

## **Statistical Analysis Plan**

### **A Phase 4 Double-blind Study to Evaluate the Shedding and Immunogenicity of Trivalent and Quadrivalent Formulations of FluMist in Children 24 to < 48 Months of Age**

**Protocol Number:** D2560C00013

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## List of Abbreviations

<b>Abbreviation or Specialized Term</b>	<b>Definition</b>
AE	adverse event
ATP	as treated population
AUC	area under the curve
CMH	Cochran-Mantel-Haenszel
CI	confidence interval
eCRF	electronic case report form
ECG	electrocardiogram
GMFR	geometric mean fold rise
GMR	geometric mean response
HAI	serum hemagglutination inhibition
Ig	immunoglobulin
IM	immunogenicity
ITT	intent-to-treat
IV	intravenous
IWRS	interactive web response system
LLOQ	lower limit of quantification
LOD	limit of detection
PK	pharmacokinetics
qRT-PCR	quantitative real-time polymerase chain reaction
SAE	serious adverse event
SAP	statistical analysis plan
SC	subject identification
SID	subject identification
WHO	World Health Organization

## **1 INTRODUCTION**

This document describes the statistical analysis for Protocol D2560C00013, a phase 4 double-blind study to evaluate the shedding and immunogenicity of trivalent and quadrivalent formulations of FluMist in children 24 to < 48 months of age.

As background information, an overview of the study design is provided. The main portion of the document details the statistical summaries relating to each study objective as well as describing the general conventions and definitions that will be used.

## **2 STUDY OVERVIEW**

### **2.1 Study Objectives**

#### **2.1.1 Primary Study Objective(s)**

To describe the level of serum hemagglutination inhibition (HAI) antibody responses induced by trivalent and quadrivalent formulations of FluMist against antigenically matched influenza strains.

#### **2.1.2 Secondary Study Objectives**

The secondary objectives of this study are:

1. To describe the proportion of individuals who shed vaccine viruses
2. To describe the duration of shedding of vaccine viruses
3. To quantify the titer of shed vaccine viruses
4. To describe the level of serum neutralizing antibody responses induced by trivalent and quadrivalent formulations of FluMist against antigenically matched influenza strains
5. To describe the level of mucosal antibody responses induced by trivalent and quadrivalent formulations of FluMist against antigenically matched influenza strains.
6. To describe the safety and tolerability of trivalent and quadrivalent formulations of FluMist.

#### **2.1.3 Exploratory Study Objectives**

The exploratory objectives of this study are:

1. To further characterize the serum and mucosal immune responses following vaccination
2. To compare results of shedding analyses using culture-based methods and quantitative real-time polymerase chain reaction (qRT-PCR).

## 2.2 Study Design

This randomized, double-blind, multi-center study will enroll approximately 200 children 24 to < 48 months of age at screening. Subjects will be randomized (~65 subjects per group) at 1:1:1 ratio to receive two doses of either 1) a quadrivalent formulation of FluMist®. Strains included in the vaccine will be based on the World Health Organization (WHO) and Vaccines and Related Biological Products Advisory Committee (VRBPAC) 2017-2018 Northern Hemisphere recommendations and will include a replacement H1N1 strain (A/Slovenia/2903/2015) and A/New Caledonia/7/2014(H3N2), B/Phuket/3073/2013 (B/Yamagata-lineage), and B/Brisbane/60/2008 (B/Victoria-lineage); 2) a second quadrivalent formulation of FluMist. Strains included in the vaccine will be based on the 2015-2016 licensed formulation of FluMist: A/Bolivia/559/2013 (H1N1), A/Switzerland/9715293/2013 (H3N2), B/Phuket/3073/2013 (B/Yamagata-lineage), and B/Brisbane/60/2008 (B/Victoria-lineage); or 3) a trivalent formulation of FluMist. Strains included in the vaccine will be based on the strains recommended by the WHO and VRBPAC for the 2015-2016 season: A/Bolivia/559/2013 (H1N1), A/Switzerland/9715293/2013 (H3N2), and B/Phuket/3073/2013 (B/Yamagata-lineage).

Subjects will be screened for the study within 75 days prior to randomization. Randomization will be stratified according to whether the subject ever received prior influenza vaccination (Yes/No). It is intended that approximately 50% of randomized subjects will not have been previously vaccinated. All subjects will receive an initial dose of investigational product at the site on Study Day 1. Subjects will receive a second dose on Study Day 28.

