

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Belshe RB, Edwards KM, Vesikari T, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. *N Engl J Med* 2007;356:685-96.

Supplemental Material

Methods

Identification of Influenza Virus Positive Cultures and Determination of Strain Matching

Nasal swabs for influenza virus culture were to be collected from children within 24 hours of qualifying symptoms or as soon as possible thereafter. Specimens were stabilized in viral transport media within 4 hours of collection and shipped at 2-8°C within 24-72 hours to one of four designated central virology laboratories: Quest Diagnostics Clinical Trials, Van Nuys, California, USA; Department of Microbiology, Prince of Wales Hospital, Hong Kong; Department of Virology, University of Turku, Turku, Finland; ERNVL, RVU Health Protection Agency, London, United Kingdom. Cultures were incubated at 33°C for at least 14 days, and identification of positive samples was performed according to each laboratory's standard procedures. Supernatants from positive cultures were stored at -70°C and shipped to MedImmune for strain-matching by hemagglutination inhibition (HAI) identification assay, and for genotyping and subtyping by PCR. Viruses were characterized as either antigenically similar or as not well matched to the vaccine strain. Reference antisera provided by the CDC were used to antigenically characterize isolates and a ≥ 4 -fold difference in HAI titers was considered indicative of antigenic variation between 2 viruses.

Statistics

Relative efficacy refers to the relative reduction in attack rates of culture-confirmed influenza and was defined as $(1 - RR) \times 100$; where RR is defined as the observed rate in the live attenuated vaccine group divided by the observed rate in the inactivated vaccine group.

Confidence intervals were constructed using an exact binomial method for multiple strata, conditioned upon the total number of cases, with mid-probability adjustment.¹ This method estimates the common relative risk across all strata and provides a conditional maximum likelihood point estimate with an exact confidence interval, adjusting for duration of follow-up. Data were further stratified by age at first dose (6–23, 24–35, and 36–59 months), prior influenza vaccination status (yes/no), a previous history of recurrent wheezing (yes/no), and country of residence. If the lower bound of the 95 percent confidence interval was > -30 percent, then non-inferior relative efficacy was demonstrated, and if non-inferior relative efficacy was observed, then an assessment of statistically superior relative efficacy was performed. Statistically superior relative efficacy would be shown if the lower bound of the 95 percent confidence interval was >0 percent. The primary analysis was conducted using the prespecified according-to-protocol population (randomized children who did not have a major protocol violation; Web only Supplementary Appendix Figure 1) and included all positive cultures occurring ≥ 14 days following the last required vaccination dose. Analysis of the primary end point was also done using the intent-to-treat population.

The safety population included all children who received at least one dose of study vaccine. The safety comparison of primary interest was occurrence of medically significant wheezing (MSW) from receipt of dose 1 (of either vaccine) through 42 days after the last dose. MSW was assessed by constructing a 2-sided 95 percent confidence interval for the rate difference of live attenuated vaccine minus inactivated vaccine. Confidence intervals and p-values were computed using the asymptotic method based on inverting a score test for the common rate difference across strata.² Constrained estimates of variability were constructed for each stratum and were combined to form a weighted chi-square value. The strata were weighted using an iterative technique to

produce weights proportional to the amount of comparative information within each stratum. MSW and other safety end points were summarized for both treatments by prior vaccination history and by dose. P-values for post-hoc safety comparisons (e.g. hospitalizations) were calculated by inverting two 1-sided tests based on asymptotic methods using StatXact PROCs v6.2.

References to Statistics

1. Guess HA, Lydick EG, Small RD, Miller LP. Epidemiologic programs for computers and calculators: exact binomial confidence intervals for the relative risk in follow-up studies with sparsely stratified incidence density data. *Am J Epidemiol* 1987;125:340-7.
2. Miettinen O, Nurminen M. Comparative analysis of two rates. *Stat Med* 1985;4:213-26.

