved toward combining several immunostimulants in the form of Adjuvant System(s) (AS), rather than relying on a single immunostimulant. AS have enabled the development of new vaccines targeting diseases and/or populations with special challenges that were previously not feasible using classical vaccine technology.

What is new? In the last 13 years, several AS-containing vaccines have been studied targeting different diseases and populations. Over this period, overall vaccine safety has been monitored and real-life safety profiles have been assessed following routine use in the general population in many countries. Moreover, new methods for safety assessment, such as a better determination of the mode of action, have been implemented in order to help understand the safety characteristics of AS-containing vaccines.

Abbreviations: AE, adverse event; AESI, adverse events of special interest; AS, Adjuvant System; CI, confidence interval; CRPS, Complex Regional Pain Syndrome; EMA, European Medicines Agency; GBS, Guillain-Barré syndrome; H1N1/AS03, AS03-adjuvanted pandemic influenza vaccine; HBV, hepatitis B virus; HBV/AS04, hepatitis B vaccine adjuvanted with AS04; HIV, human immunodeficiency virus; HPV, human papillomavirus; AS04-HPV-16/18, HPV types 16 and 18 AS04-adjuvanted; IFN, interferon; MPL, 3-*O*-desacyl-4′- monophosphoryl lipid A; NF-κB, nuclear factor κB; OR, odds ratio; pIMD, potential immune-mediated disease; POTS, Postural Orthostatic Tachycardia Syndrome; RTS,S/AS01, malaria vaccine containing the RTS,S hybrid protein adjuvanted with AS01; RZV, recombinant zoster vaccine; SAE, serious adverse event; TLR, toll-like receptor; VZV, varicella zoster virus; WHO, World Health Organization.

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What is the impact? New standards and safety experience accumulated over the last decade can guide and help support the safety assessment of new candidate vaccines during development.

Contents

1. Why we needed new adjuvants

Vaccination is an important and successful public health intervention in the prevention of infectious diseases. The World Health Organization (WHO) estimates that from 2001 to 2020, vaccination will prevent 20 million deaths in low- and middle-income countries alone, leading to social and economic benefits worth 280 billion US dollars [1]. The global benefits of a century of vaccine use in terms of lives saved and number of people living without disabilities are unquestionable. Nonetheless, infectious diseases remain the leading cause of childhood death worldwide [2]. This is partly because classical vaccine technologies are sometimes not sufficient for preventing diseases caused by complex pathogens. Other challenges in vaccine development are how to improve immune responses in populations with hyporesponsive immune systems; how to improve the immunogenicity to purified recombinant antigens; and how to maximize vaccine production in situations of a finite antigen source [3]. Innovative approaches and new technologies that could potentially address these needs include viral vector vaccines, DNA vaccines, use of alternate delivery routes (intra-dermal, intra-nasal) and novel adjuvants.

Adjuvants are substances added to vaccines to enhance their immunogenicity. Aluminum was the first and only adjuvant used routinely in human vaccines for more than 50 years [4]. In the 1990s, several new adjuvants were introduced for human vaccines [5]. But it became apparent that vaccines formulated with single adjuvants could not always induce the required immune response to overcome vaccine development challenges. This led to the concept of combining two or more different immunostimulants to achieve the desired response (the Adjuvant Systems [AS] approach) [6] (Box 1).

Box 1 The discovery and development of Adjuvant Systems.

Early vaccines were discovered by trial and error because the necessary technology and knowledge about how the immune system responds to pathogens needed for rational vaccine design were not available. By the 1990′s vaccine knowledge and technologies were expanding rapidly, allowing development of more rational approaches to vaccine design. In the search for more effective adjuvants to protect against difficult pathogens, different combinations of immune-stimulatory molecules and classical adjuvants were explored. These molecules were selected based on previous experience with other antigens and on their known immuno-enhancing properties and physio-chemical characteristics. For example, MPL (3-O-desacyl-4′-monophosphoryl lipid A), and QS-21 (*Quillaja saponaria* Molina: fraction 21) had been known since the 1980′s and had been used previously in humans. These immune-stimulatory molecules were combined with adjuvants or formulations already in use, such as aluminum, liposomes and oil-in-water emulsions, with the expectation of achieving additive effects on the innate and adaptive immune response. Once the desired immune response was achieved in pre-clinical studies, the AS was tested in clinical trials. The history of the development of AS is reviewed by Garcon et al, 2017 [6].

From numerous families of AS that were designed by mixing and matching different immune-stimulatory molecules with classical adjuvants or formulations, three AS families have been licensed in prophylactic vaccines containing recombinant antigens: AS04, AS03 and AS01 [6] (Fig. 1). AS04 is used in the hepatitis B (HBV) vaccine for individuals with chronic renal failure or undergoing renal dialysis (HBV/AS04; *Fendrix*, GSK) and the human papillomavirus (HPV) vaccine, used in girls and young women

(AS04-HPV-16/18; *Cervarix*, GSK). AS03 was widely used in pandemic influenza vaccines (H1N1/AS03; *Pandemrix*, *Arepanrix*, GSK) and (pre)pandemic influenza vaccines (H5N1/AS03) for stockpiling and pandemic preparedness. AS01 is used in the malaria vaccine (RTS,S/AS01; GSK) and recombinant zoster vaccine (RZV; *Shingrix*, GSK). Each AS contains a range of molecules with varying immune-enhancing properties (Box 2), and different doses of AS can be used in individual vaccine formulations.

