

Vaccines and Related Biological Products Advisory Committee

Meeting Date: July 26 and 27, 2001

FDA Briefing Document for

Aviron

Cold Adapted Influenza Vaccine, Trivalent - FluMistä

Clinical Section

ChrisAnna M. Mink M.D.

Wasima N. Rida, Ph.D.

Antonia Geber, M.D.

Summary of Clinical Trials Performed with FluMist.

PEDIATRIC TRIALS					
Protocol Number	Study Goal	Age Range	Total	FluMist	Placebo
AV002 ^a	Dose Escalation	18-71 months	238	155	83
AV002-2 ^a	Dose Escalation	18-71 months	118	79	39
AV006	Efficacy against Culture Confirmed Influenza	15-71 months	Year 1, 1602 Year 2, 1358	1070 917	532 441
AV011	Challenge of Subset of AV006 subjects with Vaccine Strain H1N1	34-91 months	222	CAIV-M	-
AV007	Lot Consistency Study	12-36 months	500	Consistency lots, n=300; efficacy lot, n=100	100
AV010	Safety in Asthmatics	9-17 years	48	24	24
AV012	Herd Immunity Trial	18 mo – 18 years	Year 1, 4298 Year 2, 1958 ^a Year 3, 5173 ^a	4298 1958 5173	- - -
AV014	Consistency from Two Manufacturing Facilities	12-42 months	225	225	-
AV015	Safety of Re-vaccination in Year 3 of Subset of AV006 Subjects	3-8 years	949	949	-
AV017 ^a	Safety of revaccination in prior FluMist and placebo recipients (1-3 doses)	1-10 years	1245	1129	116
AV018 ^a	Concomitant MMR and Varicella Immunizations with FluMist	12-15 months	73 of planned 1200	blinded	blinded
AV019	Safety Assessment in Northern California Kaiser Permanente Clinics	1-17 years	9689	6473	3216
AR001 ^{a,b}	Safety of Classical vs. Recombinant Processes for Preparation of FluMist	6 months and over	65 – children 449 - total	65	-
ADULT TRIALS					
AV001 ^a	Phase I/II spray vs. drops	18-65 years	239	181	58
AV003	Efficacy Against Investigational Challenge with Wild Type Influenza	18-40 years	103, 92 were challenged	36 (TIV=33)	34
AV004 ^a	Safety	18-65 years	20	15	5
AV005 ^a	Safety of 2 doses	18-45 years	32	16	16
AV008 ^a	Safety in elderly, high risk	≥ 65 years	200	100	100
AV009	Safety and Effectiveness in Healthy Adults	18-64 years	4561	3041	1520
AR001 ^{a,b}	As above				
VA #448 ^a	Safety and Efficacy of FluMist or Placebo when given with TIV in COPD	≥ 50 with COPD	2215	1107	1108
DMID #98-005	Safety in HIV-infected compared to HIV-negative Adults	18-40 years	111, Infected, n=57 Negative, n=54	55	56

a – Not summarized in briefing document; includes some studies still ongoing and/or complete reports not submitted to BLA.

b – Children and adults participated in this protocol

INDICATION SOUGHT BY AVIRON

Active immunization for the prevention of influenza in children, adolescents and adults 1-64 years of age. In 1-8 year old children, FluMist has been shown to reduce influenza and influenza associated illnesses including otitis media, lower respiratory illness and febrile illness. In adults 18-64 years of age, FluMist has been shown to be effective in reducing influenza-like illnesses with an associated reduction in work loss, decrease in the number of healthcare provider visits, and decrease in the use of prescription and non-prescription medications during the influenza outbreak period.

An indication for travelers to areas where influenza viruses are circulating is also being sought.

Question 1. Are the data adequate to support the efficacy of FluMist in individuals aged 12 months – 64 years?

STUDIES SUBMITTED IN SUPPORT OF EFFICACY

Study AV006: Years 1 and 2 – Clinical endpoint efficacy study against culture-confirmed influenza in children aged 15-71 months of age.

Study AV011: Efficacy against shedding of vaccine strain following challenge with a monovalent vaccine strain A/Shenzhen (H1N1) in a subset of Year 1 and 2 AV006 participants.

Study AV009: Clinical endpoint efficacy study against illness during influenza outbreak periods in adults ages 18-65.

Study AV003: Primary endpoint was protective efficacy following FluMist or inactivated influenza vaccine in healthy serosusceptible adults against intranasal challenge with wild-type influenza.

Efficacy and immunogenicity results from these studies are summarized in this section of the briefing document.

STUDIES SUBMITTED IN SUPPORT OF CONSISTENCY OF MANUFACTURING

Study AV007: Lot consistency trial, including comparison with efficacy lot using immunogenicity as measure of consistency. Performed in healthy children, ages 12-36 months.

Study AV014: Manufacturing bridging study comparing vaccines blended and filled at Aviron-PA with vaccines blended and filled at Medeva with immunogenicity as measure of consistency, performed in healthy children, ages 12- 42 months.

The immunogenicity results from these studies are also summarized in this section of the briefing document.

EFFICACY DATA BY STUDY

STUDY AV006 (Years 1 and 2)

Title: A Phase 3, Randomized, Double-blind, Placebo-controlled, Trial to Assess the Safety, Immunogenicity and Efficacy of Influenza Virus Vaccine, Trivalent, Types A&B, Live, Cold-Adapted (CAIV-T) in Healthy Children.

INTRODUCTION/ STUDY DESIGN

This is a prospective, randomized, double-blind, placebo-controlled, multi-center trial conducted over two years at 10 study sites. In Year 1, children 15-71 months of age (before their 6th birthday) were randomized 2:1 to receive vaccine or placebo. Subjects were enrolled into either a 1 or 2-dose regimen, but the study was not planned as a comparison of the 1 vs. 2 dose regimens. The plan was to enroll subjects into the 2-dose cohort until mid-October, and thereafter to enroll subjects into the 1-dose cohort. The 2-dose regimen with a 60-day interval was chosen based upon historical data using CAIV-T in infants, which suggested there was minimal immune response to H1N1 after one dose of vaccine, possibly due to interference from the other influenza strains. In comparison, the response to H3N2 was relatively robust after one dose. The 2-dose regimen was also chosen because that is the recommended schedule for inactivated influenza vaccine for first time vaccination of children less than nine years of age. No preliminary studies to evaluate the optimal dosing interval were performed. The 1-dose regimen was included because, in practice, some children may only receive 1 dose of vaccine before the influenza season. Essentially, 8 of 10 sites enrolled children in the 2-dose regimen between August and October 1996, and 2 sites (Baylor and UCLA) enrolled subjects into 1 dose regimen from mid-Sept through Dec.

In Year 2, efficacy and safety of one dose re-vaccination was assessed. Subjects remained in the same treatment group, i.e., they were not re-randomized. Thus, no assessment of long-term efficacy of FluMist was planned since all subjects who had received FluMist in Year 1, also received FluMist in Year 2.

LOCATION: United States, multi-center; Collaborative Research and Development Agreement (CRADA) with NIAID

STUDY PERIOD: 2 years: Year 1 – 1996-97 (1st enrolled 8/96), Year 2 – 1997-98

PIVOTAL TRIAL: Yes, Phase 3 Efficacy

POPULATION: Healthy children, ages 15-71 months at enrollment

SAMPLE SIZE: **Year 1:** Total - n=1602; FluMist – n= 1070; Placebo – n= 532
Two-dose regimen – n= 1304; One-dose regimen- n= 288)
Immunogenicity cohort – n=214 (209 considered valid).
Year 2: Total - n=1358; FluMist – n=917; Placebo – n=441.
Two-dose regimen – n=755; One-dose regimen – n=162.
Immunogenicity cohort – n=162 (160 considered valid).

OBJECTIVES (Year 1)

Primary Efficacy:

- To demonstrate that children receiving a 2-dose primary vaccine regimen of FluMist are protected from culture confirmed influenza illness caused by community-acquired subtypes

antigenically similar to those contained in the vaccine for the influenza season directly following vaccination.

Secondary Efficacy

- To demonstrate that children enrolled in a 2-dose primary regimen of FluMist are protected from culture confirmed influenza illness caused by community-acquired subtypes antigenically similar to those contained in the vaccine for the influenza season directly following vaccination (i.e., 2-dose regimen, as randomized).
- To estimate the efficacy of a 1-dose primary vaccination regimen of FluMist to protect children against community-acquired subtypes antigenically similar to those contained in the vaccine for the influenza season directly following vaccination.
- To demonstrate the efficacy of either a 1 or 2-dose primary vaccination regimen of FluMist to protect children against community-acquired subtypes antigenically similar to those contained in the vaccine for the influenza season directly following vaccination.
- To demonstrate the efficacy of a 2nd year's single dose of Flumist to protect children who received a 1 or 2-dose primary vaccination regimen of Flumist in the previous year against community-acquired subtypes antigenically similar to those contained in the vaccine for the influenza season directly following the 2nd year's vaccination (Year 2, Follow-on Study Cohort).

Other Objectives

Immunogenicity: To assess the immunogenicity of Dose 1 and Dose 2 of FluMist in a subset of children from each of the 10 clinical study sites. In the Follow-on study, the immunogenicity of Year 2 will be assessed in the same subset.

Relatedness: To determine the relatedness of influenza strains in the vaccine to strains circulating in the community during the study as assessed by HAI antibodies in a subset of the immunogenicity cohort.

Tolerability and Safety: To assess the safety and tolerability of FluMist in children.

Severity of Illness: To assess the efficacy of FluMist to reduce the severity of influenza illness in children as measured by reduction in 1 or more of the following: the duration and height of fever, respiratory symptoms, restrictions in activity, pneumonia, and hospitalizations for respiratory illness.

Economic impact resulting from influenza: To assess the efficacy of FluMist to reduce the economic impact of influenza in children as measured by reduction in 1 or more of the following: the number of days of outpt and ER visits for influenza; days home from daycare, pre-school and school; days of lost work time for parents or guardians because of influenza; medication for influenza.

Economic impact resulting from non-specific respiratory illness: To assess the efficacy of FluMist to reduce the economic impact of respiratory illness in children as measured by reduction in 1 or more of the following: the number of days of outpt and ER visits for respiratory illness; days home from daycare, pre-school and school; days of lost work time for parents or guardians because of influenza; medication for respiratory illness.

VACCINES

1. FluMist – Each 0.5 ml dose contained $10^{6.7}$ TCID₅₀ of each strain of virus (2 type A and 1 type B) in normal allantoic fluid (NAF) containing sucrose-phosphate-

glutamate (SPG). 1996-97 influenza strains were A/Texas/36/91 (H1N1), A/Wuhan/359/95 (H3N2) and B/Harbin/7/94-like. There were 2 lots of FluMist (CAF014 and CAF015) used in this trial. In Year 2, the 1997-98 strains were A/Shenzhen/227/95 (H1N1), A/Wuhan/359/95 (H3N2) and B/Harbin/7/94-like, filled trivalent lot #CAF023.

2. Placebo – Lot CAF013 of NAF was used in Year 1, and lot CAF025 was used in Year 2. The placebo was described as being indistinguishable from vaccine in appearance and taste.

Vaccine administration and schedule: Vaccines were administered by intranasal spray 0.5 ml (0.25 ml per nostril) using Becton-Dickson Accuspray™ device, delivering 20-100 μm droplets into the nasal passages. For the 2-dose regimen, the 2nd dose was scheduled for 60 days (\pm 14 days, range 46-74 d) later.

Inclusion/Exclusion criteria – Healthy children 15-71 months, with no chronic illnesses, no history of wheezing or bronchodilator use within 3 months, no current (< 72 hours) URI symptoms or febrile illnesses within 1 week, no allergy to eggs, no immunocompromised household members, no prior influenza vaccines, and no nasal medications in prior 10 days.

Assignment to Group. At the time of enrollment, subjects were randomized 2:1 to the FluMist or placebo groups by a computer-generated schedule. Enrollment into the 1-dose and 2-dose regimens was not random. A separate sequentially-numbered list was provided for each study site, and randomization was achieved by assigning subjects to the next number.

Vaccine Contraindication. Dose 2 was to be contraindicated in subjects who experienced a vaccine-related SAE or any vaccine-related “unusual or alarming” event post-dose 1.

Concomitant Vaccinations and Medications. No concomitant immunizations were permitted with the study vaccines. All concomitant medications taken within first 10 days postvaccination were to be recorded on the CRF. Use of aspirin (ASA) and ASA-containing meds were discouraged, because of the association of ASA and influenza with Reye Syndrome.

MONITORING

Efficacy

Active surveillance was based upon regular phone contacts with the parents/guardians of the children. Initially calls were made every 2-3 weeks starting on the 11th day after the 1st vaccination. Once an influenza outbreak was declared (defined as an identified outbreak in the community or in a study subject at that site), the calls were increased to at least once every 7-10 days. Parents were instructed to call the study site if their child had any illness consistent with influenza. The criteria, prior to influenza season, for obtaining a culture were 2 signs or symptoms in category “a” OR \geq 1 sign or symptoms from category “b”. Once influenza season began, criteria for viral culture were 1 sign or symptoms in category “a” or 2 from category “b.”

- a) Fever (\geq 101°F rectal or oral; or 100.4 °F axillary); wheezing; shortness of breath; pneumonia; or ear infection (acute otitis media), suspected or diagnosed.
- b) Runny nose or nasal congestion; sore throat (pharyngitis); cough; muscle aches; chills; headache; irritability; decreased activity; or vomiting.

Additionally, a viral culture could also be performed if the investigator thought a patient's symptom complex warranted it. However, obtaining cultures from subjects in the first 10 days post-vaccination was discouraged because of concerns for potentially unblinding the study.

Cultures: Viral cultures were performed at the study sites. Influenza positive specimens were sent to Aviron for official determination, including subtyping of all isolates and phenotyping of those isolated obtained within 28 days of vaccination. Attempts were made to obtain specimens from subjects within 4 days of onset of symptoms (though sample could be collected at anytime). Throat and nasal swabs were obtained at all sites. Some sites also obtained nasal wash samples, and these could be added to the throat swab in lieu of the nasal swab. Nasal and throat specimens were stored at 4 °C until inoculated. In the lab, RMK cells were inoculated with the nasal and throat specimens within 4 hours of collection, or as soon thereafter as possible.

A quality control assessment (blinded specimens were sent to the sites) of the sites' abilities to grow influenza virus was performed in August 1996, and a few of the sites were unsuccessful at growing type B, no additional information was provided. The investigators elected to repeat QC testing in November of 1996, and reported that all sites grew type B influenza. The assessment was that in August the viral content of the specimens was too low to successfully grow the isolates, but data about the concentrations of viruses tested was not provided.

Immunogenicity

Year 1

A subset of 214 children (~ 1st 21 enrollees at each site) had a blood sample obtained. In the 2-dose regimen, serum was obtained pre-1st dose, pre-2nd dose (60 days \pm 14 days) and \geq 28 days after the 2nd vaccination. In the 1-dose regimen, serum was obtained pre-dose and 42 days (\pm 7 days) after the dose. Serum samples were tested for anti-hemagglutinin (HA) antibodies by hemagglutinin inhibition (HAI) and IgA and IgG anti-HA by ELISA, and assays were performed at Aviron, Mountain View CA. At Vanderbilt, a small subset of subjects (n=19) had nasal wash samples obtained for IgA anti-HA by ELISA. Subjects in the immunogenicity study who were vaccinated within 42 days of influenza season were to be excluded from the immunogenicity analysis for that particular strain.

Statistical Methods

All analyses were performed by EMMES, and the sponsor states that all of the primary and secondary analyses planned in the data analysis plan (DAP) were completed. EMMES Corp. were unblinded to the full data set for Year 1 on June 30, 1997. Unblinding of the Year 1 data by group for the investigators, AVIRON and NIAID occurred on July 12, 1997. A full analysis was planned at the end of Year 1 before proceeding to Year 2. No methods to correct for multiple comparisons were used.

EFFICACY ANALYSES (YEAR 1)

Case Definition - A culture-confirmed case of influenza illness was defined by a positive culture of a wild-type virus subtype antigenically similar to one contained in the vaccine, that occurred at least 15 days after receiving the 1st dose of vaccine or placebo. "Antigenically similar" was not defined in the protocol.

Efficacy point estimates were calculated in customary fashion:

$$100 \times (1 - \text{relative risk of vaccinee becoming a case}) = 100 \times (1 - P_v / P_p)$$

where P_v = proportion of vaccinees and P_p = proportion of placebo.

Confidence intervals (CI) were calculated using the method of Koopman for the ratio of binomial proportions.

Sample size (Year 1) was based upon the following assumptions:

- True efficacy of the vaccine is 60%.
- The true event rate in the control group will be 33% in the 96-97 season.
- The case ascertainment rate is assumed to be 75%. Therefore, the observed event rate will be 25% ($3/4 \times 33\%$).
- The loss to f/u will be 10% during the study season.
- The criteria for efficacy will be that the point estimate will be above 50% and the lower bound of the 95% CI will be above 30%.

The probability, or power, that these criteria will be met is ~90%.

Amendment #3 provided for increasing the sample size to 1400 because the attack rate was lower than the predicted 25% (~16% during the study period).

YEAR 1 EFFICACY ENDPOINTS AND EFFICACY RESULTS

Primary efficacy endpoint: The first episode of culture-confirmed community-acquired influenza in a study participant occurring anytime on the day of or after receipt of the 2nd dose of FluMist or placebo.

Secondary efficacy endpoints: The 1st episode of culture-confirmed influenza illness occurring at least 15 days after the 1st dose of vaccine or placebo in a study participant following:

- Receipt of 1 or 2 doses
- Enrollment to receive 2 doses (2 dose as randomized)
- Enrollment to receive 1 dose

All of these endpoints were also investigated separately for the specific influenza strains circulating in 1996-97 season (i.e., A/H3N2 and B).

Other Efficacy Endpoints

Efficacy against all wild-type influenza strains was to be evaluated (i.e., those antigenically similar as well as those unrelated to vaccine strains). Additionally, severity of illness was examined in 2 ways:

1. By evaluating illness characteristics in subjects with culture-confirmed influenza and assessing severity in the following manner:
 - Culture-confirmed influenza accompanied by fever ($\geq 101^\circ\text{F}$ rectal or $\geq 100.4^\circ\text{F}$ axillary)
 - Culture-confirmed influenza accompanied by otitis media (**No pre-specified definition of otitis media was stated in the protocol**).
 - Culture-confirmed influenza accompanied by missed school or daycare, lost work days for the primary care provider, and the number of health care visits
2. By evaluating illness characteristics in all participants where a culture was taken or was to be obtained because of an illness, regardless of the culture results. In these subjects, severity of

illness regardless of culture result or culture obtained was analyzed as the number of episodes per participant as follows:

- Any illness events
- Any illness event and antibiotic prescribed
- Any febrile illness event
- Any febrile illness event and antibiotic prescribed
- Any otitis media
- Any otitis media and antibiotic prescribed
- Any febrile otitis media
- Any febrile otitis media and antibiotic prescribed
- Any illness accompanied by missed school or daycare, lost work days for the primary care provider, and the number of health care provider visits.

RESULTS

AV006 – Year 1 Baseline Characteristic of Study Enrollees.

	FluMist N= 1070	Placebo N= 532
Mean Age, Months (Std. Dev.)	43.0 (16.6)	41.5 (16.5)
Race/Ethnicity, %		
White	85	84
Black	9	10
Hispanic	4	3
Asian	0.9	0.8
Other	2	2
Gender, %		
Male	47	49
Female	53	51
Household Composition Mean (Std. Dev.)		
Adults	2.1 (0.6)	2.1 (0.6)
Children	2.6 (1.2)	2.6 (1.1)
Primary Caretaker Works Outside Home, %	49	50
Number of Day Care/Preschool Days/Week Expected at Time of Enrollment, Mean (Std. Dev.)	2.4 (2.1)	2.3 (2.1)

Overall, the FluMist and placebo groups had comparable demographic characteristics at enrollment. There were some differences in the demographics of subjects who received one dose vs. those who received two doses. Most subjects in the one-dose regimen, 261 of 288 (91%) were enrolled at the Baylor or UCLA sites. Sixty-eight % of these were Caucasian and 16% Hispanic. In the 2-dose regimen, 88% were Caucasian and < 1% were Hispanic. Additionally, only 43% of subjects in the 1-dose regimen had a sibling in the trial as compared to 54% in the 2-dose regimen. No statistical comparisons were performed for the 1-dose vs. 2-dose groups.

The immunogenicity cohort demographics (n=214) also had some differences compared to those subjects not in the cohort. Caucasians accounted for 73% on the immunogenicity cohort compared to 86% of the non-immunogenicity cohort, Fisher’s exact p value < 0.001.

The immunogenicity cohort was not a random sampling of the subjects, but was the first 21 subjects per site. The sponsor states this group was selected to obtain blood samples before influenza started in the community. However, there were some sites where the 1st 21 consecutive subjects were NOT the subjects enrolled into the immunogenicity cohort.

Distribution of the participants across the 10 study sites was comparable for the FluMist and placebo recipients within both dosing regimens. All participants in Houston and Los Angeles were enrolled in the 1-dose regimen, except 15 subjects in Houston who were in the 2-dose regimen and enrolled into the Immunogenicity cohort. All other participants were enrolled into the 2-dose regimen, except 2 subjects in Rochester and 25 in Bardstown who were enrolled at the end of the enrollment period and were intentionally enrolled into the 1-dose regimen.

Protocol Compliance

There were a total of 205 of 1602 participants with protocol deviations (136 in FluMist group, 69 in placebo group). Subjects missing appropriate data (not defined) for safety or efficacy analyses were excluded. Among the protocol deviations, eight Accuspray™ devices (FluMist n=5, placebo n=3) were found to be defective requiring study vaccine replacement (this occurred without unblinding of the subjects).

Ninety seven percent of subjects in the 2-dose regimen completed study visits and 93% of 2-dose subjects received dose 2 within the specified window of 46-74 days post-dose. A total of 42 subjects did not receive dose 2 (27 FluMist, 15 placebo). The most frequent reasons were refusal (n=3, FluMist n=2, placebo n=1), consent withdrawal (n=11, FluMist n=5, placebo n=6) and 4 subjects experienced adverse events (AEs) related to dose 1 (FluMist n=2, placebo n=2). The two AEs in the FluMist group were wheezing (onset 4 days post-dose 1) and allergies requiring an inhaler therapy (onset 7 days post-dose 1). An additional subject in the FluMist group developed new onset juvenile rheumatoid arthritis after dose 1 of FluMist and was not given a second dose.

For the immunogenicity subset, 209 of 214 subjects were evaluable for analyses. 5 subjects were excluded (except ITT analysis) because they had received an influenza vaccine (CAIV or TIV, trivalent inactivated vaccine, i.e., the licensed injectable influenza vaccine) previously. Ninety seven percent of the immunogenicity subset completed Year 1 of the study.

Efficacy (Year 1)

A total of 3127 viral cultures were obtained from all subjects. Of these, 139 positive cultures for influenza were reported to the statistical center by the local laboratories. However, one of these was lost in shipment to or at Quintilles, and could not be confirmed at Aviron. There were 18 positive cultures obtained within 14 days of Dose 1 and thus, not included in the primary efficacy analysis. All 18 of these isolates were phenotyped as CAIV, i.e., one of the vaccine strains (details below). One subject was initially reported as having two distinct H3N2 isolates, but Aviron could not confirm the second culture and it was eliminated from the analysis. Two sibling participants had cultures obtained on the same day, and one was positive and the other was negative for influenza. However, these samples were mislabeled and could not be definitely identified. The siblings were in different treatment groups and one positive culture was excluded from analysis (based upon a pre-determined rule for study procedures, before study unblinding).

Five subjects had 2 positive cultures (obtained 3-5 days later) for the same virus, and only the 1st isolate was counted in the analysis. Six placebo recipients were culture positive for type A/H3N2 and later culture positive for type B. These subjects contributed a single event to “any strain” analysis and a single event to both H3N2 and B analyses. Thus, the analysis included 114 positive influenza cultures [139- (1+1+18+5)] from 108 subjects for wild type influenza (types A/H3N2 and B). Of the 114 positive cultures, 14 were in FluMist recipients (n=11 for 2-dose and n=3 in 1-dose regimen) and 100 were in placebo recipients (n=86 for 2-dose and n= 14 in the 1-dose regimen).

AV006- Year 1. Efficacy of FluMist in Preventing Culture-Confirmed Influenza Illness.

Analysis Group	Strain	Estimated Efficacy % (95% CI)
Participants Receiving Two Doses	Any	93.4 (87.5, 96.5)
	H3N2	96.0 (89.4, 98.5)
	B	90.5 (78.0, 95.9)
Participants in Two Dose Regimen	Any	93.2 (87.6, 96.3)
	H3N2	95.5 (89.3, 98.1)
	B	90.5 (78.0, 95.9)
Participants in One Dose Regimen	Any	88.8 (64.5, 96.5)
	H3N2	86.9 (46.6, 96.8)
	B	91.3 (45.6, 98.6)
All Randomized Participants	Any	92.6 (87.3, 95.7)
	H3N2	94.5 (88.3, 97.4)
	B	79.5 (79.5, 95.7)

H1N1 did not circulate in the community and no field efficacy data were generated for this strain.

AV006 – Year 1. Efficacy of FluMist by Age, Gender, and Race

Category	Any Strain % (95 CI)	H3N2 % (95% CI)	B % (95% CI)
Age (mos.)			
< 24	84.7 (57.5, 94.6)	89.6 (59.1, 97.4)	71.4 (-31.6, 93.8)
24-35	96.2 (85.8, 99.0)	94.3 (78.7, 98.5)	100 (81.2, 100)
36-48	87.0 (66.8, 94.9)	88.7 (63.1, 96.5)	83.4 (27.8, 96.2)
48-60	100 (89.9, 100)	100 (84.8, 100)	100 (77.1, 100)
>60	90.6 (70.3, 97.1)	100 (79.2, 100)	83.6 (44.1, 95.2)
Gender			
Female	91.8 (83.8, 95.8)	94.0 (85.6, 97.6)	86.4 (61.1, 95.3)
Male	93.8 (85.0, 97.4)	95.5 (82.8, 98.8)	93.2 (79.0, 97.8)
Race			
White	92.3 (96.5, 95.7)	94.8 (88.8, 97.7)	89.5 (76.9, 95.2)
Non-White	94.9 (70.0, 99.2)	91.6 (47.6, 98.7)	100 (52.0, 100)

Of note, the analyses by age provided in the study report include some overlapping age ranges (48 months).