Supplemental Table 1**Relative Efficacy of Live Attenuated Vaccine to Inactivated Vaccine against Otitis Media and LRI with Culture Confirmed Influenza.**

	Live Attenuated Vaccine			Inactivated Vaccine			95% Exact CI	
	N	# of Cases	Crude Attack Rate (cases/N)	N	# of Cases	Crude Attack Rate (cases/N)	Relative Efficacy ^a	for Relative Efficacy ^a
Otitis Media								
Antigenically Similar	3916	10	0.3%	3936	10	0.3%	0.4%	-146, 59.6
Antigenically Dissimilar	3916	16	0.4%	3936	43	1.1%	61.4%	32.2, 78.8
Regardless of Antigenic Match	3916	26	0.7%	3936	54	1.4%	50.6%	21.5, 69.5
LRI								
Antigenically Similar	3916	8	0.2%	3936	11	0.3%	24.5%	-89.8, 71.0
Antigenically Dissimilar	3916	8	0.2%	3936	21	0.5%	63.4%	18.9, 84.7
Regardless of Antigenic Match	3916	18	0.5%	3936	33	0.8%	45.9%	4.4, 70.2

ATP Population

a. Relative efficacy was adjusted for country, age, prior vaccination status, and wheezing history status.

Supplemental Table 2

Description of Hospitalizations Associated with Medically Significant Wheezing During Days 0-42 After Dosing – Subjects <24

Months of Age

Country	Age	Past Medical History	MSW	# Days from Previous Dose		Duration		Chest X-ray Findings	Lab Results	Treatment Received	AE Severity/ Outcome	Relation of AE to Study Product ^a
	(mos)		Preferred Term	to	Hosp Onset	Hosp	AE					
LIVE ATTENUATED VACCINE TWO DOSE GROUP												
Israel	9	None	Wheezing	13 PD1	14 PD1	3	21	Bilateral infiltrates, hyperinflation		Inhaled bronchodilators, inhaled/systemic steroids	Severe Recovered	Probably
	M		Wheezing Pneumonia	37 PD1	41 PD1	5	13	Bilateral infiltrates		Inhaled bronchodilators, inhaled steroids	Severe Recovered Moderate Recovered	Probably not
Hong Kong	20	Wheeze history noted by parent/guardian	Broncho-spasm	36 PD1	37 PD1	6	7	No consolidation	NPA negative for influenza and RSV	Inhaled bronchodilators, oral steroids, antihistamines, O ₂	Severe Resolved w/resid	Possibly

Country	Age (mos)	Past Medical History	MSW Preferred Term	# Days from Previous Dose to		Duration (dy) of		Chest X-ray Findings	Lab Results	Treatment Received	AE Severity/ Outcome	Relation of AE to Study Product ^a
				AE Onset	Hosp Onset	Hosp	AE					
LIVE ATTENUATED VACCINE TWO DOSE GROUP (continued)												
Belgium	11	GE reflux; F Wheeze in past 12 mos	Bronchitis	35 PD2	36 PD2	13	14	No infiltrates	NPA negative for influenza, parainfluenza, RSV, rhinovirus, adenovirus, metapneumo- virus, coronavirus	Inhaled bronchodilators, inhaled steroids, decongestants	Mild Recovered	Definitely not
Belgium	21	RSV; M ≥3 wheezing illnesses	Pneumonia	1 PD2	3 PD2	4	6	Broncho- pneumonia	NPA positive for RSV	IV fluids, antipyretics, O ₂	Severe Recovered	Probably not
US	22	None	Bronchiolitis	24 PD2	24 PD2	2	5	Atelectasis, peribronchial thickening	RSV positive	Inhaled bronchodilators	Moderate Recovered	Probably not

Country	Age	Past Medical History	MSW	# Days from Previous Dose		Duration		Chest X-ray Findings	Lab Results	Treatment Received	AE Severity/ Outcome	Relation of AE to Study Product ^a
	(mos)		Preferred Term	to	AE Onset	Hosp Onset	Hosp					
INACTIVATED VACCINE TWO DOSE GROUP												
Belgium	8	RSV bronchiolitis	Bronchiolitis	8 PD2	12 PD2	5	13	No infiltrates; bronchial and peribronchial blurring	NPA positive for RSV	Inhaled bronchodilators, inhaled steroids, O ₂	Severe Recovered	Probably not
Israel	9	Wheeze in past 12 mos	Pneumonia	25 PD2	30 PD2	3	8	Bilateral infiltrates, hyperinflation	Mycoplasma pneumoniae titer 1:160	Oral steroids, antibiotics	Moderate Recovered	Probably not
Hong Kong	13	None	Bronchiolitis	0 PD2	3 PD2	4	11	Normal	NPA positive for RSV	Inhaled bronchodilators, antihistamines, antipyretics	Resolved w/resid	Definitely not

AE = adverse event; Hosp = hospitalization; IM = intramuscular; IN = intranasal; IV = intravenous; MSW = medically significant wheezing; NP = nasopharyngeal;

NPA = nasopharyngeal aspirate; PD1 = post Dose One; PD2 = post Dose Two; Resolved w/resid = resolved with residual effects; RSV = respiratory syncytial virus.

a. Relationship applies to both IN and IM products unless otherwise indicated as determined by the blinded site investigator.