Box 2 Mechanisms of action of Adjuvant Systems in licensed or approved vaccines.

AS04

- Contains MPL adsorbed onto aluminum hydroxide or aluminum phosphate.
- Aluminum does not seem to act synergistically with MPL but may prolong the cytokine response at injection site [7].
- Causes transient activation of innate immunity through TLR4, leading to cytokine and chemokine responses and increased numbers and efficiency of activated dendritic cells.
- This early response increases activation of antigen-specific T-cells, enhances the antibody response [7,8], and may be involved in the generation of cross-functional antibodies [9].

AS03

- Is an oil-in-water emulsion with squalene and alpha-tocopherol (Vitamin E) mixed in the oil phase.
- AS03 induces a rapid perturbation of lipid metabolism in monocytes, leading to endoplasmic reticulum stress and activation [10].
- The presence of the immunostimulant α-tocopherol is associated with an increased uptake of antigen by monocytes, as well as a transient increased expression of some cytokines and chemokines [11].
- T-cell mediated help of B-cell responses is augmented, which leads to a marked and persistent antibody response when targeting antigens for which antibody-mediated protection is important [12,13].

AS01

- Contains QS-21 and MPL in a liposome-based formulation.
- MPL and QS-21 act synergistically through the stimulation of TLR-4 pathways and NLRP3 inflammasome, respectively [14], to trigger specific innate signaling pathways.
- The activation of innate immunity is transient, and leads to the production of different cytokines and chemokines including early IFN- [15,16], which has been shown to play a key role in the induction of the Th1-type functional immunity and protection against malaria [17].
- Enhances the adaptive response, leading to enhanced cell-mediated and antibody responses (reviewed in [18]).

QS-21: *Quillaja saponaria* Molina: fraction 21 (QS-21, licensed by GSK from Antigenics LLC, a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation); MPL: 3-*O*-desacyl-4′-monophosphoryl lipid A.

AS combined with the vaccine antigen(s) directly impact the innate immune response to stimulate the desired humoral and cellular adaptive responses. This immune-stimulatory activity carries a theoretical but as yet unsubstantiated risk of either inducing or exacerbating potential immune-mediated diseases (pIMDs) in susceptible persons. Thus, comprehensive evaluation of the safety of adjuvant vaccines has been paramount during their development and during post-marketing surveillance [19,20] (Fig. 1). Part of this assessment is the determination of causality which can be difficult and requires more evidence than a purely temporal relationship to vaccination [19]. Decisions on causality are a matter of judgment based the absence of confounding factors (e.g. other treatments or disease states that could account for the event), the extent to which the adverse event (AE) is consistent with the mechanism of action/pharmacology of a product (or biological plausibility), the timing of the event relative to the time of product exposure (the occurrence of event after product administration does not automatically mean that product caused or contributed to the event), whether the event reoccurred with subsequent doses, whether the AE is known to be caused by related products of the same class, etc.

In this review we summarize the development of AS with special focus in describing GSK's evolving experience in monitoring the safety of vaccines containing AS and assessing safety signals as they have arisen. New methods for safety assessment of vaccines containing AS are leading the way in vaccine adjuvant science, and helping to set new standards that will guide and support novel vaccine development. Overriding principles that guide the evaluation of safety specify that conclusions from one antigen ("X"-AS formulation) cannot be extrapolated to another antigen ("Y"-AS formulation) (Box 3). Similarly, conclusions about the safety profile of one adjuvanted vaccine in a specific target population cannot be extrapolated to a different population. These principles have had far-reaching effects on how evaluation of the safety of vaccines containing AS has been approached.

Box 3 Principles that guide safety evaluation of adjuvanted vaccines.

1. All vaccine components may impact safety. The clinical safety profile of a vaccine refers to specific antigen-AS formulations.
2. Risks may be associated with any component: the adjuvant as well as the antigen, and/or the combination of both. However, because vaccines are administered as a whole, it is difficult to discern causal associations with a specific vaccine component.
3. Experience with the same adjuvant formulated with other vaccine antigens is considered as supplementary information for the safety evaluation; conclusions cannot be directly extrapolated to a different antigen(s) formulated with the same adjuvant.
4. It is not appropriate to extend conclusions regarding the safety profile between populations, for example between naÝve and primed populations, or between different age-groups.
5. Non-clinical testing of the adjuvant alone helps to identify potential adverse effects linked to adjuvant.
6. The growing understanding of the mode of action of the adjuvant can inform biological plausibility of an event being caused by vaccination (one of the key aspects in assessing the causal relationship of the event with vaccination), informs identification of potential risks or adverse events of special interest, and may provide insights for defining ''risk windows" for certain adverse events based on the nature and kinetics of the inflammatory response and the resulting adaptive immune response.

