DOI:10.1111/j.1750-2659.2008.00056.x
www.blackwellpublishing.com/influenza

Review

Current status of live attenuated influenza vaccine in the United States for seasonal and pandemic influenza

Christopher S. Ambrose,[a] Catherine Luke,[b] Kathleen Coelingh[a]

[a]MedImmune, Gaithersburg, MD, USA. [b]Laboratory of Infectious Diseases, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA.

Correspondence: Kathleen Coelingh, MedImmune, One MedImmune Way, Gaithersburg, MD 20878, USA. E-mail: coelinghk@medimmune.com

Accepted 24 September 2008. Published Online 9 December 2008.

Abstract A live attenuated influenza vaccine (LAIV) is currently approved in the United States for the prevention of influenza in individuals 2–49 years of age. This article summarizes the available data describing the safety and efficacy of LAIV for the prevention of influenza in both children and adults. LAIV is administered as an intranasal spray and has been shown to provide high levels of efficacy against influenza illness caused by both matched and mismatched strains in children and adults. In studies comparing LAIV and inactivated influenza vaccine in children, LAIV recipients experienced 35–53% fewer cases of culture-confirmed influenza illness caused by antigenically matched strains. Protection through a second influenza season against antigenically matched strains has also been seen in children. In adults, definitive comparative studies of LAIV and inactivated vaccine have not been conducted and no statistically significant differences in efficacy have been demonstrated. The most common adverse reactions with LAIV include runny nose/nasal congestion in all age groups, fever >100°F in children, and sore throat in adults. Formulations of LAIV against pandemic influenza strains, including H5N1, H9N2, and H7N3, are currently being tested in preclinical and phase I clinical studies.

Keywords Influenza, live attenuated, vaccine.

Please cite this paper as: Ambrose *et al.* (2008) Current status of live attenuated influenza vaccine in the United States for seasonal and pandemic influenza. Influenza and Other Respiratory Viruses 2(6), 193–202.

Seasonal live attenuated influenza vaccine

Live attenuated influenza vaccine (LAIV; marketed in the United States as FluMist® [Influenza Virus Vaccine Live, Intranasal]) was approved for use in the United States in 2003, becoming the first nasally administered vaccine for human use in the United States. The approval of LAIV was the culmination of more than 30 years of collaborative research and development by scientists from academia, the National Institutes of Health (NIH), and the biopharmaceutical industry (MedImmune and Wyeth Vaccines). LAIV is currently approved in the United States for use in individuals 2–49 years of age.

Live attenuated influenza vaccine was originally derived by cold adaptation of an influenza type A strain (A/Ann Arbor/6/60 H2N2) and a type B strain (B/Ann Arbor/1/66) by serial passage at sequentially lower temperatures in specific pathogen-free primary chick kidney cells.[1] During this process, the viruses acquired multiple mutations in internal protein gene segments (i.e., genes encoding ''internal'' non-glycosylated proteins) that produced the cold-adapted (*ca*), temperature-sensitive (*ts*), and attenuated (*att*) phenotype of the master donor viruses (MDVs). The MDVs represent the LAIV genetic backbone that is updated annually with hemagglutinin (HA) and neuraminidase (NA) genes from contemporary influenza viruses to produce the annual trivalent formulation.

The individual contributions of the genetic sequences of the six internal gene segments to the *ca*, *ts*, and *att* phenotype of the MDVs are not completely understood, but many of the mutations associated with the phenotypes have been identified (Table 1). For the type A MDV, at least five genetic loci in three different internal protein gene segments contribute to the *ts* and *att* phenotypes.[2,3] For the type B MDV, at least three genetic loci in two different gene segments contribute to both the *ts* and *att* properties; two additional loci in a third gene segment also contribute to the *att* property; five loci in three segments control the *ca* property.[4,5]

Because multiple loci in several genes control the *ca*, *ts*, and *att* phenotypes of LAIV vaccine viruses, it is highly improbable that LAIV would lose these phenotypes as a result of reversion.[6,7] Given the error rate of 10^{-4} to 10^{-5}

Table 1. Phenotypic characteristics and phenotype-controlling genes for MDVs and vaccine reassortants[2–5]

Phenotype	Cold adaptation (*ca*)	Temperature sensitivity (*ts*)	Attenuation (*att*)
Characteristics of phenotype	Efficient growth at 25°C	Restricted growth at 37°C (type B) or 39°C (type A)	Restricted replication in ferret respiratory tract; minimal to no illness produced
Genes associated with indicated phenotype for each MDV			
Type A MDV and vaccine strains	Genes not identified	PB2, PB1, NP	PB2, PB1, NP
Type B MDV and vaccine strains	PB2, PA, NP	PA, NP	PA, NP, M

LAIV, live attenuated influenza vaccine; MDV, master donor virus.

misincorporations per nucleotide position during influenza virus replication and the fact that at least five point mutations are responsible for the attenuated properties of each MDV,[7,8] the probability of a LAIV vaccine virus reverting to wild-type influenza, with mutations in the five attenuating loci, would be one in at least 10^{20} replication cycles. In one study of 135 vaccine strains recovered from young vaccinated children, no evidence of reversion was observed.[9]

Each of the three influenza virus strains contained in LAIV is a 6:2 genetic reassortant virus, containing six internal gene segments from *ca*, *ts*, and *att* MDVs and two gene segments (encoding the HA and NA proteins) from a wild-type influenza virus that is selected annually by the World Health Organization and the US Public Health Service. Genetic reassortant viruses are prepared using reverse genetics technology in cell culture, a technique whereby influenza viruses can be generated from DNA plasmids containing influenza genes (Figure 1). LAIV vaccine viruses were originally generated using classical reassortment, but in 2008 the process transitioned to reverse genetics technology.

Three vaccine strains are formulated together to produce a trivalent LAIV vaccine in single-dose sprayers. Bulk vaccine is currently produced in specific pathogen-free embryonated hens' eggs, and plans are ongoing to assess future manufacture in cell culture. No preservatives are used in the manufacture of LAIV.

