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Review

Safety of AS03-adjuvanted influenza vaccines: A review of the evidence

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ABSTRACT

Clinical and post-licensure data have demonstrated that AS03-adjuvanted inactivated split virion vaccines, many with reduced antigen content, are effective against influenza infection. The objective of this review is to provide a comprehensive assessment of the safety of trivalent seasonal, monovalent pre-pandemic and pandemic AS03-adjuvanted influenza vaccines, based on non-clinical, clinical and post-licensure data in various populations. Non-clinical studies on local tolerance, toxicology and safety pharmacology did not raise any safety concerns with AS03 administered alone or combined with various influenza antigens. Data from clinical trials with over 55,000 vaccinated subjects showed that AS03-adjuvanted influenza vaccines were generally well tolerated and displayed an acceptable safety profile, although the power to detect rare events was limited. Approximately 90 million doses of A/H1N1pdm09 pandemic influenza vaccines (*Pandemrix* and *Arepanrix* H1N1) were administered worldwide, which contributed post-licensure data to the collective safety data for AS03-adjuvanted influenza vaccines. An association between *Pandemrix* and narcolepsy was observed during the A/H1N1pdm09 pandemic, for which a role of a CD4 T cell mimicry sequence in the haemagglutinin protein of A/H1N1pdm09 cannot be excluded. Provided that future AS03-adjuvanted influenza vaccines do not contain this putative mimicry sequence, this extensive safety experience supports the further development and use of AS03-adjuvanted inactivated split virion candidate vaccines against seasonal and pandemic influenza infections.

Contents

Abbreviations: AE, adverse event; AESI, adverse event of special interest; CCR, chemokine (C-C motif) receptor; GBS, Guillain-Barré syndrome; HIV, human immunodeficiency virus; MAE, medically-attended adverse event; PASS, post-authorisation safety study; pIMD, potential immune-mediated disease; SAE, serious adverse event; SCCS, self-controlled case series; SOMNIA, systematic observational method for narcolepsy and influenza immunization assessment; TIV, trivalent influenza vaccine; VAESCO, vaccine adverse event surveillance and communication.

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1. Introduction

Adjuvants are substances included in vaccines to enhance the immunogenicity of antigens with insufficient immunostimulatory capabilities [1]. Better understanding of how the immune system interacts with pathogens has contributed to the development of adjuvants containing more than one immunostimulatory molecule, known as Adjuvant Systems. AS03 is an Adjuvant System containing a surfactant, polysorbate 80, and two biodegradable oils, α-tocopherol and squalene, in phosphate-buffered saline as the aqueous carrier [2]. AS03 adjuvant capabilities derive from the oil-in-water emulsion phase as well as from α-tocopherol, for which immunostimulatory properties have been described [3]. AS03 activates the innate immune response at the injection site which, after a cascade of reactions, enhances adaptive immune responses to the vaccine antigen [2] (Fig. 1).

AS03 has been evaluated in parallel with other Adjuvant Systems in the development of vaccines containing various antigens, including split influenza antigens [1]. Several formulations of AS03 were tested at preclinical stage. $AS03_A$ and $AS03_B$, two of the variants selected for further development, had similar profiles with regards to safety and toxicology. During clinical development, dose finding studies investigated the optimal balance of immunogenicity and reactogenicity in children and in adults. The final approved formulation of a split-virion with $AS03_A$ adjuvant in a 0.5 mL dose for adults contained 3.75 μg purified hemagglutinin, which is the antigen dose, and 0.69 mg squalene, 11.86 mg DL-α-tocopherol, and 4.86 mg polysorbate 80. A 0.25 mL dose for children contained half of these amounts [4–6].

AS03 enhances antibody and T-cell responses to haemagglutinin and other viral proteins in the split antigen [7,8]. This is important because of the limited immunogenicity of antigens with

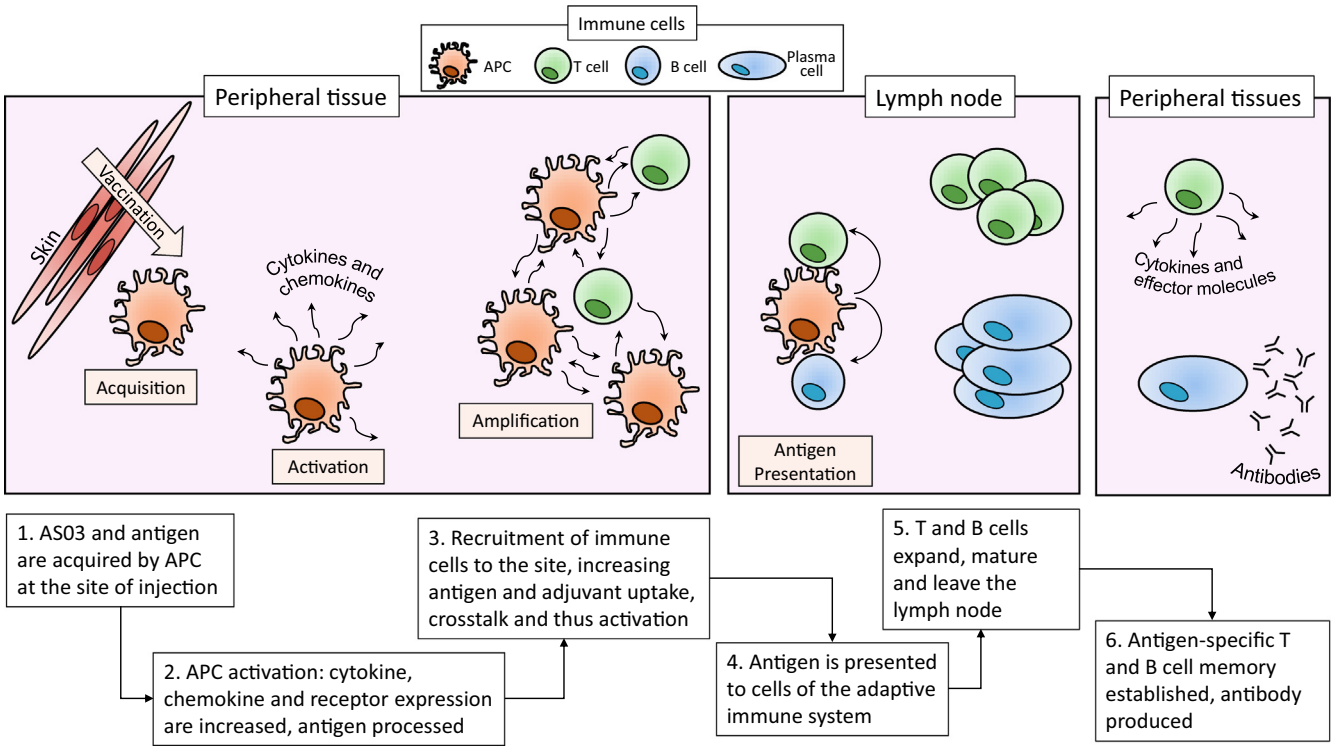

Fig. 1. AS03 mechanism of action.

pandemic potential, including those from highly pathogenic avian strains, which may result from relatively low frequencies of cognate memory B and CD4 T cells for pandemic influenza strains. This implies that vaccines containing such antigens need to mobilise naïve B and T cell repertoires [9]. Thus, with limited pre-existing immunity, induction of strong adaptive immune responses requires stronger innate immune stimulation and, hence, adjuvantation [9]. The immune enhancing properties of AS03 also result in a marked antigen-sparing effect [7], which is critical given the limited global pandemic influenza antigen manufacturing capacity.