Nasal samples for evaluation of shedding will be obtained from each subject on Days 2, 3, 4, 5, and 7 after Dose 1 and on Days 2, 4 and 6 after Dose 2. Blood and nasal samples for the assessment of immune responses will be obtained on Day 1 prior to dosing, on Day 28 (prior to dosing) and on Day 56.

Safety evaluation will consist of the collection of solicited symptoms during the 14-day period after each dose, and AEs, SAEs, and concomitant medication use through the 28-day period post each dose. On Days 1 and 28, subjects' legal representatives will be given a temperature log to record daily temperature during the 14 days post dosing. Four telephone contacts for safety follow-up will occur between Days 15-16, Days 21-23, Days 42-44 and 48-50.

The study will be conducted during the influenza "off-season" in the US. The duration of subject participation is approximately 3 to 4 months. After completion of the study all subjects will be offered and strongly encouraged to receive one or two doses (depending on

their vaccination history) of an inactivated influenza vaccine approved for use in the US for the 2017-2018 influenza season.

### **2.3 Treatment Assignment and Blinding**

An Interactive Web Response System (IWRS) will be used for randomization to a treatment group and assignment of blinded investigational product kit and sprayer numbers. A subject is considered randomized into the study when the investigator notifies the IWRS that the subject meets eligibility criteria and the IWRS provides the assignment of blinded investigational product to the subject.

Subjects will be randomized using a 1:1:1 ratio to receive either FluMist Quadrivalent 2017-2018 formulation, FluMist Quadrivalent 2015-2016 formulation, or FluMist trivalent 2015-2016 formulation. Investigational product (FluMist Quadrivalent 2017-2018 formulation, FluMist Quadrivalent 2015-2016 formulation, or FluMist trivalent 2015-2016 formulation) must be administered the same day the investigational product is assigned. If there is a delay in the administration of investigational product such that it will not be administered within the specified timeframe, the study monitor must be notified immediately.

Subjects will receive a second dose of the same vaccine 28 days after the first dose. An IWRS will be used for assignment of the blinded investigational product kit number. Subjects are assigned the blinded investigational product kit number after the investigator notifies IWRS that the subject meets eligibility criteria for receipt of Dose 2.

### **2.4 Sample Size**

#### **HAI Immunogenicity**

It is expected that for each analysis of immunogenicity, 90% of subjects will be evaluable. The resulting sample size of ~60 evaluable subjects per arm would produce a 95% confidence interval (CI) within 14 percentage points of the observed proportion of subjects seroconverting. An estimate of 40% for the proportion of subjects seroconverting was used for the purposes of CI calculations.

This is an exploratory study. Sixty evaluable subjects per arm will provide approximately 80% of power to detect a statistically significant difference in the seroconversion rate comparing the A/Bolivia H1N1 strain (2015-2016 formulation) to the replacement H1N1 strain selected for the 2017-2018 vaccine formulation (A/Slovenia/2903/2015) assuming that the seroconversion rate in the A/Bolivia H1N1 strain (2015-2016 formulation) ranged from

10% to 40%, and that the seroconversion rate for the A/Slovenia arm was 25% higher. Table 2.4-1 summarizes the study power based on various assumptions.

<b>Table 2.4-1 Power Calculations</b>				
<b>Percentage of Subjects with Immune Response</b>		<b>Absolute Difference (%)</b>	<b>N Per Group</b>	<b>Power (%)</b>
<b>A/Bolivia Strain</b>	<b>A/Slovenia Strain</b>			
10	25	15	60	58
	30	20	60	78
	35	25	60	91
	40	30	60	97
	50	40	60	99
20	35	15	60	45
	40	20	60	67
	45	25	60	84
	50	30	60	94
30	30	15	60	39
	50	20	60	61
	55	25	60	79
	60	30	60	92
40	55	15	60	37
	60	20	60	59
	65	25	60	79
	70	30	60	92

## Shedding

It is expected that for each analysis of shedding, 90% of subjects will be evaluable. The resulting sample size of ~60 evaluable subjects per arm would produce a 95% CI within 15 percentage points of the observed proportion of subjects who shed. An estimate of 50% for the proportion of subjects shedding influenza vaccine virus was used for the purposes of sample size calculations, as this value was statistically conservative (ie, had the highest variability).