Mechanism of action

Live attenuated influenza vaccine viruses replicate primarily in the ciliated epithelial cells of the nasopharyngeal mucosa to induce immune responses (via mucosal immunoglobulin [Ig]A, serum IgG antibodies, and cellular immunity), but LAIV viruses do not replicate well at the warmer temperatures found in the lower airways and lung.[7,10] During the course of replication, all LAIV viral proteins would be presented to the immune system in their native conformation and in the context of histocompatibility proteins; resultant immune responses should mimic those of natural infection with influenza virus.

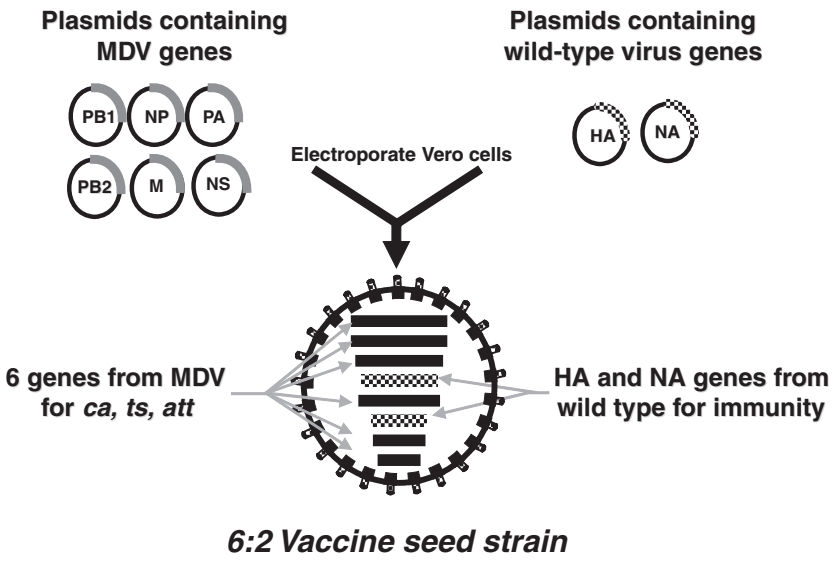

Figure 1. Schematic diagram of LAIV vaccine seed strain preparation using reverse genetics. Plasmids containing the MDV genes that control *ca*, *ts*, and *att* phenotypes and wild-type virus HA and NA genes are electroporated into Vero cells to generate the appropriate 6:2 vaccine strain. The 6:2 seed strain is used to manufacture LAIV. *att*, attenuated; *ca*, cold-adapted; HA, hemagglutinin; LAIV, live attenuated influenza vaccine; MDV, master donor virus; NA, neuraminidase; *ts*, temperature sensitive.

Table 2. LAIV efficacy in placebo-controlled pediatric studies

Study number (reference)	Region	Age range, months	Number of subjects	Influenza season	Efficacy against influenza illness % (95% CI)	
					Antigenically matched strains	All strains regardless of antigenic match
AV006[11,14]	United States	15 to <72	1602	1996	93·4 (87·5, 96·5)	92·6 (87·3, 95·7)
				1997	100 (63·1, 100)	87·1 (77·7, 92·6)
D153-P501[18]	Asia	12 to <36	3174	2000–2001	72·9 (62·8, 80·5)	70·1 (60·9, 77·3)
				2001–2002	84·3 (70·1, 92·4)	64·2 (44·2, 77·3)
D153-P502[39]	Europe	6 to <36	1784	2000–2001	85·4 (74·3, 92·2)	85·9 (76·3, 92·0)
				2001–2002	88·7 (82·0, 93·2)	85·8 (78·6, 90·9)
D153-P504[13]	South Africa, South America	6 to <36	3200	2001	73·5 (63·6, 81·0)	72·0 (61·9, 79·8)
				2002	73·6 (33·3, 91·2)	46·6 (14·9, 67·2)
D153-P513[38]	Asia	6 to <36	2172	2002	62·2 (43·6, 75·2)	48·6 (28·8, 63·3)
D153-P522[53]	Europe, Asia, Mexico	11 to <24	1233	2002–2003	78·4 (50·9, 91·3)	63·8 (36·2, 79·8)

LAIV, live attenuated influenza vaccine.

Efficacy in children

LAIV efficacy relative to placebo

The efficacy of LAIV relative to placebo has been examined in six studies in children aged 6–71 months (Table 2); LAIV is currently approved for use in children 24 months and older. Following first-year vaccination, efficacy against illness caused by antigenically matched strains (A/H1N1, A/H3N2, and B) has ranged from 62·2% to 93·4%. In studies that assessed efficacy in a second season following revaccination, efficacy ranged from 73·6% to 100%. LAIV has also been shown to reduce the incidence of all-cause febrile otitis media by 30–32% and influenza-associated otitis media by 73–98% compared with placebo.[11–13]

In addition to high levels of efficacy against matched strains, LAIV has shown protection against antigenically mismatched strains. In year 2 of study AV006, LAIV demonstrated 86% protection against the drifted variant A/Sydney/5/97 (H3N2) strain.[14] Additionally, when an influenza B variant circulated in 2000–2001, a single dose of LAIV in children 1·5–18 years of age was estimated to provide 66% (95% CI: 9, 87) protection in a large, nonrandomized, open-label study (n = 2281).[15,16] During the 2003–2004 season, LAIV was estimated to have provided 56% (95% CI: 24, 84) efficacy against the mismatched A/Fujian/411/02 (H3N2) virus (n = 1706).[17]

Although two doses of influenza vaccine are recommended in previously unvaccinated young children, three studies[11,13,18] compared one dose of LAIV with placebo in this population. Efficacy of a single dose was 58% (95% CI: 45, 68), 60% (95% CI: 31, 77), and 89% (95% CI: 65, 96), respectively. Greater efficacy is provided by two doses, and thus two doses are recommended. However, given that compliance with the two-dose regimen is low,[19] it is reassuring that a single dose of LAIV has provided clinically significant protection for vaccine-naive young children who fail to receive a second dose.