The first AS03-adjuvanted influenza vaccines targeted highly pathogenic avian H5N1 influenza strains; two licensed vaccines against the influenza A H5N1 virus are available today. Compared to unadjuvanted vaccines, these are more immunogenic, elicit cross-reactive immunity against sub-clade variants and permit antigen sparing [7,10]. Based on this experience, *Pandemrix* and *Arepanrix* H1N1, the two AS03-adjuvanted split-virion vaccines against the A/California/7/2009 H1N1 strain, were developed and licensed during the A/H1N1pdm09 pandemic. *Pandemrix* was manufactured in Germany and used mostly in Europe, while *Arepanrix* H1N1 was manufactured in Canada and used mostly in the Americas. Subsequent observational post-licensure data demonstrated the effectiveness of AS03-adjuvanted vaccines with reduced antigen content in protecting against pandemic H1N1 influenza infection and hospitalisation [11]. During 2010 to 2011, the efficacy of *Arepanrix* H1N1 relative to that of a non-adjuvanted H1N1 vaccine (same virus strain but formulated at a conventional dose) was 76.8% for prevention of H1N1 illness in children aged 6 months to 9 years in a multinational study [12]. There was also evidence from a randomised controlled trial of higher efficacy with AS03-adjuvanted trivalent influenza vaccine (TIV) as compared to non-adjuvanted TIV in preventing infection with some influenza subtypes and improved protection against death and pneumonia in persons aged 65 years or older [13]. Moreover, AS03-adjuvanted vaccines in development against other influenza A subtypes with pandemic potential (H7N1, H7N9 and H9N2) are acceptably immunogenic at antigen-sparing doses in adults [14–17].

Extensive safety data are available from the clinical development programme of AS03-adjuvanted vaccines against different influenza subtypes and from post-licensure experience, including pharmacovigilance and safety studies of AS03-adjuvanted A/H1N1pdm09 vaccines (Table 1). This comprehensive review examines these safety data, which were collected worldwide in non-clinical, clinical, pharmacovigilance and post-licensure settings in various populations, including high-risk groups.

Table 1
Scope of studies that have examined the safety of different AS03-adjuvanted influenza vaccine formulations.

Species/population assessed	Influenza subtype	HA dose (μg)	Adjuvant	Safety parameters assessed	Ref.*
Non-clinical studies					
Rabbit	N/A	N/A	$AS03_A$, AS01, AS15	Clinical, macroscopic and microscopic observations; inflammation biomarkers	[18]
	D H5N1 or Q H5N1	30 or 3.75	$AS03_A$	Clinical, macroscopic and microscopic observations; haematology, lymph node parameters	[19]
Rat	Q H5N1 or Q H1N1	0.75 or 1.5	$AS03_A$	Reproductive and developmental	[20]
	N/A	N/A	$AS03_A$ and (containing MPL) AS01, AS15	Cardiovascular and respiratory functions	[21]
	H1N1	0.75	$AS03_A$	Central nervous system toxicity, inflammation biomarkers	[22]
Beagle dog	N/A	N/A	$AS03_A$ and (containing MPL) AS01, AS04, AS15	Cardiovascular and respiratory functions	[21]
Clinical trials					
Adults (≥18 years)	D H5N1 or Q H5N1	1.9, 3.75, 7.5, 15, 30	$AS03_A$ or $AS03_B$	AEs, MAEs, SAEs, pIMDs, AESIs†	[23–25,36]
	D H1N1 or Q H1N1	1.9, 3.75	$AS03_A$ or $AS03_B$		[26–29,36]
	Q H7N1	3.75, 7.5	$AS03_A$ or $AS03_B$		[15,16]
	Q H7N9	3.75, 7.5, 15, 45	AS03 or MF59		[14]
	Q H9N2	1.9, 3.75	$AS03_A$ or $AS03_B$		[17]
Children	D H5N1 or Q H5N1	1.9, 3.75	$AS03_A$ or $AS03_B$		[30,31]
	D H1N1 or Q H1N1	1.9, 3.75	$AS03_A$ or $AS03_B$		[12,32–34]
Older adults (≥65 years)	Seasonal virus	15 (per strain)	$AS03_A$, $AS03_B$ or $AS03_C$ (with or without MPL in one study)		[13,38,39]
Children (6–35 months)	Seasonal virus	7.5 (per strain)	$AS03_B$, $AS03_C$ or $AS03_D$		[37]
Post-licensure safety studies‡					
Vaccinated population	D H1N1 (*Pandemrix*) or Q H1N1 (*Arepanrix* H1N1)	3.75**	$AS03_A$**	AEs, MAEs, SAEs, pIMDs, AESIs†, safety in special populations (pregnant women, immunocompromised individuals, solid organ transplant recipients)	[43–53,77–87,89,90,94,97–106,108–111]

AE, adverse event; AESI, adverse event of special interest; HA, haemagglutinin; MAE, medically-attended adverse event; pIMD, potential immune-mediated disease; SAE, serious adverse event.

$AS03_A$, $AS03_B$, $AS03_C$ and $AS03_D$ contain 11.86, 5.93, 2.97 and 1.48 mg of α-tocopherol per dose, respectively. D H5N1 and D H1N1 manufactured by GSK in Dresden, Germany. Q H5N1 and Q H1N1 manufactured by GSK in Quebec, Canada.

* For clinical trials: key publications discussed in this review. For list of studies that provided reactogenicity and safety data on AS03-adjuvanted influenza vaccines, see Supplementary Table 1.

† Adverse events of special interest (identified by Committee for Medicinal Products for Human Use of European Medicines Agency) include anaphylaxis, Bell's palsy, convulsion, demyelination, encephalitis, Guillain-Barré syndrome, neuritis and vasculitis.

‡ See Table 3 and Supplementary Table 2.

** Vaccine contents for individuals aged ≥10 years. Children aged 6 months to 9 years received half dose.

2. Non-clinical experience

Non-clinical local tolerance, toxicology and safety pharmacology studies were conducted with AS03 alone or combined with monovalent pandemic H5N1 or H1N1 influenza antigens (Table 2). A study of New Zealand white rabbits found that AS03 was well tolerated locally when administered alone as a single dose, with no distinct clinical signs or local reactions related to intramuscular injection detected by macroscopic examination [18]. In repeated dose toxicity studies of rabbits, there were no treatment-related effects on physiological functions following three or four intramuscular injections containing H5N1 antigen with or without AS03 [19]. In both studies, minor, transient histopathological changes at the injection site suggested an acute phase response to the adjuvanted vaccine and a subacute inflammatory response at the injection site induced by AS03 alone and in combination with H5N1 antigen [18,19]. There was no evidence of mutagenic effects in any bacterial test strain when AS03 was tested *in vitro* in the Ames test or reproductive or developmental toxicity of AS03 administered to rats alone or in combination with influenza antigens [20].

In safety pharmacology studies of rats and beagle dogs that received a single dose of AS03, another Adjuvant System or saline, there were no safety concerns, with no evidence of notable treatment-specific changes in the cardiorespiratory function of the test animals [21]. Moreover, a study conducted in cotton rats showed no central nervous system toxicity following injection of AS03 alone or combined with H1N1 [22].

These findings are in line with the likely mode of action of AS03 (Fig. 1), as determined from experiments performed in mice and with human cells [3], in which the adjuvant effect of AS03 was

Table 2
Non-clinical studies conducted with AS03 on local tolerance, toxicity and safety pharmacology.