## 3 STATISTICAL METHODS

### 3.1 General Considerations

Tabular summaries will be presented by treatment group. Categorical data will be summarized by the number and percentage of subjects falling within each category. Continuous variables will be summarized by descriptive statistics, including mean, standard

deviation or error, median, minimum, and maximum. CIs will be 2-sided, unless otherwise stated.

The data analyses will be conducted using the SAS System (SAS Institute Inc., Cary, NC) Version 9.3 or above. All SAS programs used to generate analytical results will be developed and validated according to MedImmune SAS programming standards and MedImmune SAS validation procedures.

### 3.2 Analysis Populations

The analysis populations are defined in Table 3.2-1.

**Table 3.2-1 Analysis Populations**

Population	Description
Intent-to-treat (ITT) population	Subjects who are randomized will be included in the ITT population, and subjects will be analyzed according to their randomized treatment group.
As-treated population (ATP)	Subjects who receive any study investigational product will be included in the as-treated population, and subjects will be analyzed according to the treatment they actually receive regardless of treatment assigned by randomization.
Immunogenicity population	Subjects in the ATP who have no major protocol deviations judged to have the potential to interfere with the generation or interpretation of an immune response to investigational product will be included in the immunogenicity population.

### 3.3 Study Subjects

#### 3.3.1 Subject Disposition

A summary of subject eligibility and randomization as well as treatment received (including summary of subjects randomized but not treated) will be provided. In addition, disposition of subjects throughout the study with respect to completion of treatment and follow-up will be provided.

#### 3.3.2 Demographics and Baseline Characteristics

Demographic and baseline characteristics including age (in months), sex, ethnicity, race, body length, weight, and prior vaccination status will be summarized overall and by treatment group.

#### 3.3.3 Study Drug Exposure

The number of subjects who received study vaccine, and reasons for those who did not receive study vaccine will be summarized by treatment group and dose.

### **3.3.4 Concomitant Medications**

Concomitant medications will be listed.

### **3.3.5 Medical History**

Medical history data will be listed.

## **3.4 Analyses of Immunogenicity and Shedding**

### **3.4.1 Primary Endpoint and Analyses**

The primary endpoint of the study is the proportion of subjects with strain-specific HAI antibody seroconversion, defined as  $\geq 4$ -fold increase from baseline through Days 28 and 56.

The proportion of subjects who seroconverted will be summarized descriptively at Days 28 and 56 by strain and treatment for the immunogenicity population. The 95% exact CIs of the proportions will be provided.

Although this is an exploratory study, the statistical difference in the seroconversion rates at Days 28 and 56 between the A/Bolivia H1N1 strain (2015-2016) and the replacement H1N1 strain selected for the 2017-2018 vaccine formulation will be tested by the Cochran–Mantel–Haenszel (CMH) test adjusting for the prior flu vaccination status. If it is evident that the proportion is confounded with some of other demographic and baseline disease characteristic variables, the confounding variables will be adjusted. The result between the analysis adjusting for the confounding variables and the analysis without the adjustment will be compared and explained.

### **3.4.2 Secondary Endpoints and Analyses**

Secondary endpoints of the study are:

1. The proportion of subjects who shed vaccine by treatment, strain, dose number, and baseline serostatus (by qRT-PCR)
2. The number of days of shedding by treatment, strain, dose number, and baseline serostatus (by qRT-PCR)
3. Viral titer by day, strain, dose number, and baseline serostatus (by qRT-PCR)
4. The proportion of subjects with strain-specific neutralizing antibody seroconversion rates ( $\geq 4$ -fold increase) from baseline through Days 28 and 56, by baseline serostatus
5. The proportion of subjects with strain-specific nasal Immunoglobulin A (IgA) increase ( $\geq 2$ -fold increase) from baseline through Days 28 and 56
6. The proportion of subjects with any strain-specific antibody response defined as a  $\geq 4$ -fold increase in HAI antibodies or a  $\geq 4$ -fold increase neutralizing antibodies or  $\geq 2$ -fold increase in IgA antibodies

The proportions will be summarized descriptively. The 95% exact CIs of the proportions will be provided. Serum HAI, neutralizing antibody, and nasal IgA results will be summarized by strain. Shedding will be summarized for each vaccine-type influenza strain as well as for “any strain”.

The number of days of shedding will be summarized descriptively. If individual subjects are missing a significant number of shedding samples, a data convention may be constructed to address the appropriate analytical method for those subjects.

