

# Safety and immunogenicity of Sinovac's prototype pandemic influenza H5N1 vaccines: a review on clinical trials

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**Abstract** Sinovac Biotech started to develop prototype pandemic influenza H5N1 vaccines in March 2004. On 2 April 2008, Sinovac's inactivated, aluminium-adsorbed, whole-virion prototype pandemic influenza A (H5N1) vaccine (PanFlu™) was granted production licensure by the China regulatory authority State Food and Drug Administration. The whole-virion H5N1 vaccine was manufactured in embryonated hens' eggs using the reassortant strain NIBRG-14 (A/Vietnam/1194/2004-A/PR/8/34) as vaccine virus. It showed good safety, immunogenicity and cross-reactivity in immunologically naïve adults. In primed adults,

the vaccine induced a strong booster response. Plasma from a vaccinated individual showed a beneficial effect following passive immunotherapy of an H5N1 human infection case. This article reviews the process, status and results of clinical evaluation of Sinovac's whole- and split-virion H5N1 vaccines by focusing on the whole-virion vaccine.

**Keywords** Immunogenicity, passive immunotherapy, safety, split-virion H5N1 vaccine, whole-virion H5N1 vaccine.

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## Introduction

Since the first human infection with the highly pathogenic avian influenza virus H5N1 was reported in Hong Kong in 1997, concerns of a potential human influenza pandemic caused by the virus have been growing.<sup>1</sup> The virus has become widespread in many regions since 2003, and caused 385 cases and 243 deaths in humans according to WHO's latest release.<sup>2,3</sup> Vaccine development is considered as a critical priority for preparedness against a potential influenza pandemic.

Sinovac Biotech launched the development of its prototype pandemic influenza H5N1 vaccines by collaborating with the Chinese Center for Disease Control and Prevention in March 2004. It was taken as an important part of the Chinese National Pandemic Preparedness Plan and supported by the China Ministry of Science and Technology and China Ministry of Health.

Sinovac's inactivated, aluminium-adsorbed, whole-virion prototype pandemic influenza A (H5N1) vaccine (PanFlu™, Sinovac Biotech, Beijing, China) is the only licensed H5N1 vaccine in China at present. It was granted production licensure by the China regulatory authority

State Food and Drug Administration (FDA) on 2 April 2008, after the completion of a phase II clinical trial at the end of 2007.

Sinovac has been focusing on the development of whole-virion vaccine, and simultaneously the development of a split-virion vaccine. At present, Sinovac's whole-virion H5N1 vaccine has undergone clinical evaluation for safety and immunogenicity in adults and is under evaluation in adolescents and elders. The split-virion vaccine has undergone safety observation in adults, adolescents, older adults and children, and is undergoing the assessment of immunogenicity and safety in adolescents and children. The dose ranges of whole- and split-virion vaccines are 1.25–15 and 5–30 µg respectively. Table 1 summarizes the clinical trials completed or under evaluation.

## Vaccine manufacture

The reassortant strain NIBRG-14 (A/Vietnam/1194/2004-A/PR/8/34), which was prepared by the UK National Institute for Biological Standards and Control and recommended by WHO and EU Committee for Medicinal Products for Human Use (CHMP) for the production of H5N1

**Table 1.** The clinical trials of Sinovac's H5N1 vaccines completed or under evaluation

Vaccine	Phase	Dose ( $\mu\text{g}$ )	Participant enrolled	Design	Purpose	Status	Reference
Whole virion	I	1.25, 2.5, 5, 10	120 Adults	Randomized, double-blind, placebo-controlled	Safety, immunogenicity	Completed	[6]
	I	1.25, 2.5, 5, 10	57 or 88 Adults*	Non-randomized, single-blind	Antibody persistence, booster response	Completed	[7]
	Ib	15	10 Adults	Open-labelled	Safety	Completed	Unpublished Wu J, et al.
			28 Adolescents 30 Elders				
	II	5, 10, 15	402 Adults	Randomized, double-blind	Immunogenicity, safety, cross-reactivity	Completed	Unpublished Wu J, et al.
	IIb	5	70 Adolescents	Randomized, double-blind	Immunogenicity, safety	Under evaluation	Unpublished
	IIb	10	70 Elders	Non-randomized, Open-labelled	Immunogenicity, safety	Under evaluation	Unpublished
Split virion	I	5, 10, 15, 30	40 Adults	Open-labelled	Safety	Completed	Unpublished
			40 Adolescents				
			40 Elders				
			40 Children				
	II	10, 15, 30	210 Adolescents	Randomized, double-blind	Immunogenicity, safety	Under evaluation	Unpublished
II	10, 15	140 Children	Randomized, double-blind	Immunogenicity, safety	Under evaluation	Unpublished	