Study type	Route of administration	Species	Treatment regimen	Safety parameters assessed	Main safety findings	Ref.
Single-dose toxicity/local tolerance	IM	New Zealand white rabbits	$AS03_A$, AS01, AS15, DTPw-HB, aluminium phosphate, aluminium hydroxide or saline	Clinical signs, macroscopy (injection site), microscopy (injection site), haematology, inflammatory biomarkers (CRP and fibrinogen levels) over 7 days after administration	AS03 injection associated with mild inflammation on histopathological evaluation. CRP and fibrinogen levels increased after injection of AS and DTPw-HB, peaking at day 1 and accompanied by increases in circulating heterophils	[18]
Repeat-dose toxicity	IM	New Zealand white rabbits	Experiment 1: Q H5N1 (30 μg HA) + $AS03_A$ or saline administered on days 0, 14, 28 Experiment 2: Q H5N1 (3.75 μg HA) + $AS03_A$ or saline administered on days 0, 14, 28 Experiment 3: D H5N1 (30 μg HA) + $AS03_A$, D H5N1 (30 μg HA), $AS03_A$ or saline administered on days 0, 14, 28, 42	Clinical signs, clinical chemistry and haematology parameters, lymph node weight, macroscopy (injection site), microscopy (injection site, sciatic nerve, draining lymph nodes, spleen) up to 28/29 days after last dose	Treatment-related effects restricted to mild inflammatory responses induced primarily by test articles containing AS03. Injection site inflammation mild at 3 days and minimal at 4 weeks after last injection. Signs of activation in draining lymph nodes and systemic effects in blood including transient increase of neutrophils	[19]
Reproductive and developmental toxicity	IM	Sprague Dawley rat	Experiment 1: $AS03_A$, Q H5N1 (1.5 μg HA) + $AS03_A$ Experiment 2: $AS03_A$, D H1N1 (0.75 μg HA) + $AS03_A$	Clinical signs, body weight, food consumption, gross pathology, uterine weight, numbers of corpora lutea, implantations, resorptions, live and dead foetuses, foetal weights; external, visceral, skeletal and foetal head morphology	No evidence of reproductive or developmental toxicity of AS03 alone or in combination with influenza antigens	[20]; unpublished results
CNS toxicity	IM	Cotton rat	2 or 3 injections at 2-week intervals of H1N1 (0.75 μg HA) + $AS03_A$, H1N1 (0.75 μg HA), $AS03_A$ or saline with or without preceding H1N1 influenza infection	CNS inflammation and blood brain barrier permeability parameters observed up to 3 months after the last dose	No differences between groups in CNS parameters (microglia activation, inflammatory infiltrates in the brain, albumin extravascular leakage, CSF hypocretin levels)	[22]
Safety pharmacology	IV	Anaesthetised rats	$AS03_A$, AS01, AS15 (140 times equivalent dosages in humans) or saline	Arterial pressure, heart rate, ECG, respiratory parameters from 30 min pre-dose to 120 min post-dose	No consistent treatment-related effects	[21]
	IM	Telemetered conscious beagle dogs	Saline at day 0 and $AS03_A$, AS01, AS04 or AS15 (7 times equivalent dosages in humans) on day 7	Body temperature, heart rate, arterial pressure, ECG, respiratory parameters from 1.5 h pre-dose to 72 h after AS administration. Bodyweight measured for 14 days	Decrease in food consumption and mean bodyweight with AS03 and slight increase in mean body temperature with AS01, AS03 and AS15 not considered to be adverse. No cardiovascular or respiratory effects with AS03	

CNS, central nervous system; CRP, C-reactive protein; DTPw-HB, diphtheria-tetanus-whole cell pertussis-hepatitis B vaccine; ECG; electrocardiogram; HA, haemagglutinin; IM, intramuscular; IV, intravenous.

shown to be dependent on spatial and temporal co-localisation with the vaccine influenza antigens. Overall, the non-clinical results indicated that the adjuvant effect of AS03 on antibody response after injection is limited in space and time, with an absence of systemic effects, and that AS03 contributes to enhancing antigen-specific immune responses.

3. Safety data from clinical trials

Over 55,000 subjects have been vaccinated with at least one dose of an AS03-adjuvanted influenza vaccine (approximately 25,000 with pandemic and 30,000 with seasonal influenza vaccines) since 2006 in completed and ongoing clinical trials worldwide. An overview of the design, size and results of clinical trials that provided reactogenicity and safety data is given in Supplementary Table 1.

AS03-adjuvanted influenza vaccines were generally well tolerated in clinical trials of adults and children. Dose ranging was performed in children 3–9 years of age enabling the selection of half an adult dose (i.e. 0.25 mL containing 1.9 μg HA/AS03$_B$) for subsequent development. An increase in reactogenicity, both injection site (pain mainly) and general symptoms (such as irritability and drowsiness in young children; myalgia, headache and fatigue in older children and adults), was observed following the administration of AS03-adjuvanted H1N1 or H5N1 influenza vaccines versus non-adjuvanted vaccines or placebo [12,23–33]. These symptoms were mostly mild to moderate in intensity and of short duration.

In children, especially those younger than 3 years, some reactions, including fever, tended to increase after the second dose [34]. In a phase II/III randomised, placebo-controlled, observer-blind trial of 838 vaccinated children aged 6 months to 17 years [31], rates of injection site reactions and general solicited symptoms during 7-day post-vaccination periods were higher with the AS03-adjuvanted H5N1 vaccine than with placebo. However, the study did not reveal any safety concerns, with similar frequencies between groups of unsolicited adverse events (AEs), medically-attended AEs (MAEs), serious AEs (SAEs) and potential immune-mediated diseases (pIMDs). In a large randomised comparison of AS03-adjuvanted H1N1 vaccine versus unadjuvanted H1N1 vaccine in 6145 children aged 6 months to 9 years, injection site pain was more frequent after the adjuvanted vaccine than after unadjuvanted vaccine but there was no increase in MAEs, SAEs or pIMDs relative to active control [12].

In a large study of adults conducted in North America, involving 3072 aged 18–64 years and 1489 aged 65 years or older, the AS03-adjuvanted H5N1 vaccine was more reactogenic than placebo [23]. In the phase III, placebo-controlled, randomised observer-blind study, temporary injection site reactions were more frequent and the incidence of solicited general AEs tended to be higher in the vaccine group within the 7 days of observation after vaccination. The H5N1 vaccine was, however, generally well tolerated and frequencies of unsolicited AEs, MAEs and SAEs were similar between groups. Reports of pIMDs were rare, not temporally clustered and none were considered by the investigators to be vaccine-related.

Frequencies of AEs of special interest (AESIs), as defined by the Committee for Medicinal Products for Human Use of the European Medicines Agency [35], were assessed in a pooled analysis of 22,521 adults who received AS03-adjuvanted H5N1 or H1N1 vaccines or control (non-adjuvanted vaccine or saline placebo) in 28 clinical trials [36]. The AESIs included anaphylaxis, Bell's palsy, convulsion, demyelination, encephalitis, Guillain-Barré syndrome (GBS), neuritis and vasculitis. The relative risk for experiencing any AESI (AS03-adjuvanted vaccine/control) was 1.2 (95% confidence interval [CI]: 0.9, 1.6). Overall, the pooled analysis results suggested no association between AS03-adjuvanted H5N1 and H1N1 influenza vaccines and reporting of AESIs, MAEs, SAEs or pIMDs, although statistical power was limited, especially to detect rare events such as pIMDs.

Likewise, no safety concerns were identified in studies assessing an AS03-adjuvanted seasonal TIV candidate containing half the emulsion dose used for pandemic vaccines [13,37–39]. In a phase III randomised trial of subjects aged 65 years or older in 15 countries, 21,893 received an AS03$_B$-adjuvanted TIV and 21,802 received a non-adjuvanted TIV [13]. Pain was the most frequent solicited injection site reaction and fatigue was the most frequent solicited general symptom. Reports of unsolicited AEs and MAEs were similar between groups, as were reports of pIMDs and SAEs. Nine SAEs in the group given AS03-adjuvanted TIV and six in the non-adjuvanted TIV group were considered related to vaccination by investigators. No fatal SAEs were considered related to vaccination. Overall, the results showed that the safety profile of seasonal AS03-adjuvanted candidate vaccines was clinically acceptable and generally consistent with that of AS03-adjuvanted pre-pandemic or pandemic influenza vaccines. Similarly, no safety concerns were identified in early stages of the clinical development programmes of AS03-adjuvanted vaccines against influenza caused by potential pandemic subtypes H7N1, H7N9 and H9N2 [14–17].