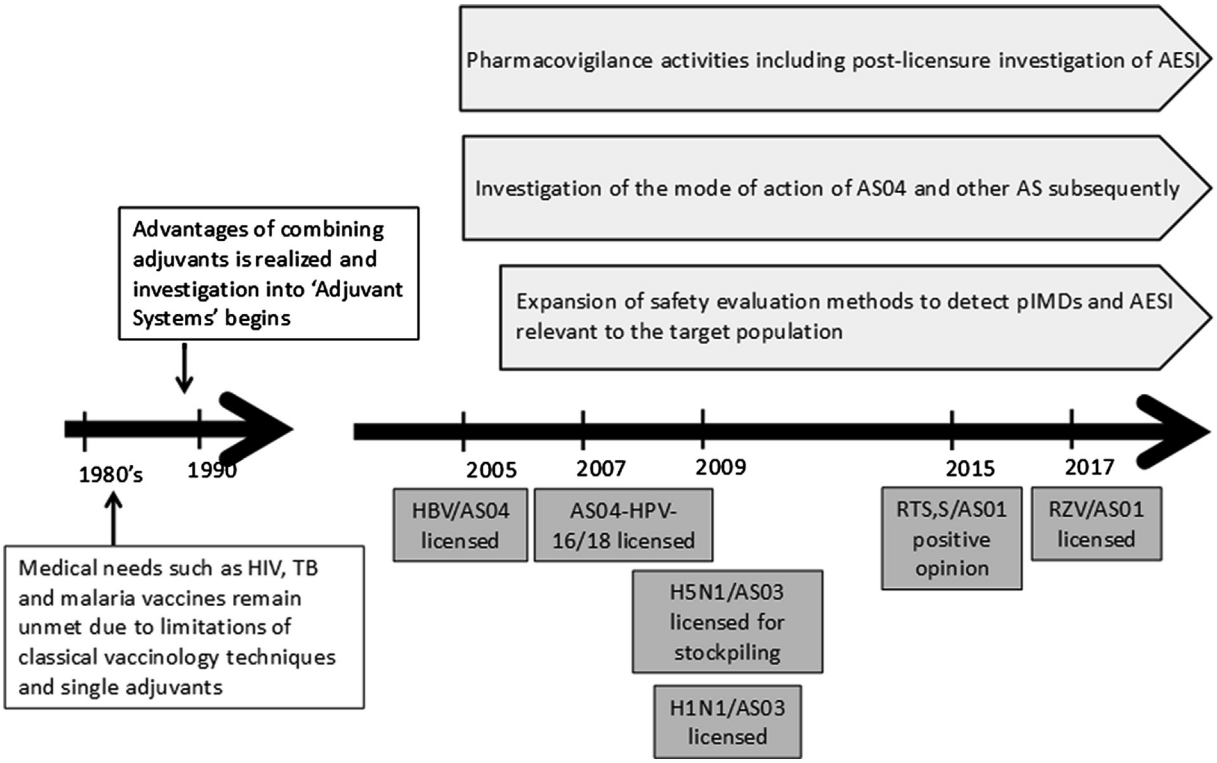

Fig. 1. Evaluation of Adjuvant Systems: timeline of development. AESI = adverse events of special interest, AS = Adjuvant Systems, HBV = hepatitis B virus, HIV = human immunodeficiency virus, HPV = human papillomavirus, pIMD = potential immune-mediated disease, TB = tuberculosis, RTS,S = malaria vaccine containing the RTS,S hybrid protein, RZV = recombinant zoster vaccine.

2. AS04: Lessons in unraveling the mode of action of adjuvants and implementing standardized methodology for the collection of AEs of special interest (AESI)

The first vaccine licensed for use that contained an AS was HBV/AS04 in 2005, followed by AS04-HPV-16/18 in 2007. AS04 contains aluminum and 3-*O*-desacyl-4′-monophosphoryl lipid A (MPL), a TLR-4 agonist (Box 2). AS04 induces increases T-cell responses and higher antibody responses than aluminum alone, and was therefore selected for use in vaccines targeting viral infections in which enhanced induction of antibody titers was necessary [21].

HBV/AS04 was designed for renal pre-dialysis and dialysis patients who respond poorly to traditional HBV vaccines. During development, GSK entered into first consultations with regulatory authorities on aspects of developing vaccines containing novel adjuvant combinations. The development of AS04-HPV-16/18 commenced soon after, which in contrast to HBV/AS04, was intended for universal mass immunization of girls and young women. The need for comprehensive evaluation of safety that included specific AESI for adjuvanted vaccines, promoted the standardization of safety methods to ensure rigorous data collection. During the licensure process for AS04-HPV-16/18, assessment of the immunological mechanisms underpinning the mode of action of the AS supported licensure and addressed questions by authorities regarding the biological plausibility of specific risks theoretically associated with the adjuvant. This was also made possible by parallel advances in new methods to assess immune cells and pathways. The experience with AS04 prompted the investigation of the mode of action of AS01 and AS03 being investigated in vaccine candidates at earlier development stages [12,18,22]. As a result of this work we have gained insights into the complex interactions that put the innate immune system at work to trigger adaptive immunity following exposure to antigen formulated with AS. Information on the mode of action of vaccines containing AS is now explored early in development, and contributes to a better understanding of the reactogenicity and safety profiles of these vaccines.

2.1. Collection of AESI and optimization of study design

Specific outcomes and AESI for AS04-HPV-16/18 were identified based on the target population of young women of child-bearing age, and theoretical concerns raised due to the immune-stimulatory activity of novel adjuvants that could induce or exacerbate immune-mediated diseases in susceptible persons. AESI in the target population included pregnancy outcomes following inadvertent exposure to AS04-HPV-16/18, investigating signals identified pre-licensure (spontaneous abortion) [23], and new onset of autoimmune diseases investigated pre- and post-licensure. Pregnancy outcomes following inadvertent exposure to AS04-HPV-16/18 were captured during clinical development and post-licensure, as reported to a Pregnancy Registry [24]. Standardized methodologies for collecting and monitoring safety data were developed and subsequently implemented in clinical trials of all AS-containing vaccines. This included the identification and evaluation of medically-attended conditions, new onset of chronic diseases of possible autoimmune etiology, and later, the development of a specific methodology for collecting pIMDs [20,25]. The latter includes autoimmune diseases and other immune-inflammatory disorders of interest, which may or may not have an autoimmune etiology. A longer safety follow-up period for pIMDs (typically at least 6–12 months) was requested by Regulatory Authorities for all vaccines containing novel adjuvants, with the intention to detect late onset events following vaccination [26,27]. The request was based on the assumption that should autoimmunity occur after vaccination, it would require several

weeks to develop, but would be less likely after 6–12 months following the last vaccine dose [25]. Standardized collection was established to improve data capture and quality. Additionally, the use of customized queries was implemented for routine signal detection purposes and periodic data analyses.