\*Participants were from two-dose primed population in previous phase I trial enrolling 120 adults. Fifty-seven and 88 adults at 6 and 12 months after the second dose respectively.

vaccines,<sup>4,5</sup> was used as a vaccine virus to manufacture Sinovac's H5N1 vaccines. The H5N1 antigen was produced on a pilot scale in embryonated hens' eggs. Two H5N1 vaccines including whole- and split-virion vaccines were developed by Sinovac, both of which were formulated with aluminium hydroxide to contain 0.25 mg aluminium/dose. The vaccines were manufactured in compliance with the Chinese Pharmacopoeia (III, 2005). The temporary validity period (expiry dating) was 24 months.

## Safety

The safety results of the whole-virion H5N1 vaccine in adults are summarized in Table 2. The whole-virion H5N1 vaccine was firstly evaluated in adults in a phase I trial enrolling 120 participants with 24 of them receiving placebo.<sup>6</sup> The results showed that the four vaccine doses (1.25, 2.5, 5 and 10  $\mu\text{g}$ ) were well tolerated with no serious adverse events reported. Most of the reported local and systemic reactions were graded as mild and transient and resolved within 72 h.

The most common local and systemic reactions were pain at injection site and fever respectively.

The incidence of adverse reactions did not occur in a dose-dependant manner. A higher incidence of adverse reactions was reported in participants receiving the 1.25  $\mu\text{g}$  dose than other doses and placebo, which was largely due to a higher frequency of pain at the injection site in this group.

In phases Ib and II trials, the safety of the whole-virion H5N1 vaccine was further evaluated in a larger population including adults, adolescents and older adults (unpublished data Wu J, et al.). A dose of 15  $\mu\text{g}$  was assessed initially in a small group of 10 adults. After the 15  $\mu\text{g}$  dose was well tolerated in adults in the phase Ib trial, the phase II trial evaluating 5, 10 and 15  $\mu\text{g}$  doses was conducted in 402 adults. The results showed that all the doses were well tolerated without immediate allergic reactions or serious adverse events. There were 34% (136/402) of participants who reported adverse reactions, all of which resolved within 72 h. Pain at the injection site and fatigue were the most common local and systemic reactions respectively. The incidence of adverse reactions did not show dose-dependency.

**Table 2.** Safety profile of whole-virion H5N1 vaccine in adults

	1.25 µg (Phase I)	2.5 µg (Phase I)	5 µg (Phases I + II)	10 µg (Phases I + II)	15 µg (Phases Ib + II)	Sum
<i>n</i>	24	24	124	226	110	508
Total	66.7 (16)	45.8 (11)	32.3 (40)	31.9 (72)	45.5 (50)	37.2 (189)
Mild	58.3 (14)	33.3 (8)	25.8 (32)	19.9 (45)	31.8 (35)	26.4 (134)
Moderate	8.3 (2)	12.5 (3)	6.5 (8)	9.3 (21)	11.8 (13)	9.3 (47)
Severe	0.0 (0)	0.0 (0)	0.0 (0)	2.7 (6)	1.8 (2)	1.6 (8)
Local	66.7 (16)	33.3 (8)	23.4 (29)	22.6 (51)	30.0 (33)	27.0 (137)
Systemic	25.0 (6)	41.7 (10)	20.2 (25)	24.3 (55)	30.9 (34)	25.6 (130)
First vaccination	62.5 (15/24)	45.8 (11/24)	28.2 (35/124)	29.2 (66/226)	40.0 (44/110)	33.7 (171/508)
Second vaccination	29.2 (7/24)	20.8 (5/24)	10.8 (13/120)	7.2 (16/221)	18.1 (19/105)	12.1 (60/494)

Data are incidences of adverse reactions (number of participants reporting adverse reactions/number of participants receiving vaccine).

The safety of the whole-virion H5N1 vaccine at a dose of 5, 10 or 15 µg was also evaluated in adolescents and older adults in a phase Ib trial. The three doses were well tolerated in older adults with only one participant reporting moderate fever in the 10 µg group. In adolescents, however, there were six of ten and five of eight participants reporting adverse reactions, of whom two and one participants respectively reported severe adverse reactions after the first dose of 10- and 15-µg vaccines. It seemed that the incidence and severity of adverse reactions were not acceptable in adolescents. However, it is too early to draw a conclusion that the whole-virion H5N1 vaccine is less safe in adolescents due to the small sample size, and further study may be needed to assess the safety profile of the whole-virion vaccine in adolescents.

