Phase I Study of the Infectivity, Safety and Immunogenicity of Two Recombinant, Live-Attenuated, Bovine/Human, Parainfluenza Virus Type 3 (B/HPIV3) Vectored Vaccines Expressing the Fusion Glycoprotein of Human Metapneumovirus (HMPV), Delivered by Nasal Spray to HPIV3-Seropositive Children 24 to <60 Months of Age

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B/HPIV3/HMPV-PreF-A
B/HPIV3/HMPV-F-B365
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SHORT TITLE	**B/HPIV3/HMPV**
PURPOSE	Assessment of the safety and immunogenicity of the live-attenuated pediatric respiratory virus vaccine candidates B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 in HPIV3-seropositive children 24 to <60 months of age
DESIGN	A blinded, randomized, placebo-controlled study design will be used to evaluate the safety and immunogenicity of the study product in HPIV3-seropositive participants. Participants will be randomized to receive B/HPIV3/HMPV-PreF-A vaccine, B/HPIV3/HMPV-F-B365 vaccine or placebo in a 2:2:1 ratio.
STUDY POPULATION	Healthy HPIV3-seropositive* children 24 to < 60 months of age *HPIV3-seropositive: HPIV3 hemagglutination-inhibition (HAI) antibody titer > 1:8, determined prior to inoculation and within the calendar year of inoculation.
SAMPLE SIZE	Approximately 25 HPIV3-seropositive children It is expected that up to 50 children will need to be serologically screened to accrue 25 HPIV3-seropositive participants.
STUDY PRODUCT	Single dose of intranasal B/HPIV3/HMPV-PreF-A vaccine, B/HPIV3/HMPV-F-B365 vaccine, or placebo, delivered by Vax300 VaxINator atomization device (Teleflex, Inc).

Table 1: Inoculation Schedule

HPIV3 Serostatus	N	Study Product	Dose
HPIV3 seropositive 24 to < 60 months-old	10	B/HPIV3/HMPV-PreF-A	$10^{5.6}$ PFU*
	10	B/HPIV3/HMPV-F-B365	$10^{5.6}$ PFU*
	5	Placebo	0

* Plaque-forming units (PFU)

HPIV3-seropositive children will have a study visit with a nasal swab on study days 0, 3, 4, 5, 6, 7, 10 and 14 ± 1 day and day 28 + 7 days. A serum specimen will be obtained pre-inoculation and post-inoculation at day 28 + 7 days. Participants will be contacted monthly between days 29 and 180 and will also have a remote follow-up on day 180 ± 7 days.

After completion of the Day 28 visit of the last participant enrolled, there will be an unblinded Data and Safety Monitoring Board (DSMB) review of safety and vaccine shedding data (Section 9.4.2).

STUDY DURATION

The expected duration of the study is about 180 days.

1 BACKGROUND

1.1 Epidemiology, Disease Burden, and the Need for a Vaccine

Human metapneumovirus (HMPV) and human parainfluenza virus type 3 (HPIV3) are two major viral pediatric pathogens, causing respiratory tract illness in infants and young children worldwide. Human parainfluenza virus type 3 (HPIV3) accounts for at least 11,000 pediatric hospitalizations annually in the United States and is an important cause of acute lower respiratory illness (ALRI) in infants and young children worldwide (1) (2) (3). Like respiratory syncytial virus (RSV), HPIV3 can cause bronchitis, croup (with hoarseness, cough and stridor), bronchiolitis, or viral pneumonia (4) and has been associated with wheezing episodes in older children with asthma (2). Although precise estimates do not exist, the outpatient burden of HPIV3 is likely substantial, since the number of emergency room and outpatient visits for HPIV3-associated illness may exceed hospitalizations by 10–80- fold. Globally, parainfluenza viruses are estimated to be associated with ~18·8 million ALRI cases, 725 000 ALRI hospital admissions, and ALRI deaths among children younger than 5 years (3).

HMPV was first reported in 2001, although serologic studies have confirmed its global circulation in humans for at least 50 years (5-8). Like RSV, HMPV causes upper respiratory illness (URI), otitis media, and LRI, including bronchiolitis, croup, pneumonia, and exacerbations of asthma in infants and young children (9, 10). The mean age of HMPV-infected infants is typically older than that of RSV-infected infants (11). In the United States, HMPV is associated with 5% to 13% of pediatric hospitalizations for respiratory illness and up to 12% of medically attended LRIs in children (10). HMPV is the second or third leading cause of viral LRI in children [(9-11) (12) and can also cause severe disease in premature infants and in elderly and immunocompromised people(11). HMPV has a single serotype with two subgroups, A and B (13). Globally, HMPV is estimated to be associated with ~14 million cases of LRI and 643,000 hospitalizations annually in children < 5 years (14).

Although HPIV3 and HMPV are major pediatric respiratory pathogens, neither a licensed HPIV3 vaccine nor a licensed HMPV vaccine exists. Development of a vaccine that could simultaneously protect against each of these pathogens could substantially reduce the global burden of acute pediatric respiratory disease.

1.2 Vaccine Descriptions

B/HPIV3/HMPV-PreF-A or B/HPIV3/HMPV-F-B365 are complementary DNA (cDNA) derived live-attenuated vaccine candidates. These candidates are based on a chimeric bovine/human parainfluenza virus type 3 (B/HPIV3) vector. B/HPIV3 is a live chimeric parainfluenza virus type 3 that contains the N, P, M and L genes of bovine PIV3 (strain Kansas), and the fusion (F) and hemagglutinin-neuraminidase (HN) genes of human PIV3 (HPIV3; strain JS) (15, 16). The HPIV3 strain JS was avirulent and poorly infectious in healthy adults with low pre-existing antibody titers to HPIV3 (17). The B/HPIV3 vector is attenuated in humans due to the host-range restriction conferred by the N, P, M, and L genes derived from bovine PIV3 (15). B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 encode stabilized versions of the fusion protein F derived from HMPV subgroup A and B, respectively, the major neutralization antigen of HMPV.

B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 were designed as bivalent live vaccines for intranasal immunization against two major pediatric respiratory pathogens, HMPV and HPIV3. The stabilized HMPV-PreF-A and HMPV-F-B365 antigens for inclusion in the B/HPIV3 vaccine vectors were selected based on their improved immunogenicity compared to their corresponding non-stabilized versions following intramuscular administration in rodents (HMPV-PreF-A) or rodents and non-human primates (HMPV-F-B365) (18).

(i) B/HPIV3/HMPV-PreF-A contains an additional gene, inserted between the N and P genes (Figure 1), encoding the fusion protein F of HMPV subgroup A with two amino acid substitutions (N46V and T160F) that stabilize this HMPV F protein in its prefusion (pre) conformation. In preclinical studies in rodents, this version of the HMPV PreF antigen was more immunogenic for induction of HMPV-neutralizing antibodies than the non-modified counterpart.

(ii) B/HPIV3/HMPV-F-B365 contains an additional gene, inserted between the N and P genes (Figure 1), which encodes the F protein of HMPV subgroup B. The HMPV F antigen encoded by B/HPIV3, F-B365, contains a 24-amino acid (aa) deletion of the F1/F2 cleavage site, which was replaced by a 6-aa amino acid linker (resulting in a net deletion of 18 aa). In addition, F-B365 contains 7 aa substitutions which result in inter- and intraprotomer stabilization of this F protein in its prefusion (PreF) conformation, strongly increasing its immunogenicity in rodents, with a moderate increase in immunogenicity detected in macaques (18).

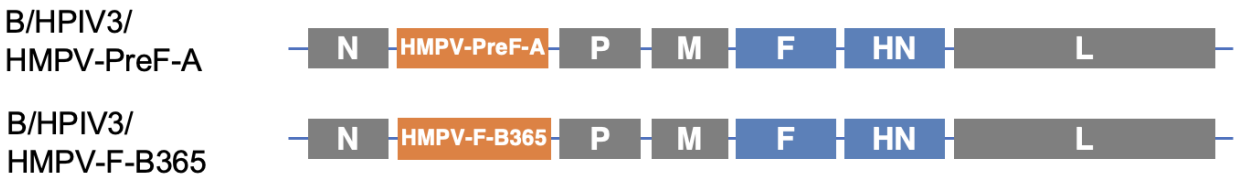

Figure 1: Diagram of the B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 Genomes

Bovine parainfluenza virus (PIV) genes are shown in grey, human PIV3 F and HN genes in blue, and HMPV-PreF-A and HMPV-F-B365 in orange.

The HMPV F antigens expressed by B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 are not incorporated into the B/HPIV3 viral particle. Since the HMPV F antigens expressed by B/HPIV3/HMPV-PreF-A or B/HPIV3/HMPV-F-B365 are stabilized in prefusion conformation, and, in case of HMPV-F-B365, include a deletion of the F1/F2 cleavage site and fusion peptide, the infectivity of these PIV3 vectors relies solely on HPIV3 F and HN. HMPV-neutralizing antibodies do not neutralize B/HPIV3/HMPV-PreF-A or B/HPIV3/HMPV-F-B365, while both candidates are effectively neutralized by HPIV3-neutralizing antibodies. This confirms that entry and membrane fusion of B/HPIV3/HMPV-PreF-A B/HPIV3/HMPV-F-B365 vectors rely solely on HPIV3 F and HN. Thus, these vaccine vectors are not expected to be blocked by pre-existing antibodies to HMPV; even in the presence of antibodies to HMPV, these B/HPIV3 vectors are expected to be immunogenic. In prior prime/boost studies of heterologous mucosal vaccines in nonhuman primates primed with live RSV, a second dose of a B/HPIV3 vector expressing the RSV fusion protein from an added gene strongly boosted RSV serum and mucosal antibody titers induced by the first dose of RSV despite preexisting antibodies established by that initial live RSV vaccination (19). By analogy, this is also expected for HMPV-experienced children who receive B/HPIV3/HMPV-PreF-A or B/HPIV3/HMPV-F-B365; HMPV antibody titers are likely to be boosted by B/HPIV3-vectored HMPV antigens.

The safety and immunogenicity of the clinical study materials of B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 was evaluated in rhesus macaques. Following administration by nasal sprayer, vaccine shedding was detectable in nasal swabs and tracheal lavages over a period of about 10 days in macaques. By day 28 after immunization, robust HPIV3 and HMPV neutralizing serum antibody titers were detected in all macaques. The HMPV neutralizing antibody titers were comparable to those induced by intranasal inoculation with HMPV. The vaccine candidates were well-tolerated in macaques. Please see the Investigator's Brochure (IB) for additional details of the assessment of infectivity and immunogenicity of these vaccines in hamsters and Rhesus macaques.

These B/HPIV3/HMPV vectored vaccines are complementary to the live attenuated RSV vaccines that are being developed in the RNA Viruses Section (RVS), Division of Intramural Research, NIAID, in reducing the overall burden of pediatric lower respiratory infections. The B/HPIV3/HMPV vaccines have the potential to be used as bivalent stand-alone vaccines, or in combination with RSV vaccines.

1.3 Prior Research

Previous efforts have been made to develop live-attenuated HPIV3 and HMPV vaccines, although to our knowledge, a combined HPIV3/HMPV has not been developed and evaluated in infants and young children. Of particular relevance to this study, we previously demonstrated that a chimeric bovine/human HPIV3 vaccine in which the bovine PIV3 hemagglutinin-neuraminidase (HN) and fusion (F) glycoproteins were replaced with their human counterparts was well-tolerated and immunogenic in a phase I clinical trial in adults, HPIV3-seropositive, and HPIV3-seronegative children (15). We have also evaluated a live-attenuated HMPV vaccine, which was well-tolerated but insufficiently infectious and immunogenic in HMPV-seronegative children (20).

1.3.1 Preclinical Studies

1.3.1.1 HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 in Rhesus Macaques

The replication, immunogenicity, and toxicity of the B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 vaccines were evaluated in rhesus macaques (*Macaca Mulatta)*. Animals in groups of 4 were immunized intranasally with these vaccine candidates. Control groups (n=4) received B/HPIV3 expressing a non-stabilized version of the fusion glycoprotein F of HMPV subgroup A, or HMPV, respectively. Vaccine candidates and controls were administered intranasally with a mucosal atomizer

device (MAD300) sprayer in a single dose 1 x 10^6 plaque forming units (PFU) per mL per animal. The rhesus study was approved by the Animal Care and Use Committee of NIAID, NIH. Combined nose/throat swabs were taken on days -2, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 14 post-inoculation to analyze virus shedding from the upper respiratory tract. Tracheal lavage was done on days 2, 4, 6, and 8, and bronchoalveolar lavage was done on days 9 and 14 to detect virus shedding from the lower respiratory tract. Blood was collected on days 4, 7, and 14 to test for viremia. Vaccine virus was detected by immunoplaque assay on Vero cell monolayer cultures. The lower limit of virus detection was 0.7 $\log_{10}$ PFU per mL for combined nose/throat swabs and viremia samples, and 1.0 $\log_{10}$ PFU per mL for tracheal and bronchial lavage fluid.

Robust viral shedding of B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 was detectable over several days in specimens obtained by nose/throat swabs, tracheal lavage, and bronchoalveolar lavage. No viremia was detected in any of the animals. B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 induced high levels of HPIV3 neutralizing serum antibodies in all animals. Both study products also induced high levels of neutralizing serum antibodies to HMPV of subgroup A. In addition, B/HPIV3/HMPV-F-B365 induced neutralizing serum antibodies to HMPV subgroup B.

No clinical signs of disease or clinical pathology associated with the vaccine candidates of B/HPIV3/HMPV-PreF-A, Lot PIV3#115A, and B/HPIV3/HMPV-F-B365, Lot PIV3#116A, were detected in macaques. The animals had no abnormal findings attributable to the test material on ophthalmologic exam, physical exam, and hematology or serum chemistry. Overall, the study products were immunogenic in rhesus macaques. Both vaccine candidates stably maintained high levels of expression of the HMPV F antigens from the additional genes through the period of vaccine shedding.

