CONFIDENTIAL

Statistical Analysis Plan
for IMPAACT 2012 and CIR 312

ClinicalTrials.gov Identifier: NCT02890381

Phase I Placebo-Controlled Study of the Infectivity, Safety and Immunogenicity of a Single Dose of a Recombinant Live-Attenuated Respiratory Syncytial Virus Vaccine, LID cp ΔM2-2, Lot RSV#009B, Delivered as Nose Drops to RSV-Seronegative Infants 6 to 24 Months of Age

This is IMPAACT 2012 SAP Version 1.0 with names of authors, names of publication writing team members and analysis timeline redacted. The Appendix includes details of the analyses that were pre-specified in the study protocol.

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1 Introduction

This document describes the interim statistical analysis of the safety data for IMPAACT 2012. The data are presented separately for the first 56 days on study and for the RSV Season Surveillance Period. The plan includes analyses that will be sent to the DSMB for review. An outline of analyses for other study objectives will be presented in a separate document.

This document and the analyses will be reviewed by the IMPAACT 2012 core team (also called the Protocol Safety Review Team or PSRT) which includes the Study Chair or Vice-Chair, DAIDS or NICHD Medical Officer, Protocol Statistician and Protocol Data Managers (PDMs) and Laboratory Data Managers (LDMs). Designees for PSRT members will be allowed in the event of their non-availability for a review.

2 Study Schema and Objectives

DESIGN: A double-blind, randomized, placebo-controlled study design will be used to evaluate the safety and immunogenicity of the vaccine in RSV-seronegative infants and children. Seronegativity refers to RSV antibody status, which is defined as a serum RSV-neutralizing antibody titer <1:40 (as determined within 42 days prior to inoculation).

SAMPLE SIZE: Approximately 33 healthy RSV-seronegative infants and children ≥6 months (180 days) to <25 months (750 days) of age will be randomized at a ratio of 2:1 to receive vaccine or placebo, respectively.

REGIMEN: A single dose of the recombinant live-attenuated respiratory syncytial virus vaccine RSV cps2 or placebo at study entry.

N	Treatment	Dose
22	Vaccine	10^5 PFU*
11	Placebo	0

*plaque forming units

Study Duration: Participants will be enrolled in the study between April 1st and October 14th (outside of RSV season) and will remain on study until they complete the post-RSV season visit between April 1st and April 30th in the calendar year following enrollment. For example, a participant enrolled on July 1st, 2016 will remain on study approximately 9-10 months (completing a final visit in April 2017) while a participant enrolled on October 14th, 2016 will remain on study approximately 6 months (also completing a final visit in April 2017).

This protocol is a companion study to CIR 312, a study being conducted by the Center for Inoculation Research (CIR, Johns Hopkins, Baltimore), and the Laboratory of Infectious Diseases (NIAID, Bethesda). The protocols have identical primary and secondary objectives; inoculation schedules; evaluation assays and schedules; safety monitoring and reporting.

OBJECTIVES:

Primary Objectives:

- Objective 1. Safety: To assess the frequency and severity of study product-related solicited and unsolicited adverse events (AEs), from Day 0 through midnight of the 28th day following inoculation, in vaccinated participants
- Objective 2. Safety: To assess study product-related serious adverse events (SAEs) from Day 0 through midnight on the 56th day following inoculation for vaccinated participants
- Objective 3. Infectivity: To determine the peak titer of vaccine virus shed and duration of virus shedding by each participant, where the primary aim is to check if the mean peak titer of shed virus in nasal washes is approximately 2.5 $\log_{10}$
- Objective 4. Infectivity: To assess the proportion of vaccinated infants infected with study vaccine, where the primary aim is to check whether >90% of vaccinees shed vaccine virus detected by infectivity assay
- Objective 5. Immunogenicity: To characterize antibody responses (Day 56) to the study vaccine

Secondary Objectives:

- Objective 1. To characterize clinical outcomes (frequency and severity of symptomatic, medically attended respiratory and febrile illness) in the vaccine and placebo recipients who experience natural infection with wild-type (wt) RSV during the subsequent RSV season
- Objective 2. To characterize antibody responses in the vaccine and placebo recipients who experience natural infection with wt RSV during the subsequent RSV season
- Objective 3. To characterize the B cell response to vaccine and the quality and epitope specificity of RSV F specific antibody, and to characterize these responses in the vaccine and placebo recipients who experience natural infection to wt RSV during the subsequent RSV season

Safety Monitoring:

Infants and children enrolled in the study will be monitored for the following (see table below which is also Table 3 from the protocol document; the tables and sections it refers to are from the protocol as well):

Table 1: AE CRF Recording Requirements (Table 3 from the protocol)