For the subset of subjects who shed, viral titers will be summarized descriptively. Area under the curve (AUC) of viral titers from the first collection date to the last collection date after each dose will be calculated using the linear trapezoidal rule.

Geometric mean response (GMRs) and geometric mean fold rises (GMFRs) of HAI antibody, neutralizing antibody, and nasal IgA will be summarized by treatment and visit for each strain.

GMR is calculated as:

$GMR = \text{anti-}\log_{10}(\text{mean}[\log_{10} X_i])$  where  $X_i$  is the assay result for subject  $i$ .  
95% CIs of GMRs will be calculated assuming log normal distribution.

GMFR is calculated as:

$GMFR = \text{anti-}\log_{10}(\text{mean}[\log_{10} Y_i / B_i])$  where  $Y_i$  is the post dose assay result for subject  $i$ ; and  $B_i$  is the baseline assay result for subject  $i$ .  
The exact 95% CIs will be provided for proportions.

For HAI antibody, neutralizing antibody, nasal IgA results reported as lower than the lower limit of quantification (LLOQ), a value equal to half of the LLOQ will be imputed in the calculation. The LLOQs for HAI titer, neutralizing antibody titer and nasal IgA antibody titer are 10, 10 and 10, respectively.

Baseline strain-specific sero-status (sero-positive/sero-negative) will be defined for HAI, neutralizing, and IgA antibodies. Seronegative is defined as HAI titer  $\leq 10$ , neutralizing antibody titer  $\leq 10$ , and IgA antibody titer  $\leq$  lower limit of quantification (LLOQ).

The correlations between the post dose assay results will be assessed by strain.

### **3.4.3 Exploratory Endpoints and Analyses**

Exploratory endpoints will be based on results from newly identified specific assays and viral shedding tested by culture-based methods. The exploratory endpoints are:

1. Endpoints determined based on newly identified specific assays to characterize immune responses
2. The proportion of subjects who shed vaccine by treatment, strain, dose number, and baseline serostatus (by culture-based methods)
3. The number of days of shedding by treatment, strain, dose number, and baseline serostatus (by culture-based methods)
4. Viral titer by day, strain, dose number, and baseline serostatus (by culture-based methods).

The analyses of the exploratory endpoints will be conducted similarly as described in Section 3.4.2.

### **3.4.4 Subgroup Analyses**

Subgroup analysis by baseline serostatus and prior flu vaccination status will be conducted for the primary and secondary endpoints. For the IgA antibody titer analyses, strain-specific subgroup analyses will be conducted by baseline HAI serostatus, and microneutralization antibody serostatus.

## **3.5 Safety Analyses**

### **3.5.1 Adverse Events and Serious Adverse Events**

Treatment-emergent adverse events (AEs), and serious adverse events (SAEs) will be coded by most recent version of MedDRA. Specific adverse events will be counted once for each subject when calculating percentages. In addition, if the same adverse event occurs multiple times within a particular subject, the highest severity and level of relationship observed will be reported. All treatment-emergent adverse events will be summarized by System organ class (SOC) and preferred term (PT), and by treatment and dose. Summaries of treatment-emergent AEs and SAEs by severity and relationship to investigational product will be provided.

### **3.5.2 Solicited Symptoms through Day 14 Post Dose**

Solicited symptoms are events that are considered likely to occur post dosing. For this study, solicited symptoms include:

- Fever  $\geq 100.4^{\circ}\text{F}$  ( $38.0^{\circ}\text{C}$ ) by any route
- Runny/stuffy nose
- Sore throat

- Cough
- Headache
- Generalized muscle aches
- Decreased activity level (lethargy) OR tiredness/weakness
- Decreased appetite

Solicited symptoms are collected for 14 days after each dose. Collection of specific solicited symptoms (sore throat, headache, generalized muscle aches) will be omitted when, according to the judgment of the investigator, the subject is too young to reliably report a particular symptom.

The following solicited symptom summaries will be provided by treatment and dose.

- The number and percentage of subjects with at least one solicited symptoms through Day 14 after each dosing;
- The number and percentage of subjects who experienced each solicited symptom by study day;
- Number of days each solicited symptom was experienced.

In addition, summary of fever will be broken down by temperature with 1°F increment.

### **3.5.3 Other Safety Evaluations**

#### **3.5.3.1 Physical Examination Including Weight, Height and Vital Signs**

The results of a physical exam including weight, height and vital signs will be listed.