Severity of Illness

The p-values are not adjusted for multiple comparisons.

Illness associated with culture positive influenza:

Efficacy was demonstrated for febrile ($\geq 101.0^{\circ}\text{F}$ oral or rectal or $\geq 100.4^{\circ}\text{F}$ ax) influenza illness of 95% (95%CI: 90, 97.5) and febrile illness associated with otitis media(OM) of 97.5% (85.5, 99.6) in the FluMist vs. placebo groups.

Illness regardless of culture status:

Flumist decreased the rate of febrile illnesses by 20% (0.78 vs. 0.98, $p < 0.01$), and for antibiotic use for febrile illnesses by 31% (0.31 vs. 0.46 events, $p < 0.01$) and for antibiotic use for febrile OM (0.14 vs. 0.22 events, $p < 0.01$) for all participants. There was not a significant decrease in OM without fever, for any OM for which antibiotics were prescribed, or for “any illness.”

AV006 – Year 1. Number of Illness Events Per Participant by Dosing Regimen and Treatment Group

	Treatment Group	N ^a	Any Illness	Febrile Illness	Otitis Media	Febrile Otitis Media
2 Doses Received	FluMist	849	2.28	0.82	0.52	0.16
	Placebo	410	2.40	0.99	0.58	0.24
	p-Value ^b		0.21	0.01	0.33	0.01
Enrolled in One-Dose Regimen	FluMist	189	1.58	0.62	0.34	0.10
	Placebo	99	1.70	0.99	0.36	0.17
	p-Value ^b		0.54	<0.01	0.91	0.17
All Participants	FluMist	1070	2.14	0.78	0.48	0.15
	Placebo	532	2.25	0.98	0.54	0.23
	p-Value ^b		0.23	<0.01	0.29	<0.01

a - All participants in analysis group

b - Wilcoxon Rank sum test

AV006 – Year 1. Number of Illness Events with Antibiotic Prescribed Per Participant by Dosing Regimen and Treatment Group

	Treatment Group	N ^a	Any Illness	Febrile Illness	Otitis Media	Febrile Otitis Media
2 Doses Received	FluMist	849	0.86	0.32	0.51	0.16
	Placebo	410	0.98	0.44	0.57	0.23
	p-Value ^b		0.14	<0.01	0.43	0.02
Enrolled in One-Dose Regimen	FluMist	189	0.68	0.29	0.34	0.10
	Placebo	99	0.84	0.49	0.35	0.16
	p-Value ^b		0.18	0.02	0.87	0.13
All Participants	FluMist	1070	0.83	0.31	0.48	0.14
	Placebo	532	0.95	0.46	0.53	0.22
	p-Value ^b		0.04	<0.01	0.36	<0.01

a - All participants in analysis group

b - Wilcoxon Rank sum test

FluMist significantly reduced the number of health-care provider visits (13.4% decrease, $p=0.02$). FluMist did not significantly reduce the number of missed school or daycare days or lost workdays for primary care provider, however, these parameters were significantly reduced ($p < .01$) when the analysis was restricted to subjects with culture-confirmed influenza.

Year 1 Immunogenicity Results

Enrollment and Compliance in Immunogenicity Substudy

Not all of the subjects in the Immunogenicity cohort had all samples obtained and not all samples were included in the analyses, and the reasons for the exclusions are not provided.

All subjects with available data were included in the post-dose 1 analysis (regardless if they were in the 1-dose or 2-dose regimen) and only subjects who received 2 doses are included in the post-dose 2 assessments. The time of obtaining the serum samples in the 1-dose and 2-dose regimens is not summarized. As noted under study design, the serum samples were to be obtained at different times for subjects in the 1-dose regimen (42 ± 7 days post-dose 1) than for subjects in the 2-dose regimen (60 ± 14 days post-dose 1 and ≥ 28 days post-dose 2 with no upper time limit stated).

AV006 – Year 1. Strain-Specific HAI Geometric Mean Titers (GMT) Following Dose One or Two in All Participants.

Strain	GMT (95% CI)					
	Pre-vaccination		Post-Dose 1		Post-Dose 2	
	FluMist	Placebo	FluMist	Placebo	FluMist	Placebo
n	136	67	131	63	115	58
H1N1	5.4 (4.2, 6.9)	4.7 (3.4, 6.5)	8.9 (6.6, 12.0)	4.6 (3.3, 6.4)	18.8 (14.2, 25.0)	5.0 (3.4, 7.1)
H3N2	9.5 (7.2, 12.5)	8.6 (6.0, 12.2)	39.5 (33.5, 46.7)	9.9 (6.8, 14.4)	43.8 (37.4, 51.3)	10.5 (6.8, 16.1)
B	4.1 (3.4, 5.0)	4.5 (3.5, 5.9)	18.3 (15.6, 21.3)	4.6 (3.5, 6.1)	25.8 (22.6, 29.4)	4.5 (3.3, 6.0)

In a subset of subjects with data available from appropriate time points, geometric mean fold rises (GMFR) were calculated from pre-vaccine to post-dose 1 and for pre-vaccine to post-dose 2. Respectively, these GMFR's were: H1N1 - 1.7 (1.4, 1.9) and 3.4 (2.7, 4.2); H3N2 – 4.3 (3.3, 5.6) and 4.7 (3.5, 6.2); and B – 4.5 (3.7, 5.6) and 6.3 (5.0, 7.8).

The percent of FluMist recipients with post-dose 2 HAI GMTs $\geq 1:32$ was: H1N1 – 52%; H3N2- 83% and B- 57%. (Although not an established correlate of immunity, HAI titers $\geq 1:32$ has been associated with protection following wild type influenza infection and after inactivated influenza vaccine).

AV006 – Year 1. Strain-Specific HAI Seroconversion Following Dose One or Dose Two in Seronegative Participants in the FluMist Group

Strain		FluMist				Converted Post-Dose Two
		Seronegative Pre-Vaccination	Initial Seroconversion at			
			Post-dose One	Post-Dose Two		
H1N1	n/N Proportion 95% CI	89/136 0.65 (0.57, 0.73)	14/86 0.16 (0.09, 0.26)	33/60 0.55 (0.42, 0.68)	45/74 0.61 (0.49, 0.72)	
H3N2	n/N Proportion 95% CI	66/136 0.49 (0.40, 0.57)	59/64 0.92 (0.83, 0.26)	3/4 0.75 (0.19, 0.99)	54/56 0.96 (0.88, >0.99)	
B	n/N Proportion 95% CI	93/136 0.68 (0.60, 0.76)	80/91 0.88 (0.79, 0.94)	6/8 0.75 (0.35, 0.97)	75/78 0.96 (0.89, 0.99)	

Among those who were seronegative at baseline in the placebo group 1/40 seroconverted to H1N1, 2/26 to H3N2, and 1/38 to B.

AV006 – Year 1. Four-Fold Rise in Strain Specific HAI Titers For All Participants in FluMist Group Following Dose One or Dose Two

FluMist				
Strain		Initial Four-Fold Rise		Overall Post-Dose Two
		Post-dose One	Post-Dose Two	
H1N1	n/N Proportion 95% CI	25/131 0.19 (0.13, 0.27)	34/88 0.39 (0.28, 0.50)	55/115 0.48 (0.38, 0.57)
H3N2	n/N Proportion 95% CI	68/131 0.52 (0.43, 0.61)	6/54 0.11 (0.04, 0.23)	61/115 0.53 (0.44, 0.62)
B	n/N Proportion 95% CI	81/131 0.62 (0.53, 0.70)	9/41 0.22 (0.11, 0.38)	78/115 0.68 (0.58, 0.76)

Proportions in columns:

1. Based on all participants in treatment group.
2. Response to Dose 2 among participants who did not seroconvert after dose 1.
3. Response following Dose 2 among participants with pre-vaccine and post-Dose 2 results.

Among placebo recipients, the numbers (%) of subjects with four fold-rises in HAI titers were 2/58 (3.4%) for H1N1, 3/56 (5.3%) for H3N2, and 2/58 (3.4%) for B.

ELISA IgG anti-HA seroconversion rates were also measured in ~202 subjects. Seroconversion was defined as a 2-fold rise in IgG anti-HA titer. Values below the assay cutoff were converted to equal the cut-off value for calculations of GMTs and ratios pre-to-post vaccination. FluMist and placebo groups had equivalent pre-titers. Less than 6% of placebo recipients had strain-specific seroconversion following Dose 1 or Dose 2. The seroconversion rates (95% CI) following FluMist were: post-dose 1 → 24% to H1N1, 55% for H3N2 and 70% for B, and post-dose 2 → 54% (44,63) for H1N1, 63% (54,72) for H3N2, and 77% (69, 85) for B, regardless of when the seroconversion occurred. The mean fold rises (95% CI) from baseline to post-dose 2 were H1N1 at 3.2 (2.4, 4.3), H3N2 at 24.6 (18.3, 33) and B at 20.8 (17, 25.3).

Nasal IgA anti-HA antibodies were measured in 19 subjects (FluMist =13 and placebo = 6) and thus, no formal comparisons could be performed.

Heterotypic immunity. Sera from 92 of the 203 children were available for assessment of heterotypic immunity. HAI antibody titers against 6 different H3N2 virus types were assessed. HAI assays were performed at Vanderbilt. Following FluMist (n=62), the seroconversion rates for the seronegative subjects (n~25) from baseline to post-dose 2 were as follows: A/Russian/269/95 - 78%; A/Johannesburg/33/94 - 52%; A/Wuhan, A/Sydney/05/97 – 96%; A/Nanchang/933/95 – 96% and A/Thessalonika/1/95 - 96%. The percent with 4-fold rises was ~50% for each strain (range of 31% for A/Johannesburg to 56% for A/Sydney). For the seronegative subjects in the placebo group (n=30), between 7 and 14% seroconverted to each of the strains. No statistical comparisons between the FluMist and placebo groups were provided.

There were nine subjects in the immunogenicity cohort (all placebo recipients) who were culture positive for H3N2 influenza during the course of the study. There were six subjects in the cohort

who were culture positive for type B (3 in the placebo group and 3 in the FluMist group). All 3 subjects in the FluMist group had 4-fold rises in anti-HA titers post-FluMist and before disease.

CAIV Culture-Positive Subjects

Within the 1st 14 days of vaccination, 116 subjects had 117 cultures performed. Subjects who had cultures met surveillance criteria, as stated above. Sixty-six of 116 were FluMist recipients and cultures were obtained after Day 11, consistent with specified date for surveillance. Thirty-eight placebo subjects also had cultures obtained, but no information is provided about these subjects. Between Day 2 and Day 11, 17 FluMist recipients had 18 samples obtained which grew 20 CAIV isolates. Sixteen of the subjects who had cultures obtained were from the Houston site (total FluMist recipients in Houston was 144), and one subject was from Maryland. Of these, 11 subjects grew type B, 5 grew type A (subtype not stated) and 2 grew both a type A and type B. Results for growth of other viruses were not provided. The symptom complexes for the FluMist subjects who were culture-positive for a CAIV were different from the FluMist subjects who were culture-negative for a CAIV strain, statistical comparisons not performed (see below).

AV006 – Year 1. Selected Reactogenicity Events (Days 0-10) for FluMist Recipients with Cultures Obtained within 14 Days of Vaccinations by Culture Results.

Participants who returned Diary Cards	Culture Results	
	Positive* N=17	Negative N=60 ^a
Event, n(%)		
Any Event	17 (100)	52 (86.7)
≥ 3 events on same day	12 (70.6)	25 (41.7)
CDC-ILI ^b	7 (41.2)	8 (13.3)
Runny nose/congestion	17 (100)	44 (73.3)
Cough	11 (64.7)	33 (55)
Irritability	9 (52.9)	19 (31.7)
Vomiting	5 (29.4)	4 (6.7)
Muscle aches	1 (5.9)	7 (11.7)
Decreased activity	9 (52.9)	15 (25)
Fever		
Temp 1: >100°F	12 (70.6)	14 (23.3)
Temp 2: >102	2 (11.8)	4 (6.7)
Temp 3: >104	1 (5.9)	0

* CAIV culture positive

a. 61 subjects were vaccinated, but only 60 returned the diary card.

b. CDC influenza like illness, defined as fever with cough or sore throat on the same day or consecutive days.

STUDY AV006 -YEAR 2

Of the 1602 subjects originally enrolled, 1561 completed Year 1 of the study and 1358 (87%) returned for Year 2. As noted above, subjects returning for Year 2 remained in their original treatment group. All enrollees were vaccinated with a single dose of study vaccine, either placebo or Flumist containing the 1997-98 strains, according to their randomization assignment in Year 1. With this, the sponsor reports potential biases included: 1) self-selection for Year 2 enrollment and 2) different immune experiences of Flumist and placebo recipients during Year 1 (e.g., placebo recipients had much higher attack rate of influenza in Year 1).

The procedures for monitoring for safety and efficacy in Year 2 were similar to those in Year 1. Participants in the Immunogenicity cohort from Year 1 who returned were offered enrollment into the Year 2 Immunogenicity substudy. Serum was obtained to characterize further the strain-specific responses to HA by HAI and IgG ELISA (assays were performed at Aviron). Pre-vaccination titers were obtained just prior to vaccination and post-titers were obtained 28 to 56 days after vaccination. At Vanderbilt, a very small subset of subjects (13 subjects of 19 from Year 1) had nasal washes obtained for IgA anti-HA by ELISA at the same time that serum samples were obtained.

VACCINES: The vaccines used are listed under Vaccines in Year 1. The A/H3N2 and B strains were the same for Year 1 and Year 2; however, the H1N1 strains were different. **In Year 1, the H1N1 was A/Texas/36/91 and in Year 2 it was A/Shenzhen/227/95.**

EFFICACY ENDPOINTS AND RESULTS FOR YEAR 2

Primary

The **primary efficacy endpoint** is the 1st episode of culture-confirmed influenza caused by subtypes of influenza antigenically similar to strains contained in the vaccine in a study child following re-vaccination with a dose of vaccine or placebo.

A **culture-confirmed case** of influenza illness is one that occurred at least 15 days after receiving a dose of vaccine or placebo and that is defined by a positive culture of a wild-type virus subtype antigenically similar to one contained in the vaccine. “Antigenically similar” is not defined in the protocol.

Secondary

The **secondary efficacy endpoints** are the 1st episode of culture-confirmed influenza illness caused by subtypes of influenza antigenically similar to strains contained in the vaccine occurring at least 15 days after re-vaccination with a dose of vaccine or placebo in a study participant who in Year 1:

- Received 2 doses
- Was enrolled to receive 2 doses
- Was enrolled to receive 1 dose

All of these endpoints were also investigated separately for the specific influenza strains circulating in 1997-98 season, including A/Sydney (H3N2), a variant strain.

Other Efficacy Endpoints

Severity of illness was examined in 2 ways: by evaluating illness characteristics in 1) subjects with culture-confirmed influenza and 2) all participants regardless if a culture was taken or using the same variables as for year 1.

RESULTS (YEAR 2)

Enrollment and Demographics.

The characteristics of the two study groups at the time of enrollment into Year 2 were similar: the mean age was 55 months and 52 months; 86% and 85% were Caucasian; and 54% and 51% were female, FluMist and placebo groups respectively.

As compared to subjects who chose not to participate, Year 2 participants were more likely to have a primary caretaker working outside of the home (51% *vs* 39%). Otherwise, the participants in Year 2 were comparable to non-participants. For the 203 subjects that did not participate in Year 2, the reasons listed for not returning were comparable between the FluMist and placebo groups. The 3 primary reasons (accounting for 73% of subjects) for not returning were withdrew consent (33% -n=66, FluMist n=35), moved away (25%- n=50, FluMist 35) and unable to contact (16%- n=33, FluMist n=24). Other reasons (accounting for less than 5% each) included: no longer meets Inc/Exc criteria, changed insurance, and "other."

For the immunogenicity cohort, 162 of 209 (78%) subjects returned for participation in Year 2. This percent of subjects who returned is lower than the percent of overall study participants who returned (78% *vs* 87%). As in Year 1, there was a lower percentage of subjects who were Caucasian in the immunogenicity cohort (75% in immunogenicity subset *vs* 85% in entire study cohort). Within the immunogenicity cohort, the FluMist (n=108) and placebo (n=54) recipients had similar demographics except more FluMist recipients had primary caretakers who worked outside of the home as compared to placebo (65% *vs* 52%).

75% of Year 2 subjects received their vaccination in Sept., 25% in October and all dosing was complete by Nov 14, 1997.

Protocol Compliance

1343 participants (99%) completed Year 2 of the study. There were a total of 170 of 1358 (13%) of participants with protocol deviations, information was provided as line listings. Most violations related to enrolling subjects (n=30, Flumist n=24) who developed exclusion criteria, such as asthma or other chronic illnesses. A total of 15 subjects (11 FluMist and 4 placebo recipients) withdrew from the study. Six subjects were withdrawn because of lack of compliance, 3 subjects moved from the study area, 3 did not return for observation, and 1 subject withdrew consent. At one site, 37 subjects received only ½ dose of study vaccinations. A missed phone call accounted for 61 protocol violations. No information about defective Accuspray devices was provided.

Efficacy

H1N1 did not circulate in the community in Year 1 or Year 2, and no field efficacy data were generated for this strain.

During Year 2 influenza season, 1808 cultures were obtained, 1188 in the FluMist group and 620 in the placebo group. Of the total, seventy-one (4%) cultures were positive for influenza, 15 (1%) of FluMist recipient cultures and 56 (9%) of placebo recipient cultures.

In the primary efficacy analysis, the sponsors have not included the type A/Sydney (H3N2) isolates and conclude that efficacy was 100% against wild-type strains “antigenically similar” to vaccine strains. The predominant circulating strain in Year 2 was A/Sydney (H3N2), which was considered to be a variant, whereas in Year 1 the circulating strain A/H3N2 was more closely matched to the A/Wuhan (H3N2) vaccine strain. **However, the protocol did not define “antigenically similar” for the purposes of the primary analysis.**

AV006 – Year 2 Overall Efficacy of FluMist in Preventing Culture-Confirmed Influenza Illness

Analysis Group	Strain	Number of Isolates		Estimated Efficacy % (95% CI)
		FluMist	Placebo	
All Year Two Participants	All community acquired strains	15	56	87.1 (77.7, 92.6)
	Strains in FluMist [A/Wuhan (H3N2), B]*	0	5	100 (63.1, 100)
	A/Sydney (H3N2)	15	51	85.9 (78.0, 91.9)
Participants Enrolled in the Two-Dose Regimen in Year One	All community acquired strains	14	52	87 (77.0, 92.6)
	Strains in FluMist [A/Wuhan (H3N2), B]	0	4	100 (53.7, 100)
	A/Sydney (H3N2)	14	48	85.9 (75.0, 92.1)
Participants in One-Dose Regimen in Year One	All community acquired strains	0	4	100 (54.9, 100)
	Strains in FluMist [A/Wuhan (H3N2), B]	0	4	100 (54.9, 100)
	A/Sydney (H3N2)	0	3	100 (39.8, 100)

*Primary analysis according to sponsor’s study report.

No participant in Year 2 had more than 1 case of influenza. The sponsor does not state the number of cultures obtained in the first 15 days post-vaccination. There were no positive cultures for CAIV. The mean number of days between vaccination and influenza was 141 for FluMist recipients and 124 for placebo recipients, p=0.05.

An additional analysis, which excluded 48 subjects with protocol violations, was provided. The protocol violations included 37 subjects (FluMist, n=21 and placebo, n=16) at one site who inadvertently received ½ dose of vaccination. The other 11 included: 7 subjects (FluMist n=1, placebo n=6) who had received TIV prior to Year 1 or Year 2 vaccination, 3 subjects (FluMist n=2) who had influenza, and 1 placebo recipient who received Vancenase (nasal steroids) within 4 days of vaccination. As noted above, there were a total of 170 protocol deviations and it is not clear why only 48 were excluded or why subjects with influenza prior the second dose in Year 1 were excluded. Nevertheless, efficacy in this analysis was similar, 87% against all circulating strains and 100% against strains similar to those in the vaccine.

Efficacy against A/H3N2 afforded by wild type infection in Year 1 was 86.1% (25.9, 97.6). which is similar to the efficacy of 86.9% against all community-acquired A/H3N2 in FluMist recipients. One of the 52 placebo recipients with wild-type A/H3N2 in Year 1 also had A/H3N2 in Year 2. This is compared to 54 of 389 placebo recipients without wild-type A/H3N2 in Year 1 who were infected in Year 2.

AV006 - Year Two. Efficacy of FluMist by Age, Gender, and Race

Category	Any Strain % (95 CI)
Age (mos.)	
< 24	NA
24-35	84.4 (35.2, 96.3)
36-48	84.5 (56.8, 94.5)
48-60	92.2 (69.0, 98.0)
≥ 60	86.9 (70.8, 94.1)
Gender	
Female	81.7 (65.3, 90.4)
Male	94.1 (82.0, 98.1)
Race	
White	87.6 (78.1, 93.0)
Non-White	75.4 (-85.6, 96.7)

Of note, the analyses provided by ages include overlapping age ranges. The number of subjects included per age group was not provided.

Severity of Illness

Illness associated with culture positive influenza:

Culture positive influenza illness was categorized into 3 symptom groups: 1) afebrile upper respiratory infection (URI) - 3 cases in FluMist group and 2 in placebo 2) febrile URI - 12 cases in the FluMist group and 54 in the placebo group and 3) lower respiratory symptoms - 0 in FluMist group, 8 in placebo (100%, with 95%CI 77, 100), events shown against “any wild-type” strain. Of note, lower respiratory symptoms were not described and case definition for this diagnosis was not provided.

Efficacy for febrile ($\geq 101.0^{\circ}\text{F}$ oral or rectal or $\geq 100.4^{\circ}\text{F}$ ax) influenza illness in the FluMist vs. placebo groups was 89.3% (80.4, 94.2) and associated with otitis media was 94.3% (78.1, 98.5). However, no efficacy was demonstrated against all otitis media. The mean duration of febrile illness due to any wild-type strain of influenza was significantly less for FluMist than placebo recipients (2.1 vs. 4.9, $p < 0.01$.)

Illness regardless of culture status:

There were significantly fewer number of illnesses per participant in the FluMist group than in the placebo group (1.46 vs. 1.61; $p = 0.01$), and fewer febrile illness (0.61 vs. 0.77; $p < 0.01$). No efficacy was demonstrated against “any otitis media” (0.32 vs. 0.34, $p = 0.16$) or episodes of febrile otitis media (0.11 vs. 0.14, $p = 0.6$) in the FluMist compared to placebo group. The number of episodes per participant of otitis media requiring antibiotics was small (0.11 in the FluMist group vs. 0.13 in the placebo group) but reached statistical significance with $p = 0.04$. There was no pre-specified definition for the diagnosis of otitis media stated in the protocol.

For economic impact (evaluation began 15 days post-vaccination), FluMist recipients missed significantly fewer school, pre-school or daycare days for “all illnesses” (0.93 vs 1.11 $p = 0.01$) and for culture-confirmed influenza (0.02 vs. 0.23; $p < 0.01$) than did placebo recipients. Also, significantly fewer lost workdays for primary care provider and HCP visits were noted for FluMist vs placebo recipients with culture-confirmed influenza ($p < 0.01$).

AV006 - Year 2 Immunogenicity Results

Of the 162 subjects who returned for Year 2, 160 subject had valid pre- and post-vaccination samples for HAI titers and 159 for IgG by ELISA. The reasons for missing data in subjects were not provided.

AV006- Year 2. Strain-Specific HAI Geometric Mean Titers (GMT)Following Revaccination in Year Two by Treatment Group.

		GMT (95% CI)			
		FluMist		Placebo	
Strain		Pre-Dose	Post-Dose	Pre-Dose	Post-Dose
N*					
H1N1 A/Texas (Yr 1 Strain)	GMT 95% CI	10.3 (7.8, 13.6)		4.1 (3.0, 5.7)	
H1N1 A/Shenzhen (Yr 2 Strain)	GMT 95% CI	8.7 (6.3, 12.1)	24.8 (19.2, 32.0)	5.0 (3.3, 7.5)	4.6 (3.1, 6.8)
H3N2	GMT 95% CI	73.4 (62.3, 86.5)	91.1 (77.9, 106.5)	21 (12.5, 35.2)	22.1 (13.1, 37.2)
B	GMT 95% CI	18.5 (15.7, 21.7)	42.4 (37.1, 48.4)	6.0 (4.3, 8.2)	6.1 (4.4, 8.5)

Table combines subjects who received one or two doses in Year 1.

*N, number of participants included was not provided.

Prior to Year 2 vaccination, all FluMist recipients were seropositive to A/H3N2, 92.4% to type B, and 57% to A/H1N1, see Table below.

AV006-Year 2. Strain-Specific HAI Seroconversion Following Re-Vaccination in Seronegative Participants in the FluMist Group

		FluMist Recipients		
Strain		Seronegative Pre-Vaccination	Seroconversion Post-dose in Year 2	Cumulative Seropositive
H1N1 A/Texas (Year 1)	n/N Percent 95% CI	46/107 43 (33, 53)	Not provided	Not provided
H1N1 A/Shenzhen (Year 2)	n/N Percent 95% CI	57/106 54 (44, 64)	38/57 67 (3, 79)	87/106 82 (73, 89)
H3N2	n/N Percent 95% CI	0/106 0 (0, 3)	- - -	106/106 100 (97, 100)
B	n/N Percent 95% CI	8/106 8 (3, 14)	7/8 88 (47, 100)	106/106 100 (97, 100)

Definitions for seronegative ($HAI \leq 1:4$) and seroconversion (4-fold rise in seronegative subjects) used in Year 1 were the same for Year 2. For the placebo group, the percent seronegative, percent seroconversion, and cumulative seropositive rates for the 3 vaccine strains were as follows: H1N1 (A/Shenzen): 72%, 0%, 26%; H3N2: 37%, 5%, 65%; B: 52%, 0%, 46%.

