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Review

A vaccine manufacturer's approach to address medical needs related to seasonal and pandemic influenza viruses

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Abstract Vaccination is considered to be one of the most effective tools to decrease morbidity as well as mortality caused by influenza viruses.
For the prevention of seasonal influenza, *Fluarix*™ and *FluLaval*™ have been marketed since 1987 and 1992, respectively. Both vaccines have consistently been shown to meet or exceed the regulatory criteria for immunogenicity against the three strains H1N1, H3N2 and B, have a good safety profile, and are recommended for vaccinating children and adults of all ages.
For the prevention of pandemic influenza, GlaxoSmithKline (GSK) has obtained licensure of a pre-pandemic vaccine, *Prepandrix*™. This split-virus H5N1 adjuvanted with AS03, a proprietary oil-in-water emulsion-based adjuvant system, has demonstrated broad immunity against drifted H5N1 strains and has been shown to be effective in preventing mortality and viral shedding in animal studies.
The influenza vaccine portfolio of GSK addresses specific medical needs related to seasonal or pandemic influenza viruses, which remain an important public health threat worldwide.

Keywords Influenza, pandemic influenza, vaccine.

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Introduction

Influenza is an acute, respiratory viral infection that is usually self-limited in healthy adults and lasts about a week. Influenza viruses circulate every winter in temperate regions and throughout the year in tropical regions. The causative agents are influenza A and influenza B viruses. The main immunogenic factors are the virus surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). There are several antigenic forms of HA and NA for influenza A which is classified into different subtypes based on various combinations of these antigens.[1–3] Only a limited number of these influenza A subtypes are known to have been associated with human disease and the ones currently in circulation in the human population are H1N1 and H3N2.[4] Other influenza A subtypes such as H5N1, H7N7 and H9N2 may sporadically cause human disease but have not been transmitted widely so far through direct human to human transmission. The influenza B virus belongs to two evolutionary lineages that are distinct at the genetic and antigenic levels and which are represented by B/Yamagata/16/88-like and B/Victoria/2/87-like viruses that have co-circulated in the population since the mid-1980s.[4–7]

The HA and NA proteins of both influenza A and influenza B viruses are subject to continuous alteration in a process of point mutations known as antigenic or genetic drift with a consequence possible escape of the host immune system by the viruses.[1,4,8,9] Antigenic drift is responsible for the yearly seasonal, otherwise known as inter-pandemic or epidemic influenza. Seasonal influenza is usually a mild disease in the healthy adult population. However, it causes significant morbidity and mortality in certain at-risk groups, i.e. elderly people aged 65 years and above, young children and people with certain underlying medical conditions.[10]

Sometimes, a more profound antigenic change can occur, and this antigenic shift can trigger the appearance of novel highly transmissible viruses bearing surface antigens previously unknown to most of the human population's immune system. The combination of these factors has potentially

lethal consequences. Antigenic shift can indeed cause pandemics, in which a large proportion of the worldwide population is affected. Three major pandemics took place during the 20th century: the 'Spanish flu' in 1918–1919, responsible for 20 to 50 million deaths worldwide, the 'Asian flu' in 1957 and the 'Hong Kong flu' in 1968. These three pandemics were caused either by reassortment of avian viruses with the circulating human virus ('Asian' and 'Hong Kong' flu) or by a direct mutation of an avian virus ('Spanish' flu). More recently, in 1997, H5N1, a new subtype of influenza appeared in South-East Asia and was transmitted from birds to humans. This new form of the virus has infected 385 individuals as of June 2008 (World Health Organization [WHO] confirmed cases),[11] resulting in 243 deaths (60% overall mortality rate), and has caused worldwide concern about the possibility of the occurrence of a new pandemic. Although H5N1 is the subtype considered most likely to cause such a pandemic, other subtypes such as H9N2, H2N2 or H7N7 are also possible candidates.

GSK influenza vaccine portfolio

Seasonal influenza

As recommended by the WHO, seasonal influenza vaccines are trivalent, containing two influenza A strains (H1N1 and H3N2) and one influenza B strain.[1] However, to ensure efficacy against new drift viruses, the vaccine strains must be updated on an annual basis for both the Northern and Southern hemisphere. To support the final strain selection, the WHO coordinates a global influenza surveillance network to identify circulating viral strains.[12] Based on epidemiology and phylogenetic analysis of HA and NA sequences of those human isolates, the WHO recommends three strains that are anticipated to become dominant during the next influenza season.[12] Although in most years the recommendations accurately predict a close antigenic match between the vaccine and circulating strains, sometimes a predominant circulating strain turns out to be antigenically different from the corresponding vaccine strain. This can have a significant negative impact on vaccine efficacy.[8,9,13,14]

For the prevention of seasonal influenza, most governments in Western countries now recommend vaccination to persons most at risk of developing complications, i.e. elderly people aged 65 years and above and people with specific underlying medical conditions. The United States (US) and Canada have recently introduced new recommendations to vaccinate all children aged 6 months to 18 years and 6–59 months, respectively, not only to decrease morbidity in the younger age group but also to decrease the transmission of influenza in the community through herd immunity. Finland has been the first country in the European Union (EU) recommending the vaccination of all children aged 6–35 months, regardless of health status, but the introduction of similar measures is being considered in Europe and in several other countries in Asia and south/central America.

