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Review of 10 years of clinical experience with Chinese domestic trivalent influenza vaccine Anflu®

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Abbreviations: ACIP, Advisory Committee on Immunization Practices; ATP, according-to-protocol; China CDC, Chinese Center for Disease Control and Prevention; CFDA, China Food and Drug Administration; CHMP, Committee for Medicinal Products for Human Use; EU, European Union; FDA, Food and Drug Administration; GMP, good manufacture practices; GMT, geometric mean titer; HA, haemagglutinin; HI, haemagglutination-inhibiting; IEC, Independent Ethics Committee; MN, microneutralization; NA, neuraminidase; TIV, trivalent influenza vaccine; US, United States; WHO, World Health Organization

Influenza viruses cause annual winter epidemics globally and influenza vaccination is most effective way to prevent the disease or severe outcomes from the illness, especially in developing countries. However, the majority of the world's total production capacity of influenza vaccine is concentrated in several large multinational manufacturers. A safe and effective preventive vaccine for the developing countries is urgent. Anflu®, a Chinese domestic preservative-free, split-virus trivalent influenza vaccine (TIV), was introduced by Sinovac Biotech Ltd. in 2006. Until now, 20.6 million doses worldwide of Anflu® were sold. Since 2003, 13 company-sponsored clinical studies investigating the immunogenicity and safety of Anflu® have been completed, in which 6642 subjects participated and were vaccinated by Anflu®. Anflu® was generally well tolerated in all age groups, and highly immunogenic in healthy adults and elderly and exceeded the licensure criteria in Europe. This review presents and discusses the experience with Anflu® during the past decade.

A new Chinese domestic, preservative-free, unadjuvanted, inactivated split-virus trivalent influenza vaccine (TIV), Anflu®, was introduced into human clinical trials in 2003 and then licensed in China in 2006. The vaccine contains 15 μg /0.5 ml hemagglutinin from each of the 3 influenza virus strains (including an H1N1 influenza A virus subtype, an H3N2 influenza A virus subtype, and an influenza B virus) that are expected to be circulating in the up-coming influenza season. The clinical data pertaining to Anflu® will be reviewed and compared with other TIVs available at present.

Influenza Epidemics and Viruses

Influenza is 1 of the most important respiratory infections of humans, responsible for 300 000–500 000 annual deaths worldwide.[1] Yearly influenza epidemics can typically infected 5–15% of worldwide population, affecting all age groups.[2] Most influenza-related hospitalizations in industrialized countries occur in young children (<5 y) and in the elderly (≥65 y) and most deaths occur among the elderly. In the United States alone, 5–20% of the population acquires influenza on an annual basis, resulting in approximately 200 000 hospitalizations and 36 000 deaths.[3,4] Taking into account work absenteeism as well as direct medical costs, the annual economic impact of influenza in the US has been estimated to be US $12–14 billion[5] and, sometimes, even up to US $87 billion.[6] In developing countries, seasonal influenza has been associated with a higher proportion of deaths, especially among remote populations.[7] In Madagascar, seasonal influenza mortality rates of 2.5% have been reported,[8] with even higher rates (15%) reported in Indonesia[9] and in the highlands of Papua New Guinea (9.5%).[10]

Influenza virus belongs to the family *Orthomyxoviridae* and is classified into 3 types: A, B, and C virus, depending on the antigenic differences of their structural proteins.[11] Influenza A and B viruses are responsible for the yearly epidemic outbreaks of human respiratory illness. The influenza type A causes moderate to severe illness in all age groups in humans while the illness caused by type B is of milder and it is primarily affects children. The type C influenza virus rarely causes illness, hence it does not cause epidemics.

Influenza A virus is subtyped based on 2 surface glycoproteins: neuraminidase (NA) and hemagglutinin (HA), which are the main antigenic determinants capable of inducing immune responses to the virus.[12-14] There are at least 15 antigenically distinct forms of HA and 9 NA antigens, the combination of which is the basis for classifying viral strains into subtypes. Epidemic human disease over the last century has only associated with H1N1, H1N2, H2N2, and H3N2. The influenza type B has a single HA and NA type and is not categorized into subtypes. Currently circulating influenza B virus can be separated into 2 distinct genetic lineages (Yamagata and Victoria). Both influenza A and B viruses maintain diversity by altering surface antigen structure from year to year in a process of point mutations called "antigenic drift"[15]. Antigenic shifts are responsible for annual

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influenza epidemics and even pandemics, with the changes in HA and NA helping the virus to overcome the immune response generated in a host population through prior infection or vaccination.

Influenza epidemics usually occur each year, generally during the winter months in temperate regions: October to April in the Northern Hemisphere and May to September in the Southern Hemisphere. World Health Organization (WHO) established the Global Influenza Network in 1952, in order to monitor antigenic drift and emerging virus strains relevant to the Northern or Southern Hemispheres. Based on these data, WHO identifies prevalent circulating strains relevant to the subsequent influenza season and then recommends 2 influenza A strains (1 H1N1 and 1 H3N2) and an influenza B strain for the influenza vaccine inclusion.[16]

Trivalent Inactivated Influenza Vaccine

Influenza immunization is most effective when circulating viruses are well-matched with vaccine viruses. The WHO estimates that globally there are about 1.2 billion people at high risk for severe influenza, and an additional 24 million health care workers who ought to be immunized annually. Currently, most commercially available influenza vaccine is trivalent inactivated vaccine (TIV). The TIVs include 3 types: whole virus, split-virus, and subunit formulations. Whole-virus vaccines, because of adverse reactions, especially in children, are not currently used. Most influenza vaccines are split-virus vaccines, produced from detergent-treated, highly purified influenza virus, or surface-antigen vaccines containing purified hemagglutinin and neuraminidase. WHO recommended TIVs should contain 15 μg each of the 2 influenza A strains (H1N1 and H3N2) and 1 influenza B strain.