After re-vaccination in Year 2 strain-specific HAI titers $\geq 1:32$ were observed in 57% (47, 66) to H1N1, 97% (92, 99) to H3N2, and 85% (77, 91) to type B of FluMist recipients.

AV006- Year 2. Four-Fold Rise in Strain Specific HAI Titers Regardless of Baseline Following Re-Vaccination in Year Two by Treatment Group

Strain		FluMist	Placebo
H1N1	n/N	43/106	0/54
	Percent	41	0
	95% CI	(31, 51)	(0, 5)
H3N2	n/N	5/106	1/54
	Percent	5	2
	95% CI	(2, 11)	(0,10)
B	n/N	32/106	0/54
	Percent	30	0
	95% CI	(22, 40)	(0, 5)

Table combines subjects who received one or two doses in Year 1.

The ELISA IgG index was considered negative if it was \leq the strain-specific cutoff values of: H1N1 = 0.04, H3N2 = 0.13, and B= 0.06. The proportion of seronegative subjects for IgG followed a similar pattern to HAI antibodies for all 3 strains. Seronegative subjects (FluMist, n=105 vs. placebo, n=54) respectively were: H1N1 (39% vs 61%), H3N2 (0% vs. 30%), and B (1% vs. 48%). Seroconversion (defined as a 2-fold rise in IgG index) occurred in 0 seropositive placebo subjects and in 1 of 33 to H1N1, 1 of 16 to H3N2, and 0 of 26 to B seronegative placebo recipients.

FluMist group – All FluMist recipients were seropositive to H3N2 in pre-titers for Year 2. For H1N1, 24 of 41 (59%) of seronegative FluMist seroconverted to IgG-HA. For B, the only seronegative FluMist subject seroconverted. After Year 2 re-vaccination, the cumulative immune responses of FluMist recipients were 84% seropositive to H1N1, 100% to H3N2 and B.

Nasal IgA anti-HA antibodies were measured in 13 subjects (FluMist = 8) and thus, no formal comparisons could be performed.

Cross-reactivity between H1N1 A/Texas/36/91 (Year 1 FluMist strain) and A/Shenzhen/227/95 (Year 2 FluMist strain) was assessed by comparing pre-vaccination titers to the two strains for 95 subjects in the immunogenicity subset. The sponsor noted that antibody titers pre-vaccination to the two strains were highly correlated (0.91, $p = 0.0001$) and concluded that cross-reactivity was suggested. However, the analysis appears to include both vaccine and placebo recipients and, therefore, if cross-reactivity is present, the current analysis does allow an assessment as to whether this might be due to an immune response to the FluMist vaccine.

STUDY – AV011

TITLE: A Phase 3, Randomized, Prospective, Open-label Challenge Study to Assess Viral Shedding of Influenza Virus Vaccine, Monovalent, Type A, Live, Cold-Adapted (CAIV-M) in Healthy Children.

INTRODUCTION/DESIGN

In Year One of study AV006 FluMist was shown to be efficacious in children ages 15-71 months against the circulating outbreak viral strains which included A/H3N2 and B strains. In Year Two, efficacy was again demonstrated against circulating outbreak strains even though the predominant strain was A/Sydney/H3N2, which was a variant strain from the A/Wuhan/359/95

vaccine strain. There were no circulating A/H1N1 strains during either influenza season and, therefore, field efficacy data against A/H1N1 strains was not obtained. Study AV011 was designed to “challenge” a subset of AV006 vaccine and placebo recipients with an intranasal dose of cold-adapted monovalent A/H1N1 vaccine strain (CAIV-M) and to compare viral shedding between prior vaccine recipients and prior placebo recipients. *The sponsor proposed that demonstrating decreased viral shedding of this H1N1 vaccine strain in previous FluMist recipients as compared to prior placebo recipients be viewed as a surrogate marker of vaccine efficacy for A/H1N1 strain.*

The study was an open-label study in which all subjects received a single challenge dose of CAIV-M A/H1N1 vaccine ~ 5 - 8 months after receipt of FluMist (CAIV-T) or placebo as part of the Year Two AV006 study. Children who had completed both years of AV006 (n=1343) were recruited for this study. AV011 was performed at the same 10 sites as AV006, and each site was to enroll ~ 20 subjects with priority for enrolling children who had been in the immunogenicity substudy to permit additional analysis of serologic data. Study sites submitted their list of proposed participants to the Statistical Center (SC) prior to enrollment and a list of enrolled subjects midway through enrollment. The SC suggested substitute participants as necessary to ensure a ratio of Flumist and placebo participants of 2:1 to 1:1. Serostatus and prior influenza infection were not considered in this selection.

STUDY PERIOD: 4/13/98 – 6/17/98
PIVOTAL: Yes
POPULATION: Children, who had completed Year 1 and 2 of AV006 (Efficacy Trial)
SAMPLE SIZE: Total - 222; FluMist – total, n=144 (Year 1 regimen: 1-dose, n = 16 and 2-dose, n= 128); Placebo – total, n=78 (Year 1 regimen: 1-dose, n = 7 and 2-dose, n = 71).

OBJECTIVES

Primary

To compare the frequency of viral shedding of CAIV, Monovalent (CAIV-M) vaccine strain A/(H1N1) in participants previously vaccinated with FluMist (1 or 2 dose regimen in Year 1, and 1 dose regimen in Year 2 in AV006) to the frequency of viral shedding in previously unvaccinated participants in AV006 (placebo recipients).

Secondary Immunogenicity and Efficacy Objectives

- To compare previously vaccinated children to children who previously received placebo for the following parameters:
 - Quantity of CAIV-M virus shed
 - Duration of CAIV-M virus shedding
- To compare the relationship between viral shedding and the following immunologic measures:
 - Quantity of pre-existing serum HAI titers and IgG titer to A/Shenzhen/H1N1
 - Quantity of pre-existing nasal IgA antibody to A/Shenzhen/H1N1

VACCINE

The challenge vaccine was CAIV-M, Type A/Shenzhen/227/95 (H1N1). Each 0.5 ml dose contained 10^7 TCID₅₀ of vaccine virus in normal allantoic fluid (NAF) containing sucrose-

phosphate-glutamate (SPG). The H1N1 lot number was CAE036, which was the same lot used for FluMist vaccine in 1997-98, though the challenge H1N1 was mixed with a different lot of NAF than used for FluMist. In the 1996-97 vaccine, the H1N1 strain was A/Texas/36/91.

PROCEDURES

Inclusion criteria

Healthy children who completed Year 1 and 2 of AV006 were eligible. The family had to have availability by telephone and be able to understand and comply with the protocol.

Exclusion criteria

Newly developed (since being in AV006) hypersensitivity to eggs or egg protein; newly diagnosed immunodeficiency or immunosuppressive treatment, or household member with newly diagnosed immunosuppression (since participation in AV006); acute febrile illness within 1 week; current URI (including common cold or nasal congestion) within 72 hours; administration of live virus vaccine within 1 month; administration of an inactivated vaccine within 2 weeks; receipt of inactivated influenza vaccine since 1996; receipt of a blood product within 3 mo prior or planned receipt within the study duration; expected administration of nasal medications during the 10 days before or after vaccination; any condition the investigator thought might interfere with vaccine evaluation. Pregnant household member or daycare provider was not an exclusion.

Vaccine Administration and Schedule

On Study Day 0, subjects had medical and vaccine histories obtained and a brief physical exam performed. Just prior to vaccination, serum samples were obtained from all subjects at all study sites and nasal wash samples were collected at 9 of 10 sites. At the Bardstown site, no nasal washes were obtained per protocol for feasibility reasons. At least 10 minutes after the nasal wash, each subject received 0.5 ml of vaccine (0.25 ml per nostril) while in the upright position. Subjects remained sitting for 30 seconds after vaccination. Only 2 sites (Saint Louis University, SLU and Vanderbilt) obtained 28-42 day post-vaccination serum samples.

Concomitant Vaccinations and Medications

No concomitant vaccinations were permitted. Use of aspirin or ASA-containing products was discouraged. All medications given in the 1st 10 days post-vaccination were recorded on CRF.

Monitoring

Immunogenicity

Serum samples were obtained prior to vaccination, and in a subset of subjects at 28-42 days post-vaccination. Sera were tested at Aviron for HAI and ELISA IgG index using HA derived from A/Shenzhen/227/95 (H1N1). Nasal wash samples (~10 ml) were obtained for testing total IgA and specific HA to A/Texas/36/91 (H1N1). HA from A/Shenzhen was not used because of technical difficulties with this antigen (details not provided). The sponsor notes that the HA from A/Texas and A/Shenzhen were cross reactive in serum HAI testing in AV006, with a high correlation, $r=0.91$, $p=0.0001$ for the 2 H1N1 antigens (See summary for AV006-Year 2).

Virology

Subjects had nasal and throat swabs obtained for culture on post-vaccination days 1, 2, 3 and 4 to assess shedding of CAIV-M vaccine strain. Both swabs were placed into the same tube of

transport medium and the inoculated media were divided into three aliquots. One aliquot of the sample was inoculated onto RMK cells, and 2 other aliquots were frozen. If the study site isolated influenza, one of the frozen aliquots was sent to SLU for quantitative assaying with results reported in plaque-forming units per milliliter (pfu/ml) of A/Shenzhen/227/95.

Statistical Analysis Plan

Efficacy Endpoints

Primary

The primary efficacy endpoint was the percent of subjects with shedding of CAIV-M on Days 1, 2, 3 or 4.

Secondary

- The number of days of viral shedding
- The 1st day of viral shedding
- Shedding by day (i.e., on which day viral shedding occurred).

Analysis of Viral Shedding

Viral shedding for the primary analysis was defined as at least 1 positive culture from Days 1,2,3, or 4 post-vaccination. Vaccine efficacy against viral shedding was calculated as: $[1 - (\text{event rate in prior vaccinees} / \text{event rate in prior placebo recipients})] \times 100\%$.

Efficacy p-values and confidence limits were computed using Koopman's method for the confidence intervals on the ratio of two binomial proportions. Number of days of shedding and distribution of plaque assay results of shedding were compared using the Kruskal-Wallis test.

Time to first day of shedding was compared using log rank test. Fisher's exact test was used to compare daily shedding results.

Analysis of Immunogenicity

Paired comparison of seronegative vs. seropositive children between HAI and nasal IgA was performed.

Determination of Sample Size

Sample size was anticipated to be ~ 200 (133 FluMist and 67 placebo recipients). The primary hypothesis was tested by means of a confidence interval (CI) for efficacy of FluMist against viral shedding. Power was calculated as the probability that the lower limit of the 2-sided 95% CI would exceed 0. Based upon HAI data from AV006, ~30% of placebo recipients were expected to have antibodies to A/H1N1, which might reduce shedding in this group.

RESULTS

Enrollment and Demographics

A total of 222 subjects (previous recipients of FluMist, n=144 and placebo, n=78) were enrolled into AV011. Of the 144 FluMist recipients, 128 had received the 2-dose regimen and 16 received the 1-dose regimen in Year 1 of study AV006. Of the 78 prior placebo recipients, 71 had received the 2-dose regimen and 7 the 1-dose regimen in Year 1. The mean interval since dosing in Year 2 was not provided, but ranged from 5-8 months.

The demographic profiles of AV011 participants were similar to AV011 non-participants as noted by comparison of the characteristics at the time of enrollment into AV006. There were no

statistically significant differences for demographics between the prior FluMist and prior placebo recipients. The ethnicity of subjects was 81 % Caucasian, 13% Black, 3% Hispanic, and 3% other. The mean age (\pm SD) of enrollees was 59.8 (\pm 17.4) month for prior FluMist and 63.1 (\pm 16.8) for prior placebo recipients. Both groups had slightly more females (FluMist – 53% and placebo – 56%). About ½ of participants had a primary caretaker who worked outside of the home. More previous placebo recipients (87%) than FluMist recipients (74.3%) spent some time (\geq 1 day) in daycare. No statistical comparisons were provided.

Protocol Compliance

All 222 subjects completed the trial. There were no withdrawals.

Protocol Deviations

There were a total of 64 protocol deviations, none of which merited ending study participation. The protocol deviations were presented as line listings. The most common deviations included, vaccine was administered too soon after nasal wash (n=24), refusal of post-vaccination blood draws (n=7), missing or delayed diary card data (n=9). For 6 subjects viral and Group A strep cultures were not obtained for illness events in the first 10 days post-vaccination as specified in the protocol. Five subjects missed one or both (nasal/throat) post-vaccination cultures on one of the Days 1-4 post-vaccination.

Specimen Acquisition

Nasal and throat swabs were obtained from 97-100% of each group of subjects on Days 1,2,3 and 4 post-vaccination. All swabs collected were analyzed. Pre-vaccination nasal wash specimens for antibodies were obtained from 199 subjects (131 FluMist and 68 placebo,) which is 100% from the 9 sites that collected nasal washes. All subjects had pre-vaccination serum samples obtained. Post-vaccination serum samples were obtained from 26/31 (84%) prior FluMist recipients and 15/17 (88%) prior placebo recipients who were expected to have samples obtained (Vanderbilt and SLU). Five serum samples (3 FluMist and 2 placebo) from the pre- or post-vaccination samples were not analyzed because of sample loss or inadequate identification.

AV011 – Efficacy as Determined by H1N1 Shedding After Challenge with CAIV-M (A/H1N1) by Treatment Group

	Prior FluMist (N= 144)	Prior Placebo (N=78)
Shedding on Any Day, N (%)	6 (4)	19 (25)
Efficacy (95% CI)	82.9% (60.2, 92.7)	
Shedding on Day, N (%)		
1	2 (1)	2 (3)
2	5 (3)	10 (13)*
3	1 (0.7)	9 (12)*
4	0 (0)	6 (8)*

* Statistically significant (p<0.05)

Table combines Year 1 one-dose and two-dose recipients.

On Day 4, 6 placebo recipients had viral shedding, but no cultures were taken after Day 4, so total duration of shedding cannot be determined. However with this in mind, the mean duration of shedding in the first 4 days post-vaccination was significantly reduced in the prior FluMist group compared to the prior placebo group (respectively 0.06 ± 0.3 vs. 0.3 ± 0.7 , p=0.0001).

Of note, shedding rates in prior placebo recipients differed between sites with a range of 0% to 62%. However, samples sizes in these groups were small, with a range of 2-13 subjects. No shedding was observed in the 16 prior vaccine recipients and 7 prior placebo recipients of the AV006 Year One “one-dose group”. The overall efficacy against shedding in the subset of AV006 Year One “two-dose group” was nearly identical to the overall efficacy for all participants listed in the table above.

Viral Shedding and Immunologic Measures

HAI titers to A/H1N1 could have resulted from FluMist vaccination or natural exposure to wild type influenza (though little A/H1N1 was circulating during the preceding 2 influenza seasons). The percent of prior FluMist recipients with HAI titers $\geq 1:8$ (considered to be seropositive) prior to vaccination with the monovalent CAIV-M vaccine was 68% (96/141) compared to 33% (25/75) of prior placebo recipients, $p < 0.0001$.

Seropositive subjects were less likely to shed CAIV-M than seronegative subjects, though the correlation was not perfect (see table below). Of the 6 prior FluMist recipients who shed CAIV-M, two were seropositive, one with a titer of 1:8 and one with a titer of 1:32 and each shed virus for 1 day. All prior placebo recipients who shed virus were seronegative. Seronegative subjects who shed virus (4 prior vaccinees and 19 prior placebo recipients) all shed virus for at least 2 days and six subjects shed virus on day 4.

Nasal IgA data are expressed as the HA-specific IgA antibody normalized to the total IgA in secretions, and a value of > 0.1 was considered to be positive. Of the 199 subjects, 88/129 (68%) of prior FluMist and 23/68 (34%) of prior placebo recipients were positive for nasal IgA antibodies pre-challenge with CAIV-M. Of the IgA-positive prior FluMist recipients, 1% shed virus vs. 12% who were nasal IgA-negative ($p=0.01$). For prior placebo recipients 13% of IgA-positive compared to 36% of IgA-negative placebo recipients shed CAIV-M ($p=0.05$). Overall, 43% nasal IgA-positive vs. 25% ($p < 0.0001$) shed CAIV-M, and the duration was statistically significantly shorter (mean number of days 0.04 vs. 0.4 days, $p=0.0001$) in nasal IgA-positive subjects vs. nasal IgA negative subjects.

The relationship of serum HAI and nasal IgA anti-HA was evaluated only in subjects who had both measurements performed and $\sim 1/3$ of subjects had discordant IgA and HAI values.

AV011. H1N1 Shedding by Serum HAI and Nasal IgA Antibody Status

Assay Results Pre CAIV-M Vaccination		Shedding on ³ 1 Days Post-Vaccination		
HAI	IgA	Yes	No	Total
Negative	Negative	20	30	50
Negative	Positive	3	27	30
Positive	Negative	1	32	33
Positive	Positive	1	79	80
	Total	25	168	193

Quantity of CAIV-M Shedding

Quantification of the amount of virus shed by plaque assay revealed that the amount of virus shed was low, and the peak viral titer (log 10 pfu/ml) shed by Flumist recipients (2.5) was similar to placebo recipients (4.2).

Durability of Antibodies

No comparisons of serum HAI antibodies pre-challenge with post-dose Year 2 in AV006 was performed.

STUDY – AV009

TITLE: A Prospective, Randomized, Double-blind, Placebo-controlled, Trial to Assess the Safety, Tolerability, and Effectiveness of Influenza Virus Vaccine, Trivalent, Types A and B, Live, Cold-Adapted (CAIV-T) in Healthy working Adults to Reduce Influenza-Like Illness, Absenteeism from Work and Health Care Costs During Influenza Outbreaks.

INTRODUCTION/DESIGN

Healthy working adults, without risk factors for influenza complications, are not targeted for annual influenza immunizations. However, if infected with influenza, healthy adults are at risk for acute illness and absenteeism from work. Immunization of working adults against influenza has been shown to reduce the cost of absenteeism, associated medical costs and employer expenses. This trial was performed to assess the safety, tolerability, and effectiveness of FluMist to support an indication for use of the vaccine in healthy working adults.

This was a prospective, randomized, double-blind, placebo-controlled, multi-center trial conducted at 13 study sites. Subjects were randomized 2:1 to receive FluMist vs. placebo.

LOCATION: United States, multi-center – 13 sites; CRADA with NIAID
STUDY PERIOD: Sept. 18, 1997 – Mar. 31, 1998
PIVOTAL TRIAL: Yes, Phase 3 Trial
POPULATION: Healthy, working adults ages 18-64 years (not had 65th birthday).
SAMPLE SIZE: Original n=3600, increased to 4,200.
Final enrollment, n=4561 (FluMist n= 3041 and placebo n=1520).

OBJECTIVES

Primary

- To show similar safety and tolerability profiles of FluMist compared to placebo, and
- To show a smaller proportion of the FluMist participants has any febrile illness (AFI) during influenza outbreaks than the placebo recipients. *In the original protocol this objective was stated as, “During influenza outbreaks, a smaller proportion of working adults randomized to CAIV-T than randomized to placebo will have an episode of influenza-like illness.” The data analysis plan (DAP) included AFI as the primary endpoint (see below).*

Secondary

- In healthy working adults, to show that during the entire influenza season (11/1/97-3/31/98) an immunization program using FluMist is cost-effective using a model of direct and indirect costs.
- During influenza outbreaks, healthy working adult CAIV-T recipients compared to placebo recipients had lower average:
 - a) number of days if illness-associated absenteeism from work,
 - b) number of days of illness-associated medically attended illnesses,
 - c) number of illness episodes, and

- d) number of days of illness episodes.
- To estimate the effect of FluMist compared to placebo for the proportion of subjects who have ≥ 1 days of illness-associated:
 - a) absenteeism from work,
 - b) medically-attended illness (MAI),
 - c) health care provider (HCP) visits,
 - d) prescription antibiotic use,
 - e) OTC med use,
 - f) Hospitalization,
 - g) Medical tests, and
 - h) Absenteeism from work or reduced work effectiveness.
- To estimate the effect of FluMist compared to placebo on the average number of days of:
 - a) Absenteeism from work or reduced work effectiveness,
 - b) OTC med use,
 - c) HCP visits,
 - d) Medical tests
 - e) Prescription antibiotic use,
 - f) Prescription med use, and
 - g) Hospitalization.

All of the illness-associated objectives were applied to the entire study period, and all of these objectives were applied to febrile URIs, severe febrile illness (SFI) and all URIs during outbreaks, as well as during the entire study period.

VACCINES

1. FluMist – Each 0.5 ml dose contained 10^7 TCID₅₀ of each strain of virus (2 type A and 1 type B) in normal allantoic fluid (NAF) containing sucrose-phosphate-glutamate (SPG). The 1997-98 strains were A/Shenzhen/227/95 (H1N1), A/Wuhan/359/95 (H3N2) and B/Harbin/7/94-like, filled trivalent lots #CAF023 (same lot as AV006 – Year 2 vaccine), CAF 024 and CAF 026 were used.
2. Placebo – 1 lot (CAF022) of NAF was used.

At the 1st visit (Study Day 0), the subjects received one dose of vaccine or placebo while in the sitting position. Vaccines were administered by intranasal spray (0.25 ml per nostril) using Becton-Dickson Accuspray™ device, and they could be self-administered or given by study personnel.

Procedures

Schedules and Vaccine Administration

Inclusion criteria – Healthy working adults ages 18-64 years were recruited. They had to work outside of the home ≥ 30 hours/week for a single employer, have health insurance and availability by telephone.

Exclusion criteria:

Significant chronic illnesses for which inactivated influenza vaccine is recommended or commonly used; receipt of TIV for the 1997-98 season; acute URI symptoms or febrile illnesses within 72 hours; allergy to eggs or egg proteins; immune deficiency or immunosuppressive therapy; participation in other investigational trials until after 3/31/98; self-reported pregnancy or risk for pregnancy within 3 months of the trial; concerns for non-compliance with the protocol.

Of note, a history of wheezing or bronchodilator use within 3 months of vaccination, concomitant immunizations, and nasal medications were not listed as exclusion criteria.

Concomitant Medications

Patients were discouraged from taking medications, except for oral contraceptive and medications for pre-existing conditions, from Day 0-7 post-vaccination. Chronic use of aspirin (ASA) or ASA-containing medications was permitted. Use of other medications was to be recorded on diary cards. Use of concomitant vaccines is not described.

Monitoring

Effectiveness (Clinical Efficacy)

Subjects were provided with monthly effectiveness diary cards (months November – March) to record illness information. Study personnel were to conduct automated telephone calls approximately every 2 weeks to remind subjects to complete and return these cards. If a diary card was misplaced, the information was to be collected over the phone and mailed to the subject for review and signature. Replacement cards for future months were mailed if needed.

The monthly diary card had 3 sections to capture effectiveness measures:

Sections 1A - queried for fever, runny nose, sore throat, cough, h/a, muscle aches, chills, and tired/weak.

Section 1B - queried for healthcare utilization or missed work because of symptoms in Part 1A as follows: “I took an OTC medication,” “I went to a healthcare provider,” “I had a medical test done,” “I took a prescription antibiotic,” “I took a non-antibiotic prescription medication,” “I was hospitalized,” “I was less effective at my job,” and “I missed work.”

Section 2 - queried for healthcare utilization for illness other than those in Part 1A, as follows: “I went to a healthcare provider (HCP),” “I had a medical test done,” “I took a prescription antibiotic,” “I took a non-antibiotic prescription,” “I was hospitalized,” and “I missed work.”

Definition of outbreak

Outbreak periods for each site were determined by the AV009 Effectiveness Committee using an pre-defined algorithm, generally by 1) select the peak week and 2) check that it contain 80% of the Cxs, if not add one adjacent week (whichever has the most positive cx's) to reach 80%. If the peak week is not unique, then the influenza surveillance data from the CDC corresponding to the local community will be used. If there are too few cases (<10) to identify an outbreak, then an independent expert (blinded to the trial) was to evaluate surveillance data (local, nearby sites, CDC or WHO) to determine the outbreak period.

Statistical Methods

Subjects who returned at least one monthly diary card during the site-specific outbreak, 14-week outbreak period, and entire 5 month period were evaluable for the effectiveness endpoints.

Effectiveness Endpoints (according to the DAP)

The definitions of illness provided in the study report are said to be interpretations of endpoint descriptions provided in the protocol. The definitions for acute febrile illness (AFI) and upper respiratory illness (URI) were modified, prior to unblinding of the study, after discussion with the protocol Effectiveness Committee to require symptoms for 2 days, rather than 1 day. AFI was the illness definition used for most analyses of efficacy. Other illness definitions applied to the data are listed following the listing of primary and secondary endpoints.

Primary effectiveness endpoint

Occurrence of any AFI. Participants were counted as reaching this endpoint if:

- They had at ≥ 2 consecutive days of symptoms in Part 1A of diary
- 1 of the symptoms on at least 1 of the days was fever, and
- There must have been at least 2 symptoms present on 1 day.

Secondary effectiveness endpoints

The secondary effectiveness objectives were to assess the effect of FluMist on complications associated with AFIs as measured by absenteeism from work and medically attended illness. The start of an AFI was the 1st day of symptoms preceded by at least 2 symptom-free days. The end of the episode was the last symptom day followed by 2 consecutive symptom-free days. The days with symptoms had to meet definition for AFI.

- Number of days of AFI-assoc. absenteeism. The number of missed work days checked in Part 1B of the diary that overlapped with AFI episodes.
- Number of days of AFI-associated medically attended illness (MAI). The number of days for which at least 1 MAI (went to a health-care provider, had a medical test done, took a prescription medication or antibiotic, or was hospitalized) occurred during an AFI was calculated.
- Number of AFI episodes. The number of AFI episodes that occurred during an outbreak for each participant.
- Days of AFI episodes. For each participant, the total number of days was counted by summing the duration of each episode, and duration was calculated as follows: start date - stop date +1.