FluLaval™

FluLaval™ is a trivalent inactivated split-virus influenza virus vaccine, containing 15 μg HA from each of the three recommended strains (H1N1, H3N2 and B). This vaccine is manufactured in Quebec, Canada, where it has been marketed since 1992 under the trade name *Fluviral*™ and is indicated for use in persons 6 months and older in Canada. In 2006, *FluLaval*™ was licensed in the US where it is indicated for use in adults aged 18 years and above. The immunogenicity and safety of *FluLaval*™ was compared to that of a registered seasonal influenza vaccine in a phase III study enrolling 1225 healthy subjects aged 50 years and above.[15] Non-inferiority of *FluLaval*™ versus the registered vaccine was demonstrated and both vaccines were well tolerated. The comparable safety profile to other marketed vaccines[15,16] taken together with the long Canadian clinical experience with this vaccine[17] supports *FluLaval*™ as an equivalent to other more widely licensed inactivated influenza vaccines.

Fluarix™

Fluarix™ is a trivalent-inactivated split-virus influenza virus vaccine, containing 15 μg HA from each of the three recommended strains (H1N1, H3N2 and B). It has been manufactured in Dresden, Germany, since 1987 and is now available in more than 100 countries worldwide.

Fluarix™ *for healthy adult and elderly populations*: In the 15 annual European registration studies conducted from 1992 to 2007,[18,19] in which a total of 2112 adult and elderly subjects were included, a single 0·5 ml dose of *Fluarix*™ was shown to be highly immunogenic, and with only a few exceptions, meeting or exceeding all three EU/CHMP (Committee for Medicinal Products for Human Use) immunogenicity criteria for each virus strain (i.e. seroconversion factor [SCF] >2·5 and >2·0, seroconversion rate [SCR] >40% and >30% and seroprotection rate [SPR] >70% and >60% in subjects aged 18–60 years and >60 years, respectively) (see Table 1). In adults aged 18–60 years and elderly subjects aged above 60 years, SPR were 69–100% and consistently exceeded 70% from 1995 onward.[18,19] The vaccine was well tolerated in all age groups and populations (Table 2). Geometric mean titers (GMT) of serum antibodies peaked 21 days after vaccination and remained above the protection level (i.e. % of vaccinees above an HI titer of 1:40) for all three strains for up to 12 months in both the adult and the elderly population.[18] In a study conducted in elderly institutionalized patients, GMTs were also shown to be higher 6 months

Table 1. Immunogenicity of *Fluarix*™ in adult populations: compliance with EU/CHMP immunogenicity criteria for each virus strain recorded 21 days post-vaccination from 1992 to 2007*

Groups of volunteers	Number of subjects	Seroconversion factor			Seroconversion rate			Seroprotection rate		
		H1N1	H3N2	B	H1N1	H3N2	B	H1N1	H3N2	B
Adults 18–60 years**	2049	17/17	17/17	16/17	17/17	17/17	16/17	17/17	16/17	17/17
Adults >60 years**	1556	16/16	16/16	16/16	14/16	15/16	15/16	16/16	16/16	16/16
Immunosuppressed cancer adult patients***	51	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Organ transplant adult patients***	89	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Diabetes mellitus type 1 adult patients***	70	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
COPD adult patients***	63	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Source: Hehme *et al.*,[18] GSK's clinical trial registry,[19] Campbell *et al.*[24] and Beran *et al.*[25]
COPD, chronic obstructive pulmonary disease; GMT, geometric mean titer of serum antibodies.
*Immunogenic data for children are discussed in the body text.
**Numbers of studies across studies (17 for healthy adults and 16 for adults >60 years) carried out between 1992 and 2007 for which EU/CHMP immunogenicity criteria for each virus strain were met or exceeded.
***As CHMP does not specify any immunogenicity criteria for patients at high risk of developing severe influenza or influenza complications, the criteria for 16–60 years of age was used to assess results of these populations. Yes: EU/CHMP criteria met or exceeded.
Seroconversion factor defined as the fold increase in serum HI GMTs post-vaccination compared to day 0.
Seroconversion rate for hemagglutinin antibody response is defined as the percentage of vaccinees who have either a pre-vaccination titer <1:10 and a post-vaccination titer ≥1:40 or a pre-vaccination titer ≥1:10 and at least a four-fold increase in post-vaccination titer.
Seroprotection rate defined as the percentage of vaccinees with a serum HI titer ≥40 after vaccination that usually is accepted as indicating protection.