Based on the AS04-HPV-16/18 experience, clinical trial designs for new vaccines containing AS have been built to provide the maximum safety information. For example, 1:1 randomization is preferred in large cohort trials, and AESI and pIMDs are identified and actively captured over a follow-up period of at least 12 months. Targeted laboratory screening and/or use of biomarkers of safety outcomes, and possibilities to collect and maintain banked serum/tissue specimens are also considered [8,28].

2.2. The mode of action of AS04-adjuvanted vaccines and links to reactogenicity and safety

Compared to an aluminum-adjuvanted vaccine, an AS04-adjuvanted vaccine induced higher levels of cytokines and chemokines at the injection site, with infiltration of higher numbers of dendritic cells and monocytes into the draining lymph node [7]. AS04 promotes antigen uptake by antigen-presenting cells when compared with aluminum, activating antigen-specific T-cells in the draining lymph node resulting in production of interferon (IFN)-γ and a Th1-biased immune response (Box 2). There was no evidence of immune dysregulation, or of mechanisms likely to cause or exacerbate immune-mediated diseases, such as direct stimulation of T-cells or an increase in the production of type 1 interferon [7,22].

The observed clinical reactogenicity and safety profile of AS04-HPV-16/18 and HBV/AS04 is consistent with what might be expected from the mode of action determined in animal models. Injection with AS04-HPV-16/18 or HBV/AS04 is characterized by reactogenicity at the injection site consistent with a local, transient inflammatory response occurring in the first hours to a few days after vaccination, and resolving within several days [29,30]. In clinical trials, local symptoms of pain, redness and swelling, and some general symptoms such as myalgia, were reported more frequently in recipients of the AS04-adjuvanted vaccines than in recipients of control vaccines. The incidence of serious AEs (SAE) and pIMDs was similar in AS04-HPV-16/18 and HBV/AS04 recipients and in controls [29,30]. Preclinical studies showed that immune factors such as direct T-cell activation, IFN-α induction and sustained levels of cytokines which are implicated in chronic or autoimmune diseases, were not observed after AS04 injection [7]. Consistent with this observation, comprehensive data from clinical trials and post-marketing evaluations do not support a causal link between AS04-adjuvanted vaccines and adverse pregnancy outcomes or the development of pIMDs (Table 1) [31–34].

Table 1
Post-marketing evaluations of vaccine safety in girls and women who received AS04-HPV-16/18.

Outcome of interest	Design	Data source	Population size and no. of cases	Main results
Pregnancy outcomes [24]	Pregnancy registry	UK, US. Voluntary enrolment through websites, GP, campaign materials	181 prospective evaluable reports: 154 live births, 14 spontaneous abortions, 1 stillbirth, 12 elective terminations, 18 infants born with a congenital anomaly	There was no cluster or constellation of congenital anomalies suggestive of possible teratogenesis. There was no evidence that vaccination with AS04-HPV-16/18 during the defined exposure period (within 60 days before conception until delivery) increases the risk of teratogenicity
Spontaneous abortion [31]	Retrospective observational cohort study	UK CPRD	207 exposed and 632 non-exposed women, with 24 and 57 cases of spontaneous abortion, respectively	No evidence of an increased risk: HR for spontaneous abortion in weeks 1–23 of gestation in women with first day of gestation from 30 days before to 45 days after any vaccine dose was 1.30 (95% CI 0.79–2.12)
Neuroinflammatory/ ophthalmic autoimmune disease and other autoimmune disease [32]	Retrospective observational cohort study	UK CPRD GOLD	65,000 exposed 9- to 25-year-old women and 195,000 non-exposed in different cohorts with 109 cases of confirmed autoimmune disease (3 neuroinflammatory/ophthalmic cases)	No evidence of increased risk: no case of confirmed neuroinflammatory/ophthalmic autoimmune disease in exposed subjects; adjusted IRR of other autoimmune disease: 1.41 (95% CI 0.86–2.31)
Autoimmune thyroiditis, GBS, IBD [35]	Meta-analysis	Data from 18 clinical trials and 4 post-licensure studies with primary and secondary data collection	154,398 exposed and 1,504,322 non-exposed subjects, age 9–25 years	Autoimmune thyroiditis variance-inverse meta-analysis OR 2.01 (95% CI 1.30–3.11) with an excess risk of 17 cases per 100,000 exposed subjects. The study does not confirm an association between vaccination and autoimmune thyroiditis. GBS: causal relationship meta-analysis 11.14 (95% CI 2.01–61.92) during the 42 days follow-up period. The study showed a small increased risk not confirmed due to the very small number of cases reported. IBD overall OR from variance-inverse meta-analysis was 1.11 (95% CI 0.75–1.66). There was no evidence of an increased risk of IBD following AS04-HPV-16/18
Potential immune-mediated events [34]	Integrated analysis of clinical trial data	Data from 42 randomized clinical trials	36,744 recipients of AS04-adjuvanted vaccines and 31,768 recipients of control vaccines, with 362 autoimmune events	Overall relative risk for developing an autoimmune disease was 0.98 (95% CI 0.80–1.21) and 0.92 (95% CI 0.70–1.22) in the AS04-HPV-16/18 analysis. There was no evidence for an increase in the relative risk of immune-mediated diseases associated with AS04 adjuvanted vaccines