With regard to duration of protection, in one placebo-controlled study LAIV demonstrated protection during a season in which influenza circulated for up to 13 months following vaccination.[17] Additionally, two placebo-controlled studies demonstrated that two doses of LAIV in year 1 provided 56% (95% CI: 31, 73) and 57% (95% CI: 6, 82) protection through a second influenza season without revaccination.[13,18]

LAIV efficacy relative to inactivated vaccine

Three studies have compared the efficacy of trivalent inactivated influenza vaccine (TIV) and LAIV in children aged 6–59 months, 6–71 months with a history of recurrent respiratory tract infections, and 6–17 years with stable, medically-treated asthma (Table 3). In these studies, LAIV was more effective than TIV, reducing culture-confirmed influenza illness caused by matched strains by 35–53%.[20–22] Across the studies, significant reductions were seen against antigenically matched A/H1N1 and B strains as well as antigenically mismatched A/H3N2 strains. LAIV also reduced influenza-associated otitis media by 51% when compared with TIV in Study MI-CP111.[22] Due to insufficient data, the current US prescribing information states that LAIV should not be administered to any individuals with asthma or children <5 years of age with recurrent wheezing because of the potential for increased risk of

Table 3. LAIV efficacy relative to TIV in active-controlled pediatric studies

Study number (reference)	Region	Age range	Number of subjects	Influenza season	Relative efficacy compared with TIV % (95% CI)	
					Against matched strains	Against all strains regardless of match
MI-CP111[22]	North America, Europe, Asia, Middle East	6 to <60 months	8475	2004–2005	44·5 (22·4, 60·0)	54·9 (45·4, 62·9)
D153-P514[20]	Europe, Israel	6 to <72 months*	2187	2002–2003	52·7 (21·6, 72·2)	52·4 (24·6, 70·5)
D153-P515[21]	Europe, Israel	6 to <18 years**	2229	2002–2003	34·7 (3·9, 56·0)	31·9 (1·1, 53·5)

LAIV, live attenuated influenza vaccine; TIV, trivalent inactivated influenza vaccine.
*The study population consisted of children with a history of recurrent respiratory tract infections.
**The study population consisted of children with stable, medically-treated asthma. Due to insufficient data, the current US prescribing information states that LAIV should not be administered to any individuals with asthma or children <5 years of age with recurrent wheezing because of the potential for increased risk of wheezing post-vaccination unless the potential benefit outweighs the potential risk.

wheezing post-vaccination unless the potential benefit outweighs the potential risk.[23]

Efficacy/effectiveness in adults

LAIV efficacy in adults 18–64 years of age

Study AV009[24] was a randomized, double-blind, placebo-controlled study in adults 18–64 years of age ($n = 4561$) that evaluated the effectiveness of LAIV in preventing any febrile illness (AFI), as well as more influenza-specific syndromes such as severe febrile illness (SFI) and febrile upper respiratory illness (FURI); prevention of influenza-like illness (ILI) as defined by the Centers for Disease Control and Prevention (CDC), and the US Department of Defense (DOD) were analyzed *post hoc*. The study was conducted during a season dominated by an antigenically mismatched A/H3N2 strain. During the season, LAIV recipients had 9·7% fewer cases of AFI compared with placebo recipients (not significant). However, LAIV recipients experienced statistically significant reductions in SFI, FURI, CDC-ILI, and DOD-ILI (Figure 2). Significant reductions in illness were seen for subjects <40 years of age as well as for subjects ≥40 years of age. However, in a *post hoc* analysis of adults 50–64 years of age ($n = 641$), effectiveness was not demonstrated; as a result, LAIV is not approved for use in the United States in adults 50 years of age and older.

From this study, which measured illness regardless of etiology and did not include laboratory diagnosis of influenza infection, it is not possible to generate an influenza-specific efficacy estimate. However, during the same season, the CDC conducted a placebo-controlled study of inactivated influenza vaccine in a similar population: healthy, working adults 18–64 years of age ($n = 1184$).[25] Although no effectiveness for inactivated vaccine was seen, results indicate

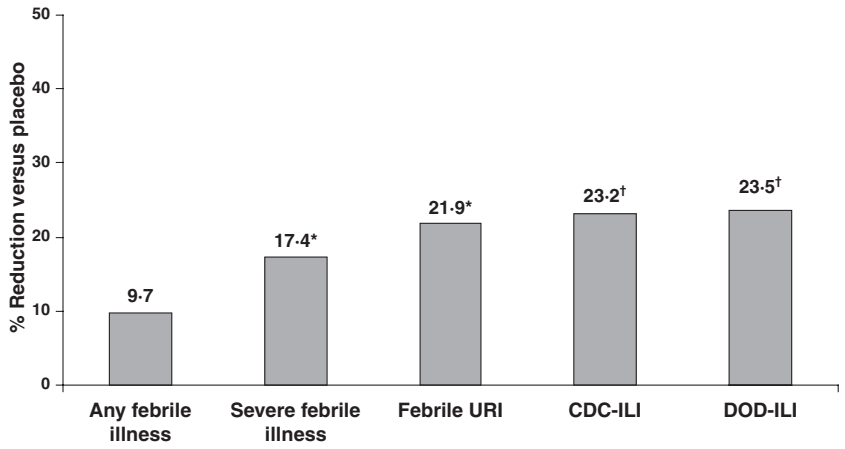

Figure 2. Effectiveness of LAIV in reducing illness in adults 18–64 years of age (study AV009). *$P < 0·05$; †$P < 0·001$. CDC-ILI, influenza-like illness as defined by the Centers for Disease Control and Prevention guidelines; DOD-ILI, influenza-like illness as defined by the US Department of Defense guidelines; LAIV, live attenuated influenza vaccine; URI, upper respiratory tract illness.

that 18% of CDC-ILI cases in placebo recipients may have been caused by influenza (4·4% laboratory-confirmed influenza, 23·8% CDC-ILI). If this ratio is applied to the rates of CDC-ILI from study AV009 (data on file), the influenza-specific efficacy for LAIV can be projected to have been 75–94%.

The first study to directly assess the efficacy of the licensed formulation of LAIV in adults was an experimental challenge study. Among seronegative adults 18–40 years of age (n = 103), LAIV demonstrated 85% (95% CI: 28, 100) efficacy against culture-confirmed influenza illness;[26] the efficacy of TIV was estimated at 71% (95% CI: 2, 97).