Table 2. *Fluarix*™: reactogenicity data* recorded within 3 days post-vaccination

Groups of volunteers	Number of subjects	Local adverse events		General adverse events	
		Redness (%)**	Pain (%)***	Fever (%)[¶]	Other (%)[§]
Healthy children 0 to <3 years[#]	160 (273 doses)	0–33	8–32	13–27	N.S.
Healthy children 3 to <6 years[#]	115 (190 doses)	7–28	16–32	11–28	0–20·7
Healthy children 6–18 years[#]	263 (386 doses)	10–29	40–63	0–5	0–25
Adults 18–60 years	665	2–26	2–20	0–4	0–23
Healthy adults >60 years	610	0–31	2–38	0–2	2–19
Immunosuppressed cancer patients	23	13	9	4	5
Organ transplant adult patients	94	0	3·2	0	0–9
Diabetes mellitus type 1 adult patients	70	1	0	1	0
COPD adult patients	70	14	4	0	10

Source: Hehme *et al.*,[18] GSK's clinical trial registry[19] and Schmidt-Ott *et al.*[26]
COPD, chronic obstructive pulmonary disease.
*Reactogenicity data were assessed using severity scales which differed before and after 1996. Only data dating from 1996 to 2007 are presented in this table except for diabetes mellitus type 1 adult patients for which data were collected in 1995. Numbers are minimal and maximal values obtained across all studies between 1996 and 2007.
**Data presented for redness in adults and in children >12 years are for reactions >20 mm in diameter and for reactions >5 mm in children <12 years.
***In adults, data for moderate and severe pain are presented. For children data for any pain are presented.
[¶]Fever was defined as a temperature >38·0°C in children ≥3 years, adults and the elderly and >38·5°C for children <3 years.
[§]Other includes malaise, fatigue, headache, myalgia and shivering.
[#]Whereas adults and children >36 months received a single 0.5 ml dose of the vaccine containing 15 μg of HA per strain, children 6–35 months received a 0·25ml dose of the vaccine, followed, for unprimed children, by a second 0·25 ml dose administered at least 4 weeks later.

after vaccination with *Fluarix*™ than before vaccination.[20] These observations suggest that the vaccine will provide protection for the whole influenza season in a high percentage of both adult and elderly persons. Other studies have shown that the vaccine induces a rapid immune response; and a significant increase in GMTs from baseline was measured 7 days after vaccination with the highest levels recorded after 21 days.[21,22] This rapid immune response suggests that vaccination during an epidemic may still be beneficial for people who are at risk of the disease because they have not been vaccinated earlier in the season. Since 2005, *Fluarix*™ has been approved by the Food and Drug Administration (FDA) for use in the US. A multicenter, randomized, double-blind study carried out in the US to obtain this licensure further supported the good reactogenicity profile of *Fluarix*™ against a placebo control.[23] The solicited symptom rates for swelling, arthralgia, fatigue, headache, chills and fever did not differ between placebo and vaccinated subjects. Only mild to moderate myalgia and injection site pain and redness were more common in vaccine than placebo recipients. Fourfold or greater increases in serum HI titers were observed in 60%, 62% and 78% of subjects and post-vaccination titers of ≥1:40 were achieved in 98%, 99% and 99% of subjects against the H1, H3 and B components of the vaccine, respectively, exceeding the pre-specified immunological criteria for acceptability for all three antigens.[23] The immunogenicity and safety of *Fluarix*™ was also compared to that of a registered influenza vaccine, in a phase III, observer-blind, randomized study, which included 1845 healthy subjects aged 18 years and above.[24] Non-inferiority of *Fluarix*™ versus the other registered influenza vaccine was demonstrated and both vaccines were well tolerated.[24] In a recent randomized, double-blind, placebo-controlled study, which included 7652 subjects aged 18 to 64 years, a statistically significant vaccine efficacy for *Fluarix*™ was demonstrated (66·9% [51·9–77·4], $P < 0·001$) against culture-confirmed influenza A and/or B cases for vaccine antigenically matched strains as well as against culture-confirmed influenza A and/or B cases, for any influenza strain (61·6% [46·0–72·8], $P < 0·001$).[25]

Fluarix™ *for high-risk adult populations*: Specific population subgroups were also studied. Five studies in high-risk adult populations (cancer, organ transplant, diabetes mellitus type 1 and chronic obstructive pulmonary disease patients) ($n = 273$) were carried out between 1992 and 2002 to assess the immunogenicity and safety of influenza vaccination. Immunogenicity in these groups exceeded the target criteria set for healthy adults (Table 1).[18,19]