Country	Age	Past	MSW	# Days from Previous		Duration		Chest X-ray	Lab	Treatment	AE	Relation of AE
	(mos)	Medical	Preferred	Dose to		(dy) of	Severity/				to Study	
Gender	History	Term	AE Onset	Hosp Onset	Hosp	AE	Findings	Results	Received	Outcome	Product ^a	
INACTIVATED VACCINE ONE DOSE GROUP												
Korea	25	None	Broncho-	33 PD1	39 PD1	4	29	Unremarkable	NPA positive	Inhaled	Moderate	Definitely not
	M		pneumonia						for RSV, negative for influenza, parainfluenza, & adenovirus	bronchodilators, decongestants/ antihistamines, IV fluids	Recovered	
Spain	51	≥3	Wheezing	4 PD1	4 PD1	4	6	Not done	Not done	Inhaled	Moderate	Probably not
	M	wheezing illnesses; diagnosed with asthma at 21 mos of age								bronchodilators, oral steroids, O ₂	Recovered	

Country	Age	Past	MSW	# Days from Previous		Duration		Chest X-ray Findings	Lab Results	Treatment Received	AE	Relation of AE
	(mos)	Medical History	Preferred Term	AE Onset	Hosp Onset	Hosp	AE				Severity/ Outcome	to Study Product ^a
INACTIVATED VACCINE TWO DOSE GROUP												
Finland	29	None	Wheezing	6 PD1	20 PD1	2	23	Atelectasis	Not done	Bronchodilators, steroids, antibiotics	Moderate Recovered	Possibly (IN) Probably not (IM)
	F											
Italy	38	None	Pneumonia	33 PD2	37 PD2	4	9	Diffuse vascular enhancement, no consolidation		Bronchodilators, inhaled steroids, antibiotics	Moderate Recovered	Probably not
	M											

AE = adverse event; Hosp = hospitalization; IM = intramuscular; IN = intranasal; IV = intravenous; MSW = medically significant wheezing; NPA = nasopharyngeal aspirate;

PD1 = post Dose One; PD2 = post Dose Two; Resolved w/resid = resolved with residual effects; RSV = respiratory syncytial virus; WBC = white blood cell.

a. Relationship applies to both IN and IM products unless otherwise indicated as determined by the blinded site investigator.

**Supplemental Table 4. Hospitalization Rates by Age Stratum Through 180 Days After Last
Vaccination**

Age (months)	Event	Live Attenuated	Inactivated Vaccine
		Vaccine n/N (%)	n/N (%)
6-11	Hospitalization for any cause	42/684 (6.1)	18/683 (2.6)
	Respiratory	22/684 (3.2)	8/683 (1.2)
	Gastrointestinal	13/684 (1.9)	8/683 (1.2)
	Hematological/Oncological	2/684 (0.3)	0/683 (0)
	Infectious	8/684 (1.2)	3/683 (0.4)
	Musculoskeletal/Trauma	2/684 (0.3)	1/683 (0.1)
	Neurological	1/684 (0.1)	0/683 (0)
	Other	1/684 (0.1)	0/683 (0)
12-59	Hospitalization for any cause	88/3495 (2.5)	101/3490 (2.9)
	Respiratory	44/3495 (1.3)	46/3490 (1.3)
	Gastrointestinal	20/3495 (0.6)	30/3490 (0.9)
	Hematological/Oncological	3/3495 (0.1)	1/3490 (0)
	Infectious	14/3495 (0.4)	5/3490 (0.1)
	Musculoskeletal/Trauma	6/3495 (0.2)	7/3490 (0.2)
	Neurological	3/3495 (0.1)	11/3490 (0.3)
	Other	5/3495 (0.1)	10/3490 (0.3)
6-59	Hospitalization for any cause	130/4179 (3.1)	119/4173 (2.9)
	Respiratory	66/4179 (1.6)	54/4173 (1.3)
	Gastrointestinal	33/4179 (0.8)	38/4173 (0.9)
	Hematological/Oncological	5/4179 (0.1)	1/4173 (0)