1.3.2 Previous Clinical Experience

This will be the first evaluation of a B/HPIV3-vectored vaccines expressing prefusion-stabilized versions of the HMPV F protein.

1.3.2.1 Phase 1 Clinical Study of a Live, Attenuated Respiratory Syncytial Virus (RSV) and PIV3 Vaccine in Seronegative Children

In a previous Phase 1 study, the safety, tolerability, infectivity, and immunogenicity of the rB/HPIV3 vector (without an additional gene encoding another respiratory virus antigen) was evaluated sequentially in an open-label study in 15 adults, followed by a randomized, blinded and placebo-controlled evaluation in HPIV3-seropositive [ages 15 – 59 months; 10 vaccinees (v) and 5 placebo recipients (p)] and seronegative children (6 – 36 months; 14 v, 7 p) (15). In this study, adults and HPIV3-seropositive children received a single intranasal dose of 0.5 mL (0.25 mL per nostril) containing 10^6 tissue-culture infectious doses ($TCID_{50}$) of rB/HPIV3. HPIV3-seronegative infants and children received a single intranasal dose of 10^5 $TCID_{50}$ of rB/HPIV3. Clinical assessments and nasal washes (NWs) were performed prior to inoculation and on days 3 – 7 and day 10 for adults and seropositive children, and safety follow-up continued through day 28. For seronegative children, clinical assessment and NWs were performed prior to inoculation and on days 3 – 7, 10, 12, 14, 17, 19, 21 and 28 with a follow-up phone call approximately 6 months after vaccination. Infection of the rB/HPIV3 virus was defined as either the isolation of vaccine virus or a ≥ 4-fold rise in serum HPIV3 HAI titers. In adults and seropositive children, replication of rB/HPIV3 was highly restricted. Increases in HAI titers greater than 4-fold were not detected in adults, and only one of 10 HPIV3-seropositive children had a ≥ 4-fold rise in serum HPIV3 HAI titers detectable, indicating that rB/HPIV3 was highly attenuated in HPIV3-seropositive participants. At a dose of 10^5 $TCID_{50}$, rB/HPIV3 was infectious in 100% of seronegative children with vaccine virus shedding detectable in 86% of vaccinees, and immunogenic, with ≥ 4-fold rises in HAI titers detectable in 93% of vaccinees. The mean peak titer of rB/HPIV3 detectable in nasal wash

specimens was $10^{2.8}$ PFU/mL, with a mean duration of shedding of 8.2 days. Upper respiratory tract and febrile illnesses were frequently observed in vaccine and placebo recipients in this cohort. A single HPIV3-seronegative vaccinee experienced lower respiratory illness (rhonchi) from days 21 – 23, but at the time of this LRI, bocavirus and coronavirus 63 were detected in nasal wash specimens; vaccine virus was not detected in this child after day 7 post immunization. Based on these results, rB/HPIV3 was selected as a vector vaccine candidate to express the surface glycoproteins of other pediatric respiratory pathogens, including the RSV F protein [(21), described below], or the HMPV F protein, yielding the vaccine candidates to be evaluated under this study protocol.

1.3.2.2 Phase 1 Clinical Study of a Live, Attenuated Respiratory Syncytial Virus (RSV) and PIV3 Vaccine in Seronegative Children

In another study, the safety and immunogenicity of a version of rB/HPIV3 expressing a non-prefusion stabilized version of the RSV F protein was evaluated in a dose-escalating study (21). In this study, 49 healthy RSV/PIV3-seronegative children 6 to 24 months of age were randomized 2:1 in three groups. The groups were enrolled sequentially and randomized 2:1 receive doses of 10^4, 10^5, or 10^6 $TCID_{50}$ of a rB/HPIV3/RSV-F vaccine or placebo, with each group receiving 3 intranasal doses of vaccine or placebo at 2-month intervals. Solicited adverse events (Solicited AEs) and unsolicited adverse events (Unsolicited AEs) were recorded during days 0 to 28 after each dose. Nasal wash samples were collected 3 times (collection windows: days 7–10, 12–18, and 28–34) after each dose. Serum was collected at baseline and 28 days after each dose. Subjects were followed for 180 days after the last dose or to the end of the RSV season.

In this study, there was no difference in the incidence of solicited AEs and AEs between vaccine and placebo arms. Solicited AEs were common in the 28 days after vaccination with 96.9% of RSV/PIV3 vaccine and 100% of placebo recipients reporting at least 1 solicited AE during the study. The majority of the AEs were mild or moderate in severity with runny/stuffy nose reported at a higher rate in the RSV/PIV3 vaccinees compared to placebo recipients. Unsolicited AEs were reported frequently 28 days after each vaccination, and rates were comparable between dosage groups. Some of the most common unsolicited AEs after RSV/PIV3 vaccination were teething, diarrhea, otitis media and increased body temperature. A majority of the unsolicited AEs reported before 28 days after vaccination were classified as mild to moderate in severity. Three severe AEs were reported for 2 participants in Group 1 (croup due to PIV1, also classified as Medically Attended (MA)-LRI, and viral gastroenteritis) and 1 participant in Group 2 (otitis media). One participant in a placebo group had a severe AE of croup due to PIV1, also classified as MA-LRI. For the placebo and vaccine recipients with croup due to PIV1 infection, the investigational drug product was considered possibly related. No serious adverse events (SAE) were reported through the 28-day observation period; however, 2 were reported in the long-term follow-up phase of the study. No SAEs were considered related to the vaccine.

Virus shedding of the rB/HPIV3/RSV-F vaccine was higher in groups 2 and 3 that had received 10^5 $TCID_{50}$ and 10^6 $TCID_{50}$ of vaccine, respectively, than in Group 1 (10^4 $TCID_{50}$ of vaccine), with the majority of shedding observed in the day 7-10 collection window after the first dose. The frequency of detection of vaccine decreased during the subsequent NW collection window (days 12–18 following immunization), and no vaccine shedding was detected in the third NW collection window (days 28–34). Peak titers in NW samples with quantifiable virus were similar across all vaccinated groups.

A dose response was observed in the RSV and HPIV3 serum antibody responses to the vaccine. A greater proportion of the study participants had a serum antibody response (as measured by a greater than 4-fold rise from baseline) in Group 3 that had received 10^6 $TCID_{50}$ compared to groups 1 and 2 that had received 10^4 $TCID_{50}$ and 10^5 $TCID_{50}$. In this study, a somewhat larger proportion of vaccinees had a seroresponse to HPIV3 than the proportion with a seroresponse to RSV (21). Retrospective analyses showed that

vaccine virus in this clinical trial product, as well as that recovered from nasal washes, contained sub-populations with mutations predicted to ablate expression of RSV F, which likely contributed to the poor RSV immunogenicity (22, 23). Overall, this vaccine had a favorable safety profile. A subsequent study showed that this vaccine was well-tolerated at the 10^6 dose in 2-month-old infants (for results from the evaluation in 2-month-old infants, see Clinicaltrials.gov: NCT00686075).

1.4 Clinical Development Plan

The investigational vaccines B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 will be evaluated initially in a cohort of HPIV3-seropositive children, although HPIV3 seronegative infants and children are the ultimate target population for this vaccine. The purpose of this initial study is to determine whether these vaccines are well-tolerated and infectious in HPIV3-seropositive children. The primary immunogenicity endpoints to be evaluated are HPIV3 and HMPV neutralizing serum antibody titers, and serum antibody titers to the HPIV3 and HMPV F proteins (measured by enzyme-linked immunosorbent assay [ELISA]). These assays will be performed at the Johns Hopkins University (JHU) Center for Immunization Research (CIR) and at the Laboratory of Infectious Diseases (LID) RVS laboratories.

Previous experience with the B/HPIV3 or a B/HPIV3-based vector expressing the RSV F protein has demonstrated that, at a dose of $10^{6.0}$ $TCID_{50}$, B/HPIV3 was highly attenuated and well tolerated in adults and HPIV3-seropositive children, and, at a dose of $10^{5.0}$ or $10^{6.0}$ $TCID_{50}$, highly attenuated in HPIV3-seronegative infants and children [(15, 21), Clinicaltrials.gov NCT00686075]. In HPIV3-seronegative children, a dose response to B/HPIV3/RSV-F was observed; a greater proportion of the study participants that had received 10^6 $TCID_{50}$ of B/HPIV3/RSV-F had a serum antibody response to vaccine (as measured by a greater than 4-fold rise from baseline) compared to participants that had received 10^4 $TCID_{50}$ and 10^5 $TCID_{50}$ (21). In preparation for the present study, the B/HPIV3/HMPV-F vaccines have been tested in nonhuman primates (NHP) at a dose of $10^{6.0}$ PFU. In the current protocol, a dose of $10^{5.6}$ PFU has been selected based on previous safety in a Phase 1 study of a $10^{6.0}$ dose of the B/HPIV3-RSV F vector vaccine in HPIV3-seronegative children as young as 2 months of age, and based on the expectation that this dose will be safe in HPIV3-seropositive children 24- to <60 months of age.

2 OBJECTIVES

2.1 Primary Objectives

Safety

1. To assess the frequency and severity of study product–related solicited and unsolicited AEs, SAEs, and LRIs from day 0 through the 28th day following inoculation.
2. To compare rates of solicited and unsolicited AEs, SAEs, and LRIs through the 28th day following inoculation.

Immunogenicity

1. To evaluate the percentage of vaccinees with a ≥4-fold rise in HPIV3-neutralizing serum antibody titers at day 28 after inoculation.
2. To evaluate the percentage of vaccinees with a ≥4-fold rise in HMPV-neutralizing serum antibody titers at day 28 after inoculation.

Infectivity

1. To determine the peak titer of vaccine virus shed by each participant.

2. To determine the proportion of vaccinees infected with vaccine virus [defined as shedding vaccine virus, detected by RT-qPCR, and/or ≥4-fold rise in HPIV3-specific and/or HMPV-specific serum antibodies, detected by neutralizing antibody assay or ELISA).

2.2 Secondary Objectives

Safety

1. To assess the frequency and severity of study product–related MAAEs and SAEs from day 0 through 180 days following inoculation.

Immunogenicity

1. To evaluate the percentage of vaccinees with a ≥4-fold rise in HPIV3 F-specific serum immunoglobulin G (IgG) titers at day 28 after inoculation.
2. To evaluate the percentage of vaccinees with a ≥4-fold rise in HMPV F-specific serum IgG titers at day 28 after inoculation.
3. To evaluate the percentage of vaccinees with a ≥4-fold rise in HPIV3 F-specific serum immunoglobulin A (IgA) titers at day 28 after inoculation.
4. To evaluate the percentage of vaccinees with a ≥4-fold rise in HMPV F-specific serum IgA titers at day 28 after inoculation.

2.3 Exploratory Objectives

1. To evaluate the association of vaccine virus shedding with AEs.
2. To assess the incidence and magnitude of vaccine virus shedding, and the incidence of infection with other respiratory pathogens, in samples collected at illness visits associated with solicited AEs on Study Days 0 through 28.
3. To evaluate the genetic stability of vaccine isolates by sequence analysis.
4. To evaluate the percentage of HMPV F expression in vaccine isolates by HPIV3/HMPV dual-staining immunoplaque assay.

Study samples may be used in comparative assays with samples from other HPIV3 vaccine studies initiated by the LID, NIAID, NIH.

3 STUDY DESIGN

The study will be performed in HPIV3-seropositive children, and will be blinded, randomized, and placebo-controlled. Subjects will be block randomized in groups of 5 (2 to receive B/HPIV3/HMPV-PreF-A, 2 to receive B/HPIV3/HMPV-F-B365, and 1 to receive placebo). Enrollment will be continuous unless Stopping Rules (Section 8.3) are met or other safety concerns occur. To ensure the unbiased clinical evaluation of subjects, all participants' parents/guardians and all staff involved in clinical assessment will remain blinded through completion. To assess the magnitude and duration of vaccine virus replication and ensure continued suitability of these vaccines for this population in this Phase 1 trial, a Scientific Investigator (Dr. Buchholz), who is not involved in clinical assessment of trial participants, will be unblinded once the final participant in each randomization block has completed the assessment phase on Day 28 (Table 17: Schedule of Events

At that time, Dr. Buchholz will review the clinical and vaccine virus shedding data for the randomized block and, if needed, consult with a clinician who is not a member of the study team. Should any concerns arise from this review, these will be shared in a blinded fashion with the Medical Monitor and Sponsor Clinical Safety Office for guidance and assistance. Additionally, this information will be reviewed unblinded by the DSMB in collaboration with Dr. Buchholz and any involved non-study-team consultants before any additional dosing or enrollments proceed.

During the enrollment period, the DSMB will perform unblinded safety reviews (Section 9.4.2) including viral shedding data if any of the following occur. Enrollment will be paused during this assessment, pending the DSMB recommendation.

a. >1 Grade 4 solicited or unsolicited AE assessed as related to the investigational vaccine within 28 days of study product administration.

b. >2 Grade 3 unsolicited AEs of the same or similar type that are assessed as related to the investigational vaccine within 28 days of study product administration.

c. Any Lower Respiratory Illness associated with vaccine virus shedding.

After completion of the Day 28 visit of the last participant, an unblinded review of all participants will be performed by the DSMB.

For the purpose of this study, HPIV3-seropositive is defined as having a serum hemagglutination-inhibition antibody (HAI) titer of >1:8. This definition has been used in previous evaluations of live-attenuated HPIV3 vaccines (24-28). In these and other previous studies, live-attenuated HPIV3 vaccines were highly restricted in replication and poorly immunogenic in children with titers > 1:8 but were far less restricted in replication and highly immunogenic in children with titers ≤ 1:8. These data suggest that this HAI antibody cutoff can distinguish effectively between HPIV3-experienced and HPIV3-naïve children.