The first domestic TIV was introduced into China in 2000, following the productions from 2 multinational manufacturers which entered the Chinese market in 1998. By 2009, there were 11 domestic and 5 multinational companies, supplying TIVs in China. In 2010, 95% of TIVs released in the Chinese market were split-virus influenza vaccines.[17] The vaccine virus strains used in China follows the WHO annual recommendations for composition of Northern Hemisphere vaccine expected to be circulating in the community in the upcoming winter. China's guidelines for seasonal influenza vaccination were adapted from the recommendations of the United States Advisory Committee on Immunization Practices (ACIP)[18] and have been issued annually by Chinese Center for Disease Control and Prevention (China CDC) since 2007.[19] The target vaccinated population recommended by China CDC included persons with chronic illness, pregnant women, individuals <5 or ≥60 y old, health care workers, and close contacts of high risk individuals. However, seasonal influenza vaccination is not included in the national immunization program and must be purchased by recipients except for a few cities where local government subsidy programs have been introduced.

Anflu®: A Preservative-free, Unadjuvanted, Split-Virus Tiv

Anflu® is developed and produced by Sinovac Biotech Co. Ltd. The vaccine includes 2 formulations: a 0.5-mL dose for children > 3 y and adults with 1-dose regimen, and a 0.25-mL dose for infants aged from 6 mo to 3 y with 2-dose regimen with 3–4 weeks apart. The influenza strains used in the preparation of Anflu® for the winter season since 2006 met the recommendations of the WHO (**Table 1**). Each of the 3 strains are produced and purified separately. Embryronated hen's eggs are inoculated with the respective virus and the virus is allowed to replicate in the allantoic fluid. After harvesting, the allantoic fluid from the eggs containing the cultured virus is concentrated and then inactivated. Following inactivation, the fluid is purified by zonal centrifugation and detergent is used to split the virus. Anflu® is formulated from each of the 3 split inactivated virus solutions, without preservatives and without adjuvants. In addition, Anflu® contain 15 μg/0.5 ml of A/H1N1-, A/H3N2-, and B-virus hemagglutinin antigens per dose.

In 2003, Sinovac Biotech designed, constructed and validated the production plant of the egg-embryonated seasonal influenza vaccine in accordance with US Food and Drug Administration (FDA) good manufacture practices (GMPs) requirements. Two years later, Sinovac Biotech obtained the GMP certification (1998 version) and production approval by CFDA for Anflu®. In March 2011, CFDA enacted the new 2010 version Chinese GMP regulation, in order to improve the quality of drug production and reach the international advanced level. In 2012, Sinovac Biotech again fulfilled the requirement for the manufacture of biological products by revised GMP regulation.

For seasonal influenza vaccines to be accepted throughout the European Union (EU), annual clinical trials must demonstrate immunogenicity and safety in at least 50 subjects between 18 and 60 y and in 50 subjects over 60 y. Vaccines must fulfill the European Medicines Agency Committee for Medicinal Products for Human Use (CHMP) efficacy criteria for each influenza strain contained in the vaccine for both age groups.[20] In China, although there is no similar mandatory requirement by China Food and Drug Administration (CFDA) on annual re-licensing influenza vaccines, Sinovac initiated annual studies to demonstrate the safety and immunogenicity of Anflu® in healthy Chinese population. Clinical data in this review on Anflu® comes from pre-market licensure trial,[21] annual trials sponsored by Sinovac Biotech[22-31] (**Table 1**) and investigator-initiated clinical trials.[32-34]

Immunogenicity

Immunogenicity data reviewed in this section have been obtained from the according-to-protocol (ATP) cohort (**Table 2**). The ATP cohort for immunogenicity included all subjects who met all eligibility criteria, complied with the procedures defined in the protocol and for whom data concerning immunogenicity endpoint measures were available.

Immunogenicity was determined by assessing the level of haemagglutination-inhibiting (HI) antibodies against all 3 HA antigen components of the vaccine in the serum of blood taken just before and after administration of the vaccine. Primary immunogenicity endpoints included: Geometric mean titer (GMT), seroprotection rate (defined as % of subjects with a postvaccination HI titer ≥1:40), and seroconversion rate (defined as % of subjects with a pre-vaccination titer <1:10, and a post-vaccination titer ≥1:40, or with a pre-vaccination titer ≥1:10 and ≥4-fold increase after vaccination). The immunogenicity endpoints of the studies were evaluated in light of the immunogenicity criteria for adults (aged 18–60 y) and the elderly (aged >60 y) for influenza vaccines set by the EU CHMP.[20] For the vaccine to be considered sufficiently immunogenic, at least 1 of the following 3 criteria has to be met for each of the antigenic strains: seroprotection rate >70%, seroconversion rate >40%, and seroconversion factor (known as increase in GMT) >2.5, for adults; seroprotection rate >60%, seroconversion rate >30%, and seroconversion factor >2, for the elderly.