Subsequently, investigators at the University of Michigan initiated a 3-year study of the efficacies of TIV and LAIV compared with placebo in healthy adults 18–48 years of age.[27] In year 1 (n = 1247), the absolute efficacies of TIV and LAIV against culture-confirmed illness were 77% (95% CI: 37, 92) and 57% (95% CI: −3, 82), respectively; results for culture- or PCR-confirmed illness were 75% (95% CI: 42, 90) and 48% (95% CI: −7, 74). The vaccines had similar efficacy against influenza A/H3N2 but LAIV had lower efficacy against influenza B; this may have resulted from poor immunity against influenza B or the chance occurrence of increased infections in LAIV recipients caused by influenza B viruses from the alternate genetic lineage, given that no influenza vaccine has demonstrated efficacy against cross-lineage B strains.[28,29] Analysis of results from year 2 of the study (2005–2006, n = 2058) were complicated by a low influenza attack rate; efficacies of the two vaccines were similar and highly variable across different analysis methods.[30]

Another study described the efficacy of LAIV and TIV in young healthy adults (US military trainees) in 2005–2006.[31] Using a methodology that compared influenza incidence within the 2 weeks following vaccination (before the development of adaptive immune responses) to the incidence 2 weeks or more after vaccination, researchers concluded that the overall efficacy for the influenza vaccines was 92% (95% CI: 85, 96); efficacy was 95% at a site that used LAIV exclusively.

LAIV experience in adults ≥50 years of age

Although LAIV is not approved for use in adults 50 years or older, several studies have been conducted in adults ≥50 years of age; these data are presented below for completeness.

In adults 60 years or older, two randomized controlled studies have been conducted, one placebo-controlled and one TIV-controlled. Study D153-P507 (n = 3242) was a randomized, double-blind study conducted in South Africa in 2001. LAIV efficacy against antigenically matched strains was 42·3% (95% CI: 21·6, 57·8) compared with placebo, with 52·5% (95% CI: 32·1, 67·2) efficacy against A/H3N2 and no efficacy against influenza B (1·4% LAIV, 1·3% placebo).[32] Study D153-P516 (n = 3009) was a randomized, open-label, TIV-controlled study conducted in South Africa in 2002[33] from which no efficacy conclusions could be drawn because of the few cases of culture-confirmed influenza illness. In these studies, rates of runny nose/nasal congestion, cough, sore throat, headache, muscle ache, tiredness, decreased appetite, and use of fever medication were increased in LAIV recipients.

The efficacy of LAIV in simultaneous combination with TIV has also been studied in the elderly. In a double-blind field trial conducted over a 3-year period in nursing home residents aged ≥65 years, 523 residents (mean age, 84 years) received TIV and either monovalent A/H3N2 LAIV or placebo.[34] TIV+LAIV recipients experienced 61% (95% CI: 18, 82) fewer cases of laboratory-documented influenza A compared with TIV+placebo. A later study with a monovalent B LAIV suggested a similar trend, but attack rates were too low to allow definitive conclusions.[35] A third study was conducted among 2215 individuals ≥50 years of age with COPD within the Veterans Affairs medical system.[36] In this study, the relative efficacy of TIV+LAIV compared with TIV+placebo in the prevention of laboratory-documented influenza illness was 16% (95% CI: −22, 43) for any influenza strain, 26% (95% CI: −17, 53) for A/H3N2, and −5% (95% CI: −113, 48) for B. However, TIV+LAIV recipients were shown to have an improvement in chronic lung disease severity index scores over the course of the study.[37] TIV+LAIV recipients reported higher rates of increased sputum, stuffy/runny nose, increased shortness of breath, chills, and itchiness at the intramuscular injection site compared with TIV+placebo recipients.

Role of antibody responses to LAIV in predicting protection

It is important to note that, unlike TIV, no general immune correlates of protection have been established for LAIV. Serum antibody responses, mucosal antibody responses, and cellular responses have been observed in vaccine recipients; one study in young children demonstrated a correlation between interferon-gamma ELISPOT responses and protection from culture-confirmed influenza illness.[38] However, no similar correlation has been seen for serum antibody responses. Serum antibody responses are generally only detected in individuals with low titers of pre-existing serum antibody. Overall, studies have demonstrated that the proportion of individuals experiencing at least a fourfold rise in serum HA inhibition (HAI) antibody titer is often correlated with protective efficacy[11,18,39,40]; however, studies have also shown protection in the absence of significant antibody responses.[26,41] In the challenge study in healthy adults described above, LAIV

provided 85% protection against influenza illness despite the fact that only 24% of LAIV recipients experienced a ≥4-fold rise in HAI titer following vaccination. Interestingly, this study also suggested that serologic endpoints in influenza vaccine efficacy trials may underestimate influenza infections in TIV recipients (thus overestimating efficacy) because none of the four TIV recipients who developed influenza illness after wild-type challenge experienced a ≥4-fold rise in HAI titer with illness (pre-challenge, post-vaccination titers were <4, 32, 128, and 128).

LAIV safety profile

The safety of LAIV has been evaluated in approximately 49 000 individuals in 48 completed studies, including more than 18 000 children younger than 5 years. Additionally, more than 10 million doses have been commercially distributed in the United States since licensure. In clinical studies, the most common adverse reactions (≥10% in LAIV recipients and at least 5% greater than in controls) were runny nose or nasal congestion in all ages, fever >100°F in children 2–6 years of age, and sore throat in adults (Tables 4 and 5).[23] Rates of reactogenicity events generally decline upon revaccination with LAIV, either in the same or subsequent seasons.

Study MI-CP111[22] was prospectively designed to evaluate wheezing in children 6–59 months of age. The incidence of medically significant wheezing (MSW) was analyzed through 42 days after vaccination with LAIV or TIV; MSW was defined as a medical diagnosis of wheezing associated with other respiratory findings (e.g., hypoxemia, respiratory distress, or initiation of daily bronchodilator therapy). MSW was reported in more subjects 6–23 months of age who had received LAIV than in those given TIV (LAIV, 5·9%; TIV, 3·8%; $P = 0·002$). Among children aged 24–59 months, rates of MSW were comparable in LAIV and TIV recipients (LAIV, 2·1%; TIV, 2·5%; $P = 0·38$). In this same study, hospitalization rates were higher in LAIV recipients 6–23 months of age compared with TIV recipients (4·2% versus 3·2%, $P = 0·09$) with no increase among children 24–59 months of age (2·1% versus 2·5%; $P = 0·33$). The increase in hospitalizations in children <2 years was driven by children 6–11 months of age (LAIV, 6·1%; TIV, 2·6%; $P = 0·002$) and most of the hospitalizations occurred >42 days after the last dose, were not temporally clustered, and were accounted for by events commonly expected in this population (e.g., respiratory tract and gastrointestinal tract infections). A biological rationale for this increase in late-occurring hospitalizations cannot be readily explained. In older subgroups of children 12–23 and 24–59 months of age, hospitalization rates were not increased in LAIV versus TIV recipients.