Fluarix™ *for the paediatric population*: Nine studies in children aged 6 months to 18 years ($n = 776$) were also conducted between 1992 and 2006 to assess the immunogenicity and safety of influenza vaccination in this specific population.[18,19,26] At least one criterion set by CHMP for adults aged 18–60 years (CHMP does not specify any immunogenicity criteria for children) was met in all trials after vaccination of individuals who had not been previously vaccinated with one 0·25 or 0·5 ml dose.[18,19,26] Several studies showed a marked benefit of a second dose in infants and toddlers who had not been previously vaccinated, as well as in children 3–6 years of age: after a second dose, all CHMP criteria (adult thresholds) were usually met for the three strains contained in the vaccine.[18,19,26] A second vaccine dose also substantially increased the immune response in children aged 6–9 years for the A/H1N1 and the B strains, underlining the overall benefit of a second dose to children <9 years of age.[26] The results from safety evaluations showed that *Fluarix*™ is well tolerated and associated with a good safety profile in children (Table 2). No serious adverse events (SAEs) considered as related to vaccination were reported by investigators.

Based on clinical documentation throughout different seasons, GSK Biologicals has been granted a license for its thiomersal-free *Fluarix*™ formulation in Europe in early 2008. The immunogenicity of the thiomersal-free formulation of *Fluarix*™ has also been evaluated in children receiving two doses, and the vaccine was shown to fulfill all three CHMP criteria defined for adults (i.e. SCF >2·5, SCR >40% and SPR >70%) both in children aged 6–35 months and in children aged 36–71 months and for all three strains.[19]

New generation influenza vaccine

It is well known in the medical community that there is a medical need to improve the protective effects of vaccination in the elderly. The efficacy of vaccination tends to decline with age. Indeed, although vaccine efficacy against laboratory-confirmed influenza illness has been shown to be between 70% and 90% in healthy adults,[27] it decreases to 50–60% in community-dwelling elderly people over the age of 65.[28,29] The protective effects of vaccination in the elderly can be improved using several approaches, including adjuvantation of vaccines. Candidate seasonal influenza vaccines developed by GSK are currently undergoing clinical evaluation with the aim of enhancing vaccine response in elderly and immunocompromised subjects.

Pandemic influenza

Influenza viruses constantly mutate and reassort. Sometimes, this can result in the appearance of a novel strain of highly pathogenic influenza, completely unknown to the human immune system, and therefore with high mortality potential. The appearance in 1997 of the H5N1 strain of the influenza virus, which was transmitted from birds to humans and caused high mortality in infected subjects, and

the consequent ongoing global human and avian activity means that the WHO Pandemic Alert Phase is now at level 3 on a scale of 1–6 (humans being regularly infected by birds, i.e. just one level short of human to human transmission).[30] There are major concerns that either H5N1 or another highly virulent subtype of the virus could at any time reassort or mutate and thus acquire the property of human to human transmission leading to a worldwide pandemic. As we can neither predict the evolution of the H5 HA nor which strain will trigger a pandemic, it will not be possible to develop a vaccine matching the actual pandemic strain until 4–6 months after its emergence. This means that advance stockpiling of vaccine, a potentially vital aspect of pandemic preparedness,[31] is only useful if the stockpiled vaccine can elicit broadly cross-protective immunity against different H5N1 viruses, including newly emerged strains. Phylogenetic and antigenic analyses of the HA of H5N1 viruses collected since 1997 indicate that they have evolved into different sublineages or clades.[32] Analysis of the HA sequences of H5N1 isolates collected between August 2006 and March 2007 indicates that the majority belong to clades 1 and 2.[33] Clade 1 viruses and 5 subclades of clade 2 have been distinguished, three of which (clades 2·1, 2·2 and 2·3) have so far been largely responsible for the recorded human cases.[32,33]

Because the threat of a global influenza pandemic is constant and real, many governments as well as the WHO and the European Centre for Disease Prevention and Control (ECDC) are making preparations to attempt to minimize the impact of such a pandemic. The WHO's Pandemic Preparedness Plan includes vaccine use, as well as other measures such as implementation of hygiene measures, limiting contact and stockpiling of antiviral drugs. In order to speed up the availability of pandemic flu vaccines, new European regulatory procedures were put in place, allowing manufacturers to submit 'mock-up' dossiers, for vaccines identical in composition and manufacturing method to the eventual pandemic vaccine, but containing, instead of the still unidentified pandemic strain, another strain unknown to the human immune system. The marketing authorization thus obtained could then quickly be changed in the event of a pandemic to include the responsible virus strain. GSK was the first company to submit a 'mock-up' dossier for a pandemic-inactivated whole-virus vaccine with traditional alum adjuvant[34–36] to EMEA in 2005. This vaccine, *Daronrix*™, received approval in March 2007. Although whole-virus vaccines are usually more immunogenic than split-virus vaccines,[37] split-virus vaccines are in general less reactogenic. GSK has developed adjuvant systems associated with a good safety profile that allow strong and broad immune responses when combined with split-virus antigens.[38,39] Therefore, a second-generation split-virus pandemic vaccine adjuvanted with AS03 (GSK proprietary oil-in-water emulsion-based adjuvant system) was developed, called *Pandemrix*™, for which GSK now holds a provisional license.