In phases I, Ib and II trials of whole-virion vaccine, it was found that the incidences of adverse reactions in adults were remarkably lower after the second dose than those after the first dose as shown in Table 2.

The safety of the booster (third) dose of whole-virion vaccine was evaluated in 57 adults 12 months after the second dose.<sup>7</sup> The results showed that the third dose was well tolerated with no serious adverse events reported. Only 5.3% (three of 57) of participants reported adverse reactions. By comparison with the first and second doses (33.7% and 12.1% respectively), the incidence of adverse reactions after the third dose was significantly lower.

The safety of split-virion vaccine including 5, 10, 15 and 30 µg doses was evaluated in 160 participants including adults, adolescents, older adults and children. No serious adverse events were reported. There were 31.9% (51/160) of participants who reported adverse reactions, all of which were transient. Most of the adverse reactions were mild (34/160, 21.3%) or moderate (14/160, 8.8%). The incidences of adverse reactions in adults, adolescents, older adults and children were 35%, 32.5%, 20% and 40%

respectively. Pain at the injection site and fever were the most common local and systemic reactions respectively. The incidences of adverse reactions showed dose-dependency. The results indicated that the split-virion H5N1 vaccine was well tolerated.

## Immunogenicity

### Immunogenicity in an unprimed population

Immunogenicity of the whole-virion H5N1 vaccine has been assessed in adults in phases I and II clinical trials where all participants received two priming doses at the regimens of days 0–28 or 0–14. The phase I trial showed that all four doses (1.25, 2.5, 5 and 10 µg) induced haemagglutination-inhibition (HI) and microneutralization (MN) antibody responses after the first dose. The second dose significantly boosted the response. A dose–response relationship was seen across the four vaccination groups. Haemagglutination-inhibition geometric mean titres (GMTs) of 13.9–57.4 and MN GMTs of 15.7–45.1 were developed in the four groups 14 days after the second dose. The highest immune response of 78% HI seroconversion and seroprotection rates and a 11.5 post-to-pre-vaccination GMT ratio was elicited after two doses of 10 µg vaccine, meeting all three EU CHMP licensure criteria for HI GMT ratio (>2.5), seroconversion rate (>40%) and seroprotection rate (>70%).<sup>4,6</sup>

A larger phase II trial conducted in 402 participants with 100 or 101 participants per group, confirmed that the aluminium-adjuvanted, whole-virion H5N1 vaccine had good immunogenicity in adults (unpublished data). After two doses of 10 µg vaccine, an immune response (12.4 GMT ratio, 78% seroconversion and seroprotection rates) was induced, which met all three EU CHMP criteria. An obvious dose–response relation was seen. A higher immune response was induced in participants receiving

the 15- $\mu$ g dose. For the licensure of H5N1 vaccine, the US FDA has released more stringent criteria for HI seroconversion rate (lower limit of two-sided 95% CI  $\geq 40\%$ ) and seroprotection rate (lower limit of two-sided 95% CI  $\geq 70\%$ ).<sup>8</sup> After the second dose, the 10- $\mu$ g vaccine induced immune response meeting US FDA criterion for seroconversion rate and the 15- $\mu$ g vaccine met the two US FDA criteria. In addition, it was found that the days 0–28 regimen induced a higher immune response than days 0–14 regimen did.

### Antibody persistence

To detect antibody persistence after priming vaccination with the whole-virion H5N1 vaccine, blood samples from volunteers who had received two doses of this vaccine in previous phase I trial were collected 6 and 12 months after two priming doses.<sup>7</sup> Haemagglutination-inhibition and MN assays showed that both the antibody levels declined noticeably. Six months after the second dose, 14.8% (13/88) and 15.9% (14/88) of participants remained HI and MN titres  $\geq 1:40$  respectively. Twelve months after the second dose, 8.8% (5/57) and 14.0% (8/57) of participants retained HI and MN titres  $\geq 1:40$  respectively. Another study on non-adjuvanted, split-virion H5N1 vaccine has also shown that both HI and MN titres declined substantially after two-dose priming.<sup>9</sup> These results suggested that antibody titres after priming vaccinations of H5N1 vaccines were not very persistent and that a booster (third) dose was needed to maintain a seroprotective titre. The antibody persistence after two priming doses is shown in Figure 1.