Study Phase at the time of event onset	Calendar Date	AEs to record on CRFs	Concomitant Medications to record on CRFs
Days 0 through midnight of 28th day following inoculation (Acute Phase)	ANY	• All SAEs • All solicited AEs that meet Appendix IV criteria • All unsolicited AEs (Grades 1 to 4), with the exception of the following conditions if not treated with prescription medication or OTC medications with antipyretic properties: diaper rashes, teething pain, and spitting up. Note: SAEs and LRIs must be reported via DAIDS Adverse Experience Reporting System (DAERS; see Section 7.3.4).	• All cough and cold remedies including decongestants, cough suppressants, expectorants • All nasal sprays (except saline spray) • All antihistamines • All antipyretics • All prescription medications For SAE and LRIs: • All concomitant medications related to the recorded event
From 12:01 am on 29th day after inoculation to midnight of the 56th day following inoculation (Post-Acute Phase)	ANY	• All SAEs Note: SAEs must be reported via DAERS (see Section 7.3.4).	• All concomitant medications related to the recorded event
After Day 56 Visit and until RSV Season Surveillance Period	Up to October 31st in year of inoculation	• Grade ≥3 AE or SAE that is deemed related to Pre-RSV Season Study Visit procedures.	• All concomitant medications related to the recorded event
RSV Season Surveillance Period	November 1st to March 31st following inoculation	• Fever, LRI, URI, and/or otitis media that are medically attended • All SAEs Note: these events do not need to meet the Appendix IV criteria. SAEs and medically attended LRIs must be reported via DAERS (see Section 7.3.4).	For SAE and LRIs: • All concomitant medications related to the recorded event Medications related to recorded medically attended illness should be documented in source notes.
Post-RSV Season	April 1st-April 30th in the year after the inoculation	• Grade ≥3 AE or SAE that is deemed related to Post-RSV Season Study Visit procedures.	• All concomitant medications related to the recorded event
Throughout study	ANY	• Unresolved AE or SAE with onset date during Day 0 to midnight on the 28th day after inoculation • Unresolved SAE with onset date prior to midnight on the 56th day following inoculation • Unresolved SAE with onset date during RSV Surveillance Period or related to the Pre- or Post-RSV Season Study Visit	• All concomitant medications related to the recorded event

3 Analysis principles

As requested by the DSMB for previous studies (P1114 and 2000), the data for this review will be summarized with the treatment arms pooled, so blinding will be maintained. All subjects who received inoculation (vaccine or placebo) will be included in these safety analyses.

4 Accrual

The following tables will be presented or described in text (based on the *status* study dataset).

a. Table with number enrolled
b. Table with enrollment by month
c. Table with enrollment by site

5 Study status

a. Table including number enrolled, number off treatment and still on study, and number off study with reasons for early discontinuation (based on the *pstat1* variable from the *status* study dataset)

The following tables will be restricted to only the participants who received inoculation.

6 Selected characteristics of participants at baseline

a. Table showing n and % for gender, race, ethnicity (from the *status* study dataset)
b. Table showing mean, standard deviation (SD), median, 25^{th}-75^{th} percentile, min, max for age (in months) at randomization

7 Days between randomization and inoculation

a. Table showing the number of days between randomization and inoculation, to check if the inoculation was received as requested, within 3 days of randomization.

8 Safety Primary Objectives

Objective 1: Safety: To assess the frequency and severity of study product-related solicited and unsolicited adverse events (AEs), from Day 0 through midnight of the 28^{th} day following inoculation, in vaccinated participants

Objective 2: Safety: To assess study product-related serious adverse events (SAEs) from Day 0 through midnight on the 56^{th} day following inoculation for vaccinated participants

Notes:

1. The protocol requires grading of events according to The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009), which is available on the RSC website: http://rsc.tech-res.com/safetyandpharmacovigilance

   With the exception of the solicited events which will be graded using a study-specific grading table (see Tables 2-4 at the end of this document).

2. The protocol does not require the collection of any laboratory evaluations, but abnormal values reported from non-study mandated blood draws will be recorded in the database. The protocol requires all adverse events and solicited events to be recorded on CRFs and reported according to Table 1 above (Table 3 in the protocol). Solicited events are: fever, acute otitis media, systemic illness, URI (rhinorrhea, cough without lower respiratory tract illness, hoarseness, pharyngitis), LRI (wheezing, rhonchi, rales, pneumonia, laryngotracheobronchitis (croup)).