At Vanderbilt University Medical Center (VUMC), participants will be screened and enrolled under this protocol. If eligible, participants will be randomized and will receive study product. At the CIR and the University of Rochester Medical Center, a separate screening protocol may be used to determine eligibility. Signed and witnessed informed consent will be obtained prior to enrollment. Enrollment will occur at the time of inoculation with study product on Day 0. Participants will remain enrolled in the study until they complete remote follow-up at Day 180 ± 7 days.

The first 28 days after inoculation are considered the Acute Phase of the study, and the participants' parents/guardians will be contacted daily during the Acute Phase. These contacts will consist either of an in-person evaluation of interim medical history, clinical assessment, and nasal swab, or an interim medical history conducted by a mutually agreed upon communication method. If a child develops a respiratory or febrile illness or otitis media during the acute phase, an in-person evaluation will be performed. During the Acute Phase, the participants will be evaluated for AEs or SAEs. Between Days 29 and 180 parents/guardians will be asked to contact study staff to report Medically Attended Adverse Events (MAAEs), which will be reported per protocol. Between days 29-180, study staff will contact the participants' parents/guardians monthly and on Day 180 (±7 days) after inoculation to capture any previously unreported MAAEs.

Clinical assessments will be completed and nasal swabs will be obtained on study days 0, 3, 4, 5, 6, 7, 10, 14 and 28 and whenever febrile or respiratory illnesses or otitis media (solicited AEs) are reported during the first 28 days following inoculation. The nasal swab will be tested for vaccine virus, for respiratory pathogens that are considered adventitious agents, including wild-type HPIV3 and HMPV, and for mucosal IgG and IgA antibody as listed in the schedule of events. The schedules of evaluations during the Acute Phase are shown in Table 17: Schedule of Events
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4 STUDY POPULATION

Approximately 25 HPIV3-seropositive children ≥ 24 months to < 60 months of age will be enrolled; each subject will receive a single dose of $10^{5.6}$ PFU of B/HPIV3/HMPV-PreF-A, $10^{5.6}$ PFU of B/HPIV3/HMPV-F-B365 vaccine, or placebo in a 2:2:1 ratio. These numbers were chosen based on experience with phase I evaluation of other live-attenuated respiratory virus candidate vaccines (24-28) and statistical considerations (Section 9.1).

4.1 Recruitment Process

Research staff will recruit participants from pediatric practices and clinics in the surrounding area. At the CIR and the University of Rochester, an institutional review board (IRB)-approved screening protocol may be used for this study. At VUMC, children will be screened under this protocol. Upon referral by the participants' primary care provider or the provider's staff, staff will contact parents/guardians in person or remotely as appropriate, via telephone, email, or text message, send them IRB-approved informational brochures, and obtain contact information. Study staff will follow up with interested parents/guardians to elaborate upon the details and requirements of the screening and study process.

Research staff may also recruit potential participants from local pediatric practices and clinics through mail, email, or electronic medical record messaging (e.g., MyChart), and via mail to households in local zip codes containing age- appropriate children. In addition, research staff may recruit participants through group gatherings such as health fairs and by social media posting IRB-approved recruitment materials.

If parents/guardians are interested in having their child participate and the child meets the minimum inclusion and exclusion criteria, then the study staff will schedule a screening visit to determine the child's eligibility.

4.2 Study Visits

An overview of the study visits, evaluation schedule, and specimen collection is provided in Appendix I (Table 17). This section contains additional information on visit-specific study procedures.

Study visits, except inoculation, may be performed at one of the clinical sites or at a mutually agreed-upon location. Inoculation must be performed at one of the clinical sites. Sites will follow relevant CDC and institutional guidance regarding in-person visits and use of personal protective equipment. Unless otherwise specified, visits may be split, with required procedures performed on more than one day within the allowable visit window. All clinical tasks will be performed by an appropriately trained and credentialed medical professional. The physical examination will include temperature, heart rate, respiratory rate, assessment of ears, eyes, nose, and throat (EENT), lungs, heart, and skin, as well as abdominal, musculoskeletal, and as appropriate, neurological exams. A focused clinical assessment will include temperature, heart rate, respiratory rate, and evaluation of EENT, lung, heart, and lymph nodes.

All specimens will be obtained, processed, stored and transported per the Manual of Procedures (MOP).

In addition to the protocol-specified procedures listed in this section, study staff may complete other tasks consistent with standard operating procedures (SOPs), including but not limited to collecting, reviewing, and updating demographic and locator information; reviewing elements of informed consent; scheduling telephone (or text/email) contacts and visits; providing instructions for contacting study staff between visits; providing visit reminders; and following up on missed visits. All visit procedures will be documented in accordance with the MOP. Refer to Section 10 for more information on documentation requirements and completion of case report forms (CRFs). The study's medical professional will inform parent/guardian of any significant abnormal physical findings and, after obtaining parental release of information, will make appropriate referrals back to the child's primary caregiver, if necessary.

4.3 Consenting Process

At the CIR and the University of Rochester, the screening process may be completed under a separate screening protocol and consent. At VUMC, screening will be performed under this protocol. The parent/guardian must complete the informed consent process and sign the informed consent document (ICD) before screening or study procedures are performed. The consenting process for the study may take place during the screening visit or may be conducted at a separate visit. During the consenting process, the child's parent will review the ICD, be encouraged to ask questions, and complete a comprehension assessment to evaluate consent understanding. Study staff will use incorrect answers from the comprehension assessment to identify areas of the ICD that need further review with the parent/guardian. This will help ensure that the parent/guardian has sufficient understanding of the process before the ICD is signed. Parent/guardian will be provided with a copy of the ICD.

During the consenting process, the parent will be given the opportunity to provide permission for use of their child's picture in advertisement flyers, articles, or presentations. In addition, the parent will be given the opportunity to provide permission for storing their child's specimens for future respiratory virus vaccine studies and for research purposes.

A parent/guardian will complete a Health Insurance Portability and Accountability Act (HIPAA) medical record release to allow study staff to review medical history and immunization records, and to allow review of AEs during the study. The portions of the medical record that are pertinent to the study will be maintained in the study chart.

4.4 Screening Visit

The screening process will include reviewing the medical record, obtaining a medical history from parent/guardian, and conducting a physical examination. The physical examination will occur on the day of screening or the day of inoculation. The medical record review should include review of history related to the study eligibility criteria, the immunization record, and growth chart data. The medical history from the parent should include demographics, prior diagnoses, current medications, signs and symptoms, developmental status, age of household members, day care attendance, and use of medications prior to inoculation.

The screening visit will include obtaining a serum sample to test for the presence of HPIV3 antibodies. Approximately 3-5 mL of blood is sufficient quantity for the screening and pre-inoculation assays. The procedure will be performed and documented per the MOP. Hematologic and metabolic screening laboratory tests will not be performed on the participant. Such tests are not routinely performed as part of well child care, given that the risk of undiagnosed hepatic, metabolic, and renal diseases is much lower in children than in adults.

The screening serum sample must be obtained within the calendar year of inoculation. If the screening sample was obtained more than 42 days before inoculation, an additional serum sample will be obtained prior to inoculation to serve as the preinoculation sample. Study staff should consider potential randomization and inoculation dates when scheduling the participant screening visits.

Table 2: Screening Visit Procedures

Screening Visit Procedures		
Administrative Tasks		□ Conduct consenting process □ Confirm parent/guardian's informed consent comprehension □ Obtain release of medical records as required per HIPAA □ Obtain available medical records □ Assess eligibility
Clinical Tasks		□ Obtain, review, and document medical history □ Perform physical examination or defer until study day 0 □ Address any concerns □ Document findings □ Obtain and review participant's immunization record □ Review participant's medical records to determine age-appropriate developmental assessment, and participant's weight and length
Laboratory Tasks	**Blood**	*Collect blood for*: □ Screening sample for HPIV3 serum antibody testing and pre-inoculation sample[1] for HMPV and HPIV3 serum antibody testing
Follow-up Preparation		□ Schedule enrollment visit if eligible

[1]Obtained no more than 42 days prior to inoculation.

4.5 Randomization

Randomization numbers will be assigned by Research Electronic Data Capture (REDCap) and will be forwarded to the DSMB Executive Secretary. A copy of the randomization code will be retained by the unblinded dispenser.

Randomization will be implemented using the Randomization Module within Vanderbilt's REDCap database. The investigational pharmacy at each of the 3 institutions will distribute the assigned study product. Pediatric subjects will be block-randomized in blocks of 5 (2 vaccine recipients for each vaccine and 1 placebo recipient per block). Detailed procedures for randomization and unblinding will be placed in the MOP.

Unblinded study personnel will label the vaccine syringes and complete the appropriate parts of the vaccine administration record (VAR). After the participant has been inoculated, the vaccine administrator and verifier will initial the label and place the label in the subject's study chart after inoculation. The inoculation time will be documented on the VAR and a copy will be sent to the pharmacy. All syringes will be appropriately disposed of by the study staff.

4.6 Inoculation (Day 0)

Study product administration occurs on study day 0 and must be completed at a clinic or medical office site or pediatric practice where emergency supplies are available in case of an adverse event (e.g., allergic reaction). Prior to inoculation, an authorized prescriber will provide a request for study product to the unblinded dispenser. The request must include the information outlined in the MOP.

Informed consent will be obtained from the parent/guardian of each child who participates in this study prior to the performance of any study procedures or inoculation. At the VUMC, study-specific informed consent will be obtained prior to screening, whereas at Johns Hopkins CIR and the University of Rochester, screening may be performed under a separate protocol and study-specific consent will be obtained prior to inoculation. The child's parent/guardian will be encouraged to ask questions and complete a comprehension assessment to evaluate understanding of the study. Study staff will use incorrect answers from the comprehension assessment to identify those areas of the ICD that need further review with the parent/guardian. This will help ensure that the parent/guardian has sufficient understanding of the study process before the ICD is signed.

If the participant is noted to have any of the following on the study visit day, inoculation must be deferred:

- fever (temporal or rectal temperature of ≥ 100.4°F), or
- URI or LRI symptoms or signs (including but not limited to rhinorrhea, cough, or pharyngitis), or
- nasal congestion significant enough to interfere with successful inoculation, or
- otitis media

Table 3: Enrollment Visit Procedures

Enrollment Visit Procedures *(Day 0)*		
Administrative Tasks		• Confirm study ICD is completed and signed and dated by both parent/guardian and study staff who completed the consenting process • Complete eligibility determination and confirmation • Complete paper-based eligibility checklist • Obtain release of medical records as required per HIPAA
Clinical Tasks		• Obtain interim history from parent/guardian • Complete physical exam if deferred at screening, OR Focused physical exam if physical completed at screening • Address any concerns • Document findings • Confirm eligibility
Laboratory Tasks	**Blood**	*If insufficient volume obtained at screening or screening was > 42 days prior to enrollment, collect blood for*: • Pre-inoculation HPIV3 and HMPV antibody titers
	Nasal Swab	*Collect nasal swab for:* • Vaccine virus detection and quantification • rRT-PCR for adventitious agents • Mucosal IgG and IgA Antibodies
Study Product Administration		• Administer study product and maintain participant in an upright position for 1 minute • Observe for approximately 30 minutes after inoculation to evaluate for immediate hypersensitivity reactions • Any malfunction of the VaxInNator device will be documented.
Follow-up Preparation		• Confirm contact method • Complete teaching • Schedule next visit

Following the inoculation day visit, the parent/guardian will record the infant/child's temperatures and signs of illness on the temperature card and provide these to study personnel during an in-person research visit or non-visit day contact. New rectal thermometers will be given, and temporal artery thermometers will be provided to parent/guardian for use during the study. For temperature measurements, parent/guardian will be instructed to use the study-provided temporal artery thermometer to screen for elevated temporal artery temperatures. This device is used to minimize the number of rectal temperature measurements and has been shown to be an effective screening tool for rectal fever (29). The parent/guardian will measure temporal artery temperatures following the manufacturer's directions. If any temporal artery temperature is ≥ 100.0°F, parent/guardian will be asked to measure a rectal temperature within 20 minutes (29). For study-specific management and grading of temperatures, see Section 8.2.2, Table 14: Grading for Fever.

4.7 Study Phases

The Acute Phase begins with inoculation and ends at midnight on the 28th day after inoculation. During the Acute Phase of the study, a study healthcare professional will be available by telephone 24 hours a day for consultation with parent/guardian regarding any illnesses that may occur.

Study personnel will have daily contact with participants' parents/guardians for the first 28 days after inoculation. Minimal to absent viral shedding has been observed for HPIV3 positive participants in previous studies of live-attenuated HPIV3 vaccines (24-28); additional days are added to comply with regulatory requirements.

On non-visit days, study staff will contact the parent/guardian and will record the parent/guardian-provided temperatures and signs of illness. Participants with illness may have additional in-person research visits to assess the illness (Section 4.8).

Timing and amounts of study compensation will vary according to site and are included in the site-specific consent forms.

4.7.1 Schedule of Visits

4.7.1.1 Acute Phase: In-person Study Visit Days

An in-person study visit and clinical assessment performed by a healthcare professional will be completed during visits on Days 3, 4, 5, 6, 7, 10 and 14 ± 1 day. If parents/guardians report that a participant has a febrile or respiratory illnesses or otitis media between Days 14-28, an in-person research illness visit will be scheduled to assess the severity of the illness and obtain a nasal swab for testing for vaccine virus and adventitious agents.