GBS = Guillain-Barré Syndrome, IBD = Inflammatory Bowel Disease, CI = confidence interval, HR = Hazard ratio, UK CPRD = United Kingdom Clinical Practice Research Datalink, GP = general practitioner, IRR = incidence risk ratio, OR = odds ratio.

A large observational cohort study and a pregnancy registry in the UK and US did not show any evidence for an increased risk of spontaneous abortion in women inadvertently exposed to AS04-HPV-16/18 during pregnancy [24,31]. Additionally, there was no increase in the risk of teratogenicity in the offspring of women vaccinated within 60 days before conception until delivery [24,31].

A meta-analysis of clinical trial data was conducted to examine the risk of autoimmune thyroiditis, Guillain-Barré Syndrome (GBS) and Inflammatory Bowel Disease (IBD) in recipients of AS04-HPV-16/18 versus recipients of control vaccines [35]. There was a small increased risk of autoimmune thyroiditis in the cohort of AS04-HPV-16/18 recipients versus controls (OR 2.01, 95% CI 1.30–3.11), which equates to an excess risk of 17 cases per 100,000 exposed subjects. However, the meta-analysis concluded that there was insufficient evidence to prove a causal relationship with AS04-HPV-16/18 vaccination [35]. The analysis suggested an increased risk of GBS after AS04-HPV-16/18 (OR 11.14, 95% CI 2.01–61.92), which equates to an excess risk of <1 per 100,000 exposed subjects. But a conclusion could not be drawn because of the low number of cases and wide CIs around the estimate. The study showed no association between vaccination with AS04-HPV-16/18 and IBD (OR 1.11, 95% CI 0.75–1.66). It was concluded that the anticipated benefit of vaccination in preventing cervical cancer as reported in earlier efficacy studies and in more recent effectiveness studies [36] outweighs the potential risks. The European Committee for Medicinal Products for Human Use recently confirmed the favorable benefit/risk and the acceptable safety profile of AS04-HPV-16/18 following review of this analysis [37].

An observational cohort study showed no evidence of an increased risk of confirmed neuro-inflammatory/ophthalmic autoimmune diseases in subjects exposed to AS04-HPV-16/18 [32], and a meta-analysis of >68,000 individuals vaccinated with an AS04-formulated vaccine showed no increased risk of autoimmune events after vaccination compared to controls [34]. These results are consistent with the mode of action of AS04 which is local and transient.

2.3. Post-licensure challenges

The presence of a novel adjuvant drew media attention and invigorated public debate about theoretical safety concerns. In 2013, the Japanese health authority temporarily suspended vaccination with both available HPV vaccines (AS04-HPV-16/18 and the quadrivalent HPV vaccine) after reports of Complex Regional Pain Syndrome (CRPS) and Postural Orthostatic Tachycardia Syndrome (POTS) occurring after HPV vaccination. All AEs following immunization submitted to GSK through spontaneous reporting from worldwide sources are coded into the safety database using the International Conference on Harmonisation Medical Dictionary for Regulatory Activities (MedDRA). Comprehensive evaluation of potential CRPS cases in the database did not show any increase in CRPS after vaccination and no safety concern was identified [38]. Additional reviews conducted by European Medicines Agency (EMA) and the WHO Global Advisory Committee on Vaccine Safety confirmed that there was no evidence supporting a causal link between HPV vaccine (from either manufacturer) and the development of CRPS or POTS [39,40]. US and Australian agencies have since endorsed the conclusions of the EMA and WHO, and continue to actively recommend HPV vaccination based on the positive benefit-risk ratio [39–42]. In Japan, coverage of the first dose of HPV vaccine decreased from approximately 73–77% to <1% after the suspension of the vaccine recommendation [43]. Pervasive negative media coverage and the ongoing government suspension of proactive recommendations continue to undermine confidence in HPV vaccines in Japan [43].

3. AS03: Lessons on how vaccine mode of action and nonclinical research inform safety evaluations

The number of influenza vaccines available for production is limited by the amount of available antigen. Therefore, antigen sparing (produce the maximum possible number of doses with the least antigen content needed to be effective) is potentially critical for a global response necessary in the event of an influenza pandemic. To this end, an H5N1/AS03 vaccine was developed as part of pandemic preparedness advocated by the WHO [44]. AS03 contains α-Tocopherol (Vitamin E), squalene and polysorbate 80 in an oil-in-water emulsion (Box 2). AS03 was selected for use in the pre-pandemic H5N1 influenza vaccine because H5N1/AS03 induced higher neutralizing antibody responses, allowed antigen sparing and induced cross-clade protection compared to unadjuvanted influenza vaccine [12]. Furthermore, AS03 is relatively easy to manufacture and large quantities can be produced rapidly. H5N1/AS03 was evaluated in clinical trials conducted in >10,000 adults and children [12], and was licensed in Europe in 2009 for use in an officially declared pandemic situation [45], and in 2013 in the US for prevention of H5N1 disease [46]. When the A/California/07/2009 (H1N1) pandemic was declared in April 2009, AS03 was selected for the H1N1 vaccine based on the experience with H5N1/AS03. By the time H1N1/AS03 was deployed, a large clinical database using AS03 with pre-pandemic H5N1, H1N1 and seasonal influenza vaccines in different populations was available, showing an acceptable safety profile of AS03-adjuvanted influenza vaccines.