In the University of Michigan multi-year comparative study of LAIV and TIV in adults <50 years of age,[27,30] year

Table 4. Incidence of reactogenicity events within 10 days after administration of doses 1 and 2 of LAIV or placebo to children 2–6 years of age (studies AV006, D153-P501)

Reactogenicity events, *n*/*N* (%)	Dose 1			Dose 2		
	LAIV (*n* = 876–1759)	Placebo (*n* = 424–1034)	*P*	LAIV (*n* = 702–1490)	Placebo (*n* = 330–868)	*P*
Runny/stuffy nose, nasal congestion	1022/1759 (58·1)	513/1034 (49·6)	<0·001	717/1490 (48·1)	378/868 (43·5)	0·032
Sore throat	93/879 (10·6)	37/425 (8·7)	0·324	46/702 (6·6)	25/331 (7·6)	0·598
Cough	522/1757 (29·7)	351/1029 (34·1)	0·016	525/1488 (35·3)	280/866 (32·3)	0·150
Vomiting	161/1754 (9·2)	113/1028 (11·0)	0·130	132/1485 (8·9)	72/864 (8·3)	0·704
Headache	82/879 (9·3)	30/424 (7·1)	0·205	41/702 (5·8)	22/331 (6·6)	0·676
Muscle aches	53/878 (6·0)	12/424 (2·8)	0·014	22/702 (3·1)	7/330 (2·1)	0·424
Chills	39/878 (4·4)	14/424 (3·3)	0·372	23/703 (3·3)	12/331 (3·6)	0·854
Decreased activity (lethargy)	254/1755 (14·5)	108/1028 (10·5)	0·003	148/1489 (9·9)	84/866 (9·7)	0·886
Irritability	366/1755 (20·9)	190/1029 (18·5)	0·141	205/1487 (13·8)	119/864 (13·8)	>0·99
Decreased appetite	188/876 (21·5)	105/604 (17·4)	0·055	113/783 (14·4)	99/534 (18·5)	0·048
Use of fever medication	168/876 (19·2)	101/604 (16·7)	0·244	91/783 (11·6)	72/534 (13·5)	0·349
Fever						
>100°F oral or equivalent	281/1744 (16·1)	114/1020 (11·2)	<0·001	156/1466 (10·6)	82/858 (9·6)	0·436
>101°F oral or equivalent	124/1744 (7·1)	53/1020 (5·2)	0·053	76/1466 (5·2)	46/858 (5·4)	0·848
>102°F oral or equivalent	51/1744 (2·9)	25/1020 (2·5)	0·547	41/1466 (2·8)	20/858 (2·3)	0·591

LAIV, live attenuated influenza vaccine. *n*, number of subjects reporting the event; *N*, number of evaluable subjects (those who returned diary cards) for each event. Range in *N* reflects differences in data collection between the two pooled studies.

Table 5. Summary of solicited events observed within 7 days after one dose of LAIV or placebo to adults 18–64 years of age (study AV009)

Event, *n*/*N* (%)	LAIV (*n* = 3264)	Placebo (*n* = 1619)
Cough	426/3208 (13·3)	167/1589 (10·5)
Runny nose	1399/3208 (43·6)	429/1589 (27·0)
Sore throat	827/3208 (25·8)	262/1589 (16·5)
Headache	1165/2960 (39·4)	548/1476 (37·1)
Chills	258/3208 (8·0)	95/1589 (6·0)
Muscle aches	503/3208 (15·7)	228/1589 (14·3)
Tiredness/weakness	724/2960 (24·5)	304/1476 (20·6)
Fever		
>100°F	42/3208 (1·3)	24/1589 (1·5)
>102°F	3/3208 (0·1)	2/1589 (0·1)
>104°F	0/3208 (0)	0/1589 (0)

LAIV, live attenuated influenza vaccine.

1 reactogenicity results showed an increased rate of arm soreness in TIV recipients and increased rates of runny nose/congestion, cough, and headache in LAIV recipients. In year 2, TIV recipients had increased rates of arm soreness, arm redness, muscle aches, as well as trouble breathing and red eyes (reported in other studies as ocular respiratory syndrome); LAIV recipients had increased rates of sore throat and runny nose/congestion.

The vaccine viruses in LAIV replicate in the cells of the nasal mucosa and can at times be isolated from nasal secretions post-vaccination. Based on two prospective studies, shedding of vaccine virus on at least 1 day is frequent in young children (e.g., 89% and 69% of subjects aged 6–23 and 24–59 months, respectively) and decreases with advancing age (44%, 27%, and 17% in individuals aged 5–8, 9–17, and 18–49 years, respectively).[42,43] In these studies, peak shedding incidence occurred on day 2, with the average subject with shedding having virus recoverable for 1·5–3 days and with titers below 1·5 $\log_{10}$ $TCID_{50}$/ml after 13, 10, and 6 days post-vaccination for individuals 6–23 months, 2–8 years, and ≥9 years of age.