Table 4: Acute Phase In-Person Visit Procedures

Days 3, 4, 5, 6, 7, 10 and 14 In-person Visit Procedures *(each visit ± 1 days)*		
Clinical Tasks		• Document the participant's previous days' interim history as reported from the parent/guardian, include: ▪ medications and/or immunizations ▪ signs and symptoms of illness ▪ highest temperature reading, indicate temperature method • Perform focused clinical examination • Address any concerns • Review safety data • Document findings
Laboratory Tasks	**Nasal Swab**	*Collect nasal swab for:* • Vaccine virus detection and quantification • Mucosal IgG and IgA antibodies (only on Days 10 and 14)
Follow-up Preparation		• Schedule non-visit day contacts and follow-up in-person visits

During an Acute Phase study visit, if the participant is diagnosed with or suspected of having URI, LRI, otitis media or fever (as defined in Appendix II), testing of the nasal swab specimen for adventitious agents will be performed as described in the MOP.

4.7.1.2 Acute Phase: Non-Visit Study Day Contacts

Parents/guardians will be contacted on Days 1, 2, 8, 9, 11-13 and 15-27 (each visit ± 1 day). On these non-visit days, study staff will contact the parent/guardian and will record the parent/guardian-provided temperatures and signs of illness. Participants with illness may have additional visits to assess the severity of the illness (Section 4.8).

Table 5: Acute Phase Non-Visit Contact Procedures

Days 1, 2, 8, 9, 11-13 and 15-27 Contact Procedures *(each visit ± 1 day)*	
Clinical Tasks	• Document the participant's interim history as reported from the parent/guardian, include: • medications and/or immunizations • signs and symptoms of illness • highest temperature reading, indicate temperature method • Address any concerns • Review safety data • Document findings
Follow-up Preparation	• Schedule an illness appointment if necessary

4.7.1.3 Study Day 28 Visit

The Day 28 Visit should be conducted between 28 and 35 days following inoculation. Because the Acute Phase ends on the 28th day following inoculation, only events through that time should be evaluated as having occurred during the Acute Phase.

Table 6: Day 28 Visit Procedures

Day 28 Visit *(+7 Days)*		
Administrative Tasks		• Provide study compensation
Clinical Tasks		• Document the participant's interim history as reported through the 28th day following inoculation, including: ▪ visits to medical provider/hospitalizations ▪ medications and/or immunizations ▪ signs and symptoms of illness meeting SAE criteria • Address any concerns • Review safety data • Perform focused clinical assessment* • Document findings
Laboratory Tasks	**Blood**	*Collect blood for:* • Serum antibodies to HPIV3 and HMPV
	Nasal Swab	*Collect nasal swab for:* • Vaccine virus detection and quantification • Adventitious agents* • Mucosal IgG and IgA antibodies
Follow-up Preparation		• Review MAAE and SAE criteria with participants and how to contact study personnel during Post-Acute Observation Days 29-180 if there is an MAAE or SAE • Review plans for remote follow-up on day 180 (± 7days)

*A clinical assessment is performed and adventitious testing is requested only if the participant has febrile or respiratory illness or otitis media that started prior to midnight on Day 28 and has not been addressed at a previous Illness Visit.

4.7.1.4 Study Day 29 Non-Visit Contact

There will be a non-visit contact on Day 29 to obtain interim history through midnight on the 28th day following inoculation. If the Day 28 Visit takes place on Days 29-35, it is not necessary to have an additional contact with the family on Day 29.

Table 7: Day 29 Non-Visit Procedures

Day 29 Non-Visit Procedures	
Clinical Tasks	□ Obtain and document from parent/guardian the participant's previous days' interim history through midnight on day 28 including: o medications and/or immunizations o signs and symptoms of illness o highest temperature reading, indicate temperature method □ Address any concerns □ Review safety data □ Document findings
Follow-up Preparation	□ Review MAAE and SAE criteria with participants and how to contact study personnel during Post-Acute Observation Days 29-180 if there is an MAAE or SAE

4.7.1.5 Study Days 29-180 Post-Acute Observation

Between Days 29 and 180 parents/guardians will be asked to contact study staff to report MAAEs and SAEs, which will be reported per protocol. Between days 29-180, study staff will contact the participants' parents/guardians monthly and on Day 180 (±7 days) and will record the parent/guardian-provided information regarding any previously unreported MAAEs and SAEs for the month.

Table 8: Days 29-180 Monthly Non-Visit Contact Procedures

Days 29-180 (±7 days) Non-Visit Procedures	
Clinical Tasks	☐ Obtain and document from parent/guardian the participant's previous months' interim history including: o Any MAAEs or SAEs ☐ Attempt to obtain medical record related to any MAAE or SAE ☐ Complete medical record release if needed ☐ Document findings
Follow-up Preparation	☐ Review MAAE and SAE criteria with participants and how to contact study personnel during Post-Acute Observation Days 29-180

4.8 Illness Visit

The timeframe after staff notification in which the Illness Visit must occur depends on the study phase and the grading of the fever and respiratory symptoms as described in Section 8.2. If the Illness Visit occurs on a day concurrent with an in-person study visit, a single nasal swab collection is required, and adventitious agent testing will be requested. All sites will follow relevant CDC and institutional guidance regarding in-person visits and use of personal protective equipment to ensure protection of subjects, families, and staff.

Table 9: Illness Visit Timeframe

Illness Visit Timeframe			
Phase	**Symptoms**	**Grade**	**Visit Timeframe**
Acute	Fever, otitis media or URI	1	Within 3 days
Acute	Fever, otitis media or URI	≥ 2	Within 2 days
Acute	LRI	Any	Within 1 day; a follow-up visit, including nasal swab is required one to three days after the initial assessment.

Table 10: Illness Visit Procedures

Illness Visit Procedures		
Administrative Tasks		□ Complete Adventitious Agent Assay Request for rRT-PCR on nasal swab for adventitious agents □ Complete medical record release if needed
Clinical Tasks		□ Obtain and document from parent/guardian the participant's interim history including: o medications and/or immunizations o signs and symptoms of illness o highest temperature reading, indicate temperature method □ Perform focused clinical assessment □ Address any concerns □ Review safety data □ Document findings
Laboratory Tasks	**Nasal Swab**	*Collect nasal swab for:* □ Vaccine virus detection and quantification □ rRT-PCR for adventitious agents
Follow-up Preparation		□ Schedule follow-up as appropriate

4.9 Early Discontinuation Study Visit

In the event that a child is unable to continue participation in the study, parent/guardian will be encouraged to allow the participant to complete safety monitoring through Day 28. Every effort should be made to schedule a final Early Discontinuation Visit.

Table 11: Early Discontinuation Procedures

Early Discontinuation		
Administrative Tasks		□ Record data on CRF
Clinical Tasks		□ Obtain interim history □ Address any concerns □ Review safety data □ Encourage parent/guardian to allow the participant to complete safety monitoring through Day 28 □ Document findings
Laboratory Tasks	**Blood**	*Collect blood for:* □ Serum antibodies to HPIV3 and HMPV
	Nasal Swab	*If Early Discontinuation Visit is within 14 days of inoculation (Appendix I),* *collect nasal swab for:* □ Vaccine virus detection and quantification □ Mucosal IgG and IgA antibodies

4.10 Participant Retention

Study staff will make every effort to retain participants in the study, thereby minimizing potential biases associated with loss to follow-up.

4.11 Participant Withdrawal or Termination from the Study

Participants in this study may voluntarily withdraw from the study at any time. Any participant who has received study product will be encouraged to remain in the safety evaluation for the duration of the study even if sample collection is refused.

A participant may withdraw or terminate participation in the study early for any of the following reasons:

- Withdrawal of consent – applies to a parent/guardian who verbally or in writing withdraws consent for the participant to continue in the study for any reason.
- Noncompliant with protocol – applies to a parent/guardian who does not comply with protocol-specific visits or evaluations on a consistent basis, such that adequate follow-up is not possible, and the participant's safety would be compromised by continuing in the study.
- Investigator discretion – participant withdrawal may occur if the investigator believes that it is in the best interest of the participant.
- Other – a category used when previous categories do not apply; requires an explanation.

The study may be ended for the following reasons:

- Research is terminated by sponsor or investigator – applies to the situation where the entire study is terminated by the sponsor or investigator for any reason.
- The study sponsor, CIR, the institutional review board (IRB), the Office for Human Research Protections (OHRP), NIAID, or the US Food and Drug Administration (FDA) may decide to end the study.

For any participant who withdraws or who is terminated from the study prior to completion of follow-up, study staff will document the reason for the withdrawal or termination. In the event that the circumstances that led to a participant's withdrawal or termination change, the study staff will contact the PI to discuss options for resumption of follow-up. Withdrawn subjects will not be replaced. Compensation will be given only for the visits and contacts that are completed.

4.12 Specimen Collection

Specimens will be collected for this study as indicated in the Schedule of Events. Further information on collection of blood and nasal swab specimens will also be provided in the MOP.

4.12.1 Virus Detection

Specimens for viral detection, quantification of vaccine virus shedding by rRT-qPCR, and to obtain vaccine isolates for analysis of genetic stability will be obtained by nasal swab approximately 8 times after inoculation, as shown in Appendix I. These specimens may also be tested for adventitious respiratory viruses and bacteria if needed. Additional anterior nasal swab specimens for detection of vaccine virus and adventitious respiratory viruses by rRT-qPCR will also be obtained from participants who meet illness criteria during the Acute phase (Days 0 through Day 28). If a participant becomes ill during the Acute Phase, then additional nasal swabs may be obtained to exclude infection with an adventitious virus. Laboratory testing will be performed by personnel who are not involved with clinical assessment to maintain the blinding status.

4.12.2 Immunologic Assays

For measurement of HPIV3 and HMPV serum antibodies, a pre-inoculation serum specimen will be obtained ≤ 42 days prior to inoculation (Study Day 0), and a post-inoculation specimen will be obtained on Study Day 28 (+7 days).

Serum specimens will be obtained by venipuncture, fingerstick, or heelstick. No more than 5 mL of blood will be drawn at each blood draw visit. A maximum of 10 mL of blood will be obtained from participants for study purposes from screening through the Day 28 Visit.

Nasal swabs will be obtained for mucosal IgG and IgA antibodies on Study Days 0, 10 (±1 day), 14 (±1 day) and 28 (+7 days),

4.12.3 Specimen Preparation, Testing, Storage, and Shipping

All specimens collected for this study will be labeled, transported, processed, tested, stored and/or shipped in accordance with SOPs. The frequency of specimen collection and testing will be directed by the Schedule of Evaluations (Appendix I).

4.12.4 Research Laboratories

Quantitation of the amount of vaccine virus shed, assays to measure immune responses before and after inoculation, and assessment of nasal swabs for adventitious viral agents will be performed at the CIR. Specimens may be sent to LID, NIAID for confirmatory testing, for specialized immune assays, and for sequence analysis, including deep sequencing, of vaccine isolates.

4.12.5 Plan for Use and Storage of Biological Samples

All specimens collected as part of this study may, with the parent/guardian's permission, be stored for future research as part of CIR's approved biospecimen repository for vaccine research. These samples and data may be used for future screening for respiratory virus vaccine studies and to learn more about other diseases. The parent/guardian or child will not own the blood specimens, nasal fluid, or data after it is given to the study. No financial benefit will be provided to the parent/guardian or child from any product or idea created by the investigators using the data or materials. These samples will not be sold or used to make commercial products. Genetic tests will not be performed on these samples unless separate informed consent is obtained.

Samples stored in the repository will be labeled with the study identification number of the participants that, by themselves, cannot identify study participants but are linkable to the study databases generated by the main study. The repository database will contain only the study participants' numbers. A master log linking the study participants' identification numbers to their names is maintained by the study's clinical staff in a password-protected computer system with limited access to authorized research team members and will not be shared with the laboratory.

Study participants, or their parents/guardians, may withdraw consent for future testing of their specimens at any time.

4.12.6 Biohazard Containment

As the transmission of blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel. The procedures for obtaining, shipping, and handling of all specimens for this study will follow the current recommendation of the CDC and the NIH. All infectious specimens will be transported using packaging mandated in Title 42 of the Code of Federal Regulations (CFR), Part 72 (42 CFR 72) and in accordance with individual carrier guidelines (e.g., Federal Express).

5 Inclusion/Exclusion Criteria

5.1 Inclusion Criteria

Potential participants must meet all of the following inclusion criteria to be enrolled in this study and randomized to receive study product or placebo:

5.1.1 ≥24 months of age and <60 months of age at the time of inoculation

5.1.2 HAI Screening for HPIV3 antibody is obtained within the calendar year of inoculation

5.1.3 Seropositive for HPIV3 antibody, defined as serum HPIV3 HAI titer >1:8

5.1.4 Pre-inoculation serum sample for HPIV3-neutralizing antibody specimen is obtained no more than 42 days prior to inoculation

5.1.5 In good health based on review of the medical record, history, and physical examination at the time of inoculation

5.1.6 Received routine immunizations appropriate for age based on the Advisory Committee on Immunization Practices (ACIP) Recommended Immunization Schedule for Children and Adolescents Aged 18 Years or Younger

5.1.7 Growing normally for age as demonstrated on a World Health Orgnaization (WHO) growth chart, AND has a current height and weight above the 3rd percentile for age

5.1.8 Expected to be available for the duration of the study

5.1.9 Parent/guardian is willing and able to provide written informed consent

5.2 Exclusion Criteria

Potential HPIV3-seropositive participants who meet any of the following criteria will be excluded from this study:

5.2.1. <24 months of age and >60 months of age at the time of inoculation.
5.2.2. Born at less than 34 weeks gestation.
5.2.3. Maternal history of a positive HIV test before or during pregnancy.
5.2.4. Evidence of chronic disease.
5.2.5. Known or suspected infection or impairment of immunological functions.
5.2.6. Bone marrow/solid organ transplant recipient.
5.2.7. Major congenital malformations, including congenital cleft palate or cytogenetic abnormalities.
5.2.8. Suspected or documented developmental disorder, delay, or other developmental problem.
5.2.9. Cardiac abnormality requiring treatment.
5.2.10. Lung disease or reactive airway disease.
5.2.11. More than one episode of medically diagnosed wheezing in the first year of life.
5.2.12. Wheezing episode or received bronchodilator therapy or racemic epinephrine within the past 12 months.
5.2.13. Wheezing episode or received bronchodilator therapy after the age of 12 months.