4. Post-licensure experience with AS03-adjuvanted A/H1N1pdm09 influenza vaccines

While pre-licensure clinical trials assess a vaccine's safety profile, it is essential to monitor its real-world use to capture rare AEs and accumulate safety data, including data for groups excluded from clinical trials, such as pregnant women [40]. Limited safety data in humans were available at the time A/H1N1pdm09 influenza vaccines were introduced, which was mostly based on previous experience in developing H5N1 pre-pandemic vaccines. After declaration of the pandemic by the World Health Organization, vaccination campaigns were rolled out worldwide. Guidelines were developed at a national level, with priority for vaccination given to high-risk groups, including specific age groups depending on country, pregnant women and persons with chronic respiratory disease, asthma, diabetes, immunosuppression and those with chronic heart, renal, liver or neurological disease [41]. To mitigate the limitations of clinical trials, such as limited statistical power to detect rare outcomes [42], vaccine manufacturers implemented stringent risk management plans, including passive and active surveillance activities. It was estimated that approximately 31 million doses of *Pandemrix* and 59 million doses of *Arepanrix* H1N1 were actually administered, including at least 9.5 million doses to children and 300,000 doses to pregnant women. *Pandemrix* was also administered to around 148,000 persons during the 2010–2011 influenza season in the UK, because of a shortage of seasonal TIV and continued circulation of the A/H1N1pdm09 strain. This extensive real-world use provided unique post-licensure experience with an AS03-adjuvanted influenza vaccine.

The European Medicines Agency provided recommendations on pharmacovigilance activities for prompt detection and assessment of new information on the benefits and risks of A/H1N1pdm09 vaccines, including prospective safety studies in 9000 subjects [35]. While most studies used active surveillance of large population-based registries, resulting in convenient samples, this is the only instance where the sample size was predefined. The various studies of *Pandemrix* and *Arepanrix* H1N1 focused on general safety outcomes and AESIs, safety in special populations and, later, the evaluation of emerging signals, such as narcolepsy. The results of these post-licensure studies are summarised in Table 3 and Supplementary Table 2.

Table 3
Summary of post-licensure safety studies of AS03-adjuvanted H1N1 pandemic vaccines.

Country	Design & setting	Study population	Vaccine, number of subjects	Safety endpoint(s)	Main results & conclusions	Ref.
General safety						
UK	Prospective cohort study (hypothesis-generating) 87 general practices in England	General population 7 months to 97 years	*Pandemrix* 9143 vaccinated	MAEs occurring within 31 days post-vaccination; SAEs, AESIs Follow-up 6 months	Most frequent MAEs and SAEs consistent with expected events during winter season Two AESIs above expected number: neuritis (1 case within 31 days), convulsions (8 cases within 6 months); other AESIs below expected number: Bell's palsy, Guillain-Barré syndrome, demyelination Clinically acceptable reactogenicity and safety profile in all age and risk groups	[43]
Sweden	Prospective cohort study (hypothesis-generating) Linked vaccination and health registries	General population in 3 counties All ages	*Pandemrix* 368,205 vaccinated (catchment population 750,000)	Occurrence of hospital diagnosis for AESIs in inpatient and outpatient registers Follow-up 6 months	Elevated risk for convulsion, multiple sclerosis, neuritis. Signals not confirmed in other studies [44–46]	Unpublished results (GSK internal report)
Sweden	Prospective cohort study Linked vaccination and health registries	61% of general population All ages	*Pandemrix* 3.3 million vaccinated and 2.5 million unvaccinated	Occurrence of diagnosis for > 30 neurological and immune-mediated diseasesUp to 27 months follow-up	Hazard ratios close to 1.0 for all outcomes other than expected allergic vaccine reactions and narcolepsy	[47]
Canada (Quebec, Ontario, Nova Scotia)	Web-based active surveillance	Healthcare workers 16 to 87 years	*Arepanrix* H1N1 N = 6242 (N = 7649 vaccinated with 2010–2011 seasonal TIV)	AEFIs within 8, 15, 29 days of vaccination; SAEs within 6 months	*Arepanrix* H1N1 associated with significantly more local AEs than seasonal vaccine (1% versus 0.03%, $p < 0.001$); paraesthesia frequency 0.1% versus 0%. No clustering of SAE with *Arepanrix* H1N1 in 6-month follow up	[48]
Canada (Manitoba)	Retrospective cohort study of Manitoba Health linked provincial database system (hypothesis-generating)	General population (population base 1.2 million) ≥6 months	503,241 vaccinated (288,809 *Arepanrix* H1N1; 65,885 *Arepanrix* H1N1 + TIV; 148,577 TIV) and 290,375 unvaccinated	Occurrence of AESIs and narcolepsy with hospital or physician diagnosis; AEs in subpopulations (pregnancy, autoimmune diseases, immune suppression) Follow-up 6 months	Favourable safety profile for *Arepanrix* H1N1 Multiple sclerosis and acute disseminated encephalomyelitis signals resolved through further study [45,46,50]	Unpublished results; [49]
European Union	Retrospective analysis of adverse reaction spontaneous reports to EudraVigilance (pharmacovigilance reporting system)	General population All ages	Adjuvanted (mostly *Pandemrix*) and non-adjuvanted pandemic influenza vaccines Total 46,173 and 4048 reports for adjuvanted and non-adjuvanted vaccines, respectively	Suspected autoimmune disorders based on MedDRA terms WHO causality assessment for AEFI and Brighton collaboration definitions	Reporting rates for all reports of autoimmune disorders using estimated number vaccinated as denominator 6.87 (95% CI: 6.06, 7.68) per million for adjuvanted vaccines, 9.98 (6.81, 13.16) per million for non-adjuvanted vaccines	[87]

(*continued on next page*)

Table 3 (*continued*)