Based on available information, transmission of vaccine virus from a vaccine recipient to an unvaccinated contact is likely to be a rare event, even in young children, and without negative clinical consequences. In a daycare study in children (n = 98) designed to optimize the occurrence and detection of transmission, one documented case of transmission was observed. The transmitted type B vaccine strain was only detected on 1 day, retained its *ca*, *ts* phenotype, and did not cause disease. Based on this study, the probability of transmission to a child following daycare contact with a single vaccinated child was calculated to be 0·58%.[9] Four additional type A strains from placebo recipients could not be characterized as vaccine or wild-type; inclusion of these four isolates as possible cases yields a transmission probability of 2·4%.[23]

Live attenuated influenza vaccine is not approved for use in immunocompromised individuals. However, because of concerns regarding inadvertent exposure to the vaccine, studies were conducted in relatively asymptomatic or mildly symptomatic adults and children with HIV infection. These studies demonstrated that LAIV was not associated with significant adverse events in HIV-infected individuals.[44,45] Similarly, a recent comparative safety study conducted in 243 children 5–17 years of age with HIV disease (viral load <60 000 and CD4 count >15%) demonstrated that there were no unexpected toxicities, prolonged shedding, or serious adverse events associated with either LAIV or TIV.[46]

Live attenuated vaccines against pandemic influenza

The emergence and spread of highly pathogenic avian influenza (HPAI) A/H5N1 viruses in avian populations since 2003 and the concurrent infections in humans have prompted efforts to develop vaccines for use in the event of an influenza pandemic. Most pandemic influenza vaccines currently licensed or in development target the H5N1 subtype and are inactivated injectable vaccines administered with or without adjuvant. It is important that every avenue for vaccine development be explored as part of pandemic preparedness activities. Live attenuated influenza vaccines against potential pandemic influenza A viruses are being developed in the United States and in Russia. Both approaches capitalize on the use of the technology and infrastructure already in place for seasonal LAIV, using MDVs that display *ts*, *ca*, and *att* phenotypes. Based on the experience with LAIV for seasonal influenza, it is reasonable to expect that potential advantages of using live attenuated vaccines against pandemic influenza include rapid induction of mucosal and systemic humoral and cell-mediated immune responses and broad cross-protection against antigenically distinct viruses.

In collaboration with MedImmune, the NIH (Bethesda, MD, USA), is engaged in a program to develop candidate pandemic LAIV (pLAIV) for use in the event of a pandemic. The candidate vaccines will be 6:2 reassortants, generated by reverse genetics, with the HA and NA genes of an influenza virus of pandemic potential, and the six internal protein genes of the cold-adapted MDV-A virus. The use of reverse genetics allows the removal of virulence motifs such as the multibasic cleavage site in the HA of HPAI viruses that is a known virulence factor in poultry.[47] The candidate vaccines will be thoroughly characterized in preclinical studies and evaluated for safety, infectivity, and immunogenicity in phase I clinical trials

in healthy adults. Clinical studies are being conducted in inpatients at the Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health (Baltimore, MD, USA). The studies will establish the proof of principle of the utility of such vaccines in the event of an influenza pandemic.

H5N1 pandemic LAIV

To date, NIH and MedImmune have generated three candidate pLAIV viruses of the H5N1 subtype that derive their HA and NA from H5N1 influenza viruses isolated from humans in Hong Kong in 1997, Hong Kong in 2003, and Vietnam in 2004. In all cases, the HA was modified to remove the multibasic cleavage site. As a result of this modification, all three vaccine viruses were of a low pathogenicity phenotype in chickens. The vaccine viruses were restricted in replication in the respiratory tract of mice and attenuated in ferrets, consistent with the *att* phenotype of the MDV. Toxicologic evaluation of H5N1 *ca* vaccine viruses in ferrets revealed no evidence of systemic toxicity following repeated intranasal administration.[48] Vaccination with a single dose of H5N1 AA *ca* vaccine administered intranasally protected mice against lethal challenge with wild-type virus, but two doses administered intranasally were required to elicit detectable serum HAI and neutralizing antibodies in mice and ferrets and to protect against pulmonary replication of wild-type viruses.[49] In addition, vaccination of mice with two doses of H5N1 *ca* vaccine protected mice against pulmonary replication of antigenically distinct heterologous wild-type H5N1 viruses, suggesting that such vaccines may elicit broadly cross-reactive immune responses. The H5N1 pLAIV viruses with the HA and NA from influenza A/Vietnam/1203/2004 (H5N1) and influenza A/Hong Kong/213/2003 (H5N1) have been evaluated for safety, infectivity, and immunogenicity in phase I clinical trials (data on file).

Pandemic LAIV for other avian influenza subtypes

Pandemic LAIV viruses for use against the avian influenza subtypes H9N2 and H7N3 have been generated and characterized in preclinical studies. An H9N2 influenza vaccine virus, which derived the HA and NA from the low pathogenicity avian influenza isolate A/chicken/Hong Kong/G9/1997 (H9N2), was generated by classical reassortment. The H9N2 *ca* vaccine virus did not exhibit a high pathogenicity phenotype in chickens, was restricted in replication in the upper respiratory tract of mice, and failed to replicate to detectable levels in the lower respiratory tract of mice. Despite being restricted in replication, a single dose of the H9N2 *ca* vaccine administered intranasally was immunogenic in mice and conferred complete protection against replication of homologous and heterologous wild-type H9N2 influenza viruses in the upper and lower respiratory tract.[50] This vaccine virus has been evaluated in phase I clinical trials.[51]

An H7N3 candidate pLAIV that derived its HA and NA from the low pathogenicity avian influenza H7N3 isolate A/chicken/British Columbia/CN-6/2004 was generated by reverse genetics. As with the previous avian influenza/AA *ca* vaccine viruses, the H7N3 *ca* vaccine virus was extensively characterized in pre-clinical studies[52] and was shown to be safe for evaluation in phase I clinical trials (data on file). Candidate vaccines for the other HA subtypes are currently in development.

Safety concerns with pandemic LAIV viruses

The development of live attenuated viruses with the surface glycoproteins of avian influenza viruses or other influenza viruses of pandemic potential (e.g., H2 viruses) raises the concern that these viruses may reassort with circulating seasonal influenza viruses, and thereby generate easily transmissible viruses with novel HA and NA. Although preliminary data from clinical studies suggest that the pLAIV viruses generated so far are highly restricted in replication, this remains a significant concern for regulatory authorities. During development of these vaccines, the risk of reassortment can be mitigated by conducting clinical trials in an inpatient setting during months when influenza viruses are not likely to be circulating. The clinical studies of pLAIV viruses currently being conducted in the United States are performed in an isolation facility between April and the beginning of December. In the case of the threat of an influenza pandemic, the risk of reassortment must be weighed against the benefits of administering such a vaccine to the population before proceeding with widespread use of a live vaccine. Implementation of a live attenuated pandemic influenza vaccine would be based on the recommendation of public health authorities.