5.2.14. Previous receipt of supplemental oxygen therapy in a home setting.

5.2.15. Previous receipt of an investigational HPIV3 or HMPV vaccine.

5.2.16. Previous receipt or planned administration of human immunoglobulin within the past 6 months. Receipt of licensed monoclonal antibody products for passive prophylaxis against RSV (e.g., nirsevimab) within the past 6 months is not a cause for exclusion.

5.2.17. Previous receipt of any blood products within the past 6 months.

5.2.18. Previous anaphylactic reaction.

5.2.19. Previous vaccine-associated adverse reaction that was Grade 3 or above.

5.2.20. Known hypersensitivity to any study product component.

5.2.21. Member of a household that contains an infant who is less than 12 months of age at the date of inoculation through the 10th day after inoculation.

5.2.22. Member of a household that, at the date of inoculation through the 10th day after inoculation, contains an immunocompromised individual including but not limited to:

- a person who is HIV-infected
- a person who has cancer and has received chemotherapy within the 12 months prior to enrollment
- a person living with a solid organ or bone marrow transplant

5.2.23. Attends a daycare facility that does not separate children by age and contains a child <12 months of age at the date of inoculation through the 10th day after inoculation.

5.2.24. Receipt of any of the following prior to enrollment:

- inactivated influenza vaccine within 3 days prior, or
- any other inactivated vaccine, messenger RNA (mRNA) vaccine, or live-attenuated rotavirus vaccine within the 14 days prior, or
- any live vaccine, other than rotavirus vaccine, within the 28 days prior, or
- another investigational vaccine or investigational drug within 28 days prior, or
- salicylate (aspirin) or salicylate-containing products within the past 28 days

5.2.25. Scheduled administration of any of the following after planned inoculation:

- inactivated vaccine or live-attenuated rotavirus vaccine within the 14 days after, or
- any live vaccine other than rotavirus within the 28 days after, or
- any salicylate or salicylate-containing products within the 28 days after, or
- another investigational vaccine or investigational drug in the 56 days after

5.2.26. Receipt of any of the following medications within 3 days prior to study enrollment:

- systemic antibacterial, antiviral, antifungal, anti-parasitic, or antituberculous agents, whether for treatment or prophylaxis, or
- intranasal medications, or
- other prescription medications except the permitted concomitant medications listed below.

- Permitted concomitant medications (prescription or non-prescription) include nutritional supplements, medications for gastroesophageal reflux, eye drops, and topical medications, including (but not limited to) cutaneous (topical) steroids, topical antibiotics, and topical antifungal agents.

5.2.27. Any of the following events at the time of enrollment:

- fever (temporal or rectal temperature of ≥100.4°F), or
- upper respiratory signs or symptoms (rhinorrhea, cough, or pharyngitis), or
- nasal congestion significant enough to interfere with successful inoculation, or
- otitis media, or
- contact with a person diagnosed with COVID-19 disease or active SARS-CoV-2 infection within the preceding 10 days.

Eligible siblings may be enrolled in this study provided that they are enrolled simultaneously.

5.3 Co-Enrollment Considerations

Co-enrollment in an investigational vaccine or investigational drug study is not allowed during the Acute Phase of this study. After the Acute Phase, co-enrollment may be considered at the discretion of the principal investigator (PI).

6 STUDY PRODUCT

The unblinded dispenser should consult the study MOP. Refer to the IB for further information about the study product.

- Recombinant Bivalent Live Attenuated, B/HPIV3/HMPV-PreF-A approximately $10^{5.6}$ PFU per 0.2 mL vaccine or B/HPIV3/HMPV-F-B365, approximately $10^{5.6}$ PFU per 0.2 mL vaccine
- Placebo will be Lactated Ringer's Solution for Injection, USP 0.2 mL

6.1 Study Product Regimens

Enrolled study participants will receive a single dose of B/HPIV3/HMPV-PreF-A vaccine, B/HPIV3/HMPV-F-B365 vaccine or placebo administered as nasal spray, with vaccines administered at a dose of approximately $10^{5.6}$ PFU. This dose has been selected based on previous safety of a $10^{6.0}$ dose of the B/HPIV3-RSV F vector vaccine in HPIV3-seronegative children as young as 2 months of age, and based on the expectation that this dose will be safe in HPIV3-seropositive children 24 to <60 months of age.

6.2 Study Product Formulation

6.2.1 B/HPIV3/HMPV-PreF-A

The B/HPIV3/HMPV-PreF-A vaccine is provided in a sterile 2.0-mL cryovial, each containing 0.6 mL of vaccine (Lot PIV3#115A) with a titer of approximately $10^{6.7}$ PFU/mL. The vaccine virus concentrate is diluted by trained pharmacy personnel to a dose of approximately $10^{5.6}$ PFU in a 0.2-mL volume. The vaccine vial is labeled as shown below (Figure 2).

Live Recombinant Parainfluenza Virus B/HPIV3/HMPV-PreF-A VERO GROWN VIRUS VACCINE	**Date: 27FEB2023 Vial#: XXXX Lot: PIV3#115A**
CAUTION: NEW DRUG LIMITED BY FEDERAL (USA) LAW	
TO INVESTIGATIONAL USE	
Store at –80°C ± 15°C	
Charles River Laboratories, Malvern, PA	

Figure 2: Investigational Product Label Sample

The dose will be delivered intranasally by VaxINator nasal sprayer (Vax300, Teleflex), affixed to a 1 mL syringe (Omnifix low dead space (LDS) luer lock syringe, BBraun 9167006V-02; 0.1 mL per nostril) with a dose divider.

6.2.2 B/HPIV3/HMPV-F-B365

The B/HPIV3/HMPV-F-B365 vaccine is provided in a sterile 2.0-mL cryovial, each containing 0.6 mL of vaccine (Lot PIV3#116A) with a titer of approximately $10^{6.3}$ PFU/mL. The vaccine vial is labeled as shown in Figure 3. Vaccine is prepared for delivery of a dose of approximately $10^{5.6}$ PFU in a 0.2-mL volume by trained pharmacy personnel. The dose will be delivered intranasally by VaxINator nasal sprayer (Vax300, Teleflex), affixed to a 1 mL syringe (Omnifix LDS luer lock syringe, BBraun 9167006V-02; 0.1 mL per nostril) with a dose divider.

Live Recombinant Parainfluenza Virus B/HPIV3/HMPV-F-B365 VERO GROWN VIRUS VACCINE	**Date: 27FEB2023 Vial#: XXXX Lot: PIV3#116A**
CAUTION: NEW DRUG LIMITED BY FEDERAL (USA) LAW	
TO INVESTIGATIONAL USE	
Store at –80°C ± 15°C	
Charles River Laboratories, Malvern, PA	

Figure 3: Investigational Product Label Sample

6.2.3 Diluent for Vaccine Dilutions

The diluent for vaccine dilutions is Lactated Ringer's Solution for Injection, USP.

6.2.4 Placebo for B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365

The placebo for B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 is Lactated Ringer's Solution for Injection, USP.

6.3 Study Product Storage

Vaccine will be stored in a secure freezer at -80°C ± 15°C. It must remain frozen until the time of use. Once the vaccine is thawed, it should never be refrozen for reuse. Vaccine will be prepared from new, unopened containers for each use.

Lactated Ringer's Solution for Injection, USP should be stored at room temperature as recommended by the supplier.

Procedures for managing the vaccine and diluent/placebo shipment are described in the MOP.

6.4 Study Product Preparation

The diluent for the vaccines, the placebo for the vaccines, and the B/HPIV3/HMPV vaccines must be prepared by following the detailed instruction in the MOP.
The unblinded dispenser will prepare the correct dose of study product for each participant in a biological safety cabinet (BSC) or compounding aseptic containment isolator (CACI) using aseptic technique. To preserve blinding, all prepared syringes may be covered with translucent colored tape as described in the MOP. Aliquots of frozen vaccine preparations will be titered at the CIR laboratory to ensure that preparations of appropriate potency have been delivered.

6.4.1 Placebo

Placebo is Lactated Ringer's Solution for Injection, USP.

Placebo will be drawn up in a sterile syringe to a volume of 0.2 mL and labeled per instructions in the MOP. If necessary to preserve blinding, all prepared syringes will be as described in the MOP.

6.4.2 Live B/HPIV3/HMPV-PreF-A or B/HPIV3/HMPV-F-B365

Diluent will be prepared prior to removal of vaccine from the freezer. The MOP will be followed for proper handling of the study product.

Approximately 3 vials per dose of undiluted B/HPIV3/HMPV-PreF-A vaccine will be used to prepare the administration dose. When manipulating the undiluted study product, the smallest gauge needle possible will be used to avoid loss of study product in the needle and syringe hub. Concentration of the undiluted study product is approximately $10^{6.7}$ PFU per mL for the B/HPIV3/HMPV-PreF-A vaccine. The frozen study product will be thawed and diluted with Lactated Ringer's Solution for Injection, USP to a dose of approximately $10^{5.6}$ PFU in 0.2 mL prior to administration by nasal sprayer.

Approximately 3 vials per dose of undiluted B/HPIV3/HMPV-F-B365 vaccine will be used to prepare the administration dose. When manipulating the undiluted study product, the smallest gauge needle possible will be used to avoid loss of study product in the needle and syringe hub. Concentration of the study product is approximately $10^{6.3}$ PFU per mL for the B/HPIV3/HMPV-F-B365 vaccine. The frozen study product will be thawed, and a dose of approximately $10^{5.6}$ PFU in 0.2 mL will be drawn up into a syringe for administration by nasal sprayer.

The study products will be drawn up to a volume of 0.2 mL in a sterile 1 mL syringe and labeled per instructions in the MOP. Vaccine must be administered by nasal sprayer within 4 hours of being removed from the freezer. Placebo must be administered by nasal sprayer within 4 hours of being removed from the refrigerator.

Samples of undiluted and diluted (if available) study product will be aliquoted from the remaining vaccine that has been prepared. The samples will be snap-frozen as per the MOP and stored at -80°C ± 15°C separate from the concentrated study product. Titration of vaccine will be completed to confirm the titer of the vaccine administered to the participants.

The MOP provides detailed instructions on vaccine storage, handling, preparation, labeling and transport to the clinic.

6.5 Study Product Inoculation Procedure

All study participants will receive a single dose of study product, administered by nasal spray. There is no nasal preparation prior to administration. While the participant is upright, a volume of 0.2 mL of study product will be delivered as nasal spray (approximately 0.1 mL per nostril) using a sterile, needle-less, low dead space 1 mL syringe (BBraun) with a dose divider and a VaxINator (Vax300) sprayer (Teleflex, Inc). Participant will remain upright for approximately 60 seconds following inoculation.

6.6 Study Product Acquisition

The clinical lots of B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 were generated by Charles River Laboratories using the seed virus provided by the NIH.

Lactated Ringer's Solution for Injection, USP will be used as diluent and placebo.

Vaccine virus will be stored at a NIAID designated commercial repository until formally requested by the PI/designee. Following institutional biosafety committee (IBC) approval, and prior to IRB approval, the PI/designee may request that vials of vaccine be transferred from the Sponsor to the research pharmacy at the clinical site for confirmation of the vaccine titer. However, only after receipt of IRB approval will vials of vaccine be used for administration to study subjects. Such an initial shipment may contain the full number of vials needed for implementation of the protocol, but no vaccine will be administered to participants until all necessary approvals have been received. Procedures for ordering and shipping of the study product are in the MOP.

6.7 Study Product Accountability

The unblinded dispenser is responsible for maintaining an accurate inventory and accountability record of study-product and Lactated Ringer's Solution for Injection, USP for this study. A copy of the randomization code will be retained by the unblinded dispenser as outlined in the MOP. Without written request to unblind, the randomization code may not be released. The unblinded dispenser will be responsible for maintaining the blind.

6.8 Disposition of Used/Unused Study Product

After the unblinded dispenser prepares vaccine, the label will be removed from the vaccine vials and placed in the accountability log. In this manner, monitoring personnel will be able to verify the accountability of all vaccine vials used for the study. If there is any vaccine left after the syringes have been drawn up and aliquots have been removed for titering, it will be destroyed by research personnel as per the MOP.

6.9 Final Disposition of Study Products

After study completion or termination, all unused study product will be disposed of per the sponsor's instructions.

6.10 Concomitant Medications

Permitted concomitant medications at enrollment (prescription or non-prescription) include nutritional supplements, medications for gastroesophageal reflux, gas, eye drops, and topical medications, including (but not limited to) cutaneous (topical) steroids, topical antibiotics, and topical antifungal agents.

The use of prophylactic antipyretics, decongestants, or antihistamines is discouraged during the Acute Phase: day of inoculation through the 28th day following inoculation. However, use of these medications for treatment of symptoms is allowed.

Due to the potentially confounding effect on study immunogenicity results, the following concomitant medications should be avoided after inoculation unless deemed clinically necessary.

- Licensed inactivated vaccine, mRNA vaccine or live-attenuated rotavirus vaccine within 14 days of inoculation, with the exception of inactivated influenza vaccine
- Licensed live virus vaccine, other than rotavirus vaccine, within 28 days of inoculation
- Intravenous immunoglobulins and/or any blood products within 6 months of inoculation
- Investigational drug or investigational vaccine within 56 days of inoculation

7 SAFETY ASSESSMENT, MONITORING, AND REPORTING

Participant safety will be carefully assessed, monitored, and reported throughout this study. Sections 7.1-7.4 describe safety-related roles, responsibilities, and procedures. The safety monitoring roles of the NIAID Intramural DSMB are briefly referenced in Section 7.1.4 and described in detail in Section 9.4.2.