Country	Design & setting	Study population	Vaccine, number of subjects	Safety endpoint(s)	Main results & conclusions	Ref.
Narcolepsy						
The two main reviews and meta-analysis of the association between Pandemrix / Arepanrix H1N1 and narcolepsy are described below [51–53]. Individual studies are described in Supplementary Table 2						
Finland, France, Ireland, Norway, Sweden, UK, Canada (12 national/ regional studies); 1 EU multi-country study	Review of studies published up to 2014: 11 national/regional studies (8 retrospective cohort, 1 case coverage, 2 case control) and 1 EU multi-country study (case control)	All ages, children, children and adolescents	*Pandemrix* or (Canada) *Arepanrix* H1N1 Not disclosed	Narcolepsy with/without cataplexy using American Academy of Sleep Medicine, Brighton collaboration or International Classification of Sleep Disorder criteria. Non-validated diagnostic code in 2 cohort studies	Relative risk in children and adolescents ranging from 1.6 to 25.0 (95% CI: 0.5, 61.0) Relative risk in all age groups ranging from 1.3 to 13.0 (95% CI: 0.2, infinity)	[51]
Finland, France, Ireland, Norway, Sweden, UK (11 national/regional studies); 1 EU multi-country study	Review of studies published up to 2014: 11 national/regional studies (8 retrospective cohort, 1 ecological, 1 case coverage, 1 case control) and 1 EU multi-country study (case control)	All ages, children, children and adolescents	*Pandemrix* In national studies, coverage 2–75%. In multi-country study, coverage very low to high	Narcolepsy with/without cataplexy using American Academy of Sleep Medicine, Brighton collaboration case definition or International Classification of Sleep Disorder criteria. No expert review in 3 cohort studies	Relative risk in children and adolescents ranging from 1.5 to 25.0 (95% CI: 0.3, 48.5) Relative risk in adults ranging from 1.1 to 18.8 (95% CI: 0.6, 207.4)	[52]
Finland, France, Ireland, Norway, Sweden, UK, Netherlands (11 national/regional studies)	Meta-analysis of studies published up to 2016: 7 retrospective cohort, 2 case coverage, 1 case control, 1 report	Adults, children, children and adolescents	*Pandemrix* Children and adolescents: 376 vaccinated, 95 unvaccinated narcolepsy cases Adults: 133 vaccinated, 59 unvaccinated narcolepsy cases	Narcolepsy with/without cataplexy using Brighton collaboration or International Classification of Sleep Disorder criteria	Relative risk in children and adolescents 14.3 (95% CI: 8.9, 23.0) if onset of symptoms used as index date; 5.0 (3.4, 7.5) if diagnosis used as index date. Relative risk in adults 7.0 (3.4, 14.5) if onset of symptoms used as index date; 3.0 (1.9, 4.6) if diagnosis used as index date	[53]
Afebrile convulsions, febrile seizures						
UK	Prospective cohort study (hypothesis-generating) 87 general practices in England	General population 7 months to 97 years	*Pandemrix* 9143 vaccinated	Convulsion occurring within 6 months post-vaccination	Observed number of convulsions higher than expected for the 181-day post-vaccination period (8 cases; standardised incidence rate ratio 2.65 [95% CI: 1.14, 5.22]) but not for the 30-day interval (2 cases; 3.84 [95% CI: 0.47, 13.89])	[43]
UK	SCCS General Practice Research Database	General population <10 years	*Pandemrix* 2366 vaccinated with at least one influenza vaccine (1865 doses pandemic vaccine, mostly *Pandemrix,* and 2858 seasonal TIV doses)	Risk of convulsion during 2009–2010 season (pandemic vaccine) or over 10-year surveillance period (TIV)	Incidence rate ratio in week after vaccination 0.99 (95% CI: 0.61, 1.60) for pandemic vaccine, 1.00 (0.64, 1.59) for TIV. IRR for days 1–3 post-vaccination of 3.48 (95% CI 0.86–14.07) after the second dose of pandemic vaccine consistent with increased fever post-dose 2 in some studies Overall no evidence of increased risk following TIV or single dose of *Pandemrix*	[77]
Sweden	SCCS Linked vaccination and health registries	General population 0 to 106 years	*Pandemrix* 373,398 vaccinated, 859 with a seizure episode	Hospital admission or outpatient care due to epileptic seizure	Investigation of signal from previous cohort study (unpublished results, GSK internal report). Relative incidence 1.01 (95% CI: 0.74, 1.39) in persons with previously diagnosed epilepsy; 0.67 (0.27, 1.65) for persons without epilepsy during 7-day risk period. In day 8–30 risk period: relative incidence 1.11 (0.73, 1.70) in persons without epilepsy and 1.00 (0.83, 1.21) in those with epilepsy	[44]
Norway	SCCS National linked registries	General population 0 to 45 months	*Pandemrix* 113,068 vaccinated, 656 (0.6%) with at least one febrile seizure episode	Emergency hospital admission or outpatient care due to febrile seizure	Incidence rate ratio 2.00 (95% CI: 1.15, 3.51) 1–3 days after vaccination; no increased risk 4–7 days after vaccination. Risk of febrile seizure 1–3 days after pandemic influenza diagnosis 10.12 (3.82, 26.82)	[78]

Guillain-Barré syndrome (GBS)

Canada (Quebec)	Prospective cohort study and SCCS Active hospital-based surveillance plus provincial hospital summary discharge database	General population ≥6 months	*Arepanrix* H1N1 83 confirmed GBS cases including 25 vaccinated in 4.4 million population	Risk of GBS 8, 6 and 4 weeks post-vaccination, Brighton collaboration case definition	Poisson model: adjusted relative risk 1.80 (95% CI: 1.12, 2.87) 8 weeks post-vaccination; 2.75 (1.63, 4.62) 4 weeks post-vaccination SCCS: relative risk 3.02 (95% CI: 1.64, 5.56) 4 weeks post-vaccination No evidence of increased risk in those < 50 years Number of cases attributable to vaccination ~ 2 per 1 million doses	[79]
Germany	Prospective SCCS 351 national hospitals	General population 1 to 90 years	*Pandemrix* 676 reports; 30 cases within 150 days post-vaccination	Risk of GBS 5–42 days post-vaccination Brighton collaboration case definition	Temporal association found: relative risk within 5–42 days risk period versus 43–150 days post-vaccination 4.65 (95% CI: 2.17, 9.98)	[80]
Denmark, France, Netherlands, Sweden, UK	Case-control study National healthcare databases covering 50 million population	General population Mean age 46 to 61 years	*Pandemrix* mostly 104 cases matched to 1 or more controls	Risk of GBS up to 42 days post-vaccination Brighton collaboration case definition	Odds ratio adjusted for influenza-like illness/URTI and seasonal influenza vaccination: 1.0 (95% CI: 0.3, 2.7)	[81]
Denmark, Finland, France, Netherlands, Norway, Sweden, UK	SCCS National healthcare databases covering > 50 million population	Mean age 45 to 56 years	*Pandemrix* mostly 303 cases, of which 99 vaccinated	Risk of GBS up to 42 days post-vaccination Brighton collaboration case definition	Unadjusted relative incidence 3.5 (95% CI: 2.2, 5.5); adjusted relative incidence (main finding) 1.4 (95% CI: 0.7, 2.8)	[82]
France	Case-control study 25 neurology centres (cases) and 457 general practitioners (controls)	General population Mean age 49 years	Unspecified pandemic and seasonal influenza vaccines 145 cases matched to 1080 controls	Risk of GBS within 6 weeks post-vaccination Brighton collaboration case definition	Pandemic vaccination: adjusted odds ratio 0.92 (95% CI: 0.11, 7.55). Difference in GBS rates between influenza virus circulation periods and non-circulation periods highly statistically significant (p = 0.004)	[83]
UK	Prospective cohort study British Paediatric Surveillance Unit system	General population ≤16 years	Seasonal and pandemic vaccines (Pandemrix mostly) 112 GBS cases	Actual versus expected number of pandemic and seasonal vaccinations within 6 months of GBS onset Brighton collaboration case definition	Observed numbers not significantly higher than expected. No cases with GBS received Pandemrix in 42-day risk period. Evidence of preceding infection in majority of children with GBS	[84]
UK	SCCS HES database and general practices	General population All ages	*Pandemrix* 37 vaccinated subjects, of which 9 GBS cases	Risk of GBS within 6 weeks post-vaccination ICD codes in HES	Relative incidence 1.05 (95% CI: 0.37, 2.24)	[85]
Sweden	Prospective cohort study Linked vaccination and health registries	61% of Swedish general population All ages	*Pandemrix* 3.3 million vaccinated and 2.5 million unvaccinated. Total 170 GBS events	Risk of GBS during early and late periods after vaccination, up to>1 year post-vaccination based on ICD codes	Hazard ratio 1.03 (95% CI: 0.81, 1.32)	[47]
Australia, Canada, China, Denmark, Finland, Netherlands, Singapore, Spain, UK, USA	Meta-analysis using SCCS design	Mean age 4 to 73 years	Adjuvanted (mostly *Pandemrix*) and non-adjuvanted pandemic influenza vaccines	Relative incidence of GBS within 42 days of vaccination (case finding/ascertainment method dependent on country)	Relative incidence with non-adjuvanted vaccines 3.10 (95% CI: 1.70, 5.65); with adjuvanted vaccines 1.65 (0.86, 3.19)	[86]
European Union	Retrospective analysis of adverse reaction spontaneous reports to EudraVigilance (pharmacovigilance reporting system)	General population All ages	Adjuvanted (mostly *Pandemrix*) and non-adjuvanted pandemic influenza vaccines Total 46,173 and 4048 reports for adjuvanted and non-adjuvanted vaccines, respectively	Suspected autoimmune disorders based on MedDRA terms WHO causality assessment for AEFI and Brighton collaboration definitions	Percentage of reports of autoimmune disorders (most frequently GBS) amongst all reported AEs 0.60% (95% CI: 0.53, 0.67) for adjuvanted vaccines, 0.94% (0.64, 1.24) for non-adjuvanted vaccinesReporting rates within expected background rates	[87]

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Table 3 (*continued*)