In adults and the elderly

In the pre-licensing clinical trial,[21] the study vaccine was produced by 2002–2003 influenza strain recommended by WHO. After the application for clinical trials has been approved by CFDA and the protocol and other related documents have been approved by the independent ethics committee (IEC), the double-blind, randomized and controlled trial was conducted in 2003 by Li et al. The investigators evaluated the immunogenicity of Anflu® in healthy adults and the elderly. The seroconversion rates of Anflu® in adult and elderly subjects were 77.9 and 73.7, 29.5 and 63.2, 40.5, and 65.4% for H1N1, H3N2 and B, respectively, while the proportion of subjects achieving an HI titer of 40 or more were 72.2–100.0%. The GMT against the 3 viral strains increased 2.1- to 5.6-folds in the adults and 5.4- to 6.9-folds in the elderly. Except the seroconversion rate and GMT increase folds for H3N2 in adults (it was associated with high pre-vaccination H3N2 prevalence of 99.5%), other results met the immunogenicity criteria of CHMP.

Table 1. Annual influenza virus strains used in Anflu® (Northern Hemisphere) and clinical trials sponsored by Sinovac

Season	H1N1	H3N2	B	Study site	Subjects	Objectives	Ref
2002/2003	A/New Caledonia/20/99	A/Moscow/10/99	B/Hong Kong/330/2001	Liucheng country CDC, Guangxi province	6–35 months, and 6–12, 16–60, >60 years	Safety and immunogenicity	21
2006/2007	A/New Caledonia/20/99	A/California/7/2004	B/Shang Hai/361/2002	Tianjin city CDC	18–60 and >60 years	Safety	29
				Beijing CDC	18–60 and >60 years	Safety	*
2007/2008	A/Solomon Islands/3/2006	A/Hiroshima/52/2005	B/Malaysia/2506/2004	Tianjin city CDC	18–60 years	Safety and immunogenicity	22
				Lanshan country CDC, Hunan province	>60 years	Safety and immunogenicity	23
				14 city or country CDCs	6–35 months, and 3–12, 12–17, 18–60, >60 years	Safety	30, 24, 25
2008/2009	A/Brisbane/59/2007	A/Brisbane/10/2007	B/Florida/4/2006	Qingdao city CDC, Shandong province	18–60 and >60 years	Safety and immunogenicity	26
				5 city or country CDCs	6–35 months, and 3–12, 18–60, >60 years	Safety	*
2009/2010	A /Brisbane/ 59/2007	A/ Uruguay/716/2007	B/Brisbane/60/2008	Lingchuan country CDC, Guangxi province	6–35 months	Safety and immunogenicity	27
				Beijing CDC	18–60 years	Safety and immunogenicity	31
2010/2011	A/California/7/2009	A/Victoria/210/2009	B/Brisbane/60/2008	Shenyang city CDC, Liaoning province	18–60 and >60 years	Safety and immunogenicity	28
2011/2012	A/California/7/2009	A/Perth/16/2009	B/Brisbane/60/2008	Zhengding country CDC, Hebei province	6–35 months	Safety	*
2012/2013	A/California/7/2009	A/Victoria/361/2011	B/Hubei-Wujiagang/158/2009	Yuanyang country CDC, Henan province	6–35 months, and 18–60 and >60 years	Safety and immunogenicity	*

*Unpublished.

Table 2. Immunogenicity of an inactivated split-virus trivalent seasonal influenza vaccine, Anflu®