7.1 Safety-Related Roles and Responsibilities

7.1.1 Principal Investigator

Each site PI is responsible for continuous monitoring of study participants and for alerting the protocol team if unexpected concerns arise. Trained study staff will record safety-related data on CRFs as indicated in Section 7.2. The PI is also responsible for prompt reporting to the IRBs and other applicable review bodies of any unanticipated problems (UPs) involving risks to participants or others.

7.1.2 Safety Review and Communications Plan (SRCP)

A Safety Review and Communications Plan (SRCP) was developed for the protocol. The SRCP is an internal communications document between the PI and the IND sponsor (OCRPRO) Clinical Safety Office (CSO), which delineates the safety oversight responsibilities of the PI, the CSO, and other stakeholders. The SRCP also includes the overall plan for conducting periodic safety surveillance assessments.

7.1.3 Sponsor Medical Monitor

A medical monitor representing the IND sponsor (OCRPRO) has been appointed for oversight of safety in this clinical study. The sponsor medical monitor will be responsible for performing safety assessments as outlined in a Safety Review and Communications Plan (SRCP).

7.1.4 Data and Safety Monitoring Board

An independent DSMB will monitor participant safety through routine and as-needed reviews of study data. Refer to Section 9.4.2 for more information on the composition and role of the DSMB in the monitoring of this study.

7.1.5 Sponsor Reporting

A SUSAR is a suspected adverse reaction that is both serious and unexpected, as defined in 21 CFR 312.32. SUSARs will be reported to the FDA and all participating investigators as IND Safety Reports. The Regulatory Sponsor will also submit a brief report of the progress of the investigation to the FDA on an annual basis as defined in 21 CFR 312.33.

Following notification from the PI, OCRPRO, as the IND Sponsor, will report all SAEs to the FDA within the required timelines. Fatal and life-threatening events will be reported within 7 calendar days of the sponsor's awareness and all other SAEs will be reported within 15 calendar days of the sponsor's awareness.

7.2 Safety-Related Recording on CRFs

AEs that occur during protocol-specified AE reporting periods following inoculation of study product should be considered AEs. The current section outlines which events should be collected on source documents and which should be recorded on CRFs for inclusion in the database.

AEs may be observed by the study investigator or designee, elicited or volunteered from the parent/guardian or participant, or captured on participant's temperature cards. Assessment of safety will include clinical observation and monitoring of laboratory parameters as necessary. Follow-up measures such as history, physical examination, and laboratory testing and/or treatment may be necessary if a participant experiences an AE. Details of AEs will be properly documented on the source documents, recorded on CRFs, and reported to LID investigators and the DSMB in separate semi-annual and annual reports. AEs will be provided to the IRB as defined by the IRB policy.

The AEs (solicited and unsolicited; and SAEs) to be recorded on CRFs and the study phase and the calendar dates during which they are to be reported are defined in Table 12.

Concomitant medications and AEs identified in this study will be recorded on CRFs. AEs will be recorded as signs, symptoms, laboratory test results, and diagnoses, as shown in Table 12.

Table 12: AE CRF Recording Requirements

Study Phase at the Time of Event Onset	AEs to Record on CRFs	Concomitant Medications to Record on CRFs
Day 0 through midnight of 28th day following inoculation (Acute Phase)	• All SAEs • All solicited AEs that meet Appendix II criteria • All unsolicited AEs (Grades 1 to 4) • Exception of diaper rash, teething pain, and spitting up unless treated with prescription medication or non-prescription medications with antipyretic properties	Record these medications on the CRFs regardless of whether the related event is recorded on the CRFs: • All cough and cold remedies including decongestants, cough suppressants, expectorants • All nasal sprays (except saline spray) • All antihistamines • All antipyretics • All prescription medications For SAEs and LRIs: • All medications related to the recorded event
From day 29 through day 180. (Post-Acute Observation)	• All MAAEs and SAEs	• All medications related to the recorded event
Throughout study	• Unresolved AEs or SAEs with onset date from Day 0 to 28th day after inoculation	• All medications related to the recorded event

7.3 Serious Adverse Event Reporting

SAEs are described in Section 8.1.2. All SAEs occurring between days 0 and 180 will be reviewed by a study physician within 3 days of notification, recorded and reported as specified by the CSO, through REDCap or on the Safety Expedited Report Form (SERF) and followed through to resolution. SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE CRF and the SERF or Safety Reporting Database. In addition to the SAE Reporting Category identified below, other AEs that must be reported in an expedited manner are LRIs as defined in Appendix II if they occur during study days 0 to 28.

Deaths and immediately life-threatening SAEs will be reported within 1 business day after the site becomes aware of the event. All other SAEs will be reported within 3 business days of site awareness. SAEs will be reported either by fax or e-mail to all of the following:

- Sponsor: Office of Clinical Research Policy and Regulatory Operations, NIAID, NIH: Clinical Safety Office (CSO): Phone: 301-846-5301, Fax: 301-846-6224, rchspsafety@mail.nih.gov
  REDCap: https://crimsonredcap.cc.nih.gov/redcap/index.php
- DSMB Executive Secretary: Phone 301-846-5360, Fax: 301-846-6224, niaiddsmbia@niaid.nih.gov
- LID, NIAID: Ursula Buchholz, PhD, NIAID/NIH, 301-594-1533, Fax: 301-480-1268, Email: ubuchholz@niaid.nih.gov

SAEs will also be reported to WIRB-Copernicus Group (WCG IRB) (contact information below) based on its reporting requirements:

- WIRB-Copernicus Group, 212 Carnegie Center, Suite 301, Princeton, NJ 08540 Phone: 1-855-818-2289

7.4 Reporting of Unanticipated Problems to OCRPRO

An UP is defined as any event, incident, experience, or outcome that is:

1. Unexpected in terms of nature, severity, or frequency in relation to
   a. The research procedures that are described in the IRB-approved research protocol and informed consent or other study documents; and
   b. The characteristics of the participant population being studied; and
2. Possibly, probably, or definitely related to participation in the research; and
3. Places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. Per the IND sponsor, an AE with a serious outcome will be considered increased risk.

UPs must be reported to the local IRB per their requirements. Unless otherwise specified above, UPs (as defined in this protocol, or as defined by the IRB of record, whichever is more conservative) that are also AEs or SAEs, nust be reported to the CSO (by REDCap, or by email and SERF if REDCap is not available) no later that when they are due to be reported to the IRB (no later than 7 calendar days of the PI awareness of the event).

SPONSOR CLINICAL SAFETY OFFICE CONTACT INFORMATION:

Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704
Phone 301-846-5301
Fax 301-846-6224

E-mail: rchspsafety@mail.nih.gov

UPs that are not AEs (UP nonAE) are not routinely reported to the CSO. However, an UP nonAE that may, in the opinion of the investigator, involve risk to the participant, affect others in the research study, or significantly impact the integrity of research data would be considered a non-serious UP and would be reported to the CSO. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

8 PARTICIPANT MANAGEMENT

8.1 Management of Adverse Events

An AE is any untoward medical occurrence in a participant administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the subject's participation in the research, whether or not related to the subject's participation in the research. This includes exacerbation of pre-existing conditions and intercurrent illnesses.

Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

All AEs identified in this study will be source documented, consistent with the policies and procedures referenced in Section 10. Among other details, source documentation will include the severity of each event (graded as described in Sections 8.2.1 and 8.2.2 and its relationship to study product, assessed by the study clinician according to the following categories and definitions:

Related	There is a reasonable possibility that the adverse event may be related to the study drug.
Not related	There is not a reasonable possibility that the adverse event may be related to the study drug.

There are 2 categories of AEs specific to CIR 363: solicited and unsolicited. Solicited AEs are described in Section 8.1.1. Unsolicited AEs are all other AEs. However, for infants and children, the following common events will not be recorded as AEs unless a prescribed concomitant medication is used to treat them: non-infectious rashes, teething pain, and spitting up. The use of medications will only be captured in the event of an AE or pre-existing condition.

SAEs are described in Section 8.1.2.

8.1.1 Solicited Adverse Events

Solicited AEs are predefined AEs that can occur after study product administration, may be expected to occur if the study product is insufficiently attenuated, and have protocol-specific criteria for reporting. Solicited AEs, whether identified by a parent/guardian or clinician, are only recorded on CRFs if they meet the definitions per Appendix II Individual symptoms listed in the "events" column that fail to meet the criteria in the "definition" column in Appendix II are recorded in source documents but are not recorded on the CRFs. During the Acute Phase of this study, solicited AEs meeting the criteria for reporting will be recorded on CRFs, assigned a severity grade (Section 8.2), and assessed for relationship to study product (see Section 8.1). Solicited AEs are defined in Appendix II and include the following:

1. Fever
2. URI
    a. Rhinorrhea,
    b. Pharyngitis,
    c. Cough without LRI, or
    d. Hoarseness
3. Otitis Media
4. LRI
    a. Wheezing,
    b. Pneumonia,
    c. Laryngotracheobronchitis (croup),
    d. Rhonchi, or
    e. Rales

Solicited AEs Elicited by History Unconfirmed by Clinical Assessment

With the exception of fever, solicited AEs occurring on the day of the study visit and reported by parents/guardians are recorded on the source documents but are NOT recorded on CRFs if a clinical assessment done on the day of the event(s) does/did not confirm their presence. For example, if a parent/guardian reports rhinorrhea on the day of visit, and there is/was no rhinorrhea upon exam, then the participant is considered to not have rhinorrhea that day.

If the parent/guardian report of a fever meets the "definition" column criteria in Appendix II on a day on which there was a clinical assessment, the fever will be recorded on CRFs regardless of whether the clinical assessment confirmed its presence.

Events elicited by parent/guardian history for days on which there was no clinical exam will be:

- Recorded on the CRFs as AEs if the parent/guardian description meets the "definition" column criteria in Appendix II.
- Recorded only on the source document, and NOT on the CRF, if the parent/guardian description fails to meet the "definition" column criteria in. For example, both rhinorrhea and cough must each occur on 2 consecutive days to meet the definition required for reporting per Appendix II.

8.1.2 Serious Adverse Events

A SAE is an AE, whether considered related to the study product or not, that meets one or more of the following criteria:

1. Results in death during the period of protocol-defined surveillance.
2. Is life threatening: defined as an event in which the participant was at immediate risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death were it more severe.
3. Requires inpatient hospitalization (or prolongation of existing hospitalization): defined as at least an overnight stay in the hospital or emergency ward for treatment that would have been inappropriate if administered in the outpatient setting.
4. Results in a persistent or significant disability/incapacity.
5. Is a congenital anomaly or birth defect.
6. Is an important medical event that may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed above.

8.1.3 Unexpected Adverse Event

An AE is unexpected if it is not listed in the IB or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. It is the responsibility of the IND Sponsor to make this determination.

8.2 Grading the Severity of Adverse Events

The Investigator will assess all AEs with respect to **Seriousness** (criteria listed above), **Severity** (intensity or grade), and **Causality** (relationship to study agent and relationship to research). All AEs and fever will be graded using the protocol-defined grading system outlined below.

8.2.1 AE Grading

Table 13: Grading for Adverse Events

Severity	Defined
Grade 1 Mild	No medical intervention required; may include over-the-counter medications managed by the participant or caregiver for treatment of symptoms. Does not interfere with usual activities, including eating and/or sleeping.
Grade 2 Moderate	Symptoms interfering to some degree with usual activities, including eating and/or sleeping. In most cases, symptoms severe enough to necessitate a medical care visit would likely meet this criterion; however, if medical care is sought and symptoms are assessed as only mild, the event may remain Grade 1. If prescription medication is used or recommended for symptoms, the event automatically moves to at least Grade 2.
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

8.2.2 Fever Grading

Table 14: Grading for Fever

Severity	Defined
Grade 1	≥100.4ºF but ≤101.4ºF
Grade 2	≥101.5ºF but ≤102.4ºF
Grade 3	≥102.5ºF but ≤104.8ºF
Grade 4	≥104.9ºF

8.3 Pausing and Stopping Rules

If any of the following occur in a participant during the specified period after receiving the study product, additional enrollment/inoculations will be temporarily suspended at all sites (Table 15).

Table 15: Pausing and Stopping Rules

Event	Reporting
• An SAE that cannot be attributed to an etiology unrelated to the study product. • >1 Grade 4 AE assessed as related to the vaccine that occurs within 28 days of study product administration • >2 Grade ≥3 unsolicited AEs of the same or similar type and assessed as related to the vaccine that occurs within 28 days of study product administration • Any LRI associated with vaccine virus shedding	A description of the vaccine-associated AE(s) or safety issue must be reported by the PI or study staff, within one business day of the PI's awareness, to the CSO and the DSMB by fax or email. The event will be reviewed by the DSMB prior to resuming enrollment.

The DSMB and regulatory sponsor will be informed and receive pertinent data of the event by the PI. Follow-up visits for participants already inoculated will continue as outlined in Appendix I (Table 18: Definitions of Solicited Adverse Events).

The DSMB will notify the PI (via the study sponsor) of their recommendations as to whether enrollment can resume, or if the study needs to be stopped. The regulatory sponsor (OCRPRO) will be informed about this decision. In the event of an SAE, the study may be resumed if it can be demonstrated to the DSMB that there is no proven causal relationship with the vaccine. The regulatory sponsor (OCRPRO) will determine if the FDA needs to be notified.

9 STATISTICAL CONSIDERATIONS

9.1 General Design Issues

9.1.1 General Design

The goal of this phase I, blinded, randomized, placebo-controlled vaccine trial is to assess the safety, infectivity, and immunogenicity of the B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 vaccine candidates in HPIV3-seropositive pediatric participants.

Twenty-five HPIV3-seropositive participants will be randomized in a 2:2:1 ratio (10:10:5) to receive either the B/HPIV3/HMPV-PreF-A vaccine at a dose of $10^{5.6}$ PFU, B/HPIV3/HMPV-F-B365 vaccine at a dose of $10^{5.6}$ PFU or placebo to evaluate safety and immunogenicity.