Acknowledgements

This research was supported in part by the Intramural Research Program of the NIAID, NIH. The authors thank Kanta Subbarao (NIH), and Robert Walker, Seth Toback, Robert Fuentes, and Gregory Susla (MedImmune) for helpful discussion and review of the manuscript.

References

1 Maassab HF. Plaque formation of influenza virus at 25 degrees C. Nature 1968; 219:645–646.

2 Jin H, Lu B, Zhou H *et al.* Multiple amino acid residues confer temperature sensitivity to human influenza virus vaccine strains (FluMist) derived from cold-adapted A/Ann Arbor/6/60. Virology 2003; 306:18–24.

3 Jin H, Zhou H, Lu B, Kemble G. Imparting temperature sensitivity and attenuation in ferrets to A/Puerto Rico/8/34 influenza virus by transferring the genetic signature for temperature sensitivity from cold-adapted A/Ann Arbor/6/60. J Virol 2004; 78:995–998.

4 Chen Z, Aspelund A, Kemble G, Jin H. Genetic mapping of the cold-adapted phenotype of B/Ann Arbor/1/66, the master donor virus for live attenuated influenza vaccines (FluMist). Virology 2006; 345:416–423.

5 Hoffmann E, Mahmood K, Chen Z *et al.* Multiple gene segments control the temperature sensitivity and attenuation phenotypes of ca B/Ann Arbor/1/66. J Virol 2005; 79:11014–11021.

6 Kemble G, Greenberg H. Novel generations of influenza vaccines. Vaccine 2003; 21:1789–1795.

7 Murphy BR, Coelingh K. Principles underlying the development and use of live attenuated cold-adapted influenza A and B virus vaccines. Viral Immunol 2002; 15:295–323.

8 Smith DB, Inglis SC. The mutation rate and variability of eukaryotic viruses: an analytical review. J Gen Virol 1987; 68 (Pt 11):2729–2740.

9 Vesikari T, Karvonen A, Korhonen T *et al.* A randomized double-blind study of the safety, transmissibility, and phenotypic and genotypic stability of cold-adapted influenza virus vaccine (LAIV, FluMist®). Pediatr Infect Dis J 2006; 25:590–595.

10 Gruber WC. The role of live influenza vaccines in children. Vaccine 2002; 20:S66–S73.

11 Belshe RB, Mendelman PM, Treanor J *et al.* The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine in children. N Engl J Med 1998; 338:1405–1412.

12 Belshe RB, Gruber WC. Prevention of otitis media in children with live attenuated influenza vaccine given intranasally. Pediatr Infect Dis J 2000; 19:S66–S71.

13 Bracco Neto H, Farhat CK, Tregnaghi MW *et al.* Efficacy and safety of one and two doses of live attenuated influenza vaccine in vaccine-naive children. Pediatr Infect Dis J 2009 (in press).

14 Belshe RB, Gruber WC, Mendelman PM *et al.* Efficacy of vaccination with live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine against a variant (A/Sydney) not contained in the vaccine. J Pediatr 2000; 136:168–175.

15 Gaglani MJ, Piedra PA, Herschler GB *et al.* Direct and total effectiveness of the intranasal, live-attenuated, trivalent cold-adapted influenza virus vaccine against the 2000-2001 influenza A(H1N1) and B epidemic in healthy children. Arch Pediatr Adolesc Med 2004; 158:65–73.

16 Halloran ME, Longini IM Jr, Gaglani MJ *et al.* Estimating efficacy of trivalent, cold-adapted, influenza virus vaccine (CAIV-T) against influenza A (H1N1) and B using surveillance cultures. Am J Epidemiol 2003; 158:305–311.

17 Halloran ME, Piedra PA, Longini IM Jr *et al.* Efficacy of trivalent, cold-adapted, influenza virus vaccine against influenza A (Fujian), a drift variant, during 2003-2004. Vaccine 2007; 25:4038–4045.

18 Tam JS, Capeding MR, Lum LC *et al.* Efficacy and safety of a live attenuated, cold-adapted influenza vaccine, trivalent against culture-confirmed influenza in young children in Asia. Pediatr Infect Dis J 2007; 26:619–628.

19 Jackson LA, Neuzil KM, Baggs J *et al.* Compliance with the recommendations for 2 doses of trivalent inactivated influenza vaccine in children less than 9 years of age receiving influenza vaccine for the first time: a Vaccine Safety Datalink study. Pediatrics 2006; 118:2032–2037.

20 Ashkenazi S, Vertruyen A, Aristegui J *et al.* Superior relative efficacy of live attenuated influenza vaccine compared with inactivated influenza vaccine in young children with recurrent respiratory tract infections. Pediatr Infect Dis J 2006; 25:870–879.

21 Fleming DM, Crovari P, Wahn U *et al.* Comparison of the efficacy and safety of live attenuated cold-adapted influenza vaccine, trivalent, with trivalent inactivated influenza virus vaccine in children and adolescents with asthma. Pediatr Infect Dis J 2006; 25:860–869.

22 Belshe RB, Edwards KM, Vesikari T *et al.* Live attenuated versus inactivated influenza vaccine in infants and young children. N Engl J Med 2007; 356:685–696.

23 FluMist®, Influenza Virus Vaccine Live, Intranasal. Full Prescribing Information, Gaithersburg, MD: MedImmune, 2007.

24 Nichol KL, Mendelman PM, Mallon KP *et al.* Effectiveness of live, attenuated intranasal influenza virus vaccine in healthy, working adults: a randomized controlled trial. JAMA 1999; 282:137–144.

25 Bridges CB, Thompson WW, Meltzer MI *et al.* Effectiveness and cost-benefit of influenza vaccination of healthy working adults: a randomized controlled trial. JAMA 2000; 284:1655–1663.

26 Treanor JJ, Kotloff K, Betts RF *et al.* Evaluation of trivalent, live, cold-adapted (CAIV-T) and inactivated (TIV) influenza vaccines in prevention of virus infection and illness following challenge of adults with wild-type influenza A (H1N1), A (H3N2), and B viruses. Vaccine 1999; 18:899–906.