Study	Subjects,	Geometric mean titer, (95% CI)						Seroconversion rates, % (95% CI)			Seroprotection rates, % (95% CI)		
(Season)	N	H1N1		H3N2		B		H1N1	H3N2	B	H1N1	H3N2	B
		Day 0	Day 21/28	Day 0	Day 21/28	Day 0	Day 21/28						
Subjects aged 6-35 months													
Li et al.[21] (2002/2003)	179	19	534	14	196	6	48	94.4	79.3	54.4	100	83.2	56.4
Li et al.[27] (2009/2010)	51	20.2 (13.3–30.7)	588.9 (423.5–818.9)	13.8 (8.9–21.5)	278.6 (183.2–423.7)	6.2 (5.3–7.2)	30.3 (22.9–40.1)	80.6 (72.6-94.3)	92 (79.9–98.2)	46 (33.5–62.9)	100 (91.1–100.0)	98 (88.0–100.0)	50 (35.7–64.5)
Luo et al.[32] (2010/2011)	73/40	16.8 (7.3–26.4)	93.3 (86.1-99.0)	14.6 (6.3–22.8)	83.8 (77.0–90.7)	5.8 (0–11.6)	27.8 (22.6–33.1)	70 (55.8–84.2)	72.5 (58.7–86.3)	40 (24.8–55.2)	90 (80.7–99.3)	87.5 (77.3–97.7)	67.5 (53.0–82.0)
Subjects aged 6-12 years													
Li et al.[21] (2002/2003)	232	148	2058	128	322	18	128	87.5	36.6	75.9	100	100	91.8
Luo et al.[32] (2010/2011)	74/73	17.9 (7.4–28.3)	112.7 (103.6–121.9)	15.6 (6.8–24.4)	119.6 (110.4–128.9)	7.6 (2.6–12.6)	36.7 (30.9–42.4)	64.4 (53.4–75.4)	74 (63.9–84.0)	37 (25.9–48.1)	95.9 (91.3–100.0)	97.3 (93.5–100.0)	69.9 (59.3–80.4)
Subjects aged 18-60 years													
Li et al.[21] (2002/2003)	190	244	1357	183	376	17	56	77.9	29.5	40.5	100	100	72.1
Zhang et al.[22] (2007/2008)	411	16.0–19.4	879.9–969.1	30.6–36.9	399.1–444.6	11.6–15.4	254.4–280.0	91.9–95.7	83.5–85.4	89.2–94.2	98.5–99.3	97.8–99.3	95.6–97.1
Jiang et al.[26] (2008/2009)	62	11.2 (8.7–14.3)	244.7 (172.2–347.6)	17.3 (12.6–23.8)	462.8 (339.4–631.1)	30 (24.8–36.9)	153 (125.8–186.2)	88.7 (77.5–95.5)	90.3 (79.6–96.5)	64.5 (51.3–76.2)	95.2	100	100
Wu et al.[31] (2009/2010)	49	25.4 (19.6–33.0)	668.3 (481.5–927.8)	9.9 (7.5–13.0)	302 (179.0–509.7)	36.7 (31.0–43.6)	151 (116.2–196.3)	93.8 (81.8–99.3)	85.4 (71.6–94.1)	66.7 (51.5–79.6)	100 (90.8–100.0)	89.6 (76.6–96.8)	97.9 (87.5–100.0)
Wang et al.[28] (2010/2011)	63	19.4 (13.1–28.6)	390.1 (262.2–580.3)	19.6 (13.9–27.6)	626.1 (450.6–869.8)	32.5 (25.5–41.3)	369.2 (279.3–488.0)	82.5 (70.5–90.9)	93.7 (83.7–98.6)	92.1 (81.7–97.6)	92.1 (81.7–97.6)	98.4 (90.3–100.0)	98.4 (90.3–100.0)
2012/2013 *	64	22.1 (16.0–30.5)	905.1 (655.7–1249.4)	55.2 (41.1–74.1)	769.4 (587.0–1008.4)	14.1 (11.4–17.5)	113.1 (87.3–146.5)	90.6 (80.1–96.7)	78.1 (65.7–87.4)	78.1 (65.7–87.4)	100 (92.9–100.0)	100 (92.9–100.0)	92.2 (82.0–97.7)
Subjects aged >60 years													
Li et al.[21] (2002/2003)	133	139	951	96	522	8	55	73.7	63.2	65.4	100	100	72.2
Jiang et al.[26] (2008/2009)	58	10.4 (7.9–13.6)	193.7 (137.3–273.4)	17.5 (12.8–24.0)	498 (327.5–757.2)	22.8 (18.7–27.8)	130.6 (104.1–163.8)	82.8 (70.1–91.4)	84.5 (72.1–92.7)	74.1 (60.7–84.7)	91.4	94.8	98.4
Wang et al.[28] (2010/2011)	57	8.4 (6.7–10.7)	201.6 (133.6–304.3)	18.8 (12.7–27.9)	749.6 (496.8–1131.0)	22.9 (17.7–29.5)	344.2 (244.6–484.5)	89.5 (77.8–96.2)	91.2 (80.0–97.4)	87.7 (75.7–95.0)	93 (82.2–98.4)	94.7 (84.5–99.4)	96.5 (86.8–100.0)

*Unpublished; CI, confidence interval. Li et al.[21] did not provide the CI values for GMT, seroconversion rates and seroprotection rates. Zhang et al.[22] evaluated the immunogenicity of the 3 consecutive lots of Anflu® and provided the analyzed results separately for the 3 groups, but did not provide the results from data pooled from the 3 groups. Jiang et al.[26] did not provide the CI values for seroprotection rates.

Table 2. Immunogenicity of an inactivated split-virus trivalent seasonal influenza vaccine, Anflu® (continued)

Study	Subjects,	Geometric mean titer, (95% CI)						Seroconversion rates, % (95% CI)			Seroprotection rates, % (95% CI)		
(Season)	N	H1N1		H3N2		B		H1N1	H3N2	B	H1N1	H3N2	B
		Day 0	Day 21/28	Day 0	Day 21/28	Day 0	Day 21/28						
Luo et al.[32]	75	19	108.3	28.8	117.9	7.2	36.8	62.7	60	38.7	89.3	86.7	69.3
(2010/2011)		(9.4–28.6)	(99.9–116.6)	(19.3–38.3)	(106.8–128.0)	(1.3–13.1)	(30.8–42.9)	(51.7–73.6)	(48.9–71.1)	(27.7–49.7)	(82.3–96.3)	(79.0–94.4)	(58.9–79.8)
2012/2013 *	60	9	177.5	45.7	618.2	18.4	102	83.3	83.3	66.7	86.7	100	86.7
		(6.8–12.0)	(117.1–269.1)	(32.9–63.5)	(451.1–847.2)	(15.6–21.6)	(77.1–134.9)	(71.0–91.7)	(71.0–91.7)	(53.2–78.3)	(74.9–94.1)	(92.5–100.0)	(74.9–94.1)

*Unpublished; CI, confidence interval. Li et al.[21] did not provide the CI values for GMT, seroconversion rates and seroprotection rates. Zhang et al.[22] evaluated the immunogenicity of the 3 consecutive lots of Anflu® and provided the analyzed results separately for the 3 groups, but did not provide the results from data pooled from the 3 groups. Jiang et al.[26] did not provide the CI values for seroprotection rates.