Country	Design & setting	Study population	Vaccine, number of subjects	Safety endpoint(s)	Main results & conclusions	Ref.
Multiple sclerosis and other demyelinating conditions						
Multiple sclerosis and other demyelinating conditions were included as endpoints in general safety studies described above in the UK [43], Sweden (unpublished results, GSK internal report; [47]) and Canada (unpublished results [49]). In the two unpublished studies, there were signals for multiple sclerosis (Sweden and Canada), neuritis (Sweden) and acute disseminated encephalomyelitis (Canada), which were investigated in the studies listed below						
Canada (Manitoba)	Retrospective cohort study Manitoba Health linked provincial database system	General population (population base 1.2 million) ≥6 months	341,347 *Arepanrix* H1N1 vaccinated subjects matched on propensity score to unvaccinated subjects	Incidence of multiple sclerosis and other demyelinating conditions within 12 months post-vaccination	Hazard ratio for multiple sclerosis 0.9 (95% CI: 0.6, 1.4) after one year. Hazard ratios for risk of demyelinating conditions not ultimately diagnosed as multiple sclerosis around 0.5 in all analyses	[45,46]
Canada (Manitoba)	Hospital chart review	General population All ages	*Arepanrix* H1N1 22 confirmed (out of 139) hospitalised cases of acute disseminated encephalomyelitis (ADEM), including 1 vaccinated	Incidence of ADEM Brighton collaboration case definition	4.7 (95% CI: 1.9, 11.4) times increased incidence suggested to be related to increased incidence of influenza during same period. No temporal relationship with vaccination	[50]
Anaphylaxis						
Anaphylaxis was included as an endpoint in general safety studies described above in the UK [43], Sweden (unpublished results, GSK internal report; [47]) and Canada (unpublished results [49]), none of which pointed to an association with Pandemrix or Arepanrix H1N1						
Worldwide	Spontaneous cases reported to GSK safety database	Any	*Pandemrix, Arepanrix* H1N1 97 confirmed vaccinated cases out of 395 reports	Reports of anaphylaxis Brighton collaboration case definition	Incidence per million doses 8.1 after *Pandemrix*, 6.0 after *Arepanrix* H1N1; within expected range of rates for anaphylaxis following vaccination in general (1–10 cases per million doses)	[89]
Canada (Quebec)	Cases of allergic symptoms reported to public health through passive surveillance	General population All ages	4.2 million doses of *Arepanrix* H1N1; 157,753 doses unadjuvanted vaccine	Allergic symptoms Brighton collaboration case definition	Incidence rate of anaphylaxis13 per million doses administered: in same range as reported in EU but exceeding that with seasonal influenza vaccines. Spontaneous reporting returned to baseline the following season	[90]
Solid organ transplant rejection						
UK	SCCS Clinical Practice Research Datalink and linked HES inpatient database	General population	*Pandemrix* 91 cases of rejection, of which 71 vaccinated	Occurrence of rejection of transplanted liver, kidney, lung, heart or pancreas 30- or 60-days post-vaccination	Relative incidence of rejection of any organ: 1.05 (95% CI: 0.52, 2.14) within 30 days, 0.80 (95% CI: 0.42, 1.50) within 60 days. Similar estimates for kidney only (most commonly transplanted organ)	[94]

Pregnancy outcomes						
UK	Prospective cohort study 71 general practices (sub-cohort of general safety study [43])	General population 16 to 43 years	*Pandemrix* 267 vaccinated pregnant women	Follow-up to end of pregnancy; recording of birth defects during first 6 months after birth	No evidence of increased risk of adverse pregnancy outcomes	[97]
Ireland	Retrospective cohort study Hospital discharge records of singleton deliveries before and during pandemic	General population <20 to > 40 years	698 vaccinated with *Pandemrix*; 1692 with nonadjuvanted vaccine; 606 with unknown vaccine; 3898 unvaccinated	Pregnancy outcomes at delivery: preterm birth, size for gestational age, neonatal intensive care admission, congenital anomalies, perinatal death	No association between vaccination and adverse pregnancy outcomes	[98]
Netherlands	Prospective cohort study Healthcare provider practices plus analysis of spontaneous pharmacovigilance reports	General population 17 to 45 years	10 vaccinated with *Pandemrix*; 137 with MF59-adjuvanted vaccine; 134 with unknown vaccine	Pregnancy outcomes within one week of delivery; pregnancy-related AEs reported spontaneously by healthcare providers and pregnant women	No increased risk of adverse pregnancy outcomes or neonatal problems	[99]
Denmark	Register-based, prospective, propensity-score matched cohort study	General population <20 to ≥ 40 years (mean 31 years)	*Pandemrix* Up to 7062 vaccinated and 47,523 unvaccinated women	Foetal death, spontaneous abortion, stillbirth	No evidence of increased risk of foetal death, spontaneous abortion or still birth	[100]
Norway	Register-based, prospective cohort study	General population <20 to ≥ 40 years	*Pandemrix* 25,976 children born to vaccinated women and 54,065 to unvaccinated	Foetal death	No evidence of increased risk of foetal death	[101]
Sweden	Prospective cohort study Swedish Medical Birth Register and standardised antenatal medical documents	General population <20 to ≥ 45 years	*Pandemrix* 18,612 vaccinated and 136,914 unvaccinated women	Stillbirth, preterm birth, low birth weight, congenital malformation, small for gestational age	Risk for stillbirth, preterm birth and low birth weight lower than in unvaccinated group; no difference in congenital malformation or small for gestational age	[102]
Sweden	Register-based prospective cohort study	7 healthcare regions covering 61% of the general population	*Pandemrix* Liveborn offspring of vaccinated mothers: 41,183 exposed and 234,317 unexposed (including sibling comparator)	Stillbirth, neonatal deaths, deaths up to 4.6 years of age	No association with adverse foetal outcome or child mortality, including when considered intrafamilial factors	[103]
			Pandemrix Liveborn offspring of vaccinated mothers: 14,385 exposed (first trimester) and 197,588 unexposed (including sibling comparator)	Congenital malformation in first year of life, including congenital heart disease, oral cleft, limb deficiency, based on ICD codes	Considering intrafamilial factors, no association with overall congenital malformation	[105]
Germany	Register-based, prospective cohort study	General population 15 to 49 years	90 vaccinated with *Pandemrix*; 2 with MF59-adjuvanted vaccine; 216 with non-adjuvanted vaccine; 15 with unknown vaccine; 1329 unvaccinated	Spontaneous abortion and major malformations, preeclampsia, gestational age at birth, birth weight	No increased risk observed	[104]
Canada	Retrospective cohort study Perinatal database	General population <20 to ≥ 40 years	23,340 unspecified vaccine (including *Arepanrix* H1N1); 32,230 unvaccinated	Preterm birth, small for gestational age, 5-minute Apgar score, foetal death	H1N1 vaccination associated with improved foetal and neonatal outcomes	[106]

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Table 3 (*continued*)

Country	Design & setting	Study population	Vaccine, number of subjects	Safety endpoint(s)	Main results & conclusions	Ref.
Safety in the immunocompromised population						
Ireland	Prospective study National referral centre	HIV-infected children 2 to 16 years	*Pandemrix* 24 vaccinated	Serological response and safety monitoring (AEs within 3 months post-vaccination)	Pain most frequent solicited AE. No SAEs, no impact on HIV disease progression	[108]
Germany	Prospective study Three outpatient clinics	HIV-infected patients Mean 45 years	*Pandemrix* 622 vaccinated	AEs within 7 days post-vaccination	Pain most frequent solicited AE. No SAEs, tolerability comparable to general population	[109]
France	Prospective, open-label study Two hospitals	Cancer patients on cytotoxic/ targeted therapies 33 to 85 years	*Pandemrix* 65 vaccinated	AEs within 21 days post-vaccination	Pain most frequent solicited AE. No SAEs	[110]
Sweden	Prospective study Outpatient haematology clinic	Patients with haematological disease 18 to 84 years	*Pandemrix* 31 vaccinated plus non-adjuvanted seasonal TIV 4 weeks afterwards	AEs within at least 1 year post-vaccination	Both vaccines well tolerated. No SAEs	[111]

AE, adverse event; AEFI, AE following immunisation; AESI, AE of special interest (anaphylaxis, Bell's palsy, convulsion, demyelination, encephalitis, Guillain-Barré syndrome, neuritis, vasculitis); CDC, Centers for Disease Control and Prevention; EU, European Union; HES, Hospital Episodes Statistics; MAE, medically-attended adverse event; PASS, post-authorisation safety study; SAE, serious adverse event; SCCS, self-controlled case series; TIV, trivalent influenza vaccine; URTI, upper respiratory tract infection; WHO, World Health Organization.