Other Endpoints

- Occurrence of AFI-assoc. absenteeism from work. Participants who missed work (from part in part 1B of the diary) counted once if absence occurred during an AFI episode.
- Occurrence of AFI-assoc. MAI. Participants who had a MAI (from part 1B of the diary), were counted once if the MAI was associated with an AFI.
- AFI-assoc. health care provided (HCP) visit. Participants, who checked the box, "I went to a HCP" in 1B of the diary and it overlapped with an AFI episode were counted as meeting this endpoint. Also, the total number of days of HCP visits were calculated for each participant during AFIs by summing the number of boxes checked for this endpoint.
- AFI-assoc. prescription antibiotic use. Participants were counted if they checked at least 1 box "I took a prescription antibiotic", during the time of an AFI episode (section 1B of the diary). Also, the total number of days of prescription Abx use was calculated by summing the total number of boxes checked for this endpoint.

- AFI-assoc. prescription medication use: as above if “I took a non-antibiotic prescription medication” or “I took a prescription antibiotic medication” box was checked.
- AFI-associated over-the-counter medication use: as above for OTC medication use.
- AFI-assoc. hospitalization. As above, participants were counted “was hospitalized” during AFIs box was checked. Also, the total number of days was calculated for each participant during AFIs by summing the total number of boxes checked.
- AFI-assoc. medical test. Participants were counted as meeting this endpoint if at least 1 box in Section 1B was checked for “I had a medical test done” during AFIs. Total number of days was also calculated similar to above.
- AFI-assoc. absenteeism from work or reduced work effectiveness. Participants were counted as meeting this endpoint if at least 1 box in Section 1B was checked indicating “I missed work” or “I was less effective at my job” during AFIs.

Other Illness Definitions :

Upper Respiratory Infection (URI) : participant must have had two consecutive days of URI symptoms in part 1A of effectiveness diary card.

Febrile URI: as above and in addition, fever must have been present on at least one day.

Severe Febrile Illness: If there were at least 3 days of symptoms for part 1A of effectiveness diary card, fever on at least one day, and at least 2 symptoms on all three days.

In addition to these prespecified definitions, three other definitions were applied to data in post-hoc analyses:

CDC influenza-like illness (ILI) – fever plus either cough or sore throat, must be present on the same day or on consecutive days.

DoD ILI – cough plus either fever or chills, also must be present on the same day or consecutive days.

AV ILI – 1) 2 consecutive days of symptoms, 2) at least 1 day of fever, 3) ≥ 1 URI symptom, 4) at least 1 non-febrile systemic symptoms and 5) ≥ 2 symptoms on at least 1 days.

The planned analyses of the effectiveness endpoints were applied during influenza outbreaks, during the entire study period (11/1/97 – 3/31/98), and in post-hoc analyses to site-specific influenza outbreak periods, and to a pooled, 14-week outbreak period 12/14/97-3/21/98. Key effectiveness parameters were to be evaluated across demographic subgroups – 1) men and women, 2) Caucasian and non-Caucasian, and 3) age < 40 yrs and ≥ 40 years. A cost effectiveness analysis was performed but the study report contains results of individual components of health care-utilization measures without costs applied.

Sample Size Estimates

Vaccine effectiveness was assumed to be 60% and it was assumed that 4-6% of placebo recipients would have an AFI secondary to influenza and that 70% of absenteeism would be due to influenza. (The protocol did not say how these assumptions would be verified, and cultures were not obtained). With 4200 study participants and assuming 80% of subjects were evaluable, the study would have 90% power to demonstrate 60% VE.

AV009 - RESULTS

Enrollment and Demographics

	FluMist	Placebo
Randomized, N	3041	1520
Age		
Mean	38	38
Range	18-66	18-65
Race/Ethnicity %		
White	84.7	83.5
Black	9.6	10.9
Asian	2.3	2.5
Hispanic	2.24	2.11
Native American	0.33	0.20
Other	0.85	0.79
Gender %		
Male	45.3	45.7
Female	54.7	54.3
Bachelor's degree or higher %	48	46

Protocol Compliance

Of 4561 subjects, 98% of each treatment group returned the 7 day diary card, and 97.5% provided safety information for the 1st 28 day monitoring period. There were 116 subjects who did not complete the Day 0-28 safety diary (FluMist 78, Placebo 38). The most common reason for not completing the 28 day period follow-up was lost-to-follow-up [FluMist n=65 placebo n=31]. Two subjects in the FluMist group and 1 in the placebo group withdrew due to an AE. One of the FluMist subjects withdrew due to abdominal pain and vomiting with onset 2 days post-vaccination and then diarrhea 7 days post-vaccination, which was recorded Crohns' Disease and coded as "probably not" vaccine-related. The other FluMist subject was withdrawn due to accidental death 16 days post-vaccination. The placebo subject withdrew due to a diagnosis of personality disorder, noted 17 days post-vaccination.

Approximately 95.5% of subjects (FluMist n=2902/3041 and placebo n=1453/1520) returned at least 1 monthly diary card and 88% returned 4 or more monthly diaries. For each month of the study, ~90% in each treatment group returned the effectiveness diary cards each month.

There were ~369 (8.1%) of 4561 participants with protocol deviations. The most common deviations (n=171, 3.7%) were subjects who provided the Day 28 diary data prior to Day 28 (the mean for day of return is not stated) and 86 subjects (1.9%) did not provide the diary card. Seventy-nine participants were in violation of the eligibility criteria at enrollment, most commonly because subjects had an occupation or condition for which influenza vaccine is recommended. Seventy-one percent of the vaccines were self-administered with 3.8% of subjects having some problem (no details provided). Of the 29% administered by study personnel, 2.5% reported some problem (1.84% for FluMist and 3.85% for placebo).

Effectiveness

There was no significant difference in effectiveness between the FluMist and Placebo groups in the primary analysis of effectiveness (i.e., occurrence of any febrile illness [AFI]) during Site-Specific Outbreak Periods.

AV009 - Percentage of Participants Having One or More Illness during the Site-Specific Outbreak Periods

Endpoint	FluMist	Placebo	% Reduction	p-value*
	N = 2,883	N = 1,420		
	% with	% with		
Any Febrile Illness**	13.2	14.6	9.7	0.19
Severe Febrile Illness	10.1	12.2	17.4	0.031
Febrile URI	8.5	10.8	21.9	0.011
CDC-ILI ⁺	10.7	13.9	23.2	0.0018
DoD-ILI ⁺	10.4	13.7	23.5	0.0017

* unadjusted for multiple comparisons

** primary endpoint

⁺ post-hoc analyses

Severe Febrile Illness (SFI) and febrile URIs (FURI) were less common in the FluMist than placebo group. Post-hoc analyses applied to the data revealed significantly fewer CDC-ILI and DoD-ILI in the FluMist recipients.

Similarly, the rate of AFI episodes (number of AFI episodes/1000 subject per 7-week outbreak period) was not significantly different between the FluMist and placebo groups: 151.3 FluMist vs. 168.1 placebo. However, the rates of SFI (111.0 FluMist vs. 136.7 placebo; p 0.0019) and FURI (92.4 vs. 121.0; p<0.0001) were significantly different.

The rate of AFI days during outbreaks (# of days of AFI/1,000 subjects per 7-week outbreak period) was significantly lower in FluMist group vs. placebo (1,888 vs. 1,541.2; p= 0.0001) as was the rate of SFI (1,021 vs. 1404.5 p= <0.0001) and FURI (875.7 vs. 1,164.7 p<0.0001). The rate of CDC-ILI days and DoD ILI days were significantly fewer in the FluMist group.

The rates of missed work days during outbreak periods (# of days missed/1,000 subjects) for AFI was not significantly different in the FluMist vs. placebo group (173.3 vs. 199.5; p= 0.065). There was a significant lower rate of days missed for SFI (154.7 vs. 188.3; p =0.12) and for FURI (107.0 vs 149.4; p<0.0001).

Health Resource Utilization was assessed by calculating for AFI, FURI, SFI-associated events. There was significantly less health resource utilization in the FluMist group compared to the placebo group (see table below for AFI-associated events). Similar results were seen in post-hoc analyses for CDC and DoD-ILI illness.

AV009 - Rate of AFI-associated Events during the Site-Specific Outbreak Periods

Endpoint	FluMist	Placebo	% Reduction	p-value
	N = 2,883	N = 1,420		
	(# of days or events/1000 subjects per 7-wk outbreak period)			
Days of OTC Medication use	576.9	752.3	23.3	0.0002
Days with at least 1 Health Care Provider Visit	44.0	51.5	14.7	0.055
Days taking At least One Prescription Antibiotic	195.6	342.9	42.9	<0.0001
Days taking Any Prescription Antibiotic	250.0	413.9	39.6	<0.0001

During site-specific outbreaks, <1% of subjects reported medical tests and only 4 subjects (2 FluMist and 2 placebo) had hospitalizations for AFI. Similarly, there were few of these events associated with other illnesses (SFI, FURI) and thus no analyses could be performed. Significant reductions were noted for SFI-associated events, FURI, CDC ILI and DoD ILI for days of OTC meds, days taking at least 1 prescription Abx and days taking any prescription med for the FluMist group, $p \leq 0.0002$.

The prior analyses were also performed for 14-week period, pooled outbreak period as well as the entire 5-month study period, and results were generally consistent with those presented for site-specific period. The effectiveness of FluMist was noted though the circulating strain in 1997-98 was a variant not contained in the vaccine.

Subgroups analyses

Gender - Overall, the effectiveness of FluMist was similar for men and women, though there were some differences in the rates of events. Women were slightly more likely than men to have 1 or more illness (illnesses in placebo groups: women = 15.2% and in men=13.9%). The reduction in days of illness in FluMist recipients was comparable between men and women.

Ethnicity – Of note, only 16% of enrollees were non-Caucasian. Generally, the number of and days of illness events were similar between Caucasians and non-Caucasians and the effectiveness of FluMist was similar across ethnicity and illness definitions. There was a difference in the reduction of episodes of AFI (decrease of 7.1% for Caucasians and 25.6% for non-Caucasians). A rate of missed work days was much higher for non-Caucasians in both the FluMist and placebo groups for all illness definitions. FluMist was more effective in reducing missed work days during AFIs for non-Caucasians (26.5%) than Caucasians (8% reduction). Health resource utilization was different in Caucasian compared to non-Caucasian placebo recipients. OTC medication use was higher in Caucasians, but HCP visits and prescription medication use was higher in non-Caucasians. Prescription antibiotic use was similar between the groups.

Age - Differences of illness events were noted in placebo recipients < 40 and those ≥ 40 years of age, generally rates of events were higher in the <40-year-olds. However, the effectiveness of FluMist on reducing the outcomes was generally higher in those ≥ 40 years.

Health Economic Results – a summary of economic analysis from this study is provided. For this analysis, no resource costs or hourly wage data were collected and all of the costing information came from secondary sources, primarily from Medtap (Bethesda, MD), a contract health-economic research organization. Placebo recipients were assumed to be representative of unvaccinated subjects, and event rates in the placebo recipients assumed to provide estimated of event rates in unvaccinated subjects. Thus, event rate (placebo) and relative event rates (FluMist vs. placebo) were the only economic model inputs derived from the clinical data.

The overall break even value of FluMist and administration, during an influenza season in which the predominant circulating influenza virus strain was a variant (definition for variant not provided) from the vaccine strains was found to be \$31 during AFI's and URI's. This was primarily due to the effectiveness of FluMist in reducing work and health-care provider visits during AFI and URI. The break-even cost increases to \$50 when evaluating the reduction in missed work days and health-care provider visits during any illness. Sensitivity analyses indicate that the main cost drivers were: hourly wage, the effectiveness of FluMist for reducing missed work days, and the rate of missed work days in the “unvaccinated” groups. The break-even cost of FluMist and administration was insensitive to the cost of treating the illnesses.

The validity of the assumptions and model have not been reviewed. For licensure FDA considers risk benefit analyses of vaccines and biologic products but not cost benefit analyses.

STUDY AV003

TITLE: A Phase 3 Double-blind, Placebo-Controlled Challenge Study to Assess the Efficacy of Cold-Adapted Influenza Virus Vaccine, Live Trivalent (CAIV-T, FluMist) in Healthy Adults.

LOCATION: United States, two centers; CRADA with NIAID
STUDY PERIOD: 12/11/95 – 2/15/96
PIVOTAL TRIAL: Yes, Phase 3
POPULATION: Healthy Adults
SAMPLE SIZE: Total for challenge, n= 92; Flumist, n=29; Placebo, n=31;
Inactivated Influenza, n=32

INTRODUCTION

AV003 was performed to assess the efficacy of FluMist and of the trivalent inactivated influenza vaccine (TIV) in protecting subjects from laboratory diagnosed influenza disease following a challenge with a wild-type influenza virus. This study was performed early in Aviron's clinical development of FluMist. Originally the study was performed to establish that FluMist was protective in seronegative adults and thus, merited evaluation in field trials in the pediatric population.

OBJECTIVES

1. Co-primary

- To assess the efficacy of FluMist compared to placebo post-challenge with wild type influenza against laboratory-documented influenza illness.

- To assess the efficacy of FluMist compared to trivalent inactivated influenza (TIV - Fluvirin™ manufactured by Evans Medeva) post-challenge with wild type influenza against laboratory-documented influenza illness.
- To assess the safety and immunogenicity of FluMist in adults serosusceptible to at least one of the virus strains (H1N1, H3N2, and B) contained in the influenza vaccines (FluMist and TIV).
- *Post-hoc*: To assess the reduction of viral shedding post-challenge with wild type influenza.

Laboratory-documented illness was defined: symptoms of influenza accompanied by wild-type viral shedding on one or more days and/or \geq 4-fold rise in HAI antibody to the challenge virus from Day 28 to Day 56.

Illness was defined: \geq 1 respiratory symptom of moderate or greater severity OR \geq 2 respiratory symptoms of any severity on 2 consecutive days. Respiratory symptoms include nasal stuffiness, runny nose, ear ache, sore throat, hoarseness, or difficulty breathing (not due to stuffiness). Subjects with myalgias or fever were defined as having “influenza-like illness.”

DESIGN

AV003 was a randomized, double-blind, placebo-controlled challenge study performed in healthy adults, 18-41 years old. Subjects were immunized with FluMist, TIV or placebo and then 28 days later received intranasal challenge with wild-type influenza (the strain to which the subject was serosusceptible) to assess protection.

VACCINES AND CHALLENGE STRAINS

1. Flumist – Each 0.5 ml contained 10^7 TCID₅₀ of each strain of virus (2 type A and 1 type B) in normal allantoic fluid (NAF) containing sucrose-phosphate-glutamate (SPG). The 1994-95 strains were A/Texas/36/91-like (H1N1, lot CAE002), A/Shandong/9/93 (H3N2, lot CAE006) and B/Panama/45/90 (lot CAE003). Filled trivalent lot CAF003 with NAF lot CAE008 was used.
2. Inactivated influenza vaccine (TIV) – The licensed vaccine (0.5ml dose) from Evans Medeva also contained the recommended 1994-95 strains listed under FluMist.
3. Challenge viral strains – Each 0.5 ml (0.25 ml per nostril) challenge dose contained 10^7 TCID₅₀ of one of the following strains: H1N1 A/Texas/36/91 – WT (lot E-349); H3N2 A/Shandong/9/93-WT (E-337) or B/Panama/45/90-WT. The challenge strains were delivered by nasal drops. The wild type strains were prepared by Dr. Lou Potash, under contract with NIAID.
4. Placebos
 - a. Intranasal - NAF in SPG, lot CAE008 was used.
 - b. Injectable – saline (PBS with 0.1% thimerosal)

PROCEDURES

Schedule and Vaccine Administration

There were three phases to the study, 1) screening, 2) vaccination with post-vaccination safety monitoring and 3) challenge. Screening was performed within one month of vaccination to identify subjects who were serosusceptible (defined as HAI titers \leq 1:8) to at least 1 of the vaccine strains. Upon meeting eligibility criteria (Inc/Exc criteria, signed CF and brief PE)

subjects were randomized to study group, in 1:1:1 ratio, FluMist:TIV:placebo. The study was designed to have three mutually exclusive groups, one each for those serosusceptible to H1N1, H3N2, and for type B. Some subjects in each group may have also been serosusceptible to one or both of the other strains. Subjects within each serosusceptibility group were randomly assigned to one of the three study groups. To maintain blinding, each subject received one IM injection and one intranasal immunization, as shown on Table 1.

AV003 - Vaccine Study Groups

Treatment Group	Study Vaccines	
	Intranasal Spray	IM Injection
FluMist	FluMist	Saline
TIV	NAF	TIV
Placebo	NAF	Saline

On Day 0, study vaccines were administered with the subject in the seated position. The intranasal vaccinations were delivered by spray device. Subjects remained seated for 30 seconds and stayed at the site for 15 minutes post-vaccination to observe for immediate reactions.

Participants were given vaccine diary cards to record their temperature and reactogenicity events (RE's) for seven days post-vaccination. RE's included temperature, chills, cough, sore throat, muscle aches, and runny nose. Subjects returned at 1 week to report diary card entries, which study personnel entered into the CRF. Personnel also entered any information reported by the subject during the visit. On Day 15, study personnel contacted subjects to inquire about occurrence of any adverse events (AE's) since Day 7.

Subjects were instructed to contact the study site if they experienced an AE, fever $>100^{\circ}\text{F}$, or ≥ 2 RE's listed on the diary card. A subject with an upper respiratory infection (URI) during the study, if the investigator deemed the event as clinically relevant, was to have a physical assessment and a nasopharyngeal (NP) specimen obtained for viral and bacterial cultures. If results of the viral culture were needed for medical care, they were to be revealed only to the subject's doctor (not the investigator or Aviron, until the study blind was broken).

At the end of Week 4, subjects had nasal wash samples and serum samples obtained for antibodies to influenza. Then, the subjects, who participated in the challenge phase, had a brief PE and remained at the site for 24 hour observation. These subjects received intranasal challenge with the homologous strain to which they had been serosusceptible at screening (challenge strains listed above). The participants were then sequestered for seven days. During sequestration, subjects received limited PE's, had their temperatures measured twice a day, and provided daily nasal wash samples for viral isolation.

About Day 45 (2 weeks post-challenge) study personnel called participants to check for the occurrence of any AE's. At the end of Week 8 (4 weeks post-challenge), subjects returned to the study site for final collection of blood and nasal wash samples. Brief PE and exit interviews were also performed. The total study duration was 56 days.

Inclusion/Exclusion Criteria

Inclusion criteria – Healthy adults, between the ages of 18-40 years, serosusceptible to at least 1 of the influenza strains (H1N1, H3N3, or B) and signed informed consent. Women capable of pregnancy had to practice a reliable means of birth control during the study.

Exclusion criteria – These criteria were similar to AV009.

Concomitant Vaccinations and Medications

No concomitant immunizations, aspirin-containing meds or nasal medications (for 7 days post-vaccination and post-challenge) were permitted. Use of other medications, except birth control pills, was discouraged during the study period.

Immunogenicity

Serum samples were collected at 4 time points: screening (within 1 mo of vaccination), Day 0 (vaccination day), Day 28 (challenge day), and Day 56 (4 weeks post-challenge). Serum was tested for HAI strain-specific antibodies and IgG ELISA. Nasal wash samples were obtained at the same time points for measuring IgA ELISA antibodies. Day 0 titers (not screening titers) were used for baseline data for calculating 4-fold rises and GMT ratios. Some subjects who were serosusceptible ($\leq 1:8$) at screening had titers $\geq 1:16$ at Day 0, details in Results section.

Primary Efficacy Endpoint

The primary measure of protective efficacy of FluMist was the difference between the rates of laboratory-documented influenza illness in the FluMist and placebo groups. FluMist would be considered protective if, after wild-type challenge, the rate of laboratory-documented illness in the FluMist group was significantly less than the rate in the placebo group (2 sided p-value ≤ 0.05). With the enrolled sample size (~ 30 per group), the study had about 90% power to detect a difference in rates of laboratory-documented influenza if the true rate for the placebo group and FluMist group were 50% and 10% respectively.

RESULTS

Enrollment

A total of 383 subjects were screened for study participation. 135 subjects satisfied eligibility criteria, and 103 subjects (18-41 years of age) were randomized for vaccination to assure that ~90 subjects were eligible for challenge (allowing ~10% drop-out). The dispositions of the 32 subjects not randomized are not described. Randomization by group yielded: FluMist - n= 36, TIV – n= 33, and placebo – n= 34 subjects.

Demographics

The mean age in each of the study groups was ~ 25 years and 74% of subjects were male. The ethnicity was comparable between the groups, about 52% of subjects were Caucasian, 40% Black, 4% Hispanic and 4% Asian.

The serosusceptibility of the subjects is shown in the table below. The study was designed so the challenge would include three mutually exclusive groups of ~30 subjects each, i.e., all subjects in one group would be serosusceptible to H1N1, the 2nd group to H3N2, and the 3rd group to type B. Some subjects in a group were serosusceptible to one or both of the other strains. Of note, though all participants enrolled in the vaccine phase of the protocol were serosusceptible (HAI \leq

1:8) to at least one strain at screening, seven subjects challenged with H3N2 and nine subjects challenged with type B had HAI titers \geq 1:16 on Day 0 (vaccination day). The sponsor attributed this, in part, to assay variability.

AV003 – Serosusceptibility of Study Subjects.

Time point	Study Group	Serosusceptible Subjects			
		N	H1N1	H3N2	B
Baseline	FluMist	36	24	23	10
	TIV	33	23	17	12
	Placebo	34	24	18	11
Day 28 (Prior to challenge)	FluMist	29	23	21	10
	TIV	32	23	16	11
	Placebo	31	24	17	11
Subjects challenged with the homologous strain	FluMist	29	10	9	10
	TIV	32	10	12	11
	Placebo	31	12	8	11

Protocol Compliance

Of the 103 subjects randomized, 98 completed the vaccine phase of the study. Five subjects (FluMist n=3 and placebo n=2) withdrew prior to completing the vaccination safety phase. The reasons for withdrawal included –one subject with an SAE not vaccine related (onset of hypertension 1 month post-FluMist), two subjects (1 each for FluMist and placebo) with poor compliance with the protocol and two (1 each for FluMist and placebo) withdrew for “personal reasons,” which were not stated. The sponsor states because of space limitations at the challenge facilities, 92 subjects proceeded to the challenge phase of the study, and 90 completed it. Two TIV recipients were lost to follow-up prior to completing the challenge phase, but the reasons are not provided.

Efficacy Results

Primary Efficacy Endpoint

The primary measure of protective efficacy of FluMist was the difference between the rates of laboratory-documented influenza illness in the FluMist and placebo groups.

AV003 – Percent Protective Efficacy of FluMist and TIV Relative to Placebo by Serotype for All Challenged Participants with HAI Titers < 1:8 on Day 0.

	Viral Shedding	4-Fold Rise in HAI Titers ^a	Infection ^b	Respiratory Illness	Any Illness ^c	Laboratory-confirmed influenza illness ^d
	%	%	%	%	%	%
H1N1						
FluMist	40	60	49	14	14	80
TIV	60	100	66	31	31	60
H3N2						
FluMist	-50	67	33	75	75	83
TIV	-20	100	50	40	40	70
B						
FluMist	43	24	54	-14	-14	100
TIV	100	100	100	-52	-52	100
All (95% CI)						
FluMist	25 (-159, 84)	52 (-52, 91)	42 (-32, 82)	31 (-58, 76)	31 (-58, 76)	84 (16, 100)
TIV	51 (-94, 93)	100(21, 100)	68 (9, 95)	11 (-96, 60)	11 (-96, 60)	69 (-9, 97)

A negative protective rate means the incidence in vaccinees was higher than in placebo recipients.

^a Missing 4 participants: 2 H1N1-challenged placebo recipients and 2 TIV-challenged subjects

^b Defined as viral shedding on any day and/or > 4-fold rise in HAI antibody to the challenge strain (missing 2 TIV-challenged subjects missing 4-fold rises in HAI titers).

^c Respiratory illness, systemic illness or febrile illness.

^d Defined as any influenza illness and infection (missing 1 of the 2 TIV group missing 4-fold rises in HAI titers).

For this analysis for all participants (whether or not they were serosusceptible at screening), 2/29 (7%) of FluMist, 4/32 (13%) of TIV, and 14/31 (45%) of placebo had cases of laboratory documented illness. Compared to placebo, the protective efficacy (95% CI) of FluMist was 85% (28, 100); p=0.001 and TIV was 71% (2, 97); p=0.006; Mantel-Haenszel test stratified by strain. The study was not large enough to produce precise estimates of strain-specific efficacy. The efficacy rates between FluMist and TIV were not statistically different.

Viral Shedding

Only 3 FluMist, 2 TIV and 7 placebo recipients shed virus for ≥ one day post-challenge, and these results were not statistically significantly different. Compared to placebo, FluMist was 25% (-150, 84) protective against viral shedding while TIV was 52% (-77, 94) protective. Compared to placebo, FluMist was 54% (-41, 91) protective against disease as denoted by >4-fold rises in HAI titers, while TIV was 100% (32, 100). The results were similar when the evaluations were performed with only the serosusceptible subjects.

Immunogenicity

For serosusceptible subjects, TIV induced high serum HAI GMT ratios from Day 28 (4 weeks post-vaccination) to Day 0, the values were H1N1 = 66, H3N2 = 14 and B = 20. These GMT ratios for FluMist, were all < 3 and were similar to placebo.

The percent of subjects with 4-fold rises from Day 0 to Day 28 were also high for TIV (H1N1 = 96%, H3N2 = 94% and B = 92%). These values were lower for the FluMist group (H1N1 = 29%, H3N2 = 39%, and B = 10%) and the placebo group (H1N1 = 22%, H3N2 = 11% and B = 0%). Serum IgG ELISA added little to the HAI information. The nasal IgA results were inconclusive. The H1N1 responses were higher post-TIV (GMT and percent with 2-fold rise) and H3N2 responses were higher post-FluMist, and the type B responses were similar for both groups. The post-challenge immunogenicity results showed that no TIV recipients, 21% of FluMist and 45% of placebo recipients had 4-fold rises in HAI titers from Day 28 to Day 56. Although efficacy was observed after FluMist and TIV, no correlate of protection was identified; however, the sample size was small.