Pandemic vaccines will not be available early during the pandemic and consequently will only contribute to decrease morbidity/mortality for the late phase of the epidemic. In this regard, pre-pandemic vaccination is an essential component of the Pandemic Preparedness Plan because it is the only strategy that can be proactively implemented before or in the early stages of a pandemic and is thus regarded as the most effective intervention to prevent or attenuate pandemic influenza.[40] The WHO, ECDC and several countries have already endorsed the pre-pandemic vaccine approach.[41,42] The WHO has called for development of such vaccines that use novel vaccine adjuvants, thus improving immunogenicity, to allow both antigen sparing and the induction of broadly cross-protective immunity.[43] In this context, GSK Biologicals has used its proprietary adjuvant system AS03 to develop an inactivated split-virus H5N1 vaccine containing 3·75 μg HA of the strain A/Vietnam/1194/2004 NIBRG-14, which is a recombinant H5N1 from clade 1, engineered by reverse genetics[39,44] and recommended as a prototype pandemic influenza vaccine strain by the CHMP. GSK is currently licensed to market this pre-pandemic influenza vaccine, called *Prepandrix*™, in all 27 member states of the EU.

Immunogenicity of Prepandrix™

In order to determine the appropriate dose of antigen required to induce an adequate immune response, and to evaluate the effect of the AS03-adjuvant, four antigen doses of an inactivated split virus A/Vietnam/1194/2004 NIBRG-14 formulation were studied (3·75, 7·5, 15 and 30 μg HA) with or without the AS03-adjuvant. Vaccines were administered twice 21 days apart to eight groups of 50 volunteers each, aged 18–60 years.[39] The adjuvanted formulations were significantly more immunogenic than the non-adjuvanted formulations at all antigen doses. At the lowest antigen dose (3·75 μg HA), immune responses for the adjuvanted vaccine against the homologous vaccine strain met or exceeded all immunological US FDA and EU licensure acceptance criteria. Furthermore, when assessed by the more sensitive neutralization assay (which provides an evaluation of the vaccine activity against both the HA and the NA antigens and consequently, gives a more comprehensive evaluation of the biological activity of the vaccine), 77·1% of participants receiving 3·75 μg HA of the AS03-adjuvanted H5N1 candidate vaccine showed an at least four-fold increase in neutralizing antibodies against a strain derived by reverse genetics from a drifted H5N1 isolate (A/Indonesia/5/2005, subclade 2·1) (Table 3). The breadth of this cross-clade immune response was further demonstrated by additional analyses in a subset of these subjects,[45] where a four-fold increase in neutralizing antibodies against geneti-

Table 3. Prepandemic influenza vaccines: immunogenicity data of healthy adults aged 18–60 years recorded 21 days after second vaccination