4.1. General safety

Initial observational safety data from the A/H1N1pdm09 vaccination campaigns were obtained rapidly from a prospective post-authorisation safety study (PASS) in the UK that assessed the incidence of MAEs, SAEs and AESIs following administration of *Pandemrix* [43]. Results indicated that the vaccine was generally well tolerated with a clinically acceptable safety profile. In this cohort of 9143 individuals aged 7 months to 97 years, the sample size allowed to rule out, at 95% confidence, events occurring with a frequency of 1/3000 if no event was observed (provided events occurred in all age categories). The most commonly reported MAEs and SAEs were consistent with events expected during the winter season and incidences of solicited AEs were consistent with those from clinical development. In a PASS in Sweden that also evaluated AESIs in 368,205 individuals who were vaccinated with *Pandemrix*, small increased risks following vaccination with *Pandemrix* were observed for multiple sclerosis and neuritis, and convulsions in individuals with and without epilepsy (cohort study; unpublished data). A subsequent self-controlled case series (SCCS) analysis in the same Swedish cohorts for epileptic seizures [44] and another study in Canada for multiple sclerosis [45,46] did not confirm these signals. In another Swedish cohort study of 3.3 million vaccinated and 2.5 million unvaccinated, there was no evidence of a role for *Pandemrix* in triggering any of more than 30 neurological and immune-related diseases, except narcolepsy [47]; this safety concern is discussed further below. Finally, two Canadian observational studies on common AEs following immunisation (in Quebec, Ontario and Nova Scotia) [48] and on AESIs (in Manitoba; unpublished data) [49], pointed towards a favourable safety profile for *Arepanrix* H1N1, which was confirmed through an additional investigation that excluded a risk of acute disseminated encephalomyelitis in Manitoba [50].

4.2. Narcolepsy

In the aftermath of the pandemic, the safety of AS03-adjuvanted A/H1N1pdm09 vaccine was questioned following reports of narcolepsy, a rare sleep disorder, in children and adolescents vaccinated with *Pandemrix*, initially in Sweden and Finland [51]. Studies assessing the association between *Pandemrix* and narcolepsy were conducted in several European countries [51–53]. These studies confirmed an increased risk of narcolepsy following vaccination with *Pandemrix*, with relative risk estimates ranging from 1.5 to 25.0 (95% CI range: 0.3 to 48.5) in children, and from 1.1 to 18.8 (95% CI range: 0.6 to 207.4) in adults [52]. A recent *meta*-analysis of eight European studies in children and adolescents showed the pooled relative risk of narcolepsy during the first year after vaccination ranged from 5.0 (95% CI: 3.4, 7.5), if diagnosis was used as the index date, to 14.3 (8.9, 23.0), if onset of symptoms was used as the index date [53]. The relative risk in adults, estimated from six studies, ranged from 2.9 to 7.0, depending on the index date used. The vaccine attributable risk, which could be calculated from five studies in children and adolescents, was around 1 case per 18,400 vaccine doses, and in adults, 1 per 181,000 doses (calculated from three studies) [53].

Since all countries with *Pandemrix* exposure in Europe were already engaged in the studies described above, GSK supported a study in Quebec, Canada, where *Arepanrix* H1N1 had been used, which yielded no strong evidence for an increased risk of narcolepsy after vaccination with *Arepanrix* H1N1 [54,55]. In addition, there was no signal from passive surveillance in Ontario, Canada [56].

The SOMNIA study (Systematic Observational Method for Narcolepsy and Influenza Immunization Assessment) assessed the association between adjuvanted as well as non-adjuvanted

A/H1N1pdm09 vaccines using electronic healthcare databases in nine countries across the globe. The retrospective cohort part of the study did not reveal changes in narcolepsy incidence between the period after the start of adjuvanted A/H1N1pdm09 vaccination and the period before virus circulation in any age group or country, except Sweden, an initial signalling country, and Taiwan, where the incidence increased upon virus circulation. In the case-control part of the study, there was no evidence of an increased risk of narcolepsy following adjuvanted A/H1N1pdm09 vaccines in children or adults, although this was based mostly on *Arepanrix* H1N1 use in Ontario [57].

Interpretation of the epidemiological data on the association between *Pandemrix* and narcolepsy is complexified by the use of multiple study designs and methodological limitations, including retrospective design and the inability to fully account for potential confounders [51–53]. Two recent simulation analyses have highlighted how the studies might have been impacted to some extent by bias and confounding [58,59], the most relevant of which is probably ascertainment bias. Effect modification by previous but recent A/H1N1pdm09 influenza virus infection is also a working hypothesis, based on epidemiological data from China [60–62], Quebec [54], Germany [63] and Taiwan [64] and a mathematical model of virus transmission in Norway [65], where the epidemic curve was concurrent with that in neighbouring Sweden and Finland. This suggested that over half the people vaccinated against A/H1N1pdm09 influenza virus during the pandemic had been exposed to this influenza subtype before vaccination [65]. A dual role of infection and vaccination cannot be excluded.

The aetiology of narcolepsy remains unclear. Narcolepsy is strongly associated with the HLA-DQB1*0602 allele [66], suggestive of a role of the immune system as HLA molecules are involved in antigen presentation to T cells. Other genetic polymorphisms associated with narcolepsy (e.g. *chemokine* (*C-C motif*) *receptor 1* [*CCR1*], *CCR3*, T cell receptor α) [67,68] also point towards immune system involvement, suggesting an autoimmune aetiology. One hypothesis is that T cells specific for viral or bacterial epitopes also recognise epitopes from self-proteins, exemplified by possible mimicry between a coxsackievirus B peptide and a peptide from the diabetes-associated antigen glutamic acid decarboxylase [69]. Currently, the hypothesis that CD4 T cell cross-reactivity between the A/H1N1pdm09 haemagglutinin antigen and hypocretin might have played a role in the aetiology of narcolepsy is being evaluated using specific HLA-DQB1*0602 tetramers and preliminary data indicate that antigenic mimicry might exist on the CD4 T cell level [70–72]. Whereas further evidence is needed, any CD4 T cell-based mechanism would be consistent with the importance of the DQB1*0602 allele, the lack of a direct role of the AS03 adjuvant and a potential interaction between A/H1N1pdm09 viral infection (involving the same CD4 T cell epitope) and vaccination. Immune system involvement was also highlighted in a mouse model in which haemagglutinin-specific cross-reactive T cells triggered narcolepsy-like symptoms [73]. Other research advances mechanisms based on cross-reactive antibodies between the influenza virus nucleoprotein and human hypocretin receptor 2 [74], although several aspects of this work remain controversial [75,76].

Overall, accumulated epidemiological data on the association between *Pandemrix* and narcolepsy suggest an increased risk, the mechanisms of which remain to be fully understood. Immunological data suggest a possible role of the A/H1N1pdm09 antigen through a multifactorial mechanism involving antigen mimicry.

4.3. Afebrile convulsions and febrile seizures

The pattern of reports of afebrile convulsions and febrile seizures does not suggest a greater frequency of events following vaccination with *Pandemrix* compared to background rates. This was confirmed by data from the UK prospective PASS [43] and from SCCS analyses in the UK [77] and Sweden [44], which showed no evidence of an increased risk of convulsions or epileptic seizures. In another SCCS in Norway, involving 226,889 children of whom 113,068 were vaccinated, there was a two-fold increase in febrile seizures 1–3 days following *Pandemrix* vaccination but no increased risk after 4–7 days [78]. A/H1N1pdm09 infection was associated with a 10-fold increased risk of febrile seizures in the 1–3 days period following diagnosis, which points to a favourable benefit/risk profile for the vaccine, balancing the risk of seizures with that of Influenza, which can cause fever, convulsions and potentially more serious outcomes such as pneumonia and neurological disorders.