9.1.2 Description of the Statistical Methods to be Employed

This study, like other phase I studies, is exploratory, rather than confirmatory; its purpose is to assess frequencies of AEs and patterns of immune responses. Descriptive approaches will be used to meet the protocol objectives as stated in Section 2 of this protocol, as well as formal statistical tests as outlined in Section 9.5.

9.2 Outcome Measures

9.2.1 Primary Outcome Measures

Primary Objective:
Safety:

1. To assess the frequency and severity of study product–related solicited and unsolicited AEs from day 0 through the 28th day

   Outcome measures:
   Grade 1 or higher solicited AEs as defined in Appendix II from Day 0 through Day 28
   Any LRIs as defined in Appendix II from Day 0 through Day 28

Primary Objective:
Immunogenicity:

1. To evaluate the percentage of vaccinees with a ≥4-fold rise in HPIV3 serum neutralizing antibody titers at day 28

   Outcome measure:
   ≥4-fold rise in HPIV3 neutralizing serum antibody titers from pre-study product administration (screening) to the Day 28 Visit

2. To evaluate the percentage of vaccinees with a ≥4-fold rise in HMPV-neutralizing serum antibody titers at day 28

   Outcome measure:
   ≥4-fold rise in HMPV-neutralizing serum antibody titers from pre-study product administration (screening) to the Day 28 Visit

Primary Objective:
Infectivity:

1. To determine the peak titers of vaccine virus shed by each participant
   Outcome measure:
   Peak titers of vaccine virus shed from Study Days 0-28

2. To determine the proportion of vaccinees infected with vaccine virus [defined as shedding vaccine virus, detected by immunoplaque assay, RT-qPCR, and/or ≥4-fold rise in HPIV3-specific and/or HMPV-specific serum antibodies, detected by neutralizing antibody assays or ELISA]

   Outcome measure:
   Shedding of vaccine virus, detected by immunoplaque assay and/or RT-qPCR, and/or ≥4-fold rise in HPIV3 or HMPV-specific serum antibodies, detected by neutralizing antibody assay or ELISA

9.2.2 Secondary Outcome Measures

1. To assess the frequency and severity of study product–related MAAEs and SAEs from day 0 through the 180th day following inoculation

   Outcome measure:
   MAAEs and SAEs from Day 0 through Day 180

9.2.3 Exploratory Outcome Measures

1. To assess the incidence and magnitude of vaccine virus shedding, and the incidence of other respiratory pathogens, in samples collected at Illness Visits associated with solicited AEs on Study Days 0 through 28, and with serious AEs and LRIs from Study Days 0 through 28.

   Outcome measures:
   For solicited AEs between Days 0 and 28:
   - Detection and magnitude of vaccine virus shedding detected in Illness Visit specimens as determined by RT-qPCR.
   - Detection of other respiratory pathogens in Illness Visit specimens as determined by Fast-Track and GeneXpert assays.

2. To evaluate the genetic stability of vaccine isolates by sequence analysis.

   Outcome measures:
   Frequency of mutations detected by sequence analysis in isolates collected at the peak and end of vaccine shedding.

3. To evaluate the percentage of F expression in vaccine isolates by HPIV3/HMPV dual-staining immunoplaque assay.

   Outcome measures:
   Frequency of loss of HMPV F expression in vaccine isolates, detected by immunoplaque assay.

9.3 Sample Size and Accrual

9.3.1 Sample Size and Randomization

Twenty-five HPIV3-seropositive children will be enrolled and will be randomized to receive the B/HPIV3/HMPV-PreF-A vaccine at a dose of $10^{5.6}$ PFU, B/HPIV3/HMPV-F-B365 vaccine at a dose of $10^{5.6}$ PFU or placebo at a ratio of 2:2:1. The 2:2:1 randomization ratio will be used to maximize the information obtained regarding the response of children to the B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 vaccines.

The following calculations focus on the assessment of the safety of the vaccine and, in particular, occurrence of LRI due to vaccine virus from vaccine inoculation through day 28, which because of our screening procedures, occurs very infrequently in children who participate in these types of studies but would be considered a sentinel safety event if observed in children infected with vaccine virus.

Under the proposed sample size for HPIV3-seropositive participants, we will determine rates of LRI in each vaccine arm (n=10 for each vaccine group). We have chosen to focus on this safety assessment because a significant difference in rates of LRI in vaccinees vs. placebo recipients (particularly if associated with shedding of vaccine virus) would indicate that the vaccine is insufficiently attenuated and would preclude further clinical development. Monitoring the rate of LRI during the course of the trial allows us to determine whether the observed rate might raise safety concerns. Table 16 below characterizes the exact one-sided binomial p-values associated with a given number of observed LRIs by progressive enrollment numbers and under an assumed LRI rate of either 1.0% or 2.0%. These p-values are displayed for the first 10 enrollees. The values are subjectively categorized and color-coded according to statistical strength of evidence to rule out overall LRI rates of 1.0% (left) and 2.0% (right). As an example, if one LRI event was identified within the first 5 enrollees, the p-value for the hypothesis test of a 28-day rate of 2.0% would be 0.096, falling under the subjective categorization "moderate evidence of safety concern." This chart serves primarily as a monitoring guide for the overall proportion of participants experiencing LRI within 28 days of vaccination; the p-values are not shown for all N=70 rows, although exact p-values can be computed throughout the study according to the following formula:

$$\Pr(\geq \#\text{LRI}|\text{Enrollment}) = 1 - \sum_{i=0}^{\#\text{LRI}-1} \binom{\text{Enrollment}}{i} p^i \times (1-p)^{\text{Enrollment}-i},$$

where p denotes the proposed background rate of LRI (either 1.0% or 2.0%).

In our statistical analysis, we will form vaccine-arm-specific point estimates and 95% confidence intervals (CI) for these proportions if the number of LRIs is non-zero (otherwise, we will simply report that the number of LRIs observed in a given arm was zero). We expect that there will be very little power to compare LRI event rates across vaccine arms.

Table 16: Exact One-sided Binomial p-values for Monitoring the Strength of Evidence Regarding Proportion Experiencing LRI Within 28 Days of Vaccination

	28-day rate of 1.0%		28-day rate of 2.0%		
Enrollment	Pr(≥1 LRI)	Pr(≥2 LRI)	Pr(≥1 LRI)	Pr(≥2 LRI)	Pr(≥3 LRI)
1	0.010	--	0.020	--	--
2	0.020	<0.001	0.040	<0.001	--
3	0.030	<0.001	0.059	0.001	<0.001
4	0.039	<0.001	0.078	0.002	<0.001
5	0.049	<0.001	0.096	0.004	<0.001
6	0.059	0.001	0.114	0.006	<0.001
7	0.068	0.002	0.132	0.008	<0.001
8	0.077	0.003	0.149	0.010	<0.001
9	0.086	0.003	0.166	0.013	<0.001
10	0.096	0.004	0.183	0.017	<0.001

Region of compelling evidence of safety concern
Region of sufficient evidence of safety concern
Region of moderate evidence of safety concern
Region of insufficient evidence of safety concern

In addition, we will monitor the following safety-related outcomes:

- LRIs associated with shedding of the vaccine virus, in the absence or presence of other viruses.
- LRIs associated with shedding of other viruses.
- LRIs in which a pathogen is not detected.

Our immunogenicity analysis will be primarily descriptive, and will be based on determining (and comparing) the proportion achieving a ≥ 4-fold rise in HPIV3 and HMPV neutralizing serum antibody titers 28 days following vaccination. Primarily, we will compare each of the two active vaccine arms (B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365; n=10 each) to placebo (n=5). Secondarily, we will compare B/HPIV3/HMPV-PreF-A (n=10) to B/HPIV3/HMPV-F-B365 (n=10). This study will not be powered to conduct a comparison between groups with formal hypothesis testing.

9.4 Monitoring

9.4.1 Site Monitoring Plan

As per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH-GCP) 5.18 and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID/OCRPRO will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the informed consent form (ICF) process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare REDCap data abstracts with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections, OHRP), FDA, and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms, REDCap data abstracts) and pertinent hospital or clinical records readily available for inspection by the local IRB, the FDA, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the PI and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status and regulatory obligations.

9.4.2 Monitoring by the NIAID Intramural Data and Safety Monitoring Board

The NIAID Intramural DSMB is constituted to review the safety data of Intramural NIAID clinical studies that require DSMB oversight. The NIAID Intramural DSMB includes independent experts in infectious diseases, biostatistics, and clinical research that do not have direct involvement in the conduct of the study and have no significant conflicts of interests as defined by NIAID policy. The DSMB will review the protocol prior to opening the study to enrollment. The DSMB will meet at least twice a year or on a schedule specified by the DSMB to review the completeness of the study data, the adherence to the protocol, and AE data. After completion of the Day 28 visit of the last participant enrolled, there will also be an unblinded DSMB review of safety and vaccine shedding data. Cumulative safety data (pooled across arms, with the vaccine and placebo arms presented together) will be submitted to the DSMB Executive Secretary for DSMB review. The PI will submit the written DSMB recommendations to the IRB upon receipt. All SAEs, LRIs, UPs and IND safety reports will be reported by the PI to the DSMB at the same time that they are submitted to the IRB and/or regulatory sponsor (OCRPRO). The PI will notify the DSMB of any cases of intentional or unintentional unblinding as soon as possible. The PI will notify the Board at the time a pausing or stopping criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study.

DSMB reviews will be requested by the PI if pausing and stopping rules are triggered (Section 8.3, Table 15).

- An SAE that cannot be attributed to an etiology or a cause unrelated to the study product occurs throughout the study.

- >1 Grade 4 solicited or unsolicited adverse event (AE) assessed as related to the investigational vaccine within 28 days of study product administration.

- >2 Grade 3 unsolicited AEs of the same or similar type that are assessed as related to the investigational vaccine within 28 days of study product administration

- Any Lower Respiratory Illness is associated with vaccine virus shedding

Accrual and study product administration will stop pending review.

The site will send respiratory viral samples to the JHU CIR laboratory to determine shedding of HPIV3, HMPV or adventitious viral agents, and the lab will send their results for review directly to the DSMB. Information on treatment assignment will be provided to the DSMB by the unblinded dispenser. The DSMB will review the relevant safety information and available data, including presence of vaccine virus shedding, whether other adventitious agents are present, and whether the participant received placebo or one of the candidate vaccines. The DSMB will also review whether the AE, serious AE, or LRI can be attributed to an etiology, a cause, or a diagnosis unrelated to the study vaccine, and if it is associated with shedding of vaccine virus at the time of the event (even if another pathogen is identified).

If the DSMB decides the study can continue unchanged, they will communicate their findings to the Protocol Team. Alternatively, the DSMB may recommend modifications to the study design.

9.5 Analyses

9.5.1 Assessment of Primary Objectives

Safety and immunogenicity data from all participants will be summarized.

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 6.10 within the first 28 days after inoculation may be excluded from the immunogenicity evaluations after the time of the treatment. These participants, however, will 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% CI, during Study Days 0 to 28 will be summarized. In addition, line listing of individual clinical solicited AEs and unsolicited AEs, graded by severity, will be prepared for events occurring during Study Days 0 to 28.

The proportion of participants that develop 4-fold or greater rises in HPIV3 and HMPV antibody titers following inoculation will be summarized. A line listing of the individual HPIV3 and HMPV antibody titer pre- and post-inoculation will be prepared. In addition, the geometric mean and median antibody titers will be provided for each treatment group.

The proportion of participants with infection defined as recovery of vaccine virus from a nasal swab as determined by immunoplaque assay or RT-PCR, and/or a ≥4-fold rise in neutralizing antibody titer to HPIV3 or HMPV, will be summarized. A line listing of the individual peak titer of vaccine virus shed and duration of virus shedding in nasal swabs by each individual will be prepared. In addition, the geometric mean peak titer and mean duration of virus shed will be provided.

10 DATA HANDLING AND RECORD KEEPING

10.1 Data Management and Coordination

The Data Coordinating Center for this study will be housed at VUMC. The clinical sites will maintain adequate and accurate research records containing all information pertinent to the study for all screened and enrolled participants, including CRFs and supporting source data per the MOP.

Data from source documentation for subjects enrolled in the study will be entered into the study data system. The data entry is to be completed on an ongoing basis during the study. Data entry shall be performed by authorized individuals who have a unique log-on and password. Corrections to the data system shall be tracked electronically (password protected) with time, date, individual making the correction, and what was changed.

Source documents include all recordings of observations or notations of clinical activities, and all reports and records necessary for the evaluation and reconstruction of the clinical trial. Source documents may include the subject's medical records and laboratory reports. Data will be collected directly from subjects during study visits and contacts. Data will be entered into the REDCap database developed by the Vanderbilt Coordinating Center.

10.2 Essential and Source Documents and Access to Source Data

Study-related documentation will be completed as required by the IRB, the sponsor, and regulatory authorities. Continuing review documentation will be submitted to the IRB as specified by the IRB. An annual report will be submitted by the sponsor to the FDA based on the anniversary date that the IND for the B/HPIV3/HMPV-PreF-A and B/HPIV3/HMPV-F-B365 vaccines went into effect. These reports will provide a brief description of the progress of the investigation as outlined in 21 CFR 312.33 and will include any revisions of the protocol not previously submitted to the FDA. All study records must be accessible for inspection, monitoring, and/or auditing during and after the conduct of the study by authorized representatives of the study sponsors and their contracted monitors, the FDA, regulatory authorities, IRB, OHRP, and other applicable regulatory entities. Records must be kept on-site throughout the period of study implementation; thereafter, instructions for off-site storage may be provided by NIH. No study records may be removed to an off-site location or destroyed prior to receiving approval from NIH.