Vaccine formulation	*N*	Immunogenic virus strain	Dose HA	HI Ab response						Neutralizing Ab response			
				SCF [95% CI]		SCR [95% CI]		SPR [95% CI]		GMT [95% CI]		SCR [95% CI]	
				Non-adj	Adj	Non-adj	Adj	Non-adj	Adj	Non-adj	Adj	Non-adj	Adj
Inactivated split A/Vietnam/1194/2004 NIBRG-14 vaccine containing H5 antigen with or without adjuvant	400	A/Vietnam	3·75 μg (*n* = 50)	1·2 [1·1–1·5]	27·9 [17·2–45·2]	4% [0·5–13·7]	82% [68·6–91·4]	4% [0·5–13·7]	84% [70·9–92·8]	40·7 [32·4–51·0]	314·7 [243·1–407·3]	22·0% [11·5–36·0]	85·7% [72·8–94·1]
			7·5 μg (*n* = 50)	1·7 [1·3–2·3]	38·1 [24·8–58·4]	16% [7·3–29·7]	90% [78·2–96·7]	16% [7·3–29·7]	90% [78·2–96·7]	53·4 [41·6–68·6]	343·0 [260·5–451·5]	36·7% [23·4–51·7]	86·0% [73·3–94·2]
			15 μg (*n* = 50)	2·8 [1·9–4·1]	60·5 [42·8–85·5]	35% [21·7–49·6]	96% [86·0–99·5]	35% [21·7–49·6]	96% [86·0–99·5]	80·1 [60·1–107·0]	400·1 [319·3–501·4]	53·1% [38·3–67·5]	85·7% [72·8–94·1]
			30 μg (*n* = 50)	3·9 [2·4–6·2]	36·4 [22·7–58·5]	41% [27·0–55·8]	85% [72·2–93·9]	43% [28·8–57·8]	85·0% [72·2–93·9]	113·6 [85·5–150·9]	258·2 [205·5–324·5]	64·6% [49·5–77·8]	97·9% [88·7–99·9]
		A/Indonesia	3·75 μg (*n* = 50)	1·0 [1·0–1·0]	2·0 [1·4–2·8]	0% [0·0–7·4]	20·0% [10·0–33·7]	0% [0·0–7·1]	20·0% [10·0–33·7]	14·5 [13·5–15·7]	80·3 [62·0–103·9]	2·3% [0·1–12·3]	77·1% [62·7–88·0]
		A/Turkey	3·75 μg (*n* = 20)	1·0 [1·0–1·0]	3·4 [1·9–6·1]	0% [0·0–16·8]	35·0% [15·4–59·2]	0% [0·0–16·8]	35·0% [15·4–59·2]	16·1 [13·6–19·1]	97·3 [72·5–130·6]	0% [0·0–16·8]	75·0% [50·9–91·3]
		A/Anhui	3·75 μg (*n* = 20)	1·1 [0·9–1·5]	4·7 [2·6–8·5]	5·0% [0·1–24·9]	60·0% [36·1–80·9]	5·0% [0·1–24·9]	60·0% [36·1–80·9]	16·7 [12·4–22·5]	113·2 [80·7–158·9]	0% [0·0–19·5]	85·0% [62·1–96·8]
	1206	A/Vietnam	3·75 μg (*n* = 234)* (*n* = 933)**	1·3 [1·2–1·5]	39·8 [36·8–43·1]	5·6% [3·0–9·3]	93·7% [92·0–95·2]	10·3% [6·7–14·9]	94·3% [92·6–95·7]	7·5 [6·7–8·5]	219·4 [203·3–236·9]	32·4% [21·8–44·5]	96·0% [93·0–98·0]
		A/Indonesia	3·75 μg (*n* = 234)* (*n* = 933)**	1·0 [1·0–1·1]	4·9 [4·5–5·4]	0·4% [0·0–2·4]	50·2% [46·9–53·5]	0·4% [0·0–2·4]	50·2% [46·9–53·5]	5·2 [5·0–5·4]	24·9 [22·8–27·3]	5·6% [1·6–13·8]	91·4% [87·5–94·4]

Source: Leroux-Roels *et al.*,[39;45] Chu *et al.*,[48]

N, number of volunteers; *n*, number of volunteers with available results in each vaccine group. HAI, hemagglutination inhibition; Ab, antibody; SCF, seroconversion factor (see definition in Table 1); SCR, seroconversion rate (see definition in Table 1); SPR, seroprotection rate (see definition in Table 1); GMT, geometric mean titer of serum antibodies; Non-adj, non-adjuvanted; Adj, adjuvanted.

*Number of volunteers with available results in non-adjuvanted vaccine group.

**Number of volunteers with available results in adjuvanted vaccine group.

cally modified A/turkey/Turkey/1/2005 (subclade 2·2) and against A/Anhui/1/2005 (subclade 2·3) H5N1 viruses was induced by 3·75 μg HA of the AS03-adjuvanted H5N1 vaccine in 85% and 75% of subjects, respectively. In contrast, there was no response induced against these strains in the groups receiving the non-adjuvanted vaccine formulations (Table 3). At 6 months post-vaccination, 70% and 60% of subjects who had received adjuvanted vaccine retained neutralizing antibodies against the recombinant subclade 2·2 and 2·3 strains, respectively, and 40% of these subjects retained antibodies against the recombinant subclade 2·1.[45]

Field trials to test the protective efficacy of a pre-pandemic vaccine are obviously impossible prior to the onset of a pandemic. However, evidence regarding protective efficacy can be generated in an appropriate animal model in which vaccination is followed by challenge with a live virus. One such study carried out in ferrets has shown that two doses of the AS03-adjuvanted split H5N1 vaccine A/Vietnam/1194/2004 (clade 1) containing 0·6–15 μg HA resulted in 86% (19/22 ferrets) protection from death after a lethal challenge with the homologous A/Vietnam/1194/2004 virus (94% [15/16] or 100% [11/11] protection with a dose ≥1·7 or 5 μg HA, respectively).[46] Another study in ferrets has also shown [47] that two doses of the same adjuvanted split-virus H5N1 vaccine A/Vietnam/1194/2004 vaccine containing 1·7–15 μg HA induced neutralizing antibodies in the majority of ferrets to both clade 1 (74% (17/23) responders), and clade 2 viruses (61% [14/23] responders [defined by neutralizing titers ≥1:28]), and that 96% of vaccinated animals survived lethal challenge with wild-type virus A/Indonesia/5/2005 (clade 2). Full protection (100%, 17/17) was seen in ferrets vaccinated with two doses containing ≥3·75 μg HA. Moreover, lung virus loads and viral shedding in the upper respiratory tract were reduced in vaccinated animals. This study[47] therefore not only demonstrated the cross-clade protection against lethal H5N1 challenge in ferrets with the AS03-adjuvanted H5N1 influenza vaccine but also suggested that vaccination could markedly attenuate virus shedding during an infection, thus reducing the risk of viral transmission.