STUDY AV007

TITLE: A Prospective, Randomized, Double-Blind, Placebo-Controlled Trial to Assess the Safety, Tolerability, and Immunogenicity of Three Manufacturing Lots of Influenza Virus Vaccine, Trivalent Types A and B, Cold-Adapted (CAIV-T).

DESIGN

This study was performed to demonstrate consistency of lot production. The study was a randomized, double-blind, placebo controlled study in healthy children, ages 12-36 months, from the Kaiser-Permanente Southern California outpatient clinics. Participants were randomized to one of five treatment groups (3 lot consistency lots, the AV006 efficacy lot, one placebo lot) and administered two doses of vaccine (28-60 days apart) in a blinded fashion. The participants returned for a final study visit 28-42 days after their second dose.

LOCATION: United States, multi-center; CRADA with NIAID
STUDY PERIOD: 4/21/97 – 11/08/97
PIVOTAL: Yes
POPULATION: Children age 12-36 months.
SAMPLE SIZE: n=500 (100 each for 3 consistency lots, Flumist lot used in AV006 and placebo).

OBJECTIVES

Primary

To compare the safety, tolerability, and immunogenicity of 2 doses (given 28-60 days apart) of 3 consistency lots of Flumist in healthy children 12-36 months of age. Consistency of manufacturing is to be concluded if a 4-fold or greater range in the post-dose 2 geometric mean fold rise across lots is ruled out with 95% confidence.

Secondary

To compare the safety of the 3 consistency lots of Flumist to placebo and to Flumist used in AV006 (FM-AV006).

Other Objectives

- To compare the immunogenicity of the consistency lots with that of the efficacy lot.
- To calculate post-Dose 2 seroconversion rates for recipients of each lots, to assess the consistency of those lots, and compare them with the rates for recipients of the efficacy lot.

- To assess the immunogenicity post-Dose 1 and to compare the immunogenicity at this timepoint two post-Dose 2 results in a subset of 100 subjects.
- To compare the responses to H1N1 strains A/Shenzhen/227/95 in the consistency lots and A/Texas/36/91 in the efficacy lot.

VACCINES

- Flumist – Each 0.5 ml dose contained $10^{6.7-7}$ TCID₅₀ of each strain of virus (2 type A and 1 type B) in normal allantoic fluid (NAF) containing sucrose-phosphate-glutamate (SPG). The strains and lots used are listed in the table.
- Placebo – 1 lot of NAF with SPG stabilizer was used.

AV007 – Study Vaccines and Lots Used

Treatment Group	Filled Trivalent Lots	Monovalent Harvest	Strain Reference
Placebo	CAF013	CAE013	NAF
FluMist – Lot 1	CAF016	CAE034 CAE026 CAE024 CAE019	A/Shenzhen (H1N1) A/Wuhan (H3N2) B/Harbin-like NAF
FluMist – Lot 2	CAF017	CAE035 CAE027 CAE025 CAE022	A/Shenzhen (H1N1) A/Wuhan (H3N2) B/Harbin-like NAF
FluMist – Lot 3	CAF020	CAE036 CAE033 CAE029 CAE023	A/Shenzhen (H1N1) A/Wuhan (H3N2) B/Harbin-like NAF
AV006 Efficacy Lot	CAF014	CAE014 CAE017 CAE016	A/Texas(H1N1) A/Wuhan (H3N2) B/Harbin-like
	CAF015	CAE018 CAE017 CAE016	A/Texas(H1N1) A/Wuhan (H3N2) B/Harbin-like

PROCEDURES

Inclusion criteria:

Healthy children between 12 to 36 months of age, inclusive, and had a telephone in the home.

Exclusion criteria:

Hypersensitivity to eggs or egg protein; significant chronic illness for which the CDC ACIP recommends inactivated influenza vaccines; immune deficiency disease, immunosuppressive treatment, or lived in the same household as an immunosuppressed individual; acute febrile illness within 1 week; current URI (including common cold or nasal congestion) within 72 hours; experienced wheezing within one week or had used a bronchodilator medication within 2 weeks of vaccination in the study; past receipt of cold adapted or inactivated influenza vaccine; administration of live virus vaccine within 1 month of vaccination.; administration of an inactivated vaccine within 2 weeks; receipt of a blood product within 3 mo prior or planned receipt within the study duration; expected administration of nasal medications during the 10

days before or after vaccination. Pregnant household member or daycare provider was not an exclusion criterion.

Vaccine Administration and Schedule

On Study Day 0, enrollees had a health assessment and a brief PE performed. While in upright position, each subject was given 0.5ml (0.25 ml per nostril) of study vaccine intranasally administered with an Accuspray™ device. Between Day 28 and 60, subjects were to return for Dose 2 vaccination. The same study vaccine was given for Dose 1 and Dose 2. However, subjects who experienced a “vaccine-related AE” after Dose 1 were not to receive Dose 2.

Concomitant Vaccinations and Medications

No concomitant vaccinations were permitted. Use of aspirin or ASA-containing products was prohibited. All medications given in the 1st 10 days post-vaccination were recorded on CRF.

Monitoring

Immunogenicity

All subjects had pre-vaccine and post-dose two (28-42 days post-dose 2) blood samples obtained. A subset of subjects, supposedly the 1st 100 subjects enrolled, were to also have serum obtained just prior to Dose 2 (28-60 days after Dose 1). These samples were to be assayed for strain-specific HAI titers. Subjects were considered to be seronegative if serum HAI titer $\leq 1:4$. A 4-fold rise in titer was considered to be seroconversion.

Lab Methods

The influenza HAI assays were performed at Aviron. The H1N1 assays were performed with HA from A/Shenzhen/227/95 for FluMist recipients in the consistency lots, and with the HA from A/Texas/36/91 for the FM-AV006 recipients.

Statistical Analysis Plan

Efficacy Endpoints

Primary

- Lot-to-lot consistency for the three vaccine lots required similar geometric mean titer (GMT) ratios (post-dose 2 to baseline) for each strain in each of the three lots. The null hypothesis is that the absolute value of 1 or more of the 9 GMT fold-differences (3 influenza strains from 3 lots of vaccines) is at least 4. The study protocol and data analysis plan (DAP) specified that if all 9 confidence intervals (CI's) excluded 0.25 and 4, the consistency lots would be declared consistent.

Secondary

- The percent of seronegative participants with ≥ 4 -fold rise in HAI titers would be similar among the three lots.
- To assess the immunogenicity post-dose 1 and to compare this to the immunogenicity post-dose 2 in a subset of 100 subjects,
- To compare the responses to the H1N1 strains in the consistency lots (A/Shenzhen/227/95) with the H1N1 in the FM-AV006 vaccine lot (A/Texas/36/91).

Equivalence

- The combined consistency lots and the FM-AV006 vaccines were to be declared similar if the strain-specific GMT ratio for the 3 lots combined were similar to the GMT ratio elicited by FM-AV006.
- The strain-specific titer ratios elicited by the 3 consistency lots were compared to titer ratios elicited by FM-AV006.
- H1N1 strain in the consistency lots (A/Shenzhen) was different than the H1N1 used in FM-AV006 (A/Texas). Observed differences between the consistency lot and FM-AV006 may be due to use of different strains.

Sample size

Sample size was calculated based upon the following assumptions:

- Drop-out was not greater than 10%
- Age of enrollees is uniformly distributed between 12 and 36 mo
- Variability of post-dose 2 antibodies is similar to post-dose 1 antibodies observed in previous Aviron trials with CAIV-T
- The percent of seronegative subjects at 12, 24, and 36 mo is similar to that seen in earlier trials
- Antibody responses are independent over strains.

For the primary outcome, the estimated GMTs after Dose 2 were expected to have 95% CI for all strains, not exceeding $\pm 25\%$ of the GMT for a single lot and not exceeding $\pm 13\%$ for the 3 consistency lots combined.

The secondary outcome, seroconversion after Dose 2, was measured by the percent of seronegative participants with ≥ 4 -fold rise in titer. The study was expected to provide 95% assurance that the true seroconversion rates for all strains and all lots were within $\pm 12\%$ of measured rates. The study was not designed with adequate power to show equivalence in seroconversion rates between lots.

RESULTS

Enrollment and Demographics

Five hundred subjects were randomized, 100 to each of the five treatment groups. Demographic characteristics were similar among all five groups. Mean age was 21-22 months in all groups except in the efficacy lot, it was (24 months ± 7).

Protocol Compliance

Twenty-six subjects withdrew prior to receiving the 2nd dose and 33 subjects did not complete the study. Withdrawals were evenly distributed among groups (Lot 1, n=7; Lot 2, n= 5; Lot 3, n= 5; Efficacy Lot, n= 6; and placebo, n= 9). The most common reasons were parent's request (n=23) and unsatisfactory compliance (n=6).

There were 76 protocol violations. Twenty of these were violations of the exclusion criteria (8 with asthma, 5 with a respiratory illness, 3 had received varicella vaccine, 1 had previous flu vaccine, 1 with egg allergy, 1 with history of seizures, and 1 with febrile illness within 72 hours). For the other 56 violations, 47 were study visits outside of the windows, 8 subjects had illness at

the time of vaccination, and 1 subject received the wrong study number vaccine (but it was from the correct lot).

Lot Consistency

For all 3 pairs of consistency lots (Lot 1/Lot2, Lot 2/Lots 3 and Lot 1/Lot 3) for all 3 strains, the 95% CI for ratios of HAI titers were within the range of ¼ and 4.

AV007 - Comparison of Post-Dose Two HAI Titer Ratios Among Consistency Lots

	Ratio of Titer Ratios	95% CI for Ratios of HAI Titer Ratios
H1N1		
Lot 1/Lot 2	0.70	0.48, 1.03
Lot 1/Lot 3	1.49	1.01, 2.19
Lot 2/Lot 3	2.12	1.44, 3.11
H3N2		
Lot 1/Lot 2	0.79	0.49, 1.29
Lot 1/Lot 3	1.24	0.76, 2.01
Lot 2/Lot 3	1.56	0.96, 2.53
B		
Lot 1/Lot 2	1.03	0.72, 1.49
Lot 1/Lot 3	0.90	0.63, 1.30
Lot 2/Lot 3	0.88	0.61, 1.25

AV007 - Comparison of Post-Dose Two HAI Titer Ratios between Consistency Lots and Efficacy Vaccine

		Ratio of Titer Ratios	95% CI for Ratios of HAI Titer Ratios
H1N1*	Lots 1,2, 3/Efficacy	4.17	3.06, 5.69
H3N2	Lots 1,2, 3/Efficacy	1.01	0.68, 1.51
B	Lots 1,2, 3/Efficacy	1.06	0.79, 1.42

*The efficacy H1N1 vaccine lot contained H1N1 strain A/Texas/36/91, and the consistency lots contained H1N1 strain A/Shenzhen/227/95. Sera from the lot consistency groups were assayed using the relevant H1N1 strain (i.e., A/Shenzhen) and those from the efficacy lot were assayed using the relevant H1N1 antigen (i.e., A/Texas/36/91)

The strains of H3N2 and B contained in the consistency lots and FM-AV006 were the same, and the GMT ratios between the consistency lots and FM-AV006 were similar.

Seroconversion rates for all subjects and seronegative subjects were not statistically different between the consistency lots and FM-AV006 for H3N2 and B. For H1N1, higher seroconversion rates were observed in the consistency lot recipients than for the FM-AV006 recipients.

AV007 - Strain-Specific Seroconversion Rates for All Participants and for Seronegative participants by Treatment Group

Serum HAI	Lot 1	Lot 2	Lot 3	Efficacy Lot	Placebo
H1N1^a, % (95%CI)					
All	78 (68, 86)	88 (80, 94)	73 (63, 81)	37 (27, 48)	2 (0.3, 8)
Seronegative	87 (78, 93)	94 (87, 98)	79 (69, 87)	42 (31, 53)	2 (0.3, 8)
H3N2,% (95%CI)					
All	68 (58, 78)	72 (62, 81)	66 (56, 76)	65 (54, 74)	7 (2, 14)
Seronegative	100 (94, 100)	99 (92, 99.5)	100 (94, 100)	100 (94, 100)	7 (2, 17)
B, % (95%CI)					
All	68 (58, 78)	64 (53, 73)	72 (61, 80)	70 (60, 79)	1 (0.03, 6)
Seronegative	96 (87, >99.5)	100 (93, 100)	100 (93, 100)	100 (94, 100)	2 (0.05, 11)

^a HAI assays were performed using the HA antigen with which the subject was immunized.

Cross-reactivity

A subset of subjects had serum tested against both A/Shenzhen/227/95 (in the consistency lots) and A/Texas/36/91 (in FM-AV006) and generally, more responders were noted to the strain in the vaccine that the subject had received. For the consistency lots combined, from pre to post-dose 2, 80% (75, 84) of recipients had a \geq 4-fold rise to A/Shenzhen and 49% (39, 60) to A/Texas. For FM-AV006 recipients, 15% (8, 24) had \geq 4-fold rise to A/Shenzhen and 37% (27, 48) to A/Texas. Seventeen (6%) of the placebo recipients had \geq 4-fold rise in HAI titers against either strain. A similar pattern of responses was also seen with GMT ratios.

Immunogenicity after 1 dose

100 subjects had blood samples after Dose 1 (just prior to Dose 2) to assess the immunogenicity of 1 dose. For the consistency lot recipients, the percent responders and GMT (~100 for each lot) for H3N2 were equivalent after 1 dose to after 2 dose. However, GMT's were significantly higher (p=0.0001) post-dose 2 compared to post-dose 1 for both H1N1 and type B in the consistency lots. For FM-AV006 recipients, no significant increase in GMT was noted for H3N2 or B from Dose 1 to Dose 2, but there was a significant increase in the GMT to H1N1 (p=0.015).

Subgroup analyses

Analyses were performed by gender (male- n=251, female – n=216), age (12-17 mo – n=101, 18-23 mo – n=182, and 24-36 mo-n=184), ethnicity (Caucasian-n=169, and Hispanic/Central American- n=223), and time between vaccinations (28-41 days – n=406 and 42-60 days – n=55). There were too few Blacks (n=37) and other ethnic groups (n=17) to perform any comparisons. The study did not have sufficient power to test comparisons between the subgroups, so no formal statistical comparisons were made. The combined consistency lot post-dose 2 GMT's for females appeared higher than GMT's for males for all 3 strains (non-overlapping 95% CI for H1N1 and type B), and Hispanics/Central Americans appeared to have slightly higher GMTs than Whites/Caucasians for H3N2 and type B, but 95%CI did overlap. Neither age nor time between vaccines appeared to effect the immune response.

STUDY AV014

TITLE: A Randomized, Prospective, Double-Blind Trial in Healthy Children Comparing the Safety, Tolerability, and Immunogenicity of Influenza Virus Vaccine, Trivalent, Types A and B, Live, Cold-Adapted (Flumist) from Two Different Manufacturing Facilities.

LOCATION: Australia, Two study sites
STUDY PERIOD: 12/7/98 – 4/9/98
PIVOTAL TRIAL: Yes, Consistency of facilities
POPULATION: Healthy children, 12- 42 months of age
SAMPLE SIZE: Total: n= 225

DESIGN

This was a prospective, randomized, double-blind trial to compare the safety, tolerability and immunogenicity Flumist blended and filled at 2 different facilities, Medeva and Aviron-PA. All of the vaccines were manufactured at the U.K. facility, and all vaccine lots used in clinical trials in the BLA, including AV006 - Pediatric Efficacy Study, AV009 - Effectiveness in Adults, and AV007 - Lot Consistency Trial, were blended and filled at the Medeva facility.

The study was a two-dose regimen (28 – 42 days apart) and was performed in healthy children age 12-42 months, in Australia. Subjects were randomly assigned to one of the 2 treatment groups in a 3:2 ratio, Medeva to Aviron-PA. The Southern Hemisphere was chosen so not to have interference by circulating influenza during the influenza season in the Northern Hemisphere.

OBJECTIVES

Primary

To compare the immunogenicity profiles for influenza virus vaccine strains (1997-1998) of Flumist blended and filled at Medeva to Flumist blended and filled at Aviron-PA.

Secondary

To compare the safety and tolerability profiles of the Flumist blended and filled at Medeva to Flumist blended and filled at Aviron-PA.

VACCINES

Flumist – Each 0.5 ml dose contained 10^7 TCID₅₀ of each strain of virus (2 type A and 1 type B) in normal allantoic fluid (NAF) containing sucrose-phosphate-glutamate (SPG). The strains and lots used are in the table below. The 2 lots of vaccine were indistinguishable in appearance.

AV014 – Study Vaccines and Lots Used

Treatment Group	Filled Trivalent Lots	Monovalent Harvest	Strain Reference
FluMist (Aviron-PA)	CAF1009	CAE037 CAE033 CAE041 CAE047	A/Shenzhen (H1N1) A/Wuhan (H3N2) B/Harbin - like NAF
FluMist (Medeva)	CAF020*	CAE036 CAE033 CAE029 CAE023	A/Shenzhen (H1N1) A/Wuhan (H3N2) B/Harbin - like NAF

* The same lot as lot #3 in AV007, the lot consistency study.

PROCEDURES

Entry Criteria

Inclusion criteria

Children 12-42 months of age in good health, available by telephone or home visits, and had a signed informed consent by parent/guardian.

Exclusion criteria

Hypersensitivity to eggs or egg protein; significant underlying chronic illness for which the inactivated influenza vaccine is recommended; prior receipt of any influenza vaccine. Immunodeficiency, immunosuppression or household contact (or daycare provider) with immunosuppression; acute febrile (> 38°C) illness within 72 hours of enrollment; URI including wheezing or nasal congestion within 72 hours of vaccination; receipt of aspirin (ASA) or ASA-containing products within 1 month of enrollment or anticipated use during the study; administration of a live viral vaccine within 1 month or expected receipt within 1 month of study vaccination; administration of an inactivated vaccine within 2 weeks or expected receipt within 2 weeks of study vaccination; administration of any investigational drug within 1 month prior to enrollment or anticipated receipt during the study; receipt of any blood product within 3 months or anticipated during the study; any condition that the investigator thought might interfere with evaluation of the vaccines. A pregnant household contact was not an exclusion criterion.

Also, mild asthmatics who had not used therapeutic interventions (β -agonist, steroids, etc) within 30 days and met all other Inc/Exc criteria could be enrolled at the discretion of the investigator. “Mild asthmatic” was not defined.

Vaccine Schedule and Administration: Subjects received 2 doses of Flumist given 28-42 days apart. The vaccine was given intranasally 0.5ml (0.25 ml per nostril) using an Accuspray device™ with the subjects in an upright or sitting position.

Concomitant Vaccinations and Medications

No concomitant immunizations were permitted. No ASA or ASA-containing products were permitted. EMLA was permitted for venipuncture.

Immunogenicity

Serum samples were obtained just prior to vaccination at Dose 1 (Day 0) and Dose 2 (28-42 days later) and at Visit 3 (28 days \pm 5 days after Dose 2). Samples were to be tested by HAI, which

was performed at Aviron. The sponsor states that attempts were made to perform all samples from a subject simultaneously.

Statistical Methods

Immunogenicity Endpoints

Co-primary endpoints:

- Seroconversion rate → strain-specific seroconversion rate post-dose 2 in baseline seronegative subjects. Similarity between the two groups was defined as rates within 20 percentage points.
- Geometric mean titers (GMT) → strain-specific HAI GMT's post-dose 2 in all participants regardless of baseline titers. Testing of similarity was based on the 90% two-sided CI's for the GMT ratio. If the confidence intervals for all three strains were included within the range of $\frac{1}{4}$ and 4 then the two treatment groups would be declared similar.

Secondary endpoints:

- Post-dose 2 seroresponse in all subjects.
- Post-dose 2 strain-specific HAI GMT in baseline seronegative subjects.
- Post-dose 1 seroconversion in baseline seronegative subjects and all subjects.
- Post-dose 1 strain-specific HAI GMT in baseline seronegative and all subjects.
- Post-dose 1 strain-specific HAI GMT in seroresponders.
- Post-dose 2 strain-specific HAI GMT in seroresponders.

Definitions:

- Seronegative → defined as strain-specific HAI titers $\leq 1:4$.
- Seroconversion → ≥ 4 -fold rise in strain-specific HAI titers in seronegative subjects.
- Seroresponder → ≥ 4 -fold rise in HAI titer regardless of baseline serostatus.
- For statistical analysis, HAI titers $< 1:4$ were set as equal to 1:2.

Sample Size

The proportion of baseline seronegative subjects and seroconversion rates were estimated from Study AV007, as it was assumed that the rates would be similar. If the true seroconversion rates were 85% for Aviron-PA and 95% for Medeva, and 65% of subjects were seronegative, there was a 33% probability for observing $\geq 20\%$ difference. For GMT ratios, assuming that the true degree of GMT ratio between the two groups was 2-fold, the study had 99% power to show that the GMT ratios for all 3 strains were within 4-fold (and would be declared similar). Power to show similarity within 3-fold was lower, ranging from 72%-98%.

RESULTS

Enrollment

225 subjects were randomized 3:2 (Medeva, n=135: Aviron-PA, n=90) into the study.

Demographics

Generally, the 2 study groups had similar baseline characteristics. In both groups, the mean age was 2.5 years, and ~57% were male. In the Aviron-PA group, 94% were Caucasian, 1% was Asian, 1% was Polynesian/Maori, and 3% other. In the Medeva group, 89% were Caucasian, with 2% each Asian and Polynesian/Maori and 7% were other ethnic groups.

Protocol Compliance

There were 3 “voluntary withdrawals ” after Dose 1 but prior to Dose 2, however no details were provided. There were 118 minor protocol deviations, 69 in the Medeva group and 49 in the Aviron-PA group. All of the deviations were study procedures which were performed outside of the protocol windows, most frequently was performing the Day 42 phone call late (Aviron-PA, n=37 and Medeva, n=41).

Immunogenicity

All subjects had pre-vaccine serum samples obtained and analyzed. Post-dose 2, there were six subjects (2 Aviron-PA and 4 Medeva) with samples missing from analysis. The reasons for the missing samples were not provided.

Analyses of Co-Primary Endpoints

At baseline for all subjects, 99% were seronegative for H1N1, ~80% were seronegative to B, and ~40% were seronegative to H3N2. Seroconversion rates are shown in the table below.

AV014 - Seroconversion Post-Dose Two in Baseline Seronegative

Strain	N	Aviron-PA	Medeva	Difference in %	95% CI of Difference*
H1N1	88	85%	69%	16%	(5.3, 27.1)
H3N2	40	100%	100%	0	(-12,7, 9.5)
B	71	100%	100%	0	(-7.3, 4.9)

AV014 - Post-Dose Two GMT in All Participants

Strain	N	Aviron-PA GMT	Medeva GMT	GMT Ratio	90% CI for GMT Ratio *
H1N1	88	18.3	10.9	1.67	(1.27,2.17)
H3N2	88	81.7	74.2	1.10	(0.93, 1.30)
B	88	42.2	41.7	1.01	(0.87, 1.17)

Secondary Endpoints

- Post-dose 1 seroconversion in baseline seronegative subjects and all subjects.
 - Greater differences between the Aviron-PA and Medeva groups in seroconversion rates were observed post-dose 1 than had been noted post-dose 2 for all strains for all subjects and for H1N1 for the seronegative subjects. For H1N1, the difference was 25.6 (14.2, 37.4) for all subjects and 25.4 (14, 37) for seronegative subjects.

AV014 - Proportion of Participants with ³ 4-fold Rise in Strain-Specific HAI Titer by Dose and Treatment Group for All Participants

Dose	Strain	Aviron-PA		Medeva		Rate Difference 90% CI
		n/N	% Seroconversion (95% CI)	n/N	% Seroconversion (95% CI)	
Post-dose 1	H1N1	47/ 88	53 (42, 64)	37/ 133	28 (20, 36)	25.6 (14.2, 37.4)
	H3N3	41/ 88	47 (36, 58)	66/ 133	50 (41, 58)	3.0 (-15.2, 8.4)
	B	61/ 88	69 (59, 79)	90/ 133	68 (59, 76)	1.6 (-9.5, 13.9)
Post-dose 2	H1N1	75/ 88	85 (76, 92)	90/ 131	69 (60, 77)	16.5 (5.8, 27.6)
	H3N2	45/ 88	51 (40, 62)	77/ 131	59 (50, 67)	-7.8 (-19.7, 3.7)
	B	74/ 88	84 (75, 91)	111/ 131	85 (77, 90)	-0.6 (-11.6, 8.8)

Question 2. Are the data adequate to support the safety of FluMist in subjects, 12 months to 64 years of age?

Approximately 20,000 subjects have received at least one dose of FluMist in clinical trials. However, not all studies were finalized at the time of submission of the BLA and complete study reports have not been submitted for all studies. CBER's review of data from the incomplete study reports, therefore, is not complete.

The Frequency of Subjects by Age Group (in years), and Treatment Assignment for Select Protocols* Combined. (Data submitted by Aviron through April 30, 2001.)

	Age Group (in years)									Total
	<2	2.0- 5.9	6.0- 9.9	10- 12.9	13- 18.9	19- 29.9	30- 49.9	50- 64.9	≥65	
FluMist	1254	5864	4104	2522	2499	940	2241	511	111	20,046
Placebo	378	1651	906	594	572	439	1025	209	101	5875
TIV [#]	0	0	0	0	3	18	12	0	0	33

*Protocol = first administration of FluMist or placebo in protocols 98-005, AR001, AV001, AV002, AV002-2, AV003, AV004, AV005, AV006 (Year 1), AV007, AV008, AV009, AV010, AV012 (Year 1), AV012 (Year 2 new recruits), AV014, AV017 (new recruits), and AV019.

[#]Trivalent inactivated influenza vaccine, the licensed product.

Frequency of Subjects under 2 Years of Age (in months) and Treatment Assignment for Selected Protocols* Combined

	12- 15 months	16 – 19 months	20-23 months	Total
Received 1 or 2 Doses				
FluMist	200	507	547	1254
Placebo	88	142	148	378
Received 2 Doses				
FluMist	133	272	211	616
Placebo	58	90	89	237

*Protocols includes AR001, AV002, AV002-2, AV006 (Year 1), AV007, AV012 (Year 1 and new recruits Year 2), AV014, AV017 (new recruits), and AV019.