27 Ohmit SE, Victor JC, Rotthoff JR *et al.* Prevention of antigenically drifted influenza by inactivated and live attenuated vaccines. N Engl J Med 2006; 355:2513–2522.

28 Boyce TG. Influenza vaccines. N Engl J Med 2007; 356:1172; author reply 1173.

29 Lin YP, Gregory V, Bennett M, Hay A. Recent changes among human influenza viruses. Virus Res 2004; 103:47–52.

30 Ohmit SE, Victor JC, Teich ER *et al.* Prevention of symptomatic seasonal influenza in 2005-2006 by inactivated and live attenuated vaccines. J Infect Dis 2008; 198:312–317.

31 Strickler JK, Hawksworth AW, Myers C, Irvine M, Ryan MA, Russell KL. Influenza vaccine effectiveness among US military basic trainees, 2005-06 season. Emerg Infect Dis 2007; 13:617–619.

32 de Villiers PJT, Greyling M, Taylor CM *et al.* Efficacy of a live, attenuated, influenza vaccine in South African adults aged 60 years or older against community-acquired culture-confirmed influenza. Options for the Control of Influenza, 5th International Conference, October 7–11, 2003, Okinawa, Japan.

33 Data on File (Study D153-P516). Gaithersburg, MD: MedImmune, 2006.

34 Treanor JJ, Mattison HR, Dumyati G *et al.* Protective efficacy of combined live intranasal and inactivated influenza A virus vaccines in the elderly. Ann Intern Med 1992; 117:625–633.

35 Treanor JJ, Betts RF. Evaluation of live, cold-adapted influenza A and B virus vaccines in elderly and high-risk subjects. Vaccine 1998; 16:1756–1760.

36 Gorse GJ, O'Connor TZ, Young SL *et al.* Efficacy trial of live, cold-adapted and inactivated influenza virus vaccines in older adults with chronic obstructive pulmonary disease: a VA cooperative study. Vaccine 2003; 21:2133–2144.

37 Gorse GJ, O'Connor TZ, Young SL *et al.* Impact of a winter respiratory virus season on patients with COPD and association with influenza vaccination. Chest 2006; 130:1109–1116.

38 Forrest BD, Pride MW, Dunning AJ *et al.* Correlation of cellular immune responses with protection against culture-confirmed influenza virus in young children. Clin Vaccine Immunol 2008; 15:1042–1053.

39 Vesikari T, Fleming DM, Aristegui JF *et al.* Safety, efficacy, and effectiveness of cold-adapted influenza vaccine-trivalent against community-acquired, culture-confirmed influenza in young children attending day care. Pediatrics 2006; 118:2298–2312.

40 Belshe RB, Gruber WC, Mendelman PM *et al.* Correlates of immune protection induced by live, attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine. J Infect Dis 2000; 181:1133–1137.

41 Edwards KM, Dupont WD, Westrich MK, Plummer WD Jr, Palmer PS, Wright PF. A randomized controlled trial of cold-adapted and inactivated vaccines for the prevention of influenza A disease. J Infect Dis 1994; 169:68–76.

42 Block SL, Reisinger KS, for the LAIV Study Group. Shedding and safety of live attenuated influenza vaccine in healthy subjects 6 to <60 months of age. Pediatric Academic Societies' Annual Meeting, May 2–6, 2008, Honolulu, HI.

43 Block SL, Yogev R, Hayden FG, Ambrose CS, Zeng W, Walker RE. Shedding and immunogenicity of live attenuated influenza vaccine virus in subjects 5 to 49 years of age. Vaccine 2008; 26:4940–4946.

44 King JC Jr, Fast PE, Zangwill KM *et al.* Safety, vaccine virus shedding and immunogenicity of trivalent, cold-adapted, live attenuated influenza vaccine administered to human immunodeficiency virus-infected and noninfected children. Pediatr Infect Dis J 2001; 20:1124–1131.

45 King JC Jr, Treanor J, Fast PE *et al.* Comparison of the safety, vaccine virus shedding, and immunogenicity of influenza virus vaccine, trivalent, types A and B, live cold-adapted, administered to human immunodeficiency virus (HIV)-infected and non-HIV-infected adults. J Infect Dis 2000; 181:725–728.

46 Levin MJ, Song LY, Fenton T *et al.* Shedding of live vaccine virus, comparative safety, and influenza-specific antibody responses after administration of live attenuated and inactivated trivalent influenza vaccines to HIV-infected children. Vaccine 2008; 26:4210–4217.

47 Horimoto T, Kawaoka Y. Reverse genetics provides direct evidence for a correlation of hemagglutinin cleavability and virulence of an avian influenza A virus. J Virol 1994; 68:3120–3128.

48 Jin H, Manetz S, Leininger J *et al.* Toxicological evaluation of live attenuated, cold-adapted H5N1 vaccines in ferrets. Vaccine 2007; 25:8664–8672.

49 Suguitan AL Jr, McAuliffe J, Mills KL *et al.* Live, attenuated influenza A H5N1 candidate vaccines provide broad cross-protection in mice and ferrets. PLoS Med 2006; 3:e360.

50 Chen H, Matsuoka Y, Swayne D *et al.* Generation and characterization of a cold-adapted influenza A H9N2 reassortant as a live pandemic influenza virus vaccine candidate. Vaccine 2003; 21:4430–4436.

51 Karron RA, Callahan K, Luke C *et al.* A live attenuated H9N2 influenza vaccine is well-tolerated and immunogenic in healthy adults. J Infect Dis 2008 (in press).

52 Joseph T, McAuliffe J, Lu B *et al.* A live attenuated cold-adapted influenza A H7N3 virus vaccine provides protection against homologous and heterologous H7 viruses in mice and ferrets. Virology 2008; 378:123–132.

53 Lum LCS, Forrest BD, The LAIV Concomitant Vaccine Study Group. Safety, efficacy, and immunogenicity of live attenuated influenza vaccine concurrently administered with a combination mumps, measles, and rubella vaccine. 26th Annual Meeting of the European Society for Paediatric Infectious Diseases, May 13–17, 2008, Graz, Austria.