3. Analyses will be based on events reported on the following forms: PE6833 (signs/symptoms) and PE6853 (diagnoses) (and PE6813 (hematology), PE6818 (chemistry) – if any data were reported on them) and will not explicitly include events reported as SAEs to DAIDS. Before the database is finalized, the data manager will ensure that all SAE events deemed "reportable" and included in the SDMC AR001AES table are reflected on the study CRFs. The data manager will also ensure that all events recorded on the Event Evaluation form (PE6866) are also documented on the source forms (signs/symptoms, diagnosis, hematology, or chemistry forms).

4. The adverse events with date of onset or specimen date prior to the inoculation and which are still ongoing at the time of the inoculation will be presented in a separate table, and then they will be excluded from subsequent tables summarizing adverse events.

a. Signs, symptoms and diagnoses:

The tables described in this section will be generated for the first 56 days after inoculation and then separately for the RSV Season Surveillance Period.

i. Line listing of subjects with signs, symptoms or diagnoses **present at the time of inoculation** discussed by the team on conference calls (from the *events* study dataset): patient ID, date of receipt of study agent, days from inoculation to onset of AE, abnormality, grade, relationship to study treatment (from *tra*c study dataset), onset date and end date

ii. Line listing of subjects with signs, symptoms or diagnoses **occurring after inoculation** discussed by the team on conference calls (from the *events* study dataset): patient ID, date of receipt of study agent, days from inoculation to onset of AE, abnormality, grade, relationship to study treatment (from *trac* study dataset), onset date and end date

iii. Line listing of subjects with the subset of signs, symptoms or diagnoses discussed by the team on conference calls and **which were related, possibly related, or probably related to study treatment** (from the *events* study dataset): patient ID, date of receipt of study agent, days from inoculation to onset of AE, abnormality, grade, relationship to study treatment (from *trac* study dataset), onset date and end date

iv. Line listing of subjects with signs, symptoms or diagnoses discussed by the team on conference calls **of grade≥3** (from the *events* study dataset): patient ID, date of receipt of

study agent, days from inoculation to onset of AE, abnormality, grade, relationship to study treatment (from *trac* study dataset), onset date and end date

v. Summary table with n (%) of subjects experiencing at least one episode of an adverse event, counting the worst (highest) grade adverse event by MedDRA System Organ Class (SOC) and Preferred Term (PT) (applying the CBAR *eventsum* macro on the study derived events dataset)

vi. List of all serious adverse events reported to Regulatory Safety Center (RSC) and NIAID DSMB [in Appendix]

vii. Table with number (%) of missed visits/contacts during the study (separated by acute vs RSV surveillance portion)

b. Hospitalizations (if any):

i. List: patient ID, week, days in hospital, diagnosis

c. Concomitant Medications:

i. Line listing of patients and the medications received during the study (using the *pe0414* dataset): patient ID, date of inoculation, drug code, drug name, start date, stop date, drug frequency, drug indication, drug unit, adverse events occurring during this time period, onset date, resolution date.

d. Deaths (if any):

i. List: days/weeks since inoculation, site's report of primary cause of death, site's determination of relatedness to study treatment. More detailed information on each death will be included in an Appendix.

Table 2: Definitions of Solicited Adverse Events (Solicited AEs) (Appendix IV from the protocol)

Event	Defined
Fever	Temporal temperatures ≥100.0°F unconfirmed by rectal temp -or- Rectal temperature of ≥100.4°F.
Acute Otitis Media[1]	Loss of tympanic membrane landmarks, accompanied by erythema and loss of mobility. May or may not be associated with fever or other respiratory symptoms. Confirmed with tympanometry if possible.
Upper Respiratory Tract Illness (URI)	
Rhinorrhea	Two or more consecutive days of clear or purulent discharge from the nares. Note: Not associated with crying, change of room temperature, or eating and drinking.
Pharyngitis	Two or more consecutive days of pharyngeal erythema accompanied by exudate, and/or enlarged tender lymph nodes. Note: May be associated with sore throat, or painful or difficult swallowing.
Cough without LRI	Two or more consecutive days of 3 or more episodes of cough during a 15-minute timed observation period, or cough awakens child from sleep. Note: Not associated with eating, drinking or choking.
Hoarseness	An unnaturally deep or rough quality of voice.
Lower Respiratory Tract Illness (LRI)	
Wheezing[2,3]	Sustained, high pitched, musical breath sounds, especially during the expiratory phase, which do not clear with cough.
Pneumonia[1,2,3]	Rales and crackles, originating in the lower respiratory tract, usually accompanied by tachypnea, which do not clear with cough. May be confirmed by x-ray showing areas of consolidation.
Laryngotracheobronchitis (croup) [1,2,3]	Barking cough, hoarseness, and inspiratory stridor
Rhonchi[2,3]	Coarse breath sounds which are not transmitted noises from the upper airway and do not clear with cough.
Rales[2,3]	Abnormal lung sound heard through a stethoscope. Rales may be sibilant (whistling), dry (crackling) or wet (more sloshy) depending on the amount and density of fluid refluxing back and forth in the air passages.