Since 2006 when licensed in China, annual studies of Anflu® were conducted in September or October of the same year of influenza strain recommendations by the WHO. With a few exceptions (2006 and 2011), immunogenicity assays were performed in the adults aged 18–60 y and the elderly >60 y in annual studies.[22,23,26,28,31,32] According to the European Medicines Agency guidelines, >50 subjects were included in each of these age groups. Serum samples were collected immediately before and generally 21 d after vaccination. These results indicate that, all immunogenicity endpoints for Anflu® met and exceed the regulatory criteria set by the CHMP for each virus strain, although seroconversion factors were generally lower in elderly subjects than those in adults.

In 2007, a double-blind, randomized, and controlled clinical trial was conducted to evaluate the immunogenicity of 3 consecutive manufacturing lots of Anflu® (2007/2008) and the control TIV from a multinational manufacturer in healthy adult subjects.[22] Seroconversion rates of the 3 viral strains in the 4 groups were 80.3% and above with fold increases as ≥11.1, and seroprotection rate were ≥93.4%. No significant differences were found in all immunogenicity endpoints of the 3 viral strains between the 3 consecutive lots and between Anflu® and the controlled TIV.

In infants (6–35 mo) and children (6–12 y)

Li et al.[21,27] and Luo et al.[34] evaluated the immunogenicity of Anflu® in infants and children. There are no set minimum immunogenicity criteria by CHMP for infants and children. Hence, the investigators used the immunogenicity criteria for adults. Infants received 2 doses of vaccine with the doses separated by 4 weeks, and children received 1 dose of vaccine. Serum samples were collected before and 28 d after the full immunization. For infants, in these 3 trials, the seroconversion rates exceeded 40% for each virus strain and the seroprotection rates exceeded 80% for H1N1 and H3N2 antigens and exceeded 50% for B antigen.[21,27,34] For children, in 2 trials by Li et al.[21] and Luo et al.,[34] the seroconversion rates were 64.4% and 87.5% for H1N1 antigen, 36.6% and 74.0% for H3N2 antigen, 37.0% and 75.9% for B antigen, respectively; and the seroprotection rates were 95.9% and 100.0% for H1N1 antigen, 97.3% and 100.0% for H3N2 antigen, 69.9% and 91.8% for B antigen, respectively.

Rapid immune response

Rapid immune responses induced by Anflu® to healthy infants, children, and the elderly were evaluated 1 week after 1 dose. In the 2009/2010 season, vaccination of Anflu® provided 40.8–63.3% of the seroconversion rates and 73.5–93.9% of the seroprotection rates for H1N1, H3N2 and B antigens in healthy adults, with the GMT of 87.1–157.1.[31] In the 2010/2011 season, obvious immune response was observed in both children and the elderly.[32] The seroprotection rates against H1N1 and H3N2 were more than 70% in children and more than 60% in the elderly, and the seroconversion rates against the 2 strains were all more than 30%. The seroprotection rate against B antigen was more than 30% in both children and the elderly and the seroconversion rates was more than 20%.

Cross-Strain Immunogenicity

A limited number of small studies have investigated the cross-strain immunogenicity of Anflu®.

In 2009, HI assays against 2009 pandemic influenza A/H1N1 strain NYMC X-179A (A/California/07/2009) were conducted on sera from the adults (n = 59[26]) and the elderly (n = 61[26]–70[23]) who had been vaccinated with Anflu® during the seasons 2007/2008 and 2008/2009. In healthy adult subjects, vaccination of Anflu® provided 10.2% of the seroprotection rate, 5.1% of the seroconversion rate, and 1.4-fold increase of neutralizing antibodies against A/California/07/2009 strain, while providing 94.9% of the seroprotection rate, 88.1% of the seroconversion rate, and 22.0-fold increase against A/Brisbane/59/2007 strain. Similarly, Anflu® demonstrated a lower degree of cross-strain immunogenicity against 2009 pandemic influenza A/H1N1 strain in elderly subjects (seroprotection rates of 16.4– 20.0%, seroprotection rates of 8.2–14.3%, and increase in GMT of 1.7–2.0).

In a 2009/2010 season trial, the sera of infants subjects aged 6 to 23 mo (n = 27) and 24–35 mo old (n = 24) immunized with 2-dose regimen of Anflu® were tested against the 2009 pandemic

Table 3. Reactogenicity of Anflu®

Period	Investigators	Subjects	Vaccinees (N)	Number of AR	Incidence (% AR per group)	Reference
2003	Guangxi province CDC	Infants	324	9		21
		Children	298	13	4.4	
		Adults	263	8	3	
		Elderly	207	6	2.9	
2006	Tianjin city CDC	Adults	113	8	7.1	29
		Elderly	112	5	4.5	
2006	Beijing CDC	Adults	201	4	2	*
		Elderly	100	3	3	
2007	Tianjin city CDC	Adults	425	60	14.1	22
2007	Lanshan city CDC	Elderly	140	12	8.6	23
2007–2008	14 city or country CDCs	Infants	599	64	10.7	30
		Children	613	40	6.5	
		Adolescents	485	13	2.7	
		Adults	553	50	9.1	
		Elderly	544	18	3.3	
2008	Qingdao city CDC	Adults	67	11	16.4	26
		Elderly	61	13	21.3	
2008–2009	5 city or country CDCs	Infants	293	9	3.1	*
		Children	355	14	3.9	
		Elderly	334	8	2.4	
2009	Lingchuan city CDC	Infants	63	23	36.5	27
2009	Beijing city CDC	Adults	100	13	13	32
2010	Shenyang city CDC	Adults	70	1	1.4	28
		Elderly	60	2	3.3	
2010	Sanhe city CDC and Baotu city CDC	Infants	75	5	6.7	32
		Children	75	17	22.7	
		Elderly	75	11	14	
2011	Zhengding city CDC	Infants	60	7	11.7	*
2012	Zhengzhou city CDC	Infants	65	12	18.5	*
		Adults	69	8	11.6	
		Elderly	68	5	7.4	
Total			6867	472	6.9	

*Unpublished; AR, adverse reaction; N, number.

influenza A/H1N1 strain.[27] Twenty-one days after 1 vaccination, 8.3–22.2% of the infant subjects seroconverted and 8.3–25.9% achieved an anti-H1N1 HI titer of 40 or more. After 2 vaccinations (on day 42), the seroconversion rates and the seroprotection rates ranged from 20.8% to 48.1%.