Study-related documents will be maintained for a period of at least 2 years after final marketing approval of the vaccine, or at least 2 years after the formal discontinuation of clinical development of the product (or longer based upon local laws). The sponsor is required to inform the PI as to when such documents need no longer be retained. No study document should be destroyed without prior written agreement between the sponsor and the PI. Storage of all study- related documents will be such that confidentiality will be strictly maintained. These records are also to be maintained in compliance with IRB, state, and federal medical records retention requirements, whichever are longest. Should the PI wish to assign the study records to another party and/or move them to another location, the PI must provide written notification of such intent to the sponsor with the name of the person who will accept responsibility for the transferred records and/or their new location. The sponsor must be notified in writing, and written permission must be received from the sponsor prior to destruction or relocation of research records.

10.3 Investigator's Brochure

The current version of the IB comprehensively describes all the available preclinical experience with the experimental vaccine. If relevant new information becomes available during the course of the trial, the PI will receive a revised IB or an amendment to the current version.

10.4 Quality Control and Quality Assurance

Essential documents and participant research records are subject to continuous quality control and quality assurance procedures consistent with the MOP.

11 CLINICAL SITE MONITORING

Site monitors under contract to NIAID will inspect study facilities and review participant study records including ICD, CRFs, medical records, laboratory records, and study product records, to ensure protection of study participants, compliance with the IRB approved protocol, and accuracy and completeness of records. The monitors also will review essential document files to ensure compliance with all applicable regulatory requirements. Study staff will make study facilities and documents available for inspection by the monitors.

The sponsor will retain originals of the Form FDA 1572 and copies of other study documents as deemed necessary.

Statement of Compliance

The trial will be conducted in compliance with this protocol, ICH-GCP guidelines, FDA guidelines and applicable regulatory requirements. The site monitoring will be conducted according to the OCRPRO Clinical Trial Management Group's Monitoring Plan.

12 HUMAN SUBJECTS PROTECTIONS

12.1 Institutional Review Board/Ethics Committee Review and Approval

Prior to study initiation, the PI will obtain IRB review and approval of this protocol and ICDs in accordance with 45 CRF 46. In addition to the initial review and approval, the IRB must review the study at least annually. The PI must also promptly report to the IRB any changes in the study and any UPs involving risks to participants or others.

All IRB policies and procedures must be followed, and complete documentation of all correspondence to and from the IRBs must be maintained in the essential document files.

A copy of the study approval (including approval of the ICD) is to be maintained in the investigator's study document binder, and a copy will be supplied to the sponsor.

During the study, the PI is responsible for providing the IRB with all documents subject to review (i.e., protocol amendments, ICD updates, advertisements, and any written information that may be provided to the participant's parents/guardians). Study progress reports will be made to the IRB by the investigator in accordance with IRB guidelines and government regulations.

12.2 Vulnerable Participants

The NIH is mandated by law to ensure that children be included in clinical research when appropriate (30, 31). This study responds to that mandate and will provide clinical research data to inform B/P\HPIV3/HMPV vaccine infectivity, safety and immunogenicity in children. Nonetheless, the infants who take part in this study are considered vulnerable participants per the US CFR, and site IRBs/IBCs must consider the potential risks and benefits to child participants as described in 45 CFR 46 Subpart D (for children).

12.3 Informed Consent

In obtaining and documenting informed consent, the PI must comply with the applicable regulatory requirements, ICH-GCP guidelines, and ethical principles. The written ICD must be approved by the IRB prior to its use.

Written informed consent for study participation will be obtained before any study-specific procedures are performed. The informed consent process will include information exchange, detailed discussion, and assessment of understanding of all required elements of informed consent, including the potential risks, benefits, and alternatives to study participation.

As part of the informed consent process, parents/guardians will also be asked whether they agree to storage and future research testing of the biological specimens that remaining after all protocol-specified testing has been completed. Future research testing of residual specimens may be declined with no impact on other aspects of study participation.

12.4 Potential Benefits

Participants may not receive any direct vaccine-related benefit from enrollment in this study. Some children who receive vaccine may be protected against infections with wt HPIV3 and HMPV that circulate in the community. It is hoped that information gained in this study will contribute to the development of a safe and effective vaccine for the prevention of illness associated with HPIV3 and HMPV infection.

Parents/guardian may be offered child safety seat educational material and referral to community inspection stations by study staff, and may be offered certified lactation counseling services, if appropriate.

12.5 Potential Risks

12.5.1 Venipuncture

Risks occasionally associated with venipuncture include pain and bruising at the site of venipuncture, lightheadedness, infection, and rarely syncope. Before each blood draw, we will offer to use anesthetic skin cream to decrease the pain.

12.5.2 Nasal Swab

Nasal swabs can cause discomfort and on rare occasions are associated with nose bleeds.

12.5.3 Topical Anesthetic Cream

Risks occasionally associated with the use of topical anesthetic cream include temporary skin discoloration, skin irritation, rash, hives, and rarely, dizziness or drowsiness.

12.5.4 Receipt of Study Product

If the study vaccine is insufficiently attenuated, participants could experience rhinorrhea, cough, fever, otitis media, or LRI. Immediate hypersensitivity reactions including urticaria, anaphylaxis, or other immunoglobulin E (IgE)-mediated responses are possible, as with any vaccine. With any investigational vaccine, there is a theoretical possibility of risks about which we have no present knowledge. Parents/guardians will be informed of any such risks should further data become available.

The study participant's family does not pay for the study product or research visits including examinations and laboratory tests that are part of this study, including evaluation of illness, if any. NIAID agrees that the funding for appropriate acute care will be provided through the NIAID contract for any side effects that are determined to be related to the administration of vaccine.

12.6 Reimbursement/Compensation

12.6.1 Study Compensation: Acute Phase and Follow-up

Compensation will be in the form of a check or another method of payment as decided by the individual sites. The amount of compensation for study visits, contacts and study completion bonuses will be determined by the individual sites.

In addition, the parent/guardian will receive bus tokens, taxi fare, or parking passes as needed for study visits. Parent/guardian will be compensated only for those portions of the study that are completed. Compensation will be in accordance with IRB policies and procedures and will be subject to IRB approval.

Participants may receive age-appropriate books or small toys. The total value of the books or toys will not exceed $10 per participant.

12.7 Privacy and Confidentiality

All study procedures will be conducted in private, and every effort will be made to protect participant privacy and confidentiality to the extent possible. Participant information will not be released without written permission to do so except as necessary for review, monitoring, auditing or as required by law, or as described in Section 9.4 and Section 10.2.

All study-related information will be stored securely. Participant research records will be stored in locked areas with access limited to study staff. All laboratory specimens, CRFs, and other documents that may be transmitted off-site will be identified by coded number only.

All local databases must be secured with password protected access systems. Lists, logbooks, appointment books, and any other documents that link subject identification numbers to personal identifying information should be stored in a separate, locked location in an area with limited access.

12.8 Management of Incidental Findings

Study clinicians will inform parents/guardians of all clinically meaningful physical exam findings and laboratory tests. When applicable, study clinicians will provide referrals to non-study sources of medical care for further evaluation and/or treatment of these findings.

13 ADMINISTRATIVE PROCEDURES

13.1 Regulatory Oversight

CIR 363 is sponsored by the OCRPRO, DCR, NIAID, NIH.

OCRPRO is responsible for regulatory oversight of this study. Safety-related information pertaining to the study product will be distributed prior to and during the conduct of the study, in accordance with its sponsor obligations.

NIAID provides funding to the clinical research sites at which this study will be conducted. Each institute contracts with independent clinical site monitors who will perform monitoring visits as described in Section 9.4. As part of these visits, monitors will inspect study-related documentation to ensure compliance with all applicable local and US regulatory requirements.

13.2 Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, the CIR must have the protocol and the protocol ICDs approved, as appropriate, by their local IRB/IBC, local IBC, and any other applicable regulatory entity.

For any future protocol amendments, upon receiving final IRB/IBC and any other applicable regulatory entity approvals, CIR should implement the amendment immediately.

13.3 Study Implementation

This study will be conducted in accordance with the protocol, ICH guidelines, and all applicable local and US regulations. Study implementation will also be guided by the study-specific MOP and other study implementation materials.

13.4 Protocol Deviation Reporting

Protocol deviations must be documented in participant research records. Reasons for the deviations and corrective and preventive actions taken in response to the deviations should also be documented. See MOP for further instructions.

Deviations will be reported to the IRB and other applicable review bodies in accordance with the policies and procedures of these review bodies. Serious deviations that are associated with increased risk to one or more study participants and/or significant impacts on the integrity of study data must also be reported following procedures specified in the MOP.

13.5 ClinicalTrials.gov

This protocol is subject to the US Food and Drug Administration Amendments Act of 2007 (FDAAA), including registration in ClinicalTrials.gov.

14 PUBLICATIONS

Publication of the results of this trial will be governed by NIAID policies. Any presentation, abstract, or manuscript will be made available for review by the pharmaceutical and NIAID sponsors prior to submission. Publication or presentation approval will conform to any Cooperative Research and Development Agreement (CRADA) or other collaborative agreement in place.

15 ABBREVIATIONS

ACIP	Advisory Committee on Immunization Practices
AE	adverse event
ALRI	acute lower respiratory infection
BSC	biological safety cabinet
CACI	compounding aseptic containment isolator
CDC	Centers for Disease Control and Prevention
cDNA	complementary deoxyribonucleic acid
CFR	Code of Federal Regulations
CI	confidence interval
CIR	Center for Immunization Research
CRADA	Cooperative Research and Development Agreement
CRF	case report form
CSO	Clinical Safety Office
DCR	Division of Clinical Research
DMEM	Dulbecco's modified Eagle medium
DSMB	data and safety monitoring board
EENT	eyes, ears, nose, and throat
ELISA	enzyme-linked immunosorbent assay
FDA	US Food and Drug Administration
FDAAA	US Food and Drug Administration Amendments Act of 2007
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
GMT	Geometric mean titer
HAI	hemagglutination-inhibition
HIPAA	Health Insurance Portability and Accountability Act
HMPV	Human Metapneumovirus
HN	Hemagglutinin-neuraminidase
HPIV3	Human Parainfluenza Virus Type 3
IB	investigator's brochure
IBC	institutional biosafety committee
ICD	informed consent document
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IgA	immunoglobulin A
IgG	immunoglobulin G
IND	Investigational New Drug application
IRB	institutional review board
JHU	Johns Hopkins University
JHSPH	Johns Hopkins Bloomberg School of Public Health
LID	Laboratory of Infectious Diseases
LDS	Low dead space
LRI	lower respiratory infection
MA	medically attended
MAAE	medically attended adverse event
MOP	Manual of Procedures
mRNA	messenger RNA
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NW	Nasal wash

OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
PCR	polymerase chain reaction
PFU	plaque forming unit(s)
PI	principal investigator
PIV	parainfluenza virus
PRNT	plaque reduction neutralization test
rDNA	recombinant deoxyribonucleic acid
REDCap	Research Electronic Data Capture
RNA	ribonucleic acid
RSV	respiratory syncytial virus
RT-PCR	reverse transcription polymerase chain reaction
RT-qPCR	reverse transcription quantitative polymerase chain reaction
rRT-PCR	real-time reverse transcription polymerase chain reaction
RVS	RNA Viruses Section
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SERF	Safety Expedited Report Form
SOP	standard operating procedure
SRCP	Safety Review and Communications Plan
SUSAR	serious and unexpected suspected adverse reaction
TCID	Tissue-culture infectious doses
UP	unanticipated problem
URI	upper respiratory tract illness
US	United States
VAR	vaccine administration record
VUMC	Vanderbilt University Medical Center
WCG IRB	WIRB-Copernicus Group IRB
WHO	World Health Organization
wt	wild-type

Appendix I: Tables Referenced in the Background Section

Table 17: Schedule of Events

	ACUTE PHASE																	Monthly Contact	
	Screening	Day 0	Days 1-2	Day 3	Day 4	Day 5	Day 6	Day 7	Days 8-9	Day 10	Days 11-13	Day 14	Days 15-27	Day 28	Day 29	Illness Visit	Early DC	Days 29 - 180	Day 180
Study window			± 1 day											+7 days					±7 days
In person visit	X	X		X	X	X	X	X		X		X		X		X	X		
Non-visit contact			X						X		X		X		X			X	X
Informed consent	X																		
History	X																		
Interim history		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical exam (full)[b]	(X)	X																	
Clinical assessment (focused PE)		(X)		X	X	X	X	X		X		X		(X)[c]		X			
Administer study product		X																	
Blood for: immunologic assays	5mL[a]	(X)[a]												5mL[a]			5mL[a]		
Nasal swab for: Vaccine viral detection & quantification		X		X	X	X	X	X		X		X		X		X*	X		
Request adventitious agent assay		X												(X)[c]		X			
Nasal swab for mucosal antibody		X								X		X		X			X		
	*A clinical assessment is performed and a nasal swab specimen is obtained only if the participant has febrile or respiratory illness or otitis media. Timing of visit and collection are outlined in Table 9 and Table 10																		

[a] Up to 5 mL of blood may be obtained for each blood draw. Blood for HPIV3/HMPV immunological assay is obtained not more than 42 days prior to inoculation. Screening HPIV3 antibody can be obtained greater than 42 days prior to inoculation so long as it is obtained within the same calendar year. Additional blood may be drawn for immunologic assay on Day 0 if insufficient volume is obtained at screening or screening >42 days prior to enrollment.

[b] A physical exam can be completed at screening or enrollment.

[c] Clinical assessment is performed and adventitious testing is requested only if the participant has febrile or respiratory illness or otitis media that began prior to midnight on day 28 and has not been addressed at a previous illness visit.

Appendix II: Definitions of Solicited Adverse Events

Table 18: Definitions of Solicited Adverse Events

Event	Defined
Fever	Temporal or rectal temperatures ≥100.4ºF
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[1]	Pharyngeal erythema accompanied by exudate or pharyngeal erythema with 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 (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 assessments must be made by a medical professional and confirmed by a second medical professional, if possible

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