	Infectious	22/4179 (0.5)	8/4173 (0.2)
	Musculoskeletal/Trauma	8/4179 (0.2)	8/4173 (0.2)
	Neurological	4/4179 (0.1)	11/4173 (0.3)
	Other	6/4179 (0.1)	10/4173 (0.2)

Note: Medically significant wheezing and serious adverse events (defined in Methods) and hospitalizations were analyzed from the day for first vaccination through 180 days after the last vaccination. Categories of hospitalization are mutually exclusive, e.g., RSV pneumonia is listed under “Infectious” but not under “Respiratory”; however, children may have been hospitalized more than once or for multiple causes and may be counted in more than one category. “All cause” refers to the number of individuals who were hospitalized at least once.

Respiratory hospitalizations included the terms: acute sinusitis, acute tonsillitis, adenoidal disorder, asthma, bronchiolitis, bronchitis, bronchopneumonia, bronchospasm, croup infectious, influenza, laryngitis, mastoiditis, otitis media acute, pharyngitis, pharyngotonsillitis, pneumonia, pulmonary congestion, sinusitis, tonsillar hypertrophy, tonsillitis, upper respiratory tract infection, viral infection, viral upper respiratory tract infection, and wheezing. **Gastrointestinal**

hospitalizations included the terms: abdominal pain, appendicitis, appendicitis perforated, constipation, enterocolitis, gastritis, gastroenteritis, gastroenteritis rotavirus, gastroenteritis salmonella, gastroenteritis viral, giardiasis, and ileus.

Hematological/oncological hospitalizations included the terms: acute lymphocytic leukemia, blood stem cell harvest, Henoch-Schonlein purpura, leukocytosis, myeloid leukemia, neuroblastoma, pancytopenia, and petechiae. **Infectious** hospitalizations included the terms: adenovirus infection, exanthema subitum, hand-foot-mouth disease, herpangina, herpetic gingivostomatitis, herpetic stomatitis, lymph node abscess, lymphadenitis, osteomyelitis, otitis externa, periorbital cellulitis, pharyngitis

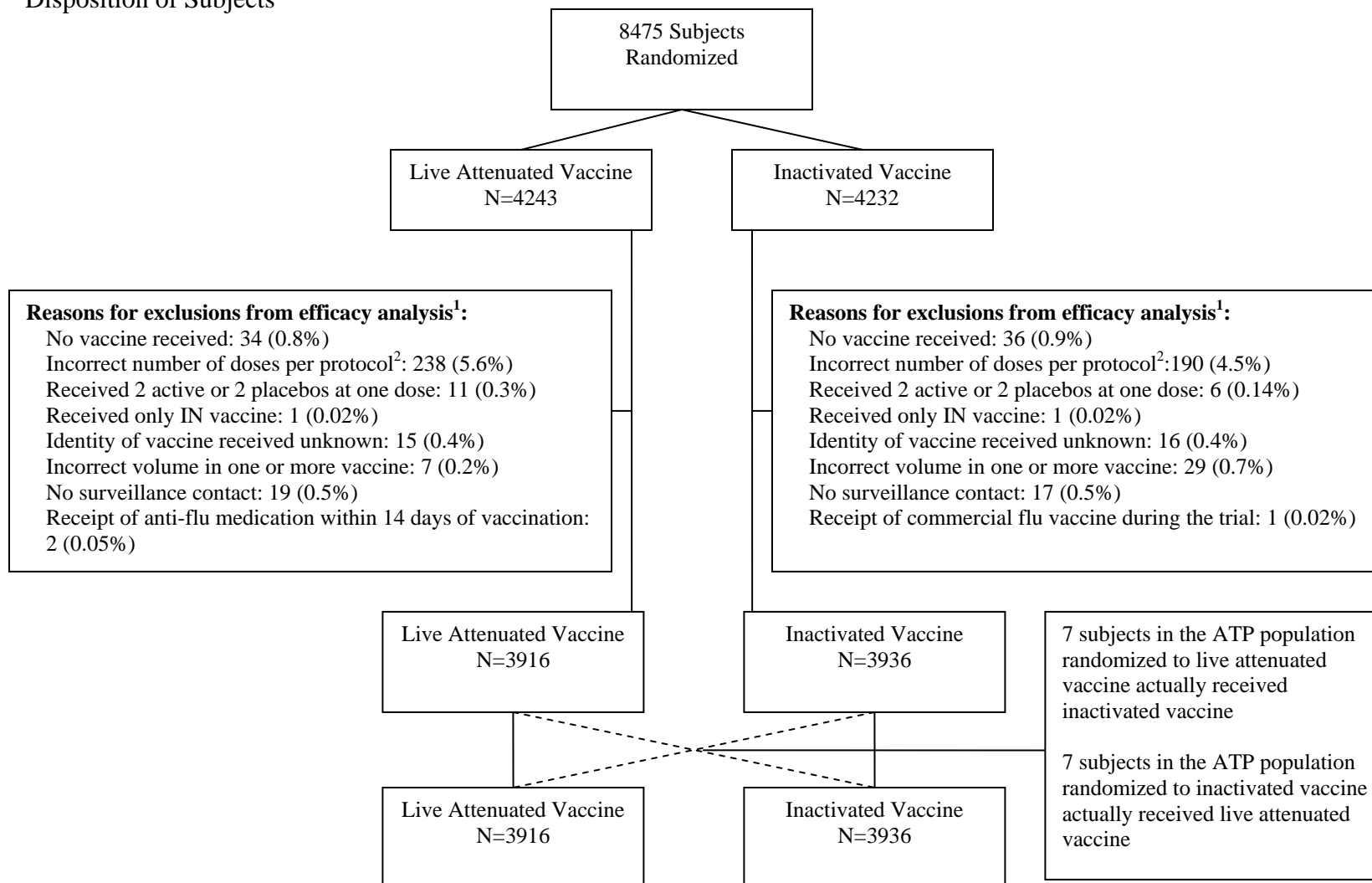
streptococcal, pneumonia respiratory syncytial viral, pyelonephritis, pyrexia, sepsis, skin bacterial infection, streptococcal infection, subcutaneous abscess, urinary tract infection, white blood cell count increased. **Musculoskeletal/trauma**