STUDIES SUBMITTED IN SUPPORT OF SAFETY

Monitoring performed in clinical trials

All trials in this section had some safety monitoring performed. Generally, Aviron monitored for three categories of adverse events (AEs): 1) solicited reactogenicity events (REs), 2) other AEs (unsolicited AEs) and 3) serious adverse events (SAEs). In all studies SAEs were defined as death, immediately life-threatening, results in permanent or substantial disability, hospitalization or prolongation of hospitalization, cancer, congenital anomaly, or is the result of an overdose (accidental or intentional). The definitions for SAEs were similar to 21 CFR 312.32. Of note, not all studies had active monitoring for all three types of AEs.

Pediatric Trials

Studies AV006 (Years 1 and 2) AV011 and AV015 (Year 3 for AV006 participants): AV006 is described above in Efficacy section.

Studies AV007 and AV014: Safety and tolerability data were compared in these studies of consistency of lot production and consistency of manufacturing, as described above.

Study AV012: Reports of SAEs in a study performed to assess herd immunity of FluMist in an HMO in a Texas community.

Study AV019: Comparison of medically-attended events and SAEs after FluMist or placebo in Children ages 1 to 17 years in Northern California Kaiser Permanente.

Adult Trials

Studies AV003 and AV009: Report of the safety profile of FluMist, compared to placebo, in adults in efficacy trials described above.

No data were submitted for the safety of annual administration of FluMist in adults.

Additional trials submitted in the BLA include Phase 1 and 2 studies (AV001, AV004 and AV005) which were performed to establish safety and dosing (spray vs. drops and 2-dose

regimen) before proceeding with product development. Study AR001, which was a comparison of FluMist prepared by classical reassortant vs. recombinant reassortant, was also submitted to the BLA. However, all clinical trials have been performed with classical reassortants, and AR001 is not discussed in this briefing document.

Subjects at “High Risk” for Influenza

Study AV010: - Evaluation of safety and tolerability of FluMist vs. placebo in children 9-17 years of age with moderate to severe asthma.

Study AV012: Evaluation of a subset of subjects in this trial, who were identified as having asthma.

DMID #98-005: Comparison of the safety and tolerability of FluMist vs. placebo in adults infected with HIV and adults without HIV infection.

Other Safety Concerns

1. Transmission of the Influenza Strains contained in FluMist

No study submitted in the BLA was specifically performed to assess transmissibility of FluMist. Aviron addressed transmissibility with the following:

- Assessment from AV006.

Transmissibility was assessed by applying statistical modeling for evaluating wild type influenza attack rate in 183 sibling pairs (1 FluMist recipient and 1 placebo recipient,) as compared to 513 FluMist and 258 placebo recipients without siblings in the trial. The sponsor's rationale was if there was a high rate of transmissibility, placebo recipients with FluMist siblings should have a lower attack rate than observed in other placebo recipients. Placebo recipients with siblings had 16% attack rate which was equivalent to the 20% in placebo recipients without siblings receiving FluMist, and the sponsor considered this as evidence of no significant transmissibility.

2. Reassortment with Wild-type Influenza Strains

- No human studies were submitted in the BLA to assess the occurrence of reassortment of influenza strains contained in FluMist with wild type influenza strains. The sponsor notes that the mutations responsible for the attenuation of FluMist are not fully defined, and that the full expression of attenuation may require that all six internal genes of Master Donor Virus work in concert.

SAFETY DATA BY STUDY

STUDY AV006 (Years 1, 2, and 3 [AV015])

TITLE: A Phase 3, Randomized, Double-blind, Placebo-controlled, Trial to Assess the Safety, Immunogenicity and Efficacy of Influenza Virus Vaccine, Trivalent, Types A&B, Live, Cold-Adapted (CAIV-T) in Healthy Children.

LOCATION: United States, multi-center

STUDY PERIOD: 2 years: Year 1 – 1996-97 (1st enrolled 8/96), Year 2 – 1997-98
PIVOTAL TRIAL: Yes
POPULATION: Healthy children, ages 15-71 months at enrollment
SAMPLE SIZE: Year 1 – Total – n=1602 (1070 FluMist, 532 Placebo)
Year 2 – Total - n=1358 (917 FluMist, 441 Placebo)

This was a prospective, randomized, double-blind, placebo-controlled, multi-center trial conducted at 10 study sites. The study was designed as a 2-year study, with the primary objective to assess efficacy of FluMist. A single cohort was recruited in Year 1 (receiving either a 1 dose or 2-dose regimen), and remaining in the same study group to be re-vaccinated with 1 dose in Year 2. In Year 1, children 15-71 months of age (not had their 6th birthday) were randomized 2:1 to receive vaccine or placebo. Subjects who completed Year One were invited to participate in the Year 2 study. Randomization is described above in the Efficacy Section. In Year three, an open label study design was followed in order to offer previous placebo recipients vaccine and to collect safety data in previous vaccinees on FluMist given in a 3rd consecutive year. Year three was designated as study AV015.

Safety Monitoring

Monitoring procedures for safety were the same for Years 1 and 2 of the study. A physical exam was performed on the day of enrollment. Subjects were observed for 30 minutes after each vaccination in Year 1 and 15 minutes in Years 2 and 3.

The three categories of AEs (REs, other AEs and SAEs) were monitored during all three years of the study. Parents/guardians were given digital thermometers and asked to maintain a diary card for 10 days post-vaccination for each dose for pre-defined RE's. These events included: temperature cough, runny nose/nasal congestion, sore throat, irritability, headache, chills, vomiting, muscle aches, and decreased activity. Study personnel contacted the parents on ~ days 4 and 10 to remind them to complete the diary cards and to return them to the study site.

The final study report indicated that all SAEs were to be reported if they occurred within 42 days of each study vaccination. After 42 days until the end of the influenza season in each year, any SAEs judged to be "related to vaccine" was to be reported to the study personnel. There is no monitoring plan specified in the protocol for the collection of SAEs either within the first 42 days post-vaccination or after that time period. In response to an FDA question, the sponsor submitted information to the BLA on 6/1/2001 stating that there had been no active monitoring specifically to capture SAEs.

RESULTS – Year 1

A total of 1070 subjects received at least 1 dose of FluMist and 532 received at least one dose of placebo. Eight hundred and eighty-one subjects received 2 doses of FluMist and 433 subjects received 2 doses placebo.

AV006 – Year 1 Solicited Reactogenicity Events with Onset Day 0 to 10 by Dose and Treatment Group

	Dose 1		Dose 2	
	FluMist, N	Placebo, N	FluMist, N	Placebo, N
Randomized	1070	532	881	433
Valid Diary Card	1056	530	850	415
	%	%	%	%
Cough	28	29	36	33
Runny nose/nasal congestion	59	48*	51	46
Sore Throat	10	8	6	7
Irritability	26	26	17	19
Headache	8	6	5	6
Chills	4	3	3	3
Vomiting	6	4*	7	5
Muscle Aches	5	3*	3	2
Decreased Activity	16	13	13	13
Fever				
Fever 1	16	12*	11	11
Fever 2	7	6	5	6
Fever 3	2	4	2	4
Fever 4	0.9	1	0.7	1
Fever 5	0.1	0.2	0.1	0.5
Any Event	74	66*	69	62*

Fever 1 – Oral > 100.0°F, rectal > 100.6°F, axillary/missing method >99.6°F

Fever 2 – Oral > 101.0°F, rectal > 101.6°F, axillary/missing method >100.6°F

Fever 3 – Oral > 102.0°F, rectal > 102.6°F, axillary/missing method >101.6°F

Fever 4 – Oral > 103.0°F, rectal > 103.6°F, axillary/missing method >102.6°F

Fever 5 – Oral > 104.0°F, rectal > 104.6°F, axillary/missing method >103.6°F

78% of subjects (parents) had axillary, 4% rectal, 10% oral temperatures, 8% the method of measurement was missing.

*p-value <0.05 based of Fisher's exact test.

Post-dose one, statistically significant differences between FluMist and placebo groups were noted for runny nose/congestion (59% vs. 48%), vomiting (6% vs 4%) and muscle aches (5% vs 3%). Runny nose/nasal congestion was also significantly different between FluMist and placebo groups after either dose (72% vs. 64%) and after both doses (35% vs 26%), but not after Dose 2. “Any RE” was reported more in FluMist than placebo groups post-dose 1, post-dose 2, after either and after both doses.

AV006 – Year 1. Selected “Other Adverse Events” with Onset Day 0 to 10 by Dose and Treatment Group

	Dose 1		Dose 2	
	FluMist	Placebo	FluMist	Placebo
Randomized, N	1070	532	881	433
	%	%	%	%
Any AE	18	15	14	15
Allergic reaction	1	0.6	0	0
Flu syndrome	0.1	0	0.1	0.5
Edema, face	0	0.2	0	0
Abdominal pain	2	0.2*	0.8	0.5
Anorexia	1	0.6	0.9	0.7
Diarrhea	4	3.2	2	2
Rash	0.1	0.8*	0.2	0
Conjunctivitis	0.5	0.4	0.6	0.7
Otitis media	2	1	3	2
Asthma	0.6	0.4	0.5	0.5
Bronchitis	0.1	0.2	0.6	0.7
Pneumonia	0.4	0	0	0.2
Sinusitis	0.2	0.4	0.4	0.5
Total respiratory	2.3	1.5	2.8	3.1
Febrile seizure	0	0	0	0.2

*p<0.05 by Fisher’s exact test.

Unsolicited AEs reported within 10 days of vaccination were coded by Body System (modified COSTART), and statistically significant differences were noted post-dose 1 for abdominal pain (FluMist 2% vs. placebo 0.2%) and rash (FluMist 0.4% vs. placebo 2.1%). For respiratory system, events included asthma, bronchitis, dyspnea, epistaxis, laryngitis, lung disease, pneumonia, sinusitis, stridor and altered voice (otitis media was included in “special senses” category).

Serious Adverse Events

As noted under trial design, there was no active monitoring plan for collecting SAEs. Five SAEs were reported within 42 days of vaccination of either dose 1 or 2. All of these were hospitalizations and occurred in FluMist recipients. All but one was judged as definitely not vaccine related. There was 1 subject with abdominal trauma from a motor vehicle accident; 1 with dehydration 23 days post-dose 1; 1 Staph infection of the foot, 16 days post-dose 2; 1 abdominal pain rule-out appendicitis 9 days post-dose 1 (this was judged probably not related [As noted above, abdominal pain occurred significantly more often in FluMist than placebo recipients.]); and 1 subject with hydrocephalus with VP shunt placement 34 days post-dose 1. Also, 1 FluMist recipient had new onset of juvenile-onset rheumatoid arthritis (JRA) within 42 days, which was not attributed to vaccination, and the sponsor did not code as an SAE. There were 5 SAEs and significant adverse events more than 42 days post-vaccination. Three of these were in the FluMist group (1 pneumonia 3 ½ months post-dose 2, 1 Henoch Schonlein Purpura (~ 3 months post dose 2), 1 dehydration (~ 3 months post-dose 2). In the placebo group there was 1 case of pneumonia ~ 2 months post-dose 1 and 1 case of croup ~1 ½ months post dose 2.

RESULTS YEAR 2

Safety

Solicited reactions were reported by vaccine group and by Year one regimen (one-dose vs. 2 doses in year one). Reaction rates in the FluMist group in Year two were similar whether subjects had received one or two doses of vaccine in Year 1. Reaction rates for all FluMist recipients and for all placebo recipients in Year 2 (i.e., regardless of dosing regimen in Year 1) are shown in the table below. There were no significant differences in reaction rates between the vaccine and placebo recipients.

AV006 – Year 2 Percent of Subjects with Post-Vaccination RE’s with Onset from Day 0 to 10 in Year Two by Treatment Group

	FluMist	Placebo
Randomized, N	917	441
Valid Diary Card, N	912	441
	%	%
Cough	24	25
Runny nose/nasal congestion	42	42
Sore throat	10	8
Irritability	14	16
Headache	9	7
Chills	3	3
Vomiting	5	4
Muscle aches	3	4
Decreased activity	11	13
Fever*		
Fever 1	11	10
Fever 2	6	3
Fever 3	3	2
Fever 4	1	0.2
Fever 5	0.2	0
Any event	58	58

*The same definitions for fever as used in Year 1 were used in Year 2.
 75% of subjects had axillary, 0.1% rectal, 1.7% oral, 5% the method was missing.

AV006- Year 2 Percent of Subjects with Selected Unsolicited Adverse Events with Onset Day 0 to 10 by Dose and Treatment Group

	FluMist	Placebo
Randomized, N	917	441
	%	%
Any AE	13	14
Allergic reaction	1	2
Edema, face	0.3	0.2
Anorexia	0.2	0.5
Diarrhea	1	3
Rash	1	0.9
Conjunctivitis	0.1	1*
Otitis media	2	1
Asthma	0.3	0.5
Bronchitis	0.1	0
Sinusitis	0.2	0.5
Total respiratory	1.7	2.7
Febrile seizure	0	0

*p<0.05 by Fisher's exact test.

For unsolicited adverse events, the only event that occurred at significantly different rates between groups (p<0.05) was conjunctivitis, which occurred in 1% of placebo and 0.01% FluMist recipients.

One 6 year old girl (with a history of allergies and chronic rhinitis) developed angioedema and urticaria within 30 min of vaccination with placebo. She was treated with Benadryl and oral prednisone, and the symptoms resolved overnight. On the next day when visited by study personnel, she was noted to have multiple pustules c/w ant bites. She was evaluated for egg allergy with scratch tests (for allantoid elements) and TIV which were negative, however intradermal testing was refused. The sponsor did not code this event as an SAE.

Serious Adverse Events

As noted, there was no active monitoring plan for capturing SAEs. Two participants (1 each FluMist and placebo) experienced an SAE within 42 days of re-vaccination in Year 2. One FluMist recipient, with a history of asthma, had onset of wheezing on day 9 post-vaccination and required hospitalization for status asthmaticus. This was judged to be “probably-not” vaccine-related by the investigator. The other event was *Salmonella* enterocolitis on day 15 post-vaccination with placebo, and this was judged as not vaccine related. Two additional SAEs occurred more than 42 days post-vaccination. These SAEs included one child who died from a malignant brain mass and malignant hyperthermia and another child who was hospitalized for pneumonia and hypoxia 5 months post-vaccination. Two other events (one child with a bee sting and another child with a lip laceration requiring an ER visit) also occurred within 10 days of vaccination.

One SAE was not reported to the investigator until 9/29/99 (the database was locked on 7/15/98). In Year 2, this subject received the third dose of FluMist on 10/14/97, and 15 days later on 10/29/97, he developed a fever of 103°F. He was taken to the emergency room, diagnosed with clinical pneumonia and treated with antibiotics for ten days. The event is not included in the

sponsor's report of SAEs in Year 2, and was not identified to CBER until completion of the inspection of the clinical site in April 2001.

AV015 Study (Year 3 of AV006)

Design

As noted above vaccine was administered in an open label fashion to AV006 study participants who had completed years 1 and 2. Subjects, who had participated in study AV011 (challenge with CAIV-monovalent H1N1) following Year 2 of AV006, were also eligible for enrollment. In AV015, prior vaccine recipients were to receive one dose of FluMist and previous placebo recipients were to receive one or 2 doses of FluMist. Thus, subjects in AV015 might have received anywhere from a total of 1-5 doses of FluMist. For subjects in the two-dose regimen of AV015, the 2nd dose was given 28-60 days post-dose 1. The choice of one or two dose regimen for prior placebo recipients was based upon primary dosing regimen (Houston was designated as a 1-dose site) or investigator/parent choice (e.g., for convenience of one dose regimen). Safety monitoring was conducted in the same manner as for the first two years. Additionally, this protocol specified that study personnel were to conduct a phone call on Day 42 to obtain any follow-up information and to inquire about SAEs. There was no placebo arm in this year of the study, and descriptive results of safety were provided.

RESULTS YEAR 3

Enrollment and Demographics

A total of 949 participants enrolled (650 prior FluMist and 299 prior placebo recipients). This represented 62% of AV006 participants who completed Year 1 and 71% of those who completed Year 2. Year 3 participants were more likely to be white/Caucasian as compared to non-participants (91% vs. 75%) which may have been partly attributed to the fact that the AV006 UCLA site, which had enrolled a predominance of Hispanics, did not participate in Year 3.

Protocol compliance

Eight subjects, all prior placebo recipients, did not complete the study. None withdrew due to an adverse event.

**AV015 (AV006-Year 3) Reactogenicity Events with Onset Day 10 Post-FluMist in Year 3
 by Treatment Group**

	Prior FluMist	Prior Placebo*	
	FluMist	1 st Dose	2 nd Dose
Vaccinated, N	650	192	186
Valid Diary Card, N	649	192	186
	%	%	%
Cough	27	24	19
Runny nose/nasal congestion	37	49	30
Sore throat	8	11	9
Irritability	10	11	11
Headache	10	14	11
Chills	2	3	5
Vomiting	5	7	6
Muscle aches	3	5	4
Decreased activity	10	13	12
Fever			
Fever 1	8	9	12
Fever 2	3	4	8
Fever 3	1	1	3
Fever 4	1	0	1
Fever 5	0	0	0
Any event	55	61	48

*For prior placebo recipients only those subjects who did not participate in Study AV011 are included i.e., only subjects who had never received a dose of CAIV.)

Fever definitions were the same as used for AV006 Years 1 and 2:

Fever 1 – Oral > 100.0°F, rectal > 100.6°F, axillary/missing method >99.6°F

Fever 2 – Oral > 101.0°F, rectal > 101.6°F, axillary/missing method >100.6°F

Fever 3 – Oral > 102.0°F, rectal > 102.6°F, axillary/missing method >101.6°F

Fever 4 – Oral > 103.0°F, rectal > 103.6°F, axillary/missing method >102.6°F

Fever 5 – Oral > 104.0°F, rectal > 104.6°F, axillary/missing method >103.6°F

75% of subjects (parents) had axillary, 0.1% rectal, 1.7% oral, 5% the method of measurement was missing.

The largest difference in adverse events following the first dose between prior FluMist and prior placebo recipients was for runny nose (37% vs. 49%).

Reactogenicity event rates were also reported for FluMist recipients across three year of vaccination, results are shown on the table below.

AV015 - Reactogenicity Events Across Three Years in Prior FluMist Recipients (Two-Doses in AV006 Year One) Excluding Those Who Participated in AV011 (H1N1 Challenge) by Year and by Dose.

	1 st Year (AV006)		2 nd Year (AV006)	3 rd Year (AV015)
	1 st Dose	2 nd Dose	1 st Dose	1 st Dose
Vaccinated, N	461	457	455	461
Valid Diary Card, N	459	456	455	461
	%	%	%	%
Cough	25	36	25	26
Runny nose/nasal congestion	57	52	40	37
Sore Throat	7	6	8	8
Irritability	24	16	15	9
Headache	7	6	7	10
Chills	2	2	2	2
Vomiting	5	7	4	6
Muscle Aches	4	3	2	4
Decreased Activity	14	12	11	10
Fever				
Fever 1	14	10	11	8
Fever 2	5	4	5	2
Fever 3	2	2	3	1
Fever 4	1	<1	2	1
Fever 5	0	0	<1	0
Any Event	73	70	56	56

Fever definitions were the same as used for AV006 Years 1 and 2, as noted in the table above.

Other Adverse Events

For unsolicited AE's, 14% of FluMist and 17% of prior placebo recipients (combined 1st dose) experienced one or more other AE.

Serious Adverse Events

Only one SAE was reported 43 days post-vaccination in a subject who underwent outpatient surgery for tonsillectomy and adenoidectomy but required hospitalization due to a prolonged response to anesthesia.

STUDIES AV007 AND AV014

The safety monitoring for these two pediatric trials was nearly identical as that performed for AV006. Active monitoring for SAEs (a study nurse called the participants 42 days post-dose 2 to question about the occurrence of SAEs) is described for AV014, but not for AV007. The results for safety, including reactogenicity profile, occurrence of "other AEs" and SAEs were also similar to those reported for AV006 Year 1.

Safety Results for AV007 (Lot Consistency Study)

A total of 500 subjects were enrolled, and 499/500 subjects returned the diary card post-dose 1, and all 474 subjects who received Dose 2 returned the diary card. After Dose 2, 285/300 participants in the consistency lots returned the diary card

After Dose 1, 76% of Flumist vaccine groups (3 consistency lots and FM-AV006) and 63% of placebo recipients had at least 1 RE (p value not given). These rates decreased after Dose 2 to 48% in both the Flumist and placebo groups. Compared to placebo, the vaccine groups had higher rates of runny nose/nasal congestion after Dose 1 (65% vs. 49%, $p < 0.003$) and chills after Dose 2 (5% Flumist vs. 0% placebo, $p < 0.002$).

SAE's

Two SAE's were reported. A 32 month old girl with a history of seizures had a seizure following a blow to the head, 23 days after receipt of the 2nd dose of FM-AV006 vaccine. This was judged not to be vaccine related. The second SAE was liver laceration post- MVA that occurred in a 25 month old female which occurred 24 days after receipt of Dose 1 of a consistency lot #2 vaccine, which was judged not to be vaccine related.

AV014

A total of 225 subjects received Dose 1 and 222 subjects received Dose 2. The sponsor did not state the number of diary cards that were returned. Seventy six percent (76%) of Aviron-PA and 71% of Medeva Flumist recipients experienced at least 1 RE after Dose 1. The most common RE's were runny nose/nasal congestion (57% Aviron-PA and 58% Medeva) and irritability (41% Aviron-PA and 37% Medeva). After Dose 1, a 10% [90% CI: -18.2, -1.8] difference in rate was only observed for vomiting (3% Aviron-PA vs. 13% Medeva).

After Dose 2, 55% of Aviron-PA and 53% of Medeva Flumist recipients experienced at least 1 RE, and again runny nose/nasal congestion (38% vs. 35%) and irritability (26% vs. 17%) Aviron-PA vs. Medeva were most common. For irritability, the difference was 9% (-1.3, 20.8). For fever- grade 1 (defined below) the rates were 6.3% (-15.7, 3.1) different, occurring in 9% of Aviron-PA and 15% of Medeva Flumist recipients.

The 90% CI for difference in event rates post-dose 1 exceeded 10% for "any RE", cough, runny nose/nasal congestion, sore throat, irritability, headache, vomiting, lethargy, fever –grade 1 (oral $>100.0^{\circ}\text{F}$, rectal or ear 100.6°F , or axillary $>99.6^{\circ}\text{F}$) and fever-grade 2 (oral $>101.0^{\circ}\text{F}$, rectal or ear 101.6°F , or axillary $>100.6^{\circ}\text{F}$). Similar findings were seen post-dose 2. No statistical analyses were provided.

SAE's

No deaths occurred in the study. 3 SAE's were reported, and all were in the Aviron-PA group. These events included: 1) a subject with a fall requiring an ER observation, occurred on 1 day post-dose 1 and was not vaccine-related, 2) a subject with a febrile illness with dehydration which required hospitalization, occurred 31 days post-dose 2 and was determined as "probably not" vaccine-related, and 3) a subject with diagnosis of Perthes disease 38 days after Dose 1, which was judged as not vaccine related.

Study AV012

TITLE: Study of Influenza Virus Vaccine, Trivalent, Types A&B, Live, Cold-Adapted (CAIV-T) in a Community-Based, Non-Randomized, Open-Label Trial in Children to Assess the Safety and Herd Immunity for the Control of Epidemic Influenza.

LOCATION: Scott & White HMO, Temple, TX - multi-center; CRADA with NIH
STUDY PERIOD: 3 Years; Year 1 – 1998-99 (1st enrolled 8/17/98 – ended 4/30/99)
PIVOTAL TRIAL: Yes, Phase 3.
POPULATION: Children, ages 18 months – 18 years (not had their 19th birthday)
SAMPLE SIZE: Total planned in 3 years – 15, 000; Year 1- Flumist n=4298

At the time of submission of the BLA, the study report was an interim report for Year 1, and all analyses were not complete.

Design

In this 3 year trial, a subject may be enrolled in Year 1, 2, 3 or any combination of years. The study is to be conducted during influenza season (August through April) of 3 consecutive years. Participants will receive 1 dose of vaccine for each year they enroll (separate CF for each year of enrollment). Children 18 mo through 18 years (before their 19th birthday) were eligible.

This is open-label, non-randomized clinical trial. Subjects residing in Temple-Belton Texas area or receiving the medical care at Scott & White (S&W) HMO were invited to participate. Unvaccinated children at S&W were used as a comparison group. The goal of the study was to enroll 50% of the 4700 children ages 18 mo – 4 years and 85% of ~15,000 children ages 5 through 18 years living in the study area. Two other populations were used for comparisons: patients at PCA (now Humana) HMO in Austin, TX and patients at S&W in Bryan-College Station TX. The sponsor did not obtain consent from the subjects in the control populations, but state that they requested IRB approval for the database searches.

Objective

Primary

The primary objective was to assess the total effectiveness of immunization of preschool and school children 18 mo – 18 yrs with Flumist (medically attended acute respiratory infection, MAARI, in vaccinated children compared to unvaccinated children in each control community with adjustments for potential co-variates.) Assessing the general safety of FluMist (essentially only SAE's were captured) was one of the secondary study objectives. Only the safety data were submitted to the BLA safety database.

VACCINES

Flumist – Each 0.5 ml dose contained 10^7 TCID₅₀ of each strain of virus (2 type A and 1 type B) in normal allantoic fluid (NAF) containing sucrose-phosphate-glutamate (SPG.) 1998-99 influenza strains were A/Beijing/262/95 (H1N1), A/Sydney/5/97 (H3N2) and B/Harbin/7/94-like. The chosen strains were representative of the FDA recommended strains for 1998-99. The filled trivalent lot numbers (CAF1003 and CAF 1004) were used in this trial.

A single dose regimen was chosen for this protocol (in contrast to the 2-dose regimen used in AV006, the Pediatric Efficacy Trial) based upon logistics for performing the study. The sponsor states that in AV006, the 1-dose regimen was efficacious.