Another randomized clinical trial was conducted to assess whether the immunogenicity of Anflu® and the pandemic (H1N1/09) influenza vaccine is affected by the order of vaccine administration.[31] 151 healthy adult volunteers were randomized into 3 groups. All groups received 1 dose of a pandemic H1N1 vaccine and Anflu®. Group 1 received the pandemic H1N1 vaccine first, followed by the seasonal vaccine 21 d later. Group 2 received vaccinations in vice versa and Group 3 received both vaccines simultaneously. Before and after each vaccination blood samples were collected to determine the immunogenicity by HI, microneutralization (MN), and B cell ELISPOT assays. All 3 vaccination strategies generated obvious immune responses. However, a significant difference in magnitude of antibody responses to pandemic H1N1 between the 3 groups was found. Prior immunization or co-immunization with seasonal influenza vaccine reduced the magnitude of GMT against pandemic H1N1 by more than 50% in Groups 2 and 3. Pre- or co-immunization of pandemic H1N1 vaccine had no effect on GMT against seasonal H1N1. MN and ELISPOT assays showed a similar effect. Vaccination with pandemic H1N1 vaccine first is recommended to avoid an associated inhibitory effect by the seasonal trivalent flu vaccine.

Comparison with other vaccines

In the pre-licensing trial[21] and the 2007/2008 annual trial,[22] the immunogenicity of Anflu® and a TIV by Sanofi Pasteur was evaluated based on seroconversion rates, seroprotection rate and GMTs for each of the 3 antigens contained in the vaccines. The enrolled subjects included infants (6–35 mo), children (6–12 y), adults (18–60 y), and the elderly (>60 y). The results suggested that both TIVs had good immunogenicity for the 3 viral strains in infants and children with no significant differences, and exceeded the CHMP acceptability criteria for immunogenicity for adults and the elderly.

In a multicenter, double-blind and randomized trial, the immunogenicity of 3 2010–2011 TIVs manufactured by Sinovac Biotech, GlaxoSmithKline, and Sanofi Pasteur was evaluated in Chinese healthy infants (6 mo to 3 y), children (6–12 y), and older adults (≥60 y).[32] All the 3 TIVs showed good and comparable immunogenicity across all age groups. After 1 or 2 (for infants) vaccinations, the seroprotection rates against both H1N1 and H3N2 strains were more than 80% and the rates against B strains ranged between 60% and 80% in all age groups.

Reactogenicity

This section reviews data relating to the reactogenicity of Anflu® from the trials reviewed in the above (**Table 3**). Generally, local and general adverse event data were solicited from the study participants for the day of vaccination and 3–7 d after. Unsolicited adverse events that occurred within 21 d after vaccination were recorded in standardized diaries. Grade 3 adverse events were defined as those that prevented normal activities,

Table 4. Percentage of participants in each age group with adverse reactions within 3 or 7 days after vaccination, based on clinical data

AR	Infants (N = 1404)	Children (N = 1266)	Adolescents (N = 485)	Adults (N = 1861)	Elderly (N = 1626)
	All grades, *n* (%)	All grades, *n* (%)	All grades, *n* (%)	All grades, *n* (%)	All grades, *n* (%)
Local adverse reactions					
Pain	1 (0.1)	13 (1.0)	2 (0.4)	85 (4.6)	15 (0.9)
Induration	0 (0.0)	1 (0.1)	0 (0.0)	11 (0.6)	3 (0.2)
Redness	2 (0.1)	3 (0.2)	1 (0.2)	7 (0.4)	5 (0.3)
Swelling	1 (0.1)	0 (0.0)	0 (0.0)	10 (0.5)	5 (0.3)
Itching	0 (0.0)	3 (0.2)	0 (0.0)	10 (0.5)	11 (0.7)
Rash	1 (0.1)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
General adverse reactions					
Fever (>37.0 °C)	99 (7.1)	45 (3.6)	5 (1.0)	41 (2.2)	39 (2.4)
Dizziness/headache	0 (0.0)	6 (0.5)	2 (0.4)	33 (1.8)	13 (0.8)
Nausea/vomiting	15 (1.1)	8 (0.6)	4 (0.8)	5 (0.3)	6 (0.4)
Abdominal pain/diarrhea	12 (0.9)	6 (0.5)	0 (0.0)	5 (0.3)	11 (0.7)
Deceased activity	16 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fatigue	0 (0.0)	0 (0.0)	1 (0.2)	20 (1.1)	8 (0.5)
Loss of appetite	13 (0.9)	2 (0.2)	0 (0.0)	0 (0.0)	1 (0.1)
Irritability	2 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Allergy	2 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)
Myalgia	0 (0.0)	0 (0.0)	2 (0.4)	19 (1.0)	4 (0.3)
Arthralgia	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.2)	2 (0.1)
Pharyngalgia	0 (0.0)	0 (0.0)	0 (0.0)	6 (0.3)	0 (0.0)

Because Luo et al.[32] did not provide safety data according to different age groups, the data from these 225 subjects enrolled in this trial was excluded from the analysis.

redness or swelling >30 mm, or fever >39 °C. In the section, local and general adverse reactions (defined as adverse events considered to being possible, probable, or definite related to vaccination by investigators) which occurred in clinical trials were summarized in **Table 4**.