In clinical trials, adults vaccinated with H1N1/AS03 reported pain at the injection site, fatigue and myalgia, and higher rates of local and systemic symptoms than recipients of control vaccines [12]. Rates of fever were higher in children who received H1N1/AS03 than in controls, with increased rates of fever after the second dose. However, there was no difference between H1N1/AS03 vaccinees and controls in the occurrence of medically-attended AE or SAE.

After authorization, specific pharmacovigilance activities were required by the EMA. This included a clinical trial of at least 9,000 persons, special monitoring of a predefined list of AESI, specific monitoring of particular population groups (such as pregnant women) and comprehensive monthly safety reports from manufacturers [47,48].

It is estimated that >90 million doses of H1N1/AS03 were administered globally during the H1N1 pandemic [49]. Post-licensure data showed a favorable benefit-risk profile in various populations including young children, immunocompromised persons and pregnant women [50–55]. In a systematic review that included four studies of children aged 6 months to 12 years who received H1N1/AS03, injection site pain was the most frequently reported symptom (incidence 31.7–84.6%). The incidence of fever was 11.0–23.8%. Compared with non-adjuvanted vaccines, children who received H1N1/AS03 had similar rates of unsolicited AE including convulsion and SAEs after vaccination. No SAEs were considered to be vaccine-related by the investigators [56]. A systematic review that assessed the safety of H1N1/AS03 in immunocompromised persons showed that the vaccine was well tolerated, and no specific safety concerns were raised [57]. A post-authorization study that included 267 pregnant women vaccinated with H1N1/AS03 showed no increased risk of pregnancy loss, congenital malformations, preterm birth or low birth weight [52].

GBS was actively monitored during the pandemic because of a previous association between an influenza vaccine and GBS in the 1970s [58]. Investigation of spontaneous AE reporting to EudraVigilance did not suggest a difference in autoimmune disease (including GBS) reported after adjuvanted or non-adjuvanted H1N1 vaccines [59]. A large multi-country self-controlled case

series in Europe found no significant increase in GBS after pandemic H1N1 vaccination (mostly AS03-adjuvanted vaccine) [60]. Smaller self-controlled case-series in Europe and Canada suggested an association between pandemic H1N1 vaccination and GBS, but given the rarity of the event and the observed benefits of adjuvanted pandemic vaccines, did not recommend changes to existing recommendations [61,62].

Reports of narcolepsy in Sweden and Finland toward the end of the pandemic were investigated by observational studies in Europe with associations between H1N1/AS03 (specifically, *Pandemrix*, and not *Arepanrix*) and narcolepsy that varied widely in magnitude [63,64]. Epidemiological data currently available to GSK suggest an increased risk of narcolepsy following vaccination with *Pandemrix*. Due to the methodological limitations of the studies, which are all retrospective observational studies, further research was needed to evaluate a potential causal relationship [63]. However, comprehensive clinical evaluation of this signal was, and continues to be challenging. This is because use of the vaccine ceased after the 2010–2011 northern hemisphere influenza season, and because of the nature of narcolepsy itself. Narcolepsy is difficult to diagnose, has no pathognomonic symptom or test, and has a multifactorial causality including genetic susceptibility. Moreover, the estimated risk of narcolepsy was negligible for another AS03-adjuvanted vaccine manufactured slightly differently in Canada (*Arepanrix*) [65], and subsequent investigations found no evidence of any differences in the immune response induced by either vaccine [66,67]. As yet, available data and technologies do not allow understanding of the immune mechanisms that led to narcolepsy during the 2009 H1N1 epidemic, or the relative role of vaccination versus recent A/H1N1pdm09 influenza virus infection [65,68–71]. Later research showed that the mechanisms may involve A/H1N1pdm09 antigen mimicry, which is more likely to explain the increased risk of narcolepsy observed with *Pandemrix* than hypotheses that are based on a direct role for AS03 [72,73]. In 2016 the EMA concluded that based on the evidence generated to date, the benefit-risk balance for *Pandemrix*, previously determined as being favorable, remained unchanged, and GSK's commitment to investigate the signal was closed by the EMA the same year [74].

4. AS01: Implementation of previous experience and challenges linked to assessing safety in different populations and settings

AS01 is a liposome-based AS that includes two immune stimulants, MPL and QS-21, a saponin molecule extracted from the bark of *Quillaja saponaria* Molina, a tree found in South America (Box 2). MPL and QS-21 appear to work synergistically to enhance antigen-specific response through a mechanism involving an early induction of IFN-γ in the draining lymph node which in turn, promotes a strong Th-1 response with production of antigen-specific T-cells [15].

AS01 was selected for use in two vaccines targeting different pathogens and populations; RZV and the malaria vaccine RTS,S/AS01. Malaria is a parasitic disease with a complex life-cycle and the target populations for vaccination are infants and children living in Africa. By contrast, herpes zoster is caused by reactivation of the varicella zoster virus (VZV) after a previous varicella infection. Whereas malaria parasites gain access to the human host through direct inoculation via a mosquito bite, VZV lies dormant for long periods in neural sensory ganglia. Reactivation occurs with immune suppression or age-related declines in immunity, especially declines in VZV-specific T-cell immunity. While severe malaria is most common in young children, herpes zoster is most common in older adults and persons with immunosuppressive conditions.