The cross-clade immunogenicity of this AS03-adjuvanted H5N1 influenza vaccine was further demonstrated in a phase III lot-to-lot consistency study, in which a larger cohort of Asian adults (aged 18–60 years) received two doses, 21 days apart, of the H5N1 A/Vietnam/1194/2004 split virus influenza vaccine containing 3·75 μg HA adjuvanted or not with the AS03 adjuvant system.[48] Twenty-one days after second vaccination (day 42), SCR of 96% and 91·4% for neutralizing antibodies against the vaccine strain and the A/Indonesia/5/05 strain, respectively, were observed in the group receiving adjuvanted vaccine.[48] In contrast, SCR in the group receiving non-adjuvanted antigen were 32·4% and 5·6% against the vaccine strain and the A/Indonesia/5/05 strain, respectively.[48] Furthermore, despite the HI assay having a greater specificity toward the H-antigen than the neutralizing antibody assay, HI seroprotective titers against the A/Vietnam/1194/2004 and A/Indonesia/05/2005 strain were observed at day 42 in 94·3% and 50·2% of subjects in the adjuvanted group.[48] In the non-adjuvanted group, only 10·3% and 0·4% of subjects presented HI seroprotective titers against the A/Vietnam and A/Indonesia strain.[48]

Prepandrix™, the H5N1 vaccine adjuvanted with AS03, also induced marked immune responses in the elderly population.[49] In children aged 3–9 years, the vaccine containing 1·9 μg HA (A/Vietnam/1194/2004) adjuvanted with AS03 demonstrated marked cross-clade immunogenicity.[50]

Safety and reactogenicity profiles of Prepandrix™

In the study by Leroux-Roels *et al.*,[39] the most common adverse event was injection site pain, reported by 90% of subjects receiving the adjuvanted 3·75 μg HA formulation within 7 days after vaccination. Pain was reported significantly less frequently (38%) in the non-adjuvanted 3·75 μg group ($P < 0·0001$). However, no case of severe pain was reported. Other injection-site adverse events were reported by less than 30% of subjects in the adjuvanted 3·75 μg HA formulation group (Table 4). The general adverse events most frequently reported were fatigue and headache, and were also more frequent in the adjuvanted vaccine groups than in the non-adjuvanted vaccine groups. These adverse events were mild to moderate in intensity and were rarely considered as being related to vaccination (as independently assessed by the investigators). The percentage of subjects reporting at least one unsolicited symptom was similar in the adjuvanted and non-adjuvanted groups (55% versus 56% in the 3·75 μg HA formulation group) but unsolicited symptoms were more often considered to be related to vaccination in the adjuvanted than in the non-adjuvanted groups (29% versus 10% in the 3·75 μg HA formulation group). However, only a minority of unsolicited adverse events reported by subjects receiving the different antigen doses were of severe intensity, and all fully resolved.

These safety results were confirmed in a larger cohort study conducted in 1206 adults aged 18–60 years old receiving two injections, 21 days apart, of H5N1 split-virus vaccine containing 3·75 μg HA, adjuvanted or not.[48] Again, although the adjuvanted vaccine induced more local and general adverse events than the non-adjuvanted vaccine, its safety profile was favorable. No SAEs related to vaccination were reported in this study.

In a phase III, randomized safety trial, a 15 μg HA dose of the split-virus H5N1 vaccine adjuvanted with AS03 was compared with the licensed seasonal influenza vaccine Flu-

Table 4. Prepandemic influenza vaccines: solicited reactogenicity data recorded 0–6 days after one or both vaccinations in healthy adults 18–60 years [%; 95% CI]