Safety Monitoring

Subjects stayed in the clinic for 15 minutes post-vaccination to monitor for immediate reactions. Parents/guardians were given a laminated parent information card, which contained the study site's phone number, and instructions to call if the subject was hospitalized, developed a serious condition, or became pregnant. They were also given a refrigerator magnet (with study information) and a Day 42 health report card (which was to be completed, signed and returned to the study center after Day 42). To be valid, the Day 42 report card had to be signed and dated later than the 42 days post-vaccination and have 1 check box completed to indicate whether or not the subject had a new significant health problem between vaccination and Day 42. Subjects at S&W were to receive an automated "reminder call" generated by the Integrated Clinical Encounter System (ICES) on Day 42 to remind the parent to call the investigator about new health problems and to return the Day 42 report postcard.

The study site contacted parents/guardians of those subjects who were identified as having a new significant health event to determine if the event met the definition of an SAE. For subjects who did not return their Day 42 postcard or the card was not valid, study personnel tried to contact the parent/guardians by telephone. If they were unreachable by phone, a registered letter was sent with a postcard. SAEs were identified by searching S&W SMS database and through reporting by postcard or by telephone.

Database Searches

Searches of the S&W database were performed every 30 days to confirm ascertainment of all SAEs. The search checked for any hospitalization in study subjects. These searches did not capture events for subjects who did not receive their care at S&W. In the Temple-Belton area, ~20% of the local physicians do not participate in S&W HMO, and these physicians were also informed about the study and requested to help identify SAEs that may be related to study vaccination.

SAEs were defined as in AV006, consistent with CFR. Investigators were to follow all SAEs until resolution. Any SAE occurring within 42 days of vaccination were to be reported to Aviron and IRB within 1 day of notification. After 42 days, only SAEs that were "vaccine-related" were to be reported.

Vaccine-relatedness of an adverse event was to be based upon the type of event, the temporal relationship of the event and vaccine administration, the known biology of the vaccine viruses, and the medical judgement of the investigator. The investigator may contact the Medical Monitor at Aviron, the Study Chair, or the DSMB chair, as needed. Categories of relationship were definitely not, probably not, possibly, probably, and definitely related. Vaccine related refers to an AE that is either possible, probably or definitely related to administration of vaccine.

Enrollment

4298 participants were enrolled between 8/17/98 and 12/19/98. 53% of participants reported that they were members of S&W HMO and 79% stated that S&W was their primary health care facility (e.g., some subjects had more than 1 insurer). Eleven percent (11%) had Medicaid. The mean age was 8.6 years (range 18 mo – 18 years) and 55% were 18mo – 9 yrs, 44% 9-17 yrs and 1% was 18 yrs old. 3 subjects, ages 1.4-1.5 years, were enrolled. Caucasians accounted for 71%, Hispanics 17% and Blacks 7% of enrollees. There were equal numbers of males (50.4%) and females (49.5%).

Subjects with Asthma

531 of 4298 subjects (12.4%) gave some history of asthma, reactive airway disease (RAD) or wheezing. 156 were identified through review of the eligibility questionnaire only, 232 through review of S&W database only, and 143 by both methods.

Of 4298 participants, 4063 either returned a valid postcard or received a successful Day 42 phone contact, for a follow-up rate of 94.5%. 3056 (71.1%) returned a valid postcard. 1006 (81%) of 1242 subjects who did not return a valid postcard had successful Day 42 phone contacts. 235 (5.4%) subjects were lost to follow-up. Of the 235 lost to follow-up, 82 were self-identified as members of S&W. So with these 82, 4145 (96%) were evaluable for safety based upon the Shared Management System (SMS) database for self-identified S&W members, postcards and/or telephone calls.

SAEs

8 SAEs were identified, and none were considered to be definitely or possibly vaccine-related. 2 of the SAEs occurred in the subjects with a history of wheezing – one was a groin abscess and one was a MVA. The other 6 SAEs included fever with hip pain, vomiting, fever and vomiting, croup, and aseptic meningitis, all of which occurred 21 or more days post-vaccination. 1 subject had onset of depression at 3 days post-vaccination considered as definitely not vaccine-related. No deaths occurred.

Other AE's

149 of 4063 subjects (3.7%) with Day 42 data reported onset of a 1 or more significant new health problem. All problems reported by the parents and new problems identified through S&W database search were evaluated by study staff for clinical significance, and if judged significant, they were recorded on the CRF. 87 such events occurred for 78 subjects, and though exact numbers are not available, most were respiratory (URI, sinusitis, OM, bronchitis, asthma exacerbation and pneumonia). These events were not presented in tabular form, but as narratives in a line listing.

STUDY AV019

TITLE: A Prospective, Double-blind, Randomized, Placebo-Controlled Trial to Assess the Safety of Frozen FluMist in Health Children and Adolescents.

LOCATION: United States, Northern California Kaiser Permanente
STUDY PERIOD: 10/2/00 - ongoing
PIVOTAL TRIAL: No, supportive for safety
POPULATION: Healthy children, age 18 months to 17 years (before their 18th birthday)
SAMPLE SIZE: Total, originally planned 15,000; at the time of submission, n=9689 with FluMist n=6473 and placebo n=3216

DESIGN

AV019 is an ongoing study intended to provide additional safety data with FluMist in children. The study is being performed in 32 outpatient clinics in the Northern California Kaiser Permanente (NCKP) system. In a double-blind fashion, children ages 18 months to 18 years were randomized in a 2:1 ratio to receive FluMist or placebo. Healthy children less than 9 years of age received 2 doses of study vaccine (28-42 days later) and children 9-17 years of age received one dose of study vaccine. Medically-attended events (MAEs) and serious adverse events (SAEs) were monitored by database searches. There was no active monitoring for post-vaccination solicited reactogenicity events by the participants.

This report contains the results from a planned interim analysis performed to present preliminary data to CBER and the advisory committee. The preliminary report was provide to CBER on 4/30/01, and the review is ongoing.

OBJECTIVES

Primary

To estimate the rates of MAEs, including SAEs and selected groups of MAEs, following the administration of FluMist compared to placebo.

VACCINES

1. FluMist – Each 0.5 ml dose contained 10^7 TCID₅₀ of each strain of virus (2 type A and 1 type B) in normal allantoic fluid (NAF) containing sucrose-phosphate-glutamate (SPG). The strains used were the 1999/2000 strains, A/Beijing/262/95 (H1N1), A/Sydney/05/95 (H3N2) and B/Yamanashi/166/98. For 2000-2001, the B strains were the same; however, the recommended strains for 2000-2001 were different from the ones used for both of the type A strains.
2. Placebo – NAF in SPG was used.

PROCEDURES

The procedures for this study for enrollment, inclusion and exclusion criteria, and dosing interval (28-42 days) were similar to AV007, except a dosing interval of 28-60 days was used in AV007.

Concomitant Immunizations and Medications

No concomitant immunizations, aspirin-containing meds or nasal medications (for 7 days post-vaccination and post-challenge) were permitted.

Monitoring

All subjects were considered evaluable for safety unless they withdrew informed consent. The safety assessment period for each participant began at the time of 1st dose (Day 0) and ended on date of 2nd dose of vaccine (for 2-dose regimen) or 42 days after vaccination, whichever came earlier. The primary method of ascertainment of safety outcomes was extraction of records from the KP computerized health care utilization databases. Outcomes were also collected via spontaneous reporting of parents/guardians. The database is managed and analyzed by the investigation team at the KP Vaccine Study Center. One or more MAEs may be assigned for a single encounter.

SAEs were defined as in AV006, 007 and other Aviron studies. The SAEs were captured by extraction of KP database, spontaneous reporting and by review of the State of California mortality tapes. MAEs were defined as an encounter with a health care provider, especially a visit to a medical clinic or emergency department, or a hospital admission. These events were also captured by extraction from the computerized databases or spontaneous reports of parents/guardians. The MAEs reflect the coded diagnoses assigned by the health care provider who saw the child at presentation, as recorded onto the standardized NCKP encounter forms.

About 2-5% NCKP members discontinue their healthcare coverage annually. Although infrequent, participants who discontinue KP coverage may not be identified until several months later, and the exact date of termination may not be known.

Statistical Methods

Relative risk estimates and confidence intervals (CI) were the primary method of evaluating outcomes. No formal hypothesis testing was planned.

MAEs were analyzed by individual coded diagnosis within the following four pre-specified classes of events: acute respiratory events, systemic bacterial infections, acute gastrointestinal events and rare, potentially wild type influenza-related events.

The sponsor states that because of the large sample size and range of potential MAEs, expect many observed MAEs. Therefore, a two-stage approach was used for the analysis of MAEs:

- a) Binomial screening – in this analysis, participants experiencing multiple encounters for the same MAE will be counted once. The relative risk (RR) with 2-sided 90% CI will be presented. $RR = \text{rate in FluMist} / \text{rate in placebo}$, and the CI were constructed using mid-probability binomial method, adjusted for follow-up time. If the lower bound is greater than 1, further statistical evaluation (Poisson regression) and medical review will be performed.
- b) Poisson regression - for all MAEs with lower bound of the 90% CI exceeds 1 (as above) Poisson regression will be performed. The number of encounters per participant for each MAE will be modeled as a Poisson random variable with treatment group, age category (1-17years, 3-8 years, 18 to < 36 months and 12 to < 18 months), gender and race included as classification variables. The RR for treatment group with 90% CI will be calculated. If the lower bound exceeds 1, additional statistical and medical investigation was planned. Results are present separately for clinic visits, ED visits, and hospitalizations.

The sponsor states that due to the exploratory nature of the study and lack of formal hypotheses testing, a large number of CI will be constructed without multiplicity adjustments.

RESULTS

Enrollment and Demographics

Between 10/2/00 and 12/22/00, 9734 participants were enrolled and 9689 (FluMist n=6473 and placebo n=3216) were evaluable for safety. One subject, between the ages of 1 and 8 years, was lost to follow-up, and due to an administrative error could not be identified. Also, 44 subjects could not be included in the analysis because the study vaccine given at dose one was uncertain due to “dosing discrepancies,” which were not described. Post-dose 2 data for 47 subjects were not included because the study vaccine received at dose 2 was different than at dose 1. Of the enrollees, 5637 were from 1-8 years and 4052 were from 9-17 years old. Originally, the study enrollment was planned for ~15,000 subjects.

The database was locked on 12/31/00 for the interim report. As of that date, all subjects had received at least 1 dose of study vaccine and 64% of subjects in the 2-dose regimen had received both doses of study vaccine. Approximately 72% of total expected follow-up for the study and ~43% of post-dose 2 follow-up was complete by 12/31/00.

For both the 1-8 year age group and 9-17 year age groups, the study population was equally distributed by gender and ethnicity; ~55% of subjects were Caucasian, 20% Hispanic, 10% Asian or Pacific Islander, 6% African American, 4.5% multiethnic and 4% other. At enrollment, 261 (3%) of subjects were 12 to <18 mo, 1097 (11%) were 18 to < 36 mo, 4279 (44%) were 36 mo to 8 years, and 4052 were 9-17years of age.

SAEs

There were 20 SAEs reported through April 15, 2001, the time of submission to CBER. No deaths were reported. Thirteen SAEs occurred in the FluMist group and seven SAEs occurred in the placebo group. The relationship to which vaccine doses (i.e., 1st or 2nd) is not reported. Four SAEs in the FluMist group occurred within 14 days of vaccination and included: hemolytic uremic syndrome, onset 11 days post-vaccination in a 12 month old; acute gastroenteritis, onset 11 days post- vaccination in a 14 month old; abdominal pain/gyn disorder onset 11 days post-dose in a 16 year old; and an appendiceal abscess, onset 11 days post-dose in a 15 yo male. The other nine SAEs had onset after day 15 and included benign foot lesion, post-extubation airway obstruction (reason for intubation not stated), testicular torsion, synovial carcinoma, appendicitis (onset Day 42 in 12 year old) and 4 subjects with diagnoses of psychiatric disorders. None of these SAEs were coded as vaccine related by study investigators.

In the placebo recipients, the three SAEs in the 1st 14 days post-vaccination included: croup, onset day 4 post-dose in a 17 mo female – coded as “possibly related” to vaccine by the investigator; trauma onset day 4 post-dose in a 17 mo female; and a psychiatric disorder onset day 4 post-vaccination in a 12 year old. The SAEs with onset after day 15 included: diabetes onset day 25 post-vaccination in a 5 year old; suicide ideation, onset on day 29 in an 8 year old; cellulitis and fractured clavicle, onset day 37 post-vaccination in a ~ 5year old, and dehydration with onset on day 39 in a 6 ¾ year old. Besides the croup event, none of the SAEs were coded as related to vaccination by the investigators.

MAEs

A total of 5,850 MAEs were reported through December 31, 2000. Twenty percent (20%) of MAEs were coded as Well care/reassurance visits, 11% were URI, 7% were otitis media, 7% trauma, and 6% were psychiatric disorders. These event codes were not reported by study group.

None of the four pre-specified group diagnoses (defined above in Statistical Methods) were associated with a statistically significant increased RR in the FluMist group after Dose 1, Dose 2, or any dose in any utilization setting (clinic, ED or hospitalization) for any age group.

Increases in the rates of individual MAEs for which the sponsor stated that a relationship to FluMist was biological-plausible were observed in the clinic or ED (not hospital setting) for:

- Conjunctivitis in subject 1-17 years, 1-8 years and 18 to < 36 months (FluMist 6.6 – 14.52 and placebo 0 – 5.17 per 100 person months),
- URI in participants 1 to 17 years of age (FluMist 1.24-1.76 and placebo 0 per 1000 person months),
- Abdominal pain in participants 1 to 17 years of age (FluMist 1.19 and placebo 0.22 per 100 person months),
- Musculoskeletal pain in subjects 1-8 years and 18 to < 36 months (FluMist 4.39 – 9.04 and placebo 0 – 1.7 per 1000 person months), and
- Asthma in subjects 18 to < 36 months (FluMist 7.75 and placebo 0 per 1000 person months).

Otitis media with effusion in the clinic setting was associated with a statistically significant increase in FluMist than placebo recipients, FluMist rate 10.79 and placebo 4.09 per 1000 person months, Poisson RR 3.43 (1.64, 7.24).

There were also some MAEs that were associated with decreased rate in FluMist compared to placebo recipients, some of which were respiratory symptoms and possibly due to protection from FluMist against natural influenza. However, these decreased events could also have been due to chance alone because of the large number of comparisons performed.

TRIALS IN SUPPORT OF SAFETY IN ADULTS

STUDY AV009

TITLE: A Prospective, Randomized, Double-blind, Placebo-controlled, Trial to Assess the Safety, Tolerability, and Effectiveness of Influenza Virus Vaccine, Trivalent, Types A and B, Live, Cold-Adapted (CAIV-T) in Healthy working Adults to Reduce Influenza-Like Illness, Absenteeism from Work and Health Care Costs during Influenza Outbreaks.

LOCATION: United States, multi-center – 13 sites; CRADA with NIAID
STUDY PERIOD: Sept. 18, 1997 – Mar. 31, 1998
PIVOTAL TRIAL: Yes, Phase 3 Trial
POPULATION: Healthy working adults ages 18-64 years (not had 65th birthday).
SAMPLE SIZE: Original n=3600, increased to 4,200.
Final enrollment, n=4561 (FluMist n= 3041 and placebo n=1520).

Safety

Subjects were observed for 15 minutes after vaccination to monitor for immediate adverse events (AEs). Subjects were given thermometers and asked to maintain a diary card for 7 days post-vaccination for pre-defined RE's, which included oral temperature > 100°F, cough, runny nose or congestion, sore throat, headache, chills, muscle aches, and weak/tired. Study personnel contacted the subjects on Day 7 to remind them to complete and mail in the diary card.

Unsolicited AEs and medications were coded for consistency using the COSTART Preferred Term and the Anatomic Therapeutic Class, respectively. All SAE's were collected for the first 28 days post-vaccination and phone contact was made at day 28 post-vaccination to query about SAEs. SAEs occurring after the 28-day safety phase were not actively collected; however, any SAEs reported during the study period were summarized in the study report.

Statistical Methods

All subjects who were randomized were evaluable for any safety data obtained. The proportion of solicited AEs between FluMist and placebo recipients was compared using exact two-sided 95% confidence intervals. FluMist was judged to be equivalent to placebo if the upper limit of the 95% CI for the difference in rates was no more than 5% for fever and 10% for other solicited events. The number of days with solicited AEs with onset in the first seven days-post vaccination were compared by examining distributions in the two groups. Unsolicited AEs and SAEs were tabulated by treatment group. A subgroup analysis of safety was to be performed to assess safety (RE and SAEs) in participants with asthma who were inadvertently enrolled.

RESULTS

A total of 4,561 subjects received 1 dose of study vaccine (FluMist = 3041, placebo = 1490.) 4,475 of 4,561 subjects (98%, FluMist n=2985 and placebo n=1490) were evaluable for safety and tolerability, i.e., returned the diary card.

Compliance

Of 4561 subjects, 98% of each treatment group returned the 7 day diary card, and 97.5% provided safety information for the 1st 28 day monitoring period. There were 116 subjects for whom safety data was not completed for the Day 0-28 post-vaccination period (FluMist 78, Placebo 38). The most common reason for not completing the 28 day period follow-up was lost-to-follow-up [FluMist n=65 placebo n=31]. Two subjects in the FluMist group and 1 in the placebo group withdrew for adverse events during this period (see below).

AV009 - Solicited Reactogenicity Events (REs) by Treatment Group (onset Day 0-7)

	FluMist	Placebo	Difference in Percent	95% CI for difference**
Randomized, N	3041	1520		
Returned Diary Card	2985	1490		
Experiencing RE*	%	%	%	%
Fever				
Oral T > 100°F	1.34	1.34	-0.00	(-0.00, 0.010)
Oral T >101.2°F	0.44	0.60	-0.00	(-0.01, 0.00)
Oral T >102.0°F	0.77	0.13	-0.00	(-0.01, 0.00)
Oral T > 103.0°F	0	0	0.00	(-0.00, 0.00)
Runny nose	44.32	26.64	0.18	(0.15, 0.21)
Sore throat	26.57	16.31	0.10	(0.08, 0.13)
Cough	13.64	10.20	0.03	(0.01, 0.06)
Headache	39.26	37.25	0.02	(-0.01, 0.05)
Muscle aches	16.11	14.50	0.02	(-0.00, 0.04)
Chills	8.28	6.11	0.02	(0.00, 0.04)
Tired/weak	24.56	20.54	0.04	(0.01, 0.07)
Any RE	70.92	61.88	0.09	(0.06, 0.12)

*Participants are counted if they experience an event at least once within 7 days following vaccination.

** CIs for the difference in proportions are based on an exact method.

Two RE's failed to meet the pre-specified equivalence criteria, those being runny nose (FluMist – 44.3% and placebo – 26.6%) and sore throat (FluMist – 26.6% and placebo – 16.3%.)

Duration of runny nose/congestion was 1-2 days for 22.6% of FluMist and 14.5% of placebo recipients, but occurred in > 5 days for 9.3% of FluMist and 7.1% of placebo recipients. The peak difference between the groups occurred on Day 2. A similar time pattern was observed for sore throat and headache. Healthcare utilization and missed workdays were not monitored for the 1st 7 days post-vaccination.

AV009 – Solicited Reactogenicity Events by Treatment Group for Participants with Asthma (Onset Day 0-7)

	FluMist	Placebo
Randomized, N	23	13
Returned Diary Card	23	13
Experiencing RE*	%	%
Fever		
Oral T > 100°F	0	0
Runny nose	43.5	23.1
Sore throat	30.4	30.8
Cough	21.7	30.8
Headache	30.4	53.8
Muscle aches	30.4	7.7
Chills	17.4	7.7
Tired/weak	39.1	15.4
Any RE	73.9	84.6

No statistical comparisons were reported.

The number of participants with asthma who were inadvertently enrolled was too small to make definitive conclusions about tolerability in this subset. Although the number is small, there was an increase in “any REs” for asthma participants (Flumist - 73.9%, placebo – 84.6%), as compared to the entire study cohort (Flumist – 70.9%, placebo – 61.7%).

Other Adverse Events

Unsolicited AEs occurring during the first seven days following vaccination were summarized by COSTART body system and preferred term.

AV009 – Percent of Subjects Experiencing Selected “Other Adverse Events” with Onset from Day 0 to 7, by Treatment Group

	FluMist	Placebo
Randomized, N	3041	1520
Returned Diary Card	2985	1490
Experiencing “other AE”	%	%
Any AE	30.2%	21.5%
Respiratory system	18.1%	7.5%
Nasal congestion	9.1%	2.3%
Rhinitis	6.1%	3.1%
Sinusitis	4.0%	2.1%

Overall, there were more unsolicited AEs in the FluMist group vs. the placebo group (respectively 30.2% vs. 21.5%, $p < 0.001$) and the difference was due primarily to respiratory system AEs (FluMist - 18.1%, placebo - 7.5%). Rates of digestive system AEs were similar in the FluMist group and placebo group both for overall events (5.59% FluMist; 6.118% placebo) and for specific events (abdominal pain, diarrhea, nausea, vomiting). Statistical comparisons were not provided.

There were 7 pregnancies (Flumist $n=5$) in the study. Five resulted in live full-term (FT) infants, and there were 2 spontaneous abortions (1 each in Flumist and placebo recipient). Five vaccine exposures were in the 1st trimester, all resulted in FT infants.

There were 16 vaccine sprayer deviations, mostly due to use off site causing use out sequence. 71% of the vaccines were self-administered with 3.8% of subjects having some problem (no details provided). Of the 29% administered by study personnel, 2.5% reported some problem (1.84% for Flumist and 3.85% for placebo).

Adverse Events Leading to Withdrawal from the Study

There were 3 AEs that led to withdrawal from the study (2 FluMist; 1 placebo). One of the FluMist subjects withdrew due to Crohn’s Disease with abdominal pain and vomiting with onset 2 days post-vaccination and then diarrhea 7 days post-vaccination, which was recorded as “probably not” vaccine-related. The other FluMist subject was withdrawn due to accidental death 16 days post-vaccination. The placebo subject withdrew due to a diagnosis of personality disorder, noted 17 days post-vaccination.

Serious Adverse Events

There were 9 serious AEs within the 28-day post-vaccination period (FluMist, n=5 and placebo, n=4). In the FluMist group these AEs were; 1 abdominal pain and cramping which led to hospitalization in a subject with Crohn's disease with onset 2 days post-vaccination; 1 hysterectomy due to severe adenomyosis/dysmenorrhea 12 days post-vaccination; 1 fatal drowning 16 days post-vaccination; 1 torn knee cartilage 18 days post-vaccination; 1 anaphylactic reaction to oral Septra 26 days post-vaccination. In the placebo group there was: 1 knee ligament repair 6 days post-vaccination; 1 hemorrhagic cystitis 8 days post-vaccination; 1 hospitalization for an AE to Tylenol and Verapamil 13 days post-vaccination; and 1 psychiatric hospitalization 17 days post-vaccination.

An additional 51 SAEs were passively reported during the remainder of the study period (FluMist, n = 30 and placebo, n=21). In the FluMist group, there were 8 injuries and/or orthopedic surgery; 1 elective jaw surgery; 3 acute tracheobronchitis/bronchitis; 1 flu; 1 renal calculus; 1 ureteral obstruction; 1 optic neuritis/cat scratch fever; 1 undiagnosed neuropathy; 1 possible panic attack; 2 hysterectomy; 4 cholecystectomy; 1 gallbladder infection; 2 leg thrombosis; 2 pancreatic cancer; 1 goiterous mass. In the placebo group there were: 7 injury and/or orthopedic surgery; 1 bacterial infection; 1 ovarian cystectomy; 2 renal calculus; 2 hysterectomy; 3 cholecystectomy; 1 appendicitis; 1 inguinal hernia repair; 1 breast cancer; and 1 fatal drug overdose.

STUDIES SUBMITTED FOR SAFETY IN HIGH RISK GROUPS

STUDY – AV010

TITLE: A Randomized, Prospective, Double-Blind, Placebo-Controlled Trial to Assess the Safety and Tolerability of Influenza Virus Vaccine, Trivalent, Types A and B, Live, Cold-Adapted (Flumist) in Patients with Moderate to Severe Asthma between the Ages of Nine and Seventeen Years.

LOCATION: United States, multi-center; CRADA with NIAID
STUDY PERIOD: 10/3/97 – 12/4/97
PIVOTAL TRIAL: No, supportive for safety
POPULATION: Children with asthma, ages 9-17 years old
SAMPLE SIZE: Total - 48; FluMist – n= 24; Placebo – n=24

OBJECTIVE

To investigate the safety and tolerability of FluMist compared to placebo when administered intranasally to children and adolescents with moderate to severe asthma.

DESIGN

The study was randomized, double-blind, prospective, placebo-controlled to assess safety and tolerability of FluMist in 9-17 year olds with moderate to severe asthma, as defined by National Heart, Blood and Lung Institute (NHBLI). According to the NHBLI Expert Panel Report II: Guidelines for the diagnosis and management of asthma: *Asthma is a chronic disorder of the airways that causes recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning. These episodes are usually associated*

with widespread but variable airflow obstruction that is often reversible whether spontaneously or with treatment (NHBLI, 1997). However, the sponsor did not provide specific definitions for “moderate” and “severe.”

Subjects were randomized 1:1 to receive FluMist or placebo. Subjects were assigned to the next available sequential identification number, affixed on each vaccine sprayer. The sponsor, study personnel, and participants remained blinded to the treatment group assignments until the study was completed. The total study duration was 35 days (from 7 days prior to 28 days post-vaccination). No immunogenicity evaluations were performed.

VACCINES

1. FluMist – Each 0.5 ml dose contained 10^7 TCID₅₀ of each strain (2 type A and type B) of virus in NAF containing SPG. The 1997-98 strains were A/Shenzhen/227/95 (H1N1, lot CAE036), A/Wuhan/359/95 (H3N2, lot CAE033) and B/Harbin/7/94-like (lot CAE 029.) Filled trivalent lot CAF026 with NAF lot CAE023 was used. The lots are the same as those in CAF020, which was Lot #3 in the Lot Consistency trial (AV007).
2. Placebo – NAF in SPG, lot CAE019 was used.
3. Inactivated influenza vaccine (TIV) – This was optional for participants, and was administered according to the manufacturer guidelines. This was offered to subjects at any time after completion of the 10 day post-vaccination monitoring period or sooner, if culture-confirmed cases were identified in the community.