### Booster response

A booster (third) dose of the whole-virion H5N1 vaccine given 12 months after the second dose significantly boosted the immune response in primed adults.<sup>7</sup> Thirty days after the third dose, 29%, 31%, 79% and 90% of participants developed HI titre  $\geq 1:40$ , and 53%, 81%, 93% and 100% of participants developed MN titre  $\geq 1:40$  in 1.25, 2.5, 5 and 10  $\mu$ g groups respectively. Both 5- and 10- $\mu$ g doses met all EU licensure criteria. The kinetics of immune responses after the first, second and third doses are shown in Figure 1.

### Cross-reactivity

The vaccine virus is a reassortant of the A/Vietnam/1194/2004 strain, which phylogenetically belongs to clade 1. Cross-reactivity assays were performed against three heterologous H5N1 drifted strains including IBCDC-RG2 (A/Indonesia/5/2005-A/PR/8/34), NIBRG-23 (A/Turkey/Turkey/1/2005-A/PR/8/34) and IBCDC-RG5 (A/Anhui/1/2005-A/PR/8/34), which belong to clade 2.1, clade 2.2 and clade 2.3 respectively.<sup>5</sup> The three heterologous

strains were reassortants derived by reverse genetics and recommended by WHO as H5N1 candidate vaccine viruses. Both HI and MN assays showed strong cross-reactive responses against the recombinant Indonesia and Anhui strains. After two doses of 10  $\mu$ g vaccine, 65% of participants had developed HI titre  $\geq 1:40$ , and 98% and 87% of participants had developed MN titre  $\geq 1:40$  against recombinant Indonesia and Anhui strains respectively (unpublished data). However, the cross-reactive response against the recombinant Turkey strain was relatively lower.

### Passive immunotherapy

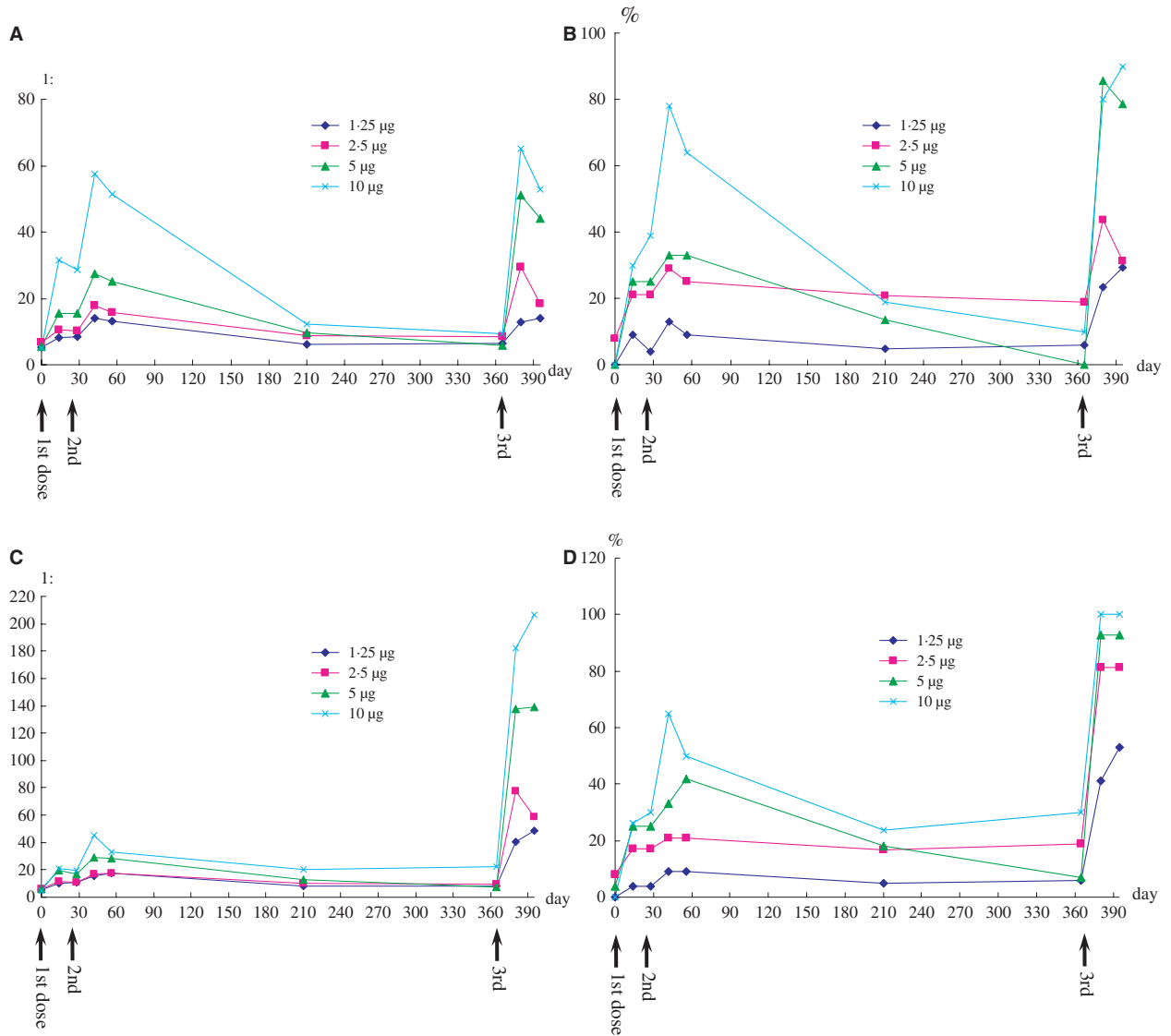
Passive immunotherapy for H5N1 infection using post-vaccination anti-H5N1 plasma has shown positive and exciting result.<sup>10</sup> In December 2007, a family cluster of two individuals infected with H5N1 virus was identified in Jiangsu Province, China. The 24-year-old index case died, and the second case, his 52-year-old father, survived after receiving early antiviral treatment and transfusion of post-vaccination anti-H5N1 plasma. The plasma was donated by a volunteer 280 days after she received two doses of the whole-virion H5N1 vaccine at days 0–28 regimen in the phase I clinical trial. The plasma showed 1:40 MN titre against homologous NIBRG-14 and 1:20 against the second case's strain A/Jiangsu/2/2007. The low MN titres were mainly due to the long interval between the second vaccination and the bleeding when the antibody titre was undergoing decline.