In adults and the elderly

Since 2006, annual trials were conducted to evaluate the reactogenicity and safety of the vaccine in adults and the elderly. A total of 1861 adult subjects and 1701 elderly subjects were enrolled and vaccinated. In solicited local adverse reactions, pain was the most common, with the incidence of 4.6% in adults and 0.9% in the elderly. Other local reactions, including induration, redness, swelling, and itching, occurred in 0.2–0.7% of Anflu® recipients. Solicited general adverse events included fever, dizziness/headache, nausea/vomiting, abdominal pain/diarrhea, fatigue, myalgia, arthralgia, and pharyngalgia. Fever is the most general reactions, with the incidence of 2.2% in adults and 2.4% in the elderly. The profile of general adverse reactions after Anflu® administration in adults was similar to that in the elderly, although the incidence of dizziness/headache, fatigue, and myalgia tended to be numerically lower in the elderly than in adults. Totally, most local and general adverse reactions were mild to moderate in severity.

In 1 observational trial, the authors compared the reactogenicity of Anflu® in adults with that in the elderly.[29] No significant differences in either the overall incidence or the alone incidence of local reactions was observed between adult and elderly recipients. Although the overall incidence of general reactions was found significantly higher in the adults than in the elderly, the alone incidence of the reactions in adults was similar to that in the elderly. There were 5 adults and 23 elderly subjects with chronic disease history, including cardiovascular disease, diabetes, and rheumatoid, none of which reported any adverse reactions after vaccination.

In children and adolescents

Limited clinical trials on the safety and tolerability of Anflu® in children and adolescents were conducted. Totally, the safety profile was evaluated in 1341 child subjects in 4 clinical trials and in 485 adolescent subjects in 1 trial, respectively. Fever is the most common adverse reactions, with the incidence ranging 1.0–3.6%. The incidences of other local and general reactions were almost no more than 1%.

In infants

Vaccine reactogenicity in infants was evaluated in 6 clinical trials between 2006 and 2012, most of which are uncontrolled, observational trials. A total of 1479 pediatric subjects were vaccinated and Anflu® was generally well tolerated. The incidences of solicited local adverse reactions in infants were less than 0.3%. Considering that infant subjects could not complain their discomforts, the list of solicited general adverse events for infants

has been modified, including fever, nausea/vomiting, diarrhea, decreased activities, loss of appetite, irritability, and allergy. Similar to adults and the elderly, fever is the most reported general adverse reactions, occurring in 7.1% of infants. In addition, nausea/vomiting and loss of appetite are frequently reported, with the incidence above 1%. Generally, adverse reaction rates were lower after the second injection than after the first injection.

In 2009, a double-blind, randomized, controlled clinical trial was conducted to evaluate the safety of Anflu® with Panflu.1®, a monovalent inactivated, split-virion, 2009 pandemic influenza A/H1N1 vaccine, in infants.[27] The results indicated that no significant differences in the incidences of adverse reactions were observed between vaccine groups. Fever and gastrointestinal disorders were the most commonly reported systemic reactions across vaccine groups. Severe adverse reactions were reported by 0.8–3.3%, and 3.2% of participants in the H1N1 vaccine groups and the TIV group, respectively. The most common severe reactions were fever and diarrhea.

Comparison with other vaccines

In 2010/2011 season, a multicenter, double-blind and randomized trial assess the safety of 3 TIVs manufactured by GlaxoSmithKline, Sanofi Pasteur, and Sinovac Biotech in infants, children and older adults.[32] Overall, 15.1% participants reported adverse event with mild and moderate adverse events being 12.8% and 2.3%. No severe adverse events were reported. The proportions of participants having adverse events among the 3 vaccine arms were 15.3%, 15.1%, and 14.7% with no significant difference. Across the 3 age groups, pain (5.9%) was the most common injection-site adverse events, and fever (6.1%) was the most common systemic adverse events followed by fatigue (1.7%). Other solicited adverse events were less common. During the study period, no unsolicited adverse events causally related to vaccination were reported.

Post-market safety monitoring

Between 2006 and June 2013, approximately 12 million people received 15.89 million doses of Anflu®. According to post-market self-reporting safety monitoring system by Sinovac Biotech, total 102 vaccinees reported adverse reactions/events, including 49 severe adverse reactions/events (3.1 per million doses). Among reported severe adverse reactions/events, fever and allergic reactions were most common. There were 3 deaths occurring in 2 female infants and 1 older woman, which were judged to be possibly unrelated to the vaccine by clinical physicians. All other patients recovered or rehabilitation after treatments.