Procedures

Schedules

Informed consent was obtained at Visit 1 (Day minus 7) and eligibility criteria were reviewed. A brief PE was performed and the subjects had to demonstrate a forced expiratory volume in 1 second (FEV₁) < 80% of predicted after having withheld albuterol for 8 hours with reversibility of > 12% within 30 minutes after 2 administrations (180 µg) of albuterol MDI. Subjects who met this criteria were given a diary card and a peak flow meter and asked to record their peak expiratory flow rates each morning at about the same time and before bronchodilator treatment.

Daily asthma scores, nighttime awakening scores, use of rescue medication (albuterol MDI) and all medications taken (including OTC meds) from Day minus 7 to Day 0 were recorded. Participants returned on Day 0 for repeat pulmonary function tests (PFT's) and, if they had not experienced an asthma exacerbation during the week of screening, they were then randomized for vaccination (FluMist or placebo).

After vaccination, participants were given a thermometer and a diary card. They were asked to record daily temps and the daily asthma assessments as done from Day -7 to Day 0. Subjects were also asked to record reactogenicity events (RE's) similar to other Aviron trials, including cough, sore throat, runny nose, headache, chills, muscle aches, and tiredness, as well as the occurrence of any other adverse events (AEs).

During the 35 day study, the subjects were asked to complete 4 diary cards, one for each of these time periods:

- Visit 1 to Visit 2 (Day -7 to Day 0)
- Visit 2 to Visit 3 (Day 0 to Day 2-5)
- Visit 3 to Visit 4 (Day 2-5 to Day 10-14)
- Visit 4 to Visit 5 (Day 10-14 to Day 28-30).

Study personnel telephoned the participants (on Days 2-3, 7-8, and 21-23) to remind them to complete the diary cards and to bring them to the next visit. However, the timing for the phone calls was not consistent with the timing for completing the diary cards.

Inclusion Criteria

- a. 9-17 years of age, inclusive.
- b. Male or non-pregnant female. Females had to have used a form of birth control considered acceptable to the investigator (not defined).
- c. Diagnosis of asthma in accordance with NHBLI, 1997.
- d. Demonstrated (FEV_1) < 80% of predicted at Day minus 7 (Visit 1) after withholding albuterol MDI for 8 hours and, if applicable, salmeterol xinafoate (Serevent™) for 24 hours. Predicted FEV_1 values were obtained using normal equations of Polgar (ages 9-17 years).
- e. Demonstrated reversibility defined as an increase in $FEV_1 \geq 12\%$ within 30 min following administration of 2 administrations (180 μ g) of albuterol MDI.
- f. Stable asthma defined as:
 - No asthma exacerbations in the prior 4 weeks. An exacerbation was defined as a worsening of asthma that was not controlled by a maximum of 6 puffs of albuterol MDI over 8 hours and/or required additional therapy such as oral steroids or additional nebulized bronchodilators.
 - No hospitalization, ER visits or unscheduled medically attended visit for the treatment of asthma in the prior 4 weeks.
 - No use of an oral steroid burst for the treatment of asthma in the prior 4 weeks.
- g. On a fixed dosage maintenance regimen of asthma medications during the prior 4 weeks.
- h. A non-smoker who had not used any tobacco products on a regular basis within the prior 6 months.
- i. Able to perform spirometry effectively (assessed by the maximum respiratory flow volume loop curves) and peak flow measurements (assessed visually by observation of peak expiratory flow technique) and able to comply with the accurate completion of diary cards.

Exclusion criteria

- a. Hx of hypersensitivity to eggs or egg protein.
- b. An acute febrile illness within 1 week prior to vaccination.
- c. Current upper respiratory illness, including the common cold or nasal congestion within 72 hours.
- d. Hx of previous CAIV.
- e. Receipt of an investigational drug within 1 month of vaccination.
- f. Signs or syxs of immunosuppression or immunodeficiency.
- g. Clinically significant hx or current evidence of bronchiectasis, CF, bronchiolitis, or other obstructive pulmonary disease other than asthma.
- h. Receipt of any live virus vaccine within 1 month prior to study vaccine.
- i. Receipt of any inactivated vaccine within 2 weeks prior to study vaccine.
- j. Receipt of any blood products, including immunoglobulin in the prior 6 months.

- k. Concurrent need for nasal steroids for the 1st 10 days post-vaccination.
- l. Underlying condition in the investigators opinion might interfere with evaluation of the vaccine.
- m. Presence of nasal polyps.
- n. Use of any anti-viral drug in the 2 weeks before or during the clinical trial, including Amantadine, Rimantadine, Acyclovir, Ganciclovir, and Famcyclovir.

Concomitant Vaccinations and Medications

No concurrent immunizations were permitted in this trial. Participants were asked to record all medications taken, including non-prescription meds, on the diary cards for the entire study duration (Day -7 to Day 28). The protocol states that use of nasal steroids was not permitted in the 10 day post-vaccination period.

Vaccine Administration

Subjects received 0.5 ml of study vaccine (0.25 ml in each nostril) by intranasal spray while in the seated position. Vaccine administration could be by self-administration under observation, by parents/guardians under observation, or by the study personnel. Subjects remained seated for 30 seconds, and remained at the clinical site for 15 minutes to observe for immediate reactions.

Monitoring

Vaccine safety was monitored for the duration of the study. Safety and tolerability measurements included asthma stability, REs, other AEs (unsolicited AEs) and SAEs.

Asthma Stability

- a. The primary measure of asthma stability was the mean percent change in percent predicted FEV₁ from baseline to post-vaccination.
- b. Other measures of asthma stability included:
 - Mean percent change from baseline in forced vital capacity (FVC), FEV₁/FVC, and forced expiratory flow during the middle half of FVC (FEF_{25%-75%}).
 - Proportion of participants with more than a 15% absolute decrease in FEV₁
 - Mean number of days with a decrease in PEFR $\geq 30\%$ or ≥ 2 SD from the baseline mean.
 - Proportion of subjects with a decrease in PEFR $\geq 30\%$ or ≥ 2 SD from the baseline mean.
 - Mean number of days with a decrease in PEFR $\geq 15\%$ or ≥ 2 SD from the baseline mean.
 - Proportion of subjects with a decrease in PEFR $\geq 15\%$ or ≥ 2 SD from the baseline mean.
 - Daily asthma scores.
 - Nighttime awakening scores.
 - Change from baseline in daily use of albuterol MDI.
 - Proportion of subjects with at least 1 asthma exacerbation.

Asthma stability measure definitions :

- **Spirometry**
 - Performed according to American Thoracic Society standards.

- Maneuvers were performed at the clinical site in the morning, before bronchodilator treatment, during the same 2 hour window to minimize diurnal variation in PFT's. Triplicate blows were performed, and spirometric results were deemed acceptable if the FEV₁ values were within 10% of one another. The same spirometer was used for each subject for all 3 study visits.
- FEV₁ percent predicted: During Visit 1, subjects had to demonstrate FEV₁ of < 80% predicted based upon age, sex, and height (no adjustment for race.) It was calculated using the highest FEV₁:
$$\% \text{ Predicted} = 100 \times \frac{\text{Highest FEV}_1}{\text{Predicted FEV}_1}$$

- Reversibility definition: At Visit 1, each subject had to demonstrate an increase in FEV₁ of $\geq 12\%$ within 30 minutes of 2 administrations (180 μg) of albuterol MDI.

Percent reversibility was calculated using the highest FEV₁ before and after morning bronchodilator treatment:

$$100 \times \frac{[\text{highest FEV}_1 \text{ after albuterol MDI} - (\text{highest FEV}_1 \text{ before albuterol MDI})]}{\text{highest FEV}_1 \text{ before albuterol MDI}}$$

- **PEFR**

- Each morning prior to taking any asthma meds, subjects obtained 3 PEFR readings (flow meters were provided, Personal Best®.) The highest of the 3 values was recorded on the diary card.

- **Asthma Symptom Scores**

Scores were assessed in the evenings before going to sleep, using the following scale:

- 0=no asthma syxs
- 1=occassional asthma syxs
- 2=frequent asthma syxs
- 3=continuous asthma syxs

- **Nighttime Awakenings**

Scores were assessed in the mornings for the previous nighttime awakenings:

- 0=fine
- 1=slept well, slight wheeze or cough
- 2=awake 2-3 times, wheeze or cough
- 3=bad night, awake most of the time

- **Use of Rescue Medications**

Prior to going to sleep, subjects recorded the number of puffs of albuterol MDI used in the previous 24 hours.

- **Asthma Exacerbations**

This was defined as a worsening of asthma that was not controlled by a maximum of 6 puffs of albuterol MDI over 8 hours and that required additional therapy such as oral steroids or additional nebulized bronchodilators.

Reactogenicity Events, AEs and SAEs

In the 1st 10 days post-vaccination, the solicited REs included temperature ($\geq 100.4^{\circ}\text{F}$), decreased activity, irritability, runny nose, sore throat, cough, headache (H/A), chills, tiredness and muscle aches (similar to those in AV006 and 007, except nasal congestion was not listed with runny nose and vomiting was not included). Definitions for other AEs, “vaccine-relatedness” (decided by the investigator) and SAEs similar to AV006 are provided. SAEs were reportable for the entire duration of the study.

On the routinely schedule phone calls during the 10 day monitoring period, study personnel were to ask about AEs. It is not stated that inquiries about SAEs were to be performed on the day 21-23 phone call, but study personnel were to query for SAEs at the Day 28 visit.

RESULTS

Enrollment and Demographics

48 subjects were enrolled, 24 in each study group. No explanation is provided for the study enrollment below expected.

Differences in the groups were noted for age (13.1 vs. 11.7 years), ethnicity (Caucasian 96% vs. 75%) and gender (males 58% vs. 50%) for the FluMist vs. placebo groups, respectively. No statistical analyses were provided. The ethnicity of the enrollees for FluMist were: 96% Caucasian and 4% Hispanic and for the placebo group: 75% Caucasian, 8% Black, 8% Asian, 4% Hispanic, and 4% Native American.

Protocol Compliance

All randomized subjects completed study follow-up. Study vaccine was self-administered by 71% of subjects, and the remainder was administered by study personnel.

Six deviations were noted for Inc/Exc criteria: 1 child had a stuffy nose at baseline, 3 deviations concerned screening FEV1 not being $<80\%$ (80%, 81% and 80%), 2 deviations concerned reversibility (1 child reversed at 11.6% and 1 child reversed at $>12\%$ but at 42 minutes, not within the 30 min specified in the protocol).

Receipt of TIV

12 subjects in each group (total $n=24$) received the TIV, 23/24 were after the 10 day RE monitoring. 1 subject received TIV on Day 10. Of note, only 13 of the 48 subjects (FluMist $n=6$ and placebo $n=7$) had received TIV in the previous 5 years.

Asthma Stability

Screening Measurements

29/48 subjects (60%, FluMist- $n=14$ and placebo- $n=15$) were on a maintenance dose of inhaled steroids at Visit 1 (Day -7) and had been on the regimen for the previous 4 weeks. The FluMist

and placebo groups had comparable PFTs at Visit 1. The mean \pm SD percent predicted FEV₁ for FluMist = 73.3 \pm 6.8 and placebo = 72.9 \pm 5.7. The mean \pm SD percent FEV₁ reversibility for FluMist = 23.8 \pm 12 and placebo = 21.2 \pm 9.9.

Primary Measure of Asthma Stability

The FluMist and placebo groups had similar baseline FEV₁ and percent change in FEV₁ from baseline (mean of predicted values obtained at Day -7 and Day 0) to Visit 3.

AV010 – Change in Spirometry FEV₁ from Baseline to Visit 3

	Baseline		Visit 3		Percent Change	
	FluMist	Placebo	FluMist	Placebo	FluMist	Placebo
FEV ₁ , % Predicted						
N	24	24	24	24	24	24
Mean	74.7	76	75.3	76.4	0.2	0.4*
SD	8.7	7.6	16.8	12.2	17.9	12

* p =0.78

Secondary Measures of Asthma Stability

There were no differences between the groups in the secondary measures of spirometry (FVC, FEV₁/FVC, and FEF_{25%-75%}) at baseline, or in the mean percent change from baseline to Visit 3. 3 subjects [FluMist n=2 (8.3%), placebo n=1(4.2%)] had >15% decrease in percent predicted FEV₁ from baseline to Visit 3. Difference in proportions was 0.042 (-0.214, 0.298).

There were no significant differences between the study groups for any of the analyses of PEFR (proportion with PEFR \geq 30%, \geq 2SD, \geq 30% or \geq 2SD below the baseline OR the number of days with PEFR \geq 30% or \geq 2SD below baseline). Of note, over the entire post-vaccination period (Day 1-28), 17/24 (71%) of FluMist and 12/24 (50%) of placebo recipients had a decrease in PEFR \geq 30% or \geq 2 SD below baseline. For similar analyses performed for decreases \geq 15% or \geq 2 SD below baseline, 20/24 (83.3%) of FluMist and 13/24 (56.5%) of placebo recipients had decreases, Fishers Exact test p =0.06.

Asthma exacerbations occurred in 2 of the 24 FluMist recipients and 0 placebo recipients, and both exacerbations were temporally associated with receipt of study vaccine. For 1 FluMist subject, the exacerbation occurred on Day 3 post-vaccination and was associated with sinusitis. For the other subject, the exacerbation occurred on Day 2 and was associated with runny nose and cough (reported as REs on the diary card).

In this very small study sample, there were no differences between the groups for any of the secondary measures of asthma stability, including PEFR, daily asthma scores, nighttime awakenings, daily use of rescue medications or asthma exacerbations.

REs

All 24 of FluMist recipients and 23/24 placebo recipients returned the diary card. No subjects withdrew due to an AE. Overall, 22/24 (91.6%) of FluMist and 21/24 (87.5%) of placebo recipients experienced at least 1 RE. Runny nose was the most frequently reported RE (FluMist

– 75% and placebo – 54%.) No statistically significant differences were noted between the groups, however the rates of RE’s (other than fever and chills) were > 20% in each group.

AV010 – Selected Reactogenicity Events by Treatment Group, Post-Vaccination Day 0-10

Reactogenicity Event	FluMist	Placebo
Randomized,	N= 24	N= 24
Returned Diary Card,	N= 24	N= 23
	%	%
Fever		
Oral temp > 100°F	0	17.4
Oral temp > 101 °F	0	8.7
Oral temp > 102 ≤ 103 °F	0	1
Cough	45.8	43.5
Sore throat	41.7	56.5
Runny nose	75	56.5
Muscle aches	20.8	34.8
Tiredness	33.3	43.5
Any RE	91.7	91.3

There were no statistically significant differences between the groups for REs.

Other AEs

Three (12.5%) FluMist recipients reported other AEs in the 10 day post-vaccination period. 1 subject had 3 AEs (epistaxis, sinusitis, and gastralgia), 1 subject had URI and 1 subject had gingivitis. Five (20.8%) placebo subjects experienced 1 AE, including URI, nasal congestion, allergic conjunctivitis, ear infection and dizziness. The day of occurrence post-vaccination was not provided. The AEs of epistaxis post-FluMist and URI post-placebo were deemed by the investigator as possibly vaccine-related. This placebo recipient developed fever of 101.1°F on day 5 (associated with cough, runny nose, headache chills and muscle aches), and a viral culture was obtained, which was negative for viruses, including influenza.

SAE’s

No SAEs were reported in this trial.

This small study demonstrated the FluMist and placebo vaccines were reactogenic in subjects with moderate to severe asthma, in that 91.6% of FluMist and 87.5% of placebo recipients experienced at least 1 RE. For asthma stability, 8% of FluMist recipients had > 15% in FEV₁ decrease from baseline to Visit 3, and 71% of FluMist and 50% of placebo recipients had a decrease in PEF_R ≥ 30% or ≥ 2 SD below baseline. Also, 2 of 24 FluMist subjects had asthma exacerbations within 3 days of receipt of vaccine.

STUDY DMID #98-005

TITLE: Comparison of the Safety, Vaccine Virus Shedding, and Immunogenicity of the Influenza Virus Vaccine, Trivalent, Types A & B, Cold-Adapted (CAIV-T) Administered to HIV-Infected and Non-HIV-Infected Adults.

LOCATION: United States, two-sites; CRADA with NIAID
STUDY PERIOD: 8/21/98 – 12/7/99
PIVOTAL TRIAL: No, Phase 2 exploratory
POPULATION: Adults, 18-50 years with HIV infection and HIV negative
SAMPLE SIZE: Planned n=140; Enrolled 57 HIV-infected and 54 HIV negative adults

INTRODUCTION/DESIGN

The target population for FluMist, a live viral vaccine, is healthy children and adults; however, some asymptomatic or mildly symptomatic HIV-infected individuals may inadvertently receive the vaccine. Thus, information about the safety and tolerability of the vaccine, as well as effect on HIV replication is needed. The study was performed at University of Maryland and University of Rochester. This was a randomized, double-blind, placebo-controlled trial performed to compare the safety profile of FluMist *vs.* placebo when administered to HIV-infected adults and HIV negative adults.

OBJECTIVES

Primary

To evaluate the reactogenicity profile of FluMist compared to placebo in HIV-infected adults with asymptomatic or mildly symptomatic HIV disease (CDC Class A1-2).

Secondary

To compare the reactogenicity profile of FluMist between HIV-infected adults with asymptomatic or mildly symptomatic HIV disease (CDC Class A1-2) with HIV-negative adults.

Additional secondary objectives included evaluating: shedding of vaccine virus, effects on HIV RNA levels in plasma, CD4 counts, and immunogenicity in the HIV-infected and HIV negative study groups.

VACCINES

3. FluMist – Each 0.5 ml dose contained 10^7 TCID₅₀ of each strain of virus (2 type A and 1 type B) in normal allantoic fluid (NAF) containing sucrose-phosphate-glutamate (SPG). The recommended 1997-98 strains used were A/Shenzhen/227/95 (H1N1), A/Wuhan/395/95 (H3N2) and B/Harbin/7/94-like. Filled trivalent lot #CAF021 was used.
4. Placebo – NAF in SPG, lot CAF025 was used.

PROCEDURES

HIV-infected subjects had to have CD4 counts > 200 cells/mm³, and be on stable anti-retroviral (ARV) therapy for six weeks or stable without ARV (stable was not defined). Pre-enrollment HIV RNA levels had to be $< 10,000$. Subjects were randomized within HIV stratum in a 1:1 ratio to receive FluMist or placebo. Subjects received one 0.5 ml intranasal dose of study vaccine on Day 0, and were followed for 28-35 days post-vaccination for clinical and laboratory

observations. HIV-infected subjects had 2 additional visits, at 3 and 6 months post-vaccination to obtain HIV RNA and CD4 lab studies.

Monitoring

Subjects maintained diary card for REs for 10 days post-vaccination. Solicited REs included malaise, runny nose/nasal congestion, sore throat, cough, muscle aches, nausea/vomiting, decreased appetite, abdominal pain, and headache. Other AEs were also recorded for 10 days post-vaccination. All SAEs were to be reported through Day 28 and “vaccine-related” SAEs were to be reported for the entire study duration, but mechanisms for capture are not stated.

The subjects were evaluated at the clinical site again on Days 3-5, 7-10 and 28-35 for review of diary cards, and an interval medical history and physical exam were performed. Nose and throat swabs were obtained on these visits for influenza cultures. HIV labs (viral RNA and CD4 counts) were measured at Day 0, 7-10 (viral RNA only), Day 28-35, 3mo and 6 mo.

Concomitant Immunizations and Medications

No concomitant immunizations were permitted. HIV infected subjects were to be on stable ARV for six weeks, unless they had CD4 counts > 500 cells/mm³.

Statistical Methods

This was an exploratory study. No adjustments were made for multiple comparisons. The planned enrollment was 140 subjects, with 70 HIV-infected and 70 HIV negative participants. Reactogenicity events (REs) were calculated by the proportion of FluMist and placebo subjects who experienced each event or any event using a Fisher’s Exact test and exact 95% confidence intervals. Logistic regression was used to assess the effect of FluMist on the occurrence of each RE between HIV-infected and HIV negative participants. Linear models were used to compare log change in HIV RNA in HIV-infected subjects receiving FluMist or placebo.

RESULTS

Enrollment and Demographics

A total of 111 subjects, 57 HIV-infected and 54 HIV negative adults were enrolled.

Demographic characteristics were different between the HIV-infected and HIV negative, more were African-American (74% vs. 22%) and older (40 vs. 34.5 years) subjects were in the HIV-infected group. All of the HIV negative subjects completed the study (through Day 28-35) and 79% of HIV-infected subjects completed the study through the 6 month visit. Twelve HIV-infected subjects withdrew, and seven of the 12 were voluntary withdrawals.

Safety

All subjects with available data were considered evaluable for safety. For RE’s, the only statistically significant difference (unadjusted $p < 0.05$) between FluMist and placebo recipients occurred for runny nose/nasal congestion in both the HIV-infected subjects (respectively 61% vs. 31%), and HIV negative groups (78% vs. 44%). For all study participants, regardless of HIV status, all of the solicited REs occurred at $\geq 5\%$ rate, except fever > 100.0°F in HIV negative FluMist recipients which occurred in zero subjects.

Fifteen unsolicited events (other AEs) occurred in 13 subjects, with a rate of 9/57 (15.8%) in HIV-infected and 6/54 (11.1%) in HIV negative subjects. In HIV-infected subjects, 4 FluMist subjects reported 5 AEs and 4/29 (14%) of placebo subjects reported other AEs. Five events occurred in HIV negative subjects. Four events were respiratory and considered as possibly related to vaccination; 2 events (wheezing and sinusitis) occurred in HIV-infected FluMist recipients. One HIV-infected placebo recipient had unilateral wheezing, and one HIV negative placebo recipient had bronchitis.

SAEs

One SAE occurred during the study, which was not vaccine related. Twenty-eight days after vaccination with placebo, an HIV-infected subject who routinely used an indwelling urinary catheter was hospitalized for an urinary tract infection. No deaths occurred in the study.

Viral Shedding

One HIV-infected subject shed vaccine virus (type B) on sampling on Day 5, but he had 2 subsequent negative cultures at Day 7-10 and Day 28-35. This subject had type B specific HAI titer < 1:4 prior to vaccination. The virus was not quantified because there was no growth on subsequent culture.

HIV Viral Load and CD4+ cell counts.

HIV viral load did not increase post-vaccination. Mean CD4+ cell counts decreased from 598/mm³ to 550 mm 28 days post-vaccination in the Flumist group and from 498/mm³ to 490/mm³ in the placebo group. The geometric mean CD4 count declined in the placebo recipients (36 cells/mm³), and increased slightly (17 cells/mm³) in the FluMist recipients from Day 28-35 to 3 months. It rose slightly in both groups between 3 and 6 months.

STUDY VA #448

This Veterans' Administration study evaluated the safety and efficacy against laboratory-documented influenza following one dose of FluMist or placebo, when given with TIV, in adults \geq 50 years of age with chronic obstructive pulmonary disease (COPD) with a planned enrollment of 4000 subjects. Enrollment was in a 1:1 ratio. The safety monitoring included post-vaccination REs for 7 days, other AEs and SAEs for the study duration. At 20 VA Medical Centers, a total of 2215 subjects were enrolled, 1107 received FluMist and 1108 received placebo. The mean age was 68 years, 98% were male and the 83% were Caucasian.

This study was ongoing at the time of submission of the BLA and was not included in the original October 30, 2000 submission. In March 2001 a Safety Update Report was submitted to the BLA which included a synopsis of this study. Line listings of the SAE data from the study were provided. A summary of the line listings of the deaths is provided below.

DEATHS IN ALL TRIALS INCLUDED IN BLA

A total of 64 deaths occurred in protocols performed with FluMist, and 63 (34 in FluMist recipients and 29 in placebo recipients) of these occurred in the VA study. A line listing of the deaths was provided in the March report. Eight of the 64 deaths in male subjects occurred within 28 days of receipt of study vaccine (FluMist, n=4 and placebo, n=4). The FluMist subjects

included one child (described in the next paragraph), a 63 year old with respiratory arrest 9 days post-vaccine, a 72 year old who died 13 days post-vaccination due to pneumonia and lung cancer, and a 61 year old who died due to an accident 4 days post-vaccination. In the placebo group the 4 deaths included: an 73 year old who died due to tracheobronchitis on day 3 post-vaccine, 76 year old with respiratory failure on day 25 post-vaccine, a 59 year old with an MI 7 days post-vaccination and an 83 year old who died on day 2 post-vaccination (cause not stated).

There was one death due to bronchopneumonia in an 18 mo old African American boy, who had received frozen FluMist (same preparation as the FluMist within the BLA) in a Wyeth-Lederle-sponsored trial performed to compare frozen and liquid FluMist formulations. A full study report for this trial was not submitted to the BLA. The child received the 2nd dose of FluMist on 5/5/00, and experienced RE of runny nose for 3 days post-vaccination. Also on the 5/5/00 visit, chronic otitis media was noted and treatment with Allergex was recommended. In the CRF, this medication was recorded as starting on 5/9/00. According to the CRF, the child was clinically well until 5/28/00 when he had onset of vomiting and fever. On 5/29/00, he was taken to the clinic and was diagnosed with bronchopneumonia, received treatment with antibiotics (Pen VK) and decongestants. On 5/31/00 he had respiratory difficulties, was taken to the ER where he received IV antibiotics (ampicillin and gentamicin) and he died on 6/1/00.

Question #3. Are there adequate data to support efficacy and safety of FluMist when administered with concomitant immunizations?

No data for efficacy or safety of FluMist with concomitant immunizations in any age group have been submitted.

No data have been submitted in support of efficacy or safety for concomitant use with vaccines recommended for international travelers.

Discussion point #4. What additional information in support of safety and efficacy of FluMist vaccine should be requested?