	Vaccine groups	
	Inactivated split A/Vietnam/1194/2004 NIBRG-14 vaccine containing 3·75 μg H5 antigen (*n* = 50)	**Inactivated split A/Vietnam/1194/2004 NIBRG-14 vaccine containing 3·75 μg H5 antigen with AS03 adjuvant (*n* = 51)**
Solicited local AEs		
Pain	38 [24·7–52·8]	90 [78·6–96·7]
Redness	18 [8·6–31·4]	18 [8·4–30·9]
Swelling	8 [2·2–19·2]	20 [9·8–33·1]
Induration	10 [3·3–21·8]	28 [15·9–41·7]
Ecchymosis	8 [2·2–19·2]	16 [7·0–28·6]
Solicited general AEs		
Arthralgia	10 [3·3–21·8]	28 [15·9–41·7]
Fatigue	28 [16·2–42·5]	45 [31·1–59·7]
Fever	0 [0·0–7·1]	4 [0·5–13·5]
Headache	36 [22·9–50·8]	53 [38·5–67·1]
Myalgia	16 [7·2–29·1]	39 [25·8–53·9]
Shivering	12 [4·5–24·3]	20 [9·8–33·1]
Sweating	10 [3·3–21·8]	18 [8·4–30·9]

Source: Leroux-Roels *et al.*[39]
AE, adverse event.

arix™ in healthy adults aged 18 years and above.[51] Significantly more participants in the AS03-H5N1 vaccine group reported general or local adverse events (84·3% versus 40·2% of subjects 18–60 years and 69·4% versus 34·1% of subjects >60 years, receiving adjuvanted H5N1 antigen and control, respectively).[51] Injection-site pain was the most common symptom in both treatment groups within the 7–day post-vaccination period (after first dose: 87·6% versus 64·5% of subjects 18–60 years and 57·8% versus 27·1% in subjects >60 years receiving adjuvanted recombinant H5N1 and *Fluarix*™, respectively, and after a second dose: 75·5% versus 15·7% of subjects 18–60 years and 50·4% versus 6·1% in subjects >60 years receiving adjuvanted recombinant H5N1 and placebo, respectively). No SAEs were related to vaccination.[51] The safety and reactogenicity profile of the AS03-H5N1 vaccine was shown to be clinically acceptable, although it had a four-fold higher antigenic content than *Prepandrix*™ (15 μg versus 3·75 μg HA, respectively).[51]

A safety evaluation of the candidate pre-pandemic H5N1 vaccine containing 1·9 μg HA adjuvanted with AS03 was also carried out in a pediatric population of children aged 3–9 years (n = 138) who were given two doses of either the AS03-adjuvanted H5N1 split-virus influenza vaccine containing 1·9 μg HA (H5N1/AS group) or *Fluarix*™ containing 15 μg HA of each of the three strains recommended for seasonal influenza (control group). The candidate H5N1 AS03-adjuvanted vaccine did not raise any safety concerns and the reactogenicity profile was considered to be clinically acceptable.[52,53]

Overall, no safety concern has been raised in any of our clinical trials using the H5N1 vaccine. The AS03-adjuvanted formulation of the vaccine induced superior immunogenicity and a higher incidence of adverse events, although the vast majority of these adverse events were mild to moderate in intensity and all were transient in nature.[39,48,49,51–53] No SAEs related to vaccination with AS03-adjuvanted H5N1 vaccine were reported.

Conclusion

Vaccination is considered to be the one of the most effective tools to decrease morbidity as well as mortality caused by influenza regardless of whether it is for seasonal or pandemic viruses.

Specifically, vaccination of the population with a stockpiled pre-pandemic influenza vaccine, either before or at the immediate onset of a pandemic (phase 6), may significantly reduce the impact of the disease, as shown by mathematical models.[54,55] This vaccination strategy characterized by the induction of broadly reactive sub-type immunity aims to protect against any potential H5N1 pandemic strain.[31,54–57] In this regard, GSK has obtained licensure of a pre-pandemic vaccine, *Prepandrix*™ that meets all CHMP and FDA adult and elderly licensing criteria.[39,48] This split-virus H5N1 adjuvanted with AS03, a proprietary oil-in-water emulsion-based adjuvant system, has demonstrated broad immunity against mutated H5N1 strains[45] and has been shown to be effective in preventing mortality and viral shedding in animal studies.[47]

GlaxoSmithKline also contributes to decrease the impact of seasonal influenza viruses on public health with *Fluarix*™ and *FluLaval*™. Both vaccines have consistently been shown to be immunogenic against strains of H1N1, H3N2 and B and have a good safety profile.[15–25] Although the efficacy of current trivalent inactivated vaccines has been demonstrated, GSK is pursuing additional development efforts in order to further decrease mortality/morbidity caused by influenza virus, especially in the elderly.

Author contributions

B. Baras developed the preclinical section of the manuscript. N. Bouveret developed the section about *FluLaval*™, L. Fries the ones about *FluLaval*™ and pandemic

influenza, P. Gillard the one about pandemic influenza, J.M. Devaster and R. Sänger developed the seasonal influenza section and E. Hanon developed all sections of the study.

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Conflict of interest

All authors are employees at GlaxoSmithKline Biologicals.

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