1 Diagnosis must be made by a medical professional
2 Must be sustained over 20 minutes.
3 Clinical assessment must be made by a medical professional and confirmed by a second medical professional, if possible.

Table 3: IMPAACT 2012-specific AE Grading Table for solicited adverse events (Table 4 from the protocol)

Severity	Defined
Grade (0) None	Not applicable
Grade (1) Mild	No medical intervention required; may include use of over-the-counter medications managed by the caregiver for treatment of symptoms
Grade (2) Moderate	Outpatient medical intervention by a health care provider required; may include use of over-the-counter and/or prescription medications.
Grade (3) Severe	Prolonged medical intervention and/or hospitalization required
Grade (4) Life-threatening	Illness requiring hospitalization with intensive care
Grade (5) Death	Event resulting in fatal outcome to the participant

Table 4: IMPAACT 2012-specific Fever Grading: Temperature Measurement (Table 5 from the protocol)

Severity	Defined
Grade (0)	≥100.0ºF but <100.4ºF (≥37.8ºC but <38ºC)
Grade (1)	≥100.4ºF but ≤101.4ºF (≥38ºC but <38.6ºC)
Grade (2)	≥101.5ºF but ≤102.4ºF (≥38.6ºC but ≤39.1ºC)
Grade (3)	≥102.5ºF but ≤104.8ºF (≥39.2ºC but ≤40.4ºC)
Grade (4)	≥104.9ºF (≥40.5ºC)

Appendix containing Section 9.5 from Protocol Version 1.0 dated August 8, 2016

9.5 Analyses

9.5.1 Assessment of Primary Objectives

Safety data from all study participants who have been inoculated will be summarized, including data from participants who discontinue study early or have some missed visits. In the immunogenicity analyses, those who do not provide data for the Day 56 Visit follow-up (due to early discontinuation or missed visit) will be treated as "failures". Sensitivity analyses will be performed to check if the results are consistent with those when these participants are excluded. Participants who receive any of the disallowed treatments listed in Section 5.11 after inoculation may be excluded from the immunogenicity evaluations after the time of the treatment. These participants will, however, be included in the safety evaluations for the duration of the study. These participants will not be replaced. Details of the analyses listed below will be included in the Statistical Analysis Plan.

The frequency of solicited AEs and unsolicited AEs, along with 90% confidence intervals, during Study Days 0 to 28 and of vaccine-related SAE during Study Day 0 to the Day 56 Visit will be summarized. In addition, line listing of individual clinical solicited AEs and unsolicited AEs during Study Days 0 to 28 and vaccine-related SAE during Study Day 0 to the Day 56 Visit, graded by severity, will be prepared.

The proportion of participants with infection defined as recovery of vaccine virus from a nasal wash and/or a $\geq$ fourfold rise in neutralizing antibody titer, as determined by culture and RT-PCR, will be summarized. A line listing of the individual peak titer of vaccine virus shed and duration of virus shedding in nasal washes by each individual will be prepared. In addition, the geometric mean peak titer and mean duration of virus shed will be provided, for each treatment group.

The proportion of participants that develop fourfold or greater rises in RSV-neutralizing antibody titer following vaccination will be summarized. A line listing of the individual RSV antibody titer pre- and post-vaccination will be prepared. In addition, the geometric mean and median antibody titers will be provided, for each treatment group. Line listings of individual RSV-neutralizing antibody responses as well as of antibody responses to the RSV F glycoprotein will be prepared as well.

Where appropriate, the Wilcoxon rank sum test will be used to determine the statistical significance of differences between vaccine and placebo recipients. A 1-tailed Fisher's exact test will be used to test the hypothesis that the vaccinated group will exhibit a greater proportion of participants who develop fourfold or greater rises in RSV-neutralizing antibody titer following vaccination compared to the placebo group.

These will be the only formal statistical comparisons between the vaccinated and placebo groups. These tests will be carried out at a 5% significance level.

The study results will be compared with the criteria listed in Section 1.1 to determine if this vaccine is a promising candidate for further evaluation in expanded Phase I studies or Phase II studies.

9.5.2 Assessment of Secondary Objectives

The summary of the frequency and severity of symptomatic, medically attended respiratory and febrile illness in the vaccine and placebo recipients who experience natural infection with wt RSV during the subsequent RSV season will be presented. A line listing of the individual RSV antibody titer pre- and post-RSV Season Surveillance Period will be prepared. In addition, the geometric mean and median antibody titers will be provided for each treatment group. The B cell responses to vaccine as well as the quality and epitope specificity of RSV F specific antibody will be summarized for each treatment group.