4.4. Guillain-Barré syndrome

Post-licensure safety data show no evidence of an association between AS03-adjuvanted A/H1N1pdm09 vaccines and GBS, which was under particular scrutiny due to the safety experience with the A/New Jersey/76 swine influenza vaccine. One population-based cohort study of *Arepanrix* H1N1 in Quebec [79] and an SCCS of *Pandemrix* in Germany [80] found a small increased risk. However, there was no evidence of an increased risk in the multinational VAESCO (Vaccine Adverse Event Surveillance and Communication) study, in which 104 patients with GBS were identified from a pooled population base of 50 million and matched to one or more controls [81]. This finding was confirmed in a subsequent VAESCO SCCS [82] and several national studies in France [83], the UK [84,85] and Sweden [47]. In a global study across 10 countries worldwide, immunisation with adjuvanted (mostly AS03) H1N1 vaccines yielded a relative incidence estimate for GBS of 1.65 (95% CI: 0.86, 3.19), while the relative incidence in non-adjuvanted vaccine recipients was higher at 3.10 (1.70, 5.65) [86]. Analysis of spontaneous reports from the European Union EudraVigilance database showed a higher percentage of reports of autoimmune disorders (most frequently GBS) for non-adjuvanted than for adjuvanted A/H1N1pdm09 vaccines (mainly *Pandemrix*) [87]. The reason for these observations is unclear but may be related to an effect mediated by infection, since GBS is often preceded by upper respiratory or gastrointestinal infection and is associated with some viral infections, including influenza [88].

4.5. Multiple sclerosis and other demyelinating conditions

A study in Manitoba in 341,000 vaccinated subjects above 6 months of age, matched on propensity score to 485,000 unvaccinated individuals, showed no evidence of an association between *Arepanrix* H1N1 and multiple sclerosis or other demyelinating conditions, based on a large sample size that yielded reasonably precise estimates [45,46]. Another analysis conducted in Manitoba found no temporal relationship between the incidence of acute disseminated encephalomyelitis and the administration of A/H1N1pdm09 or seasonal influenza vaccines [50].

4.6. Anaphylaxis

There was no evidence of an increased risk of anaphylaxis following administration of *Pandemrix* or *Arepanrix* H1N1 compared to other vaccines in a detailed analysis of cases reported to GSK [89]. This is supported by data from the UK [43], Manitoba [49] and Sweden (unpublished results; [47]). In a study in Quebec, the reported incidence of anaphylaxis following administration of *Arepanrix* H1N1 was higher than previously reported after TIV vaccination [90]. The authors highlighted potential under-reporting before the pandemic and reported no difference in rates of allergic

symptoms between the groups given adjuvanted and non-adjuvanted vaccines.

4.7. Solid organ transplant rejection

Cases of solid organ transplant rejection in temporal association with AS03-adjuvanted A/H1N1pdm09 vaccines were reported in Europe and Canada [91–93]. For the majority of cases, risk factors for rejection were present, such as non-compliance with the immunosuppressive regimen, acute infection or prior rejection episodes. A PASS with SCCS design was conducted to evaluate this signal, since transplanted patients are a high-risk priority group for immunisation with future pandemic influenza vaccines. Results indicated no increased risk of rejection within 30 and 60 days following *Pandemrix* vaccination [94]. Other clinical data from the pandemic support these findings, suggesting that, despite some evidence of transiently increased cellular alloreactivity, inactivated influenza vaccines have an acceptable safety profile in transplanted patients [95,96].

4.8. Safety in pregnancy

Several national pandemic immunisation programmes included vaccination of pregnant women. In the UK PASS, exposure to *Pandemrix* in the sub-cohort of 267 pregnant women did not increase the risk of adverse pregnancy outcomes, including spontaneous abortion, congenital anomalies, preterm delivery, low birth weight and maternal complications [97]. These findings have been confirmed by observational data from other European countries [98–105] and Canada [106].

4.9. Immunocompromised population

A systematic review and *meta*-analysis of publications up to January 2011 found that influenza vaccination (including seasonal influenza and H1N1 pandemic vaccines) was generally well tolerated in immunocompromised patients [107]. There was some variation in mild AEs within and between aetiological groups, which included patients with human immunodeficiency virus (HIV) infection, cancer patients and transplant recipients. Results from individual post-licensure studies of *Pandemrix* in HIV-infected patients [108,109] and patients with cancer [110] or haematological diseases [111] are consistent with this finding.

4.10. Overall evidence from post-licensure studies

Studies evaluating AS03-adjuvanted pandemic influenza vaccines in real-world settings had some limitations. Data on background incidence rates of outcomes such as pIMDs were scarce, which limited predefining sample sizes. Most studies relied on convenience samples and were guided by operational constraints related to the need for prompt evidence generation. Cohort studies in the range of 1,000,000 subjects per vaccinated and per unvaccinated group would have been required to detect, with sufficient power, risks of rare events with background incidences ranging from 1 to 5/100,000. Despite many studies including cohorts of several thousand subjects, this was achieved rarely [47], underlining the importance of robust pharmacovigilance to monitor vaccine use and identify safety signals. Although power was limited in some studies, especially those in risk groups, overall the data suggest that precision was acceptable, as illustrated by the width of the confidence intervals for the risk estimates presented in Table 3.

5. Conclusions

This review of safety data from non-clinical, clinical and post-licensure experience, based on both GSK and independent data, suggests that AS03-adjuvanted influenza vaccines are generally well tolerated with an acceptable safety profile in different groups, including special populations. Epidemiological data show an increased risk of narcolepsy following vaccination with *Pandemrix*, the mechanisms of which remain to be fully understood, but may involve A/H1N1pdm09 antigen mimicry [70].

Adjuvant Systems can significantly increase the immune response to the associated antigen, as observed following the administration of approximately 90 million doses of AS03-adjuvanted A/H1N1pdm09 vaccines to people in 58 countries during the A/H1N1pdm09 pandemic. In the large number of studies that evaluated the benefits of *Pandemrix* and *Arepanrix* H1N1, despite a 4-fold reduced antigen dose, high effectiveness was observed in the general population [11], ranging from 70% to 100% against laboratory-confirmed outcomes including hospitalisation, although, in a few studies, particularly in high-risk groups [112,113], effectiveness was lower. In the only randomised controlled trial of AS03-adjuvanted A/H1N1pdm09 vaccine, efficacy for prevention of H1N1 illness in children was 76.8% relative to unadjuvanted H1N1 vaccine [12]. A recent systematic review and *meta*-analysis showed that adjuvanted vaccines (mostly containing AS03) were more effective in children than non-adjuvanted vaccines, while in adults there were fewer differences in effectiveness between adjuvanted and non-adjuvanted vaccines [11].

AS03 should be considered as a component of future prophylactic influenza vaccines. This requires a solid scientific and public health rationale, including clinical data supporting a meaningful increase in efficacy against a pandemic strain [12] or other benefits, such as antigen-sparing capability [114]. This comprehensive review of safety data shows that influenza vaccines formulated with AS03 remain important to address pandemic threats, provided candidate vaccines do not contain the putative narcolepsy mimicry antigen of the A/H1N1pdm09 haemagglutinin protein [70]. These safety findings are highly relevant in the context of ongoing regulatory and public health discussions related to future influenza pandemics.

Trademark statement

Arepanrix H1N1 and *Pandemrix* are trademarks of the GSK group of companies.

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Potential conflicts of interest

CC, RvdM, VB, RBB, TMD, AS, FTDS and RR are employed by the GSK group of companies and hold shares in the GSK group of companies as part of their employee remuneration. NG and BLI were previously employees of the GSK group of companies and NG declares stock ownership. NG is also inventor on patents owned by the GSK group of companies.

Contributors' statement

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

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Appendix A. Supplementary material

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