Based on the different ages and characteristics of the target population as well as the antigen administered, the safety profiles of RTS,S/AS01 and RZV were expected to differ. The range of underlying medical conditions is vastly dissimilar among older adults in industrialized countries and young children living in Africa, special considerations such as frailty (RZV) and clinical trial conduct in the African setting (RTS,S/AS01) needed to be anticipated.

4.1. A malaria vaccine for infants and children living in Africa

RTS,S/AS01 contains the recombinant RTS hybrid antigen (a portion of the *Plasmodium falciparum* circumsporozoite protein fused to hepatitis B surface antigen. The fused antigen is expressed together with unfused HBsAg in yeast, RTS,S) with $AS01_E$ (25 μg of MPL and 25 μg of QS-21). The development of RTS,S/AS01 took place in African malaria endemic regions among communities with a low socio-economic level, issues with access to healthcare, and with a mixed understanding of the cause of malaria. The target population of young children had high levels of comorbidity. A markedly different approach for the assessment of safety was needed in this setting compared to RZV, such as monthly home visits by study staff to identify SAE, and verbal autopsies performed on all out-of-hospital deaths [75].

In clinical trials, the most frequently reported events after RTS,S/AS01 primary vaccination were fever (27% of vaccine doses), pain (16%), irritability (14%), and swelling (7%) [76]. Febrile convulsions within 7 days after vaccination occurred following 0.1% of primary vaccination doses and following 0.25% after the fourth vaccine dose. In the phase 3 trial, children who received RTS,S/AS01 experienced fewer SAE post vaccination than controls who received rabies or meningococcal vaccines [77,78]. There was a statistically significant imbalance in meningitis cases of any cause among 5- to 17-month-old recipients of RTS,S/AS01 versus controls who received rabies vaccine [75,79]. In total, 21 cases of meningitis were reported among 5,948 vaccine recipients followed for approximately 4 years, versus one case in 2,974 controls [75,76,79]. Interpretation of this finding continues to be discussed, but no causal relationship between RTS,S/AS01 vaccination and meningitis has been established. The majority of the meningitis cases were reported in two study sites, no temporal relationship to vaccination was established, and no pathogen was identified in >50% of cases. On one hand some experts have called for caution and further investigation prior to the commencement of large-scale roll-out programs in Africa [80,81]. On the other hand, others consider the single case in the control group an unexpected finding and have hypothesized that a protective effect of rabies vaccination might potentially explain the observed imbalance, rather than a detrimental effect of RTS,S/AS01 [82]. The EMA and WHO have considered the imbalance of meningitis cases to be likely a chance finding. Any potential risks need to be put into context of the potential benefit.

Gender-specific observations on mortality during phase 3 showed that RTS,S/AS01 appeared to be associated with a higher risk of death (all-cause) in girls, but not in boys. This was observed overall (risk ratio for all-cause mortality in girls was 1.91 [95% CI 1.30–2.79]), and for each age subgroup (risk ratio 2.00 [95% CI 1.18–3.39] in 5- to 17-month-old girls and 1.81 [95% CI 1.04–3.14] in 6- to 12-week-old girls) [83,84]. However, the percentage of deaths in the control groups was unequal between boys and girls (2.2% versus 1.3%, respectively), which makes it unclear if the signal in girls resulted from an unusually low death rate in the control group. Furthermore, potential confounding due to study site,

underlying health status and 70% reduced mortality in trial participants versus the general population was not controlled [85]. None of the deaths were considered by the investigator to be related to vaccination, and there was no obvious pattern or clustering in terms of cause of death, which included a wide range of mainly infectious, nutritional and accidental etiologies [84].

4.2. Preventing reactivation of VZV in older adults

RZV is a combination of 50 μg of VZV glycoprotein E and $AS01_B$ (50 μg of MPL and 50 μg of QS-21). In phase 3 trials, RZV showed >90% efficacy in preventing herpes zoster in all ≥ 50-year age groups, including adults aged 80 years and over [86,87]. RZV also prevented post-herpetic neuralgia, the most common complication of zoster disease, in 91.2% of adults aged ≥50 years, including 71.2% of ≥80-year adults [87,88].

Previous experience with AS04 and AS03, and with AS01 evaluated in adults [89] had suggested that RZV vaccination might be associated with local reactogenicity. The RZV clinical development was therefore adapted to assess reactogenicity in detail, including planned analyses stratifying by co-factors such as gender, age strata, ethnicity, and comorbid conditions. The potential impact of symptoms after vaccination on compliance with the second dose and on quality of life, were also examined.

In clinical trials, the reactogenicity profile of RZV was characterized by injection-site symptoms (mainly pain), and general symptoms after vaccination that were mild-to-moderate in intensity and short-lived (median duration 2–3 days) [87]. This reactogenicity profile is consistent with the mode of action of AS01 described in pre-clinical and clinical studies. These studies showed that the inflammation induced by AS01 is transient at the injection site (muscle), (peaking at Day 1 and returning to baseline by Day 2–3), in the draining lymph node and the systemic circulation [16,90].