Although it was difficult to distinguish exactly the effects of antiviral agents and plasma on the treatment of the patient, the fact that the patient still had a worsened respiratory status after 4 days treatment with antiviral agents and that the transfusions of anti-H5N1 plasma resolved the patient's fever, suggesting that post-vaccination plasma might be a desirable alternative for treatment of H5N1 infection.

### Problem and prospect

The past a few years have witnessed remarkable achievements of pandemic influenza H5N1 vaccine development. Many promising H5N1 vaccines including whole-virion, split-virion, adjuvanted and non-adjuvanted vaccines have been developed and clinically evaluated,<sup>6,11–13</sup> some of which have been granted production licensure in different regions. All these achievements arm us with a powerful, if not sufficient, defence against the next human influenza pandemic.

Even if several H5N1 vaccines are available for deployment, the demand for influenza vaccines will far outstrip the manufacturing capacity during a pandemic. Production capacity expansion and antigen-sparing strategy are encour-



**Figure 1.** Kinetics of immune responses after the first, second and third doses of whole-virion H5N1 vaccine. Geometric mean titres (A) and seroprotection rates (B) as determined by haemagglutination-inhibition assay, and geometric mean titres (C) and proportions of participants developing titre  $\geq 1:40$  (D) as determined by microneutralization assay.  $n = 94$  from days 0 to 56;  $n = 88$  at day 210;  $n = 57$  from day 365 forward.

aged. We have shown that whole-virion H5N1 vaccine elicited a satisfactory immune response using less antigen than subunit or split-virion vaccines did. Other studies have reported good immunogenicity of pandemic influenza candidate vaccines adjuvanted with oil-in-water emulsions, resulting in significant antigen sparing.<sup>13–15</sup> Further study may be necessary to evaluate the immunogenicity of whole-virion H5N1 vaccine adjuvanted with an oil-in-water emulsion, which is hoped that it will be further antigen sparing.

Cross-reactivity against genetically and antigenically distinct strains should be considered for the development of

pandemic candidate vaccines. Previous trials have reported the cross-reactivity of pandemic candidate vaccines.<sup>13,16,17</sup> Our whole-virion H5N1 vaccine has shown good cross-reactivity against the heterologous Indonesia and Anhui recombinant strains. However, there are few data about the protection efficacy and cross-protection, and it is impossible to study the protection efficacy and cross-protection in humans during the interpandemic period. The direct evidence from virus challenge by homologous and heterologous wild-type H5N1 viruses in animals will help us understand the protection efficacy and cross-protection of pandemic candidate vaccines.

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