Protective effect evaluations

In the 1-year follow-up observation study after the annual trial in 2007/2008 season, 278 vaccinated adults and 278 controlled adults unvaccinated with any influenza vaccine with past 3 years were retrospectively reviewed to the protect effect of Anflu® in adults aged 18–60 y.[33] Compared with 1 year before vaccination, the incidence of influenza-like illness (ILI, defined as a history of body temperature ≥38 °C accompanying with cough or sore throat symptoms) in the vaccinees within 1 year after vaccination decreased 15.8% and the cost with treatment for ILI reduced 43.0%; while, no significant changes were observed in the control. The efficacy of Anflu® against ILI was estimated as 51.1%. Moreover, Anflu® also provided protection against common cold (defined as a history of the symptoms with cough, sore throat, nasal congestion, runny nose, and nasopharynx redness and swelling). Compared with 1 year before vaccination, the incidence of common cold in the vaccinees within 1 year after vaccination decreased 10.0% and the cost reduced 33.3%, resulting in a vaccine efficacy of 24.3%.

Another post-licensure, case-control, observational study was performed to evaluate the vaccine effectiveness of trivalent inactivated influenza vaccine (2009/2010 season) among children aged 6–59 mo in Guangzhou city, China.[34] In this study all influenza cases from the 19 surveillance sites in Guangzhou were laboratory confirmed during 2009 and 2010. Controls were randomly selected from children aged 6 to 59 mo in the Children's Expanded Programmed Immunization Administrative Computerized System. A total 2529 cases and 4539 controls were finally enrolled. After adjusting for gender, age and area of residence, the vaccine effectiveness of full vaccination was 51.8% and 57.8% in the 2009 and 2010 influenza season, respectively. Partial vaccination provided 39.4% and 36.0% protection to children aged 24 to 59 mo in 2009 and 2010, respectively, and no protective effect was observed among younger children. However, the limitations of trial design and the bias due to high social concern on 2009 influenza (A) H1N1 pandemic limited the reference value of the report.

Domestic sale and international market expansion

During the past 6 seasons, the annual batch release amount of Anflu® was 1.4 million in 2007, 1.9 million in 2008, 3.6 million in 2009, 5.8 million in 2010, 2.4 million in 2011, and 2.4 million in 2012, respectively. It resulted in the total amount of 17.5 million, accounting for 8.1 percent of the total batch release amount of seasonal influenza vaccine in China and ranking the fifth. And, in terms of actual sales, an average of less than 20% of Anflu® supplied in the Chinese market was returned to manufacturers for destruction.

With the successful increases in domestic market, Sinovac Biotech started to explore international market opportunities with Anflu® since 2010. At present, Anflu® has been marketed in Mexico, Philippines, Mongolia, and Hong Kong (China). The annual overseas sale amount of the product was 110 thousand in 2010, 61 thousand in 2011, 63 thousand in 2012, and 75 thousand as of July 2013. No severe or serious adverse reactions have been reported to date. In addition, Anflu® is under registration in another 13 countries, most of which are developing or less developed countries in Southeast Asia, Africa, South America, and West Pacific regions.

Conclusion

Data from 8 papers reporting on 1377 vaccinees demonstrate Anflu® has acceptable immunogenicity (i.e., meeting or exceeding CHMP criteria) in healthy infants, children, adults, and the elderly. And reactogenicity data from 13 reports reporting on 6867 vaccinees suggest that the occurrence of serious or severe adverse reactions is very rare. There were no significant differences in terms of immunogenicity and reactogenicity with the other TIVs

from multinational manufacturers. Vaccination of Anflu® provided encouraging protections to healthy adults and even young children in the subsequent influenza season. However, few clinical trials conducted so far evaluated the efficacy, immunogenicity and safety of vaccine in high-risk population, such as pregnant women and those people with specific underlying medical conditions (including pulmonary and cardiac disease, diabetes, and immunosuppression). And the tolerability and reactogenicity of Anflu® in non-Chinese people has not been evaluated.

In addition to clinical supported data on the safety for post-market 7 years, in 2011, the Chinese vaccine regulatory system passed the assessment by WHO and was recognized to comply with international standards for vaccine regulation, indicating that the production quality of Chinese domestic vaccine has been recognized by the international. Sinovac Biotech has applied to WHO for prequalification of seasonal influenza vaccine in 2012 and believed that, in the coming future, Anflu® made in China will be provided to worldwide.

Though the influenza vaccination campaigns have significantly impacted and improved public health, they are not without drawbacks. Several factors contribute to the limited efficacy of current influenza vaccines, including virus mutability, failure to induce long-term protection, production hurdles, and decreased immunogenicity in high-risk groups. Some methods for improving the efficacy of the influenza vaccine have also been investigated, such as increasing the dosage and modifying the delivery method (e.g., intranasal/aerosol delivery, transdermal, or sublingual delivery). Moreover, the downfalls of embryonated chicken egg-based manufacturing system precipitated the development of cell culture-derived influenza vaccines. Another approach to improve vaccine efficacy, especially with poorly immunogenic viruses, is the use of adjuvants, which induce stronger protective immune responses and/or lower the dose of antigen required for protection, leading to antigen sparing. And, there has been a need for a quadrivalent influenza vaccine, containing influenza A/H1N1, A/H3N2, and B/Victoria and B/Yamagata lineage strains. Therefore, the improvement of vaccine production process and the development of quadrivalent influenza vaccine (QIV) had been initiated by Sinovac Biotech. Currently, the manufacturing technologies and processes for the QIV have almost been finalized and the preclinical researches will began at the end of this year. It is anticipated to provide more effective and safe influenza vaccine and amply vaccine supply to overcome the vaccine shortage problem during pandemic influenza outbreaks, especially in the developing countries.

Disclosure of Potential Conflicts of Interest

All authors were currently employed by Sinovac Biotech Co., LTD.

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