The incidences of local symptoms and general symptoms such as myalgia, fatigue and headache were more frequent among RZV recipients than in controls who received placebo, but did not appear to affect willingness of the participants to receive the second dose [91]. AE were evaluated according to main System Organ Class as well as by Preferred Term to capture the range of medical conditions common to this age group. In the large pivotal studies of RZV, the control group and the 1:1 randomization ratio were critical in facilitating interpretation of the safety results. Rates of SAE, pIMDs and deaths were similar among RZV and placebo recipients in phase 3 trials and no safety signal was identified. RZV received first approval for use in adults ≥50 years in October 2017 in Canada and the US, and is now also licensed in Europe, Japan and Australia.

There is a medical need to prevent herpes zoster in other populations, including immunocompromised individuals. A separate development program was launched for individuals aged 18 years and over with immunocompromising conditions. Due to the nature of the population being studied (patients with a wide range of underlying conditions such as HIV infection, cancer, or patients undergoing organ transplant, chemotherapy, or other immunosuppressive regimens), the set-up and execution of these studies was more complex than studies that assessed RZV in older adults. Safety monitoring needed to capture events such as deterioration in laboratory parameters used to monitor disease progression, exacerbations of the underlying condition, or transplant rejection. These safety outcomes were tailored for individual patient cohorts because of the different diseases under study. A large number of AEs during clinical trials were anticipated, many linked to the underlying condition or treatments, and a 6–12 month AE screening period was considered in some studies to collect baseline information about the underlying disease. RZV demonstrated efficacy of 68.17% (95% CI 55.56–77.53) in preventing herpes zoster in autologous hematopoietic stem cell transplant recipients [92,93]. RZV had an acceptable clinically safety profile in this population, with injection-site pain and fatigue reported most frequently after vaccination. Incidences of SAEs, pIMDs and importantly, relapses of underlying disease were similar between vaccinated and placebo groups [92].

4.3. Contrasting approaches to post-licensure/post-implementation safety monitoring

There are important differences in the post-licensure safety monitoring being undertaken for RZV and RTS,S/AS01 that reflect the populations and settings in which they are used. Initially, RZV will be used in industrialized countries where surveillance systems for monitoring vaccine safety are well-established. Aside from routine pharmacovigilance activities that include systematic review of spontaneous AE reports, aggregate safety data and relevant literature [19,20], the Risk Management Plan for RZV includes a targeted safety study to investigate the occurrence of a pre-selected list of pIMDs in ≥50-year adults. As RZV is implemented in clinical practice over the coming years, important real-world information on reactogenicity and safety will become available.

By contrast, RTS,S/AS01 is yet to be used in the field. In 2016 the WHO recommended pilot implementation of RTS,S/AS01 in children of at least 5 months of age to assess operational feasibility of vaccine provision to the target age-group, and to evaluate safety, effectiveness and impact on child mortality when used in real-life settings [94]. Background incidence rates of many diseases in sub-Saharan Africa where RTS,S/AS01 will be deployed are not known, which could prevent meaningful interpretation of post-implementation safety information [95]. The WHO-led malaria vaccine pilot implementation program is therefore using a cluster randomized design to allow concomitant comparison between vaccinated and control clusters. A large GSK-sponsored baseline study was designed to measure background rates of AESIs including meningitis, convulsions, cerebral malaria and several pIMDs, as well as AE leading to hospitalization or death (NCT02374450). These same events will be monitored in a second GSK-sponsored phase IV study after the onset of vaccination [96].

5. Conclusion

Three AS are included in licensed or approved vaccines targeting widely diverse populations living in varied economic settings. Their development and safety evaluation has many features in common with that of other vaccines. However, beginning with AS04, the development of AS-adjuvanted vaccines has required new strategies for safety evaluation, including new exploration of their mode of action. Thirteen years of post-licensure experience in a range of populations have progressed the way that adjuvanted vaccine safety is investigated and understood. A key learning has been that of the application of the mode of action to further interpret safety outcomes. Exploration of the mode of action of an adjuvant in relation to reactogenicity and safety is now a pivotal component of the safety evaluation of new adjuvanted vaccines. This has provided key information to begin unraveling the mechanisms underlying reactogenicity, and to provide evidence around the biological plausibility of relationships of certain AE with vaccination. Recent studies provide interesting evidence of potential relationships between the innate response to vaccination and subsequent reactogenicity and immunogenicity, and these continue to be explored [8,16,97]. In this space, exploration and identification of biomarkers of reactogenicity and implementation of a 'system biology' approach could revolutionize how vaccines are developed [28,98].

The benefit-risk balance for vaccines containing AS has been determined to date as being favorable. The anticipated benefit of vaccination on infectious diseases that cause significant global morbidity and mortality, either directly or through the development of cancer, greatly outweighs any potential risks [99].

A key learning during the development of vaccines adjuvanted with AS is that each vaccine requires specific strategies for safety monitoring, because experience with a specific AS-antigen combination or in a specific population/setting is not necessarily relevant to other diseases or populations.

AS have fundamentally changed the adjuvanted-vaccine landscape, allowing development of new effective vaccines not previously possible using classical adjuvants. AS are promoting new understanding in the field of immunology and the links between the immune response and safety. Standard methods developed to assess the safety of vaccines containing AS are leading the way in the evaluation of vaccine safety. These methods will guide and support new candidate vaccine development, particularly those using new adjuvants, beyond AS.

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Contributions

All authors attest they meet the ICMJE criteria for authorship. All authors contributed to assembling and interpreting the data, and contributed to the development of the manuscript. All authors critically reviewed the manuscript and approved the final version.

Declaration of Competing Interest

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