hospitalizations included the terms: animal bite, arthralgia, bone fissure, electric shock, forearm fracture, head injury, humerus fracture, injury, jaw fracture, and thermal burn. **Neurological** hospitalizations included the terms: concussion, convulsion,

epilepsy, febrile convulsion, hydrocephalus, and post-traumatic epilepsy. **Other** hospitalizations included the terms: accidental exposure, anuria, atrioventricular septal defect, catheter related complication, dehydration, drug hypersensitivity, erythema multiforme, hypoglycemia, urticaria, and urticaria generalized.

Supplemental Figure 1.

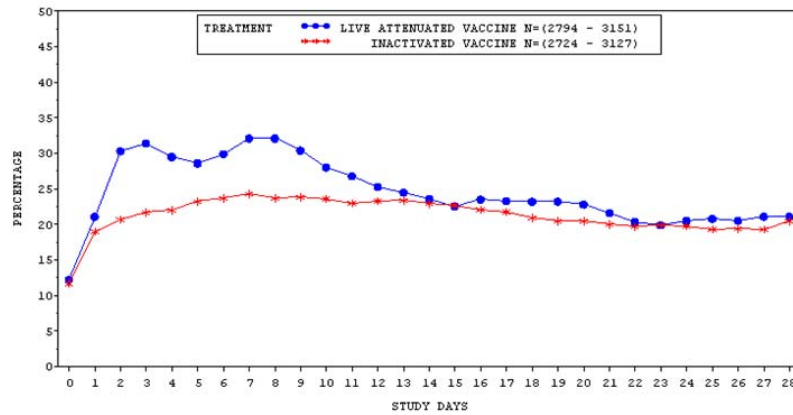
Figure 1.
Disposition of Subjects



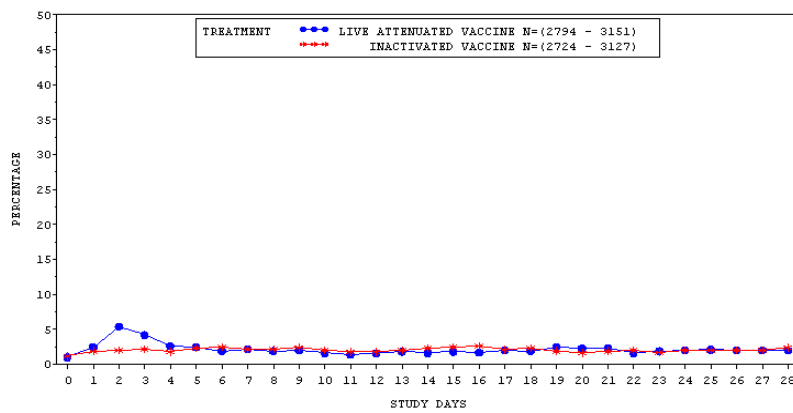
Supplemental Figure 2

The frequency and time of onset for events of runny/stuffy nose and fever for the two dose groups after dose 1 are displayed below:

Runny/Stuffy Nose

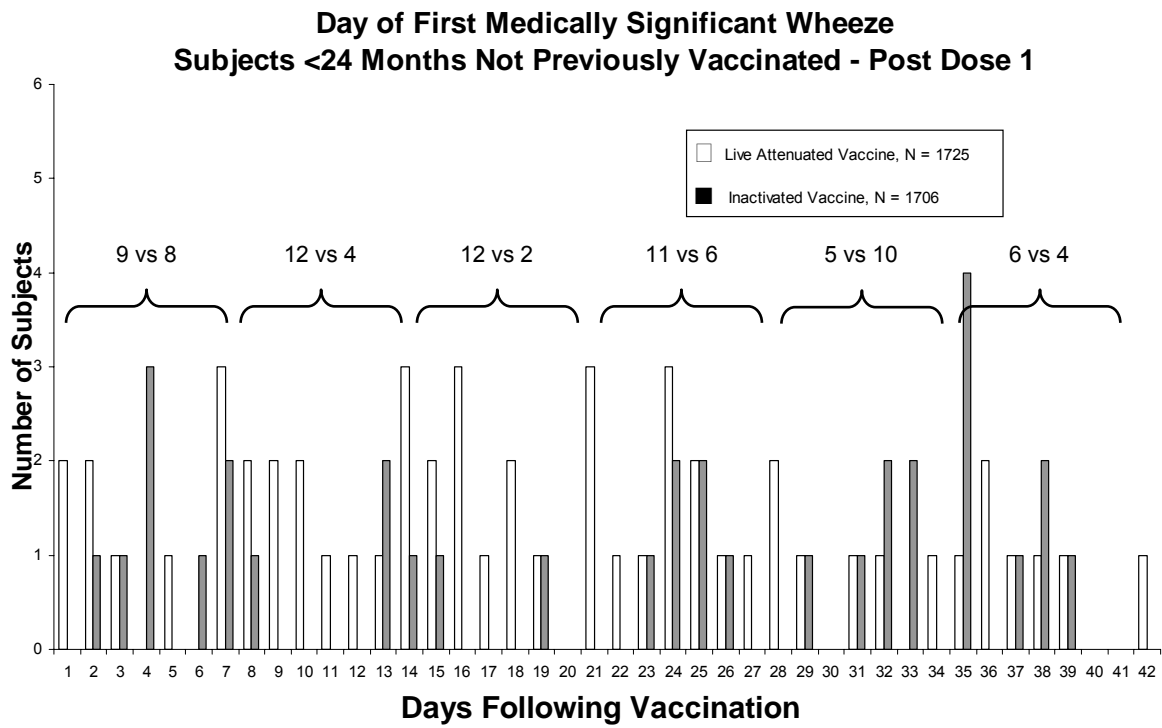


Fever (>100°F oral or equivalent)



Supplemental Figure 3

The frequency and time of onset for medically significant wheezing in children < 24 months are displayed below. Brackets and numbers represent wheezing events by week post vaccine in live attenuated vaccine vs inactivated vaccine recipients.



Supplemental Figure 4

Rates of MSW in subjects <12 months of age were 3.8% (26 subjects) and 2.1% (14 subjects) for live attenuated vaccine and inactivated vaccine (adjusted rate difference of 1.6%) respectively, compared to rates of 2.8% (29 subjects) vs. 2.0% (20 subjects) for subjects between the ages of 12 and 23 (adjusted rate difference of 0.9%). Analyses were also performed looking at the cumulative rate difference by age (e.g, rate difference for <=6 months, <=7 months, <=8 months), as displayed graphically below.

**MI-CP111: Medically Significant Wheezing (Day 0 - 42 Post Dose 1)
 Cumulative Rate Difference (Live Attenuated Vaccine - Inactivated Vaccine)
 All Subjects Not Previously Vaccinated (Safety Population)**

