Randomized, Double-Blind, Placebo-Controlled, Phase 2 Study in Healthy Volunteers to Evaluate the Efficacy and Safety of CR6261 in an H1N1 Influenza Healthy Human Challenge Model

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Influenza Virus Manufactured by
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TEAM ROSTER

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

ADA	Anti-drug Antibodies
AE	Adverse Event/Adverse Experience
ALP	Alkaline phosphatase activity
AR	Adverse Reaction
AUC	Area under the curve
BMI	Body mass index
CBC	Complete Blood Count
CCPU	Clinical Center Patient Unit
CFR	Code of Federal Regulations
CI	Confidence Interval
CMI	Cell-mediated immunity
CRF	Case Report Form
CRIMSON	Clinical Research Information Management System of the NIAID
CRIS	Clinical Research Information System
CSO	Clinical Safety Office
DCR	Division of Clinical Research
DLM	Department of Laboratory Medicine
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
ECHO	Echocardiography
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practices
HA	Hemagglutinin
HAI	Hemagglutination-inhibition
HIV	Human Immunodeficiency Virus
HRPP	Human Research Protection Program
IBC	Institutional Biosafety Committees
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
Ig	Immunoglobulin
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board
IV	Intravenous
LID	Laboratory of Infectious Disease
mAb	Monoclonal Antibody
N	Number (typically refers to participants)
NI	Neuraminidase Inhibition
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NOAEL	No observed adverse effect level
NP	Nasopharyngeal
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
OHSRP	Office of Human Subjects Research Protections
PCR	Polymerase Chain Reaction
PD	Pharmacodynamics

PI	Principal Investigator
PFT	Pulmonary Function Test
PK	Pharmacokinetics
qPCR	Quantitative PCR
RNA	Ribonucleic acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAE	Serious Adverse Event/Serious Adverse Experience
SAR	Serious Adverse Reaction
SERF	Safety Expedited Report Form
SCSU	Special Clinical Studies Unit
SMM	Sponsor Medical Monitor
SRCP	Safety Review and Communications Plan
SUSAR	Serious, Unexpected, Suspected Adverse Events
$TCID_{50}$	50% Tissue Culture Infective Dose
Th1	CD4+ T helper (Th1) cells
THC	Tetrahydrocannabinol
UBC	United Biosource Corporation
UP	Unanticipated Problem

PROTOCOL SUMMARY

Full Title:	Randomized, Double-Blind, Placebo-Controlled, Phase 2 Study in Healthy Volunteers to Evaluate the Efficacy and Safety of CR6261 in an H1N1 Influenza Healthy Human Challenge Model
Short Title:	CR6261 Influenza Challenge
IND Sponsor:	OCRPRO, DCR, NIAID, NIH
Conducted by:	LID
Principal Investigator:	Matthew J. Memoli, MD, MS
Sample Size:	122 participants
Accrual Ceiling:	200 participants
Study Population:	Healthy volunteers 18 to 45 years of age
Accrual Period:	3 years
Study Design:	This is a randomized, double-blind, placebo-controlled, single-center, phase 2 study of CR6261 administered as a 2-hour IV infusion 24 hours after intranasal challenge with the Influenza A 2009 H1N1 human challenge virus.
Study Duration:	The study will begin approximately in October 2014 and will require 1 to 2 years to complete. The length of individual subject participation is about 2 ½ months.
Study Agents	**1.** Live, recombinantly derived A/Ca/04/2009-like virus **Note:** Throughout the remainder of this document this virus will be referred to as Influenza A 2009 H1N1 human challenge virus or human challenge virus. **2.** CR6261, a human immunoglobulin (Ig)G1 monoclonal antibody (mAb) produced in PER.C6® cells that is directed against a conserved region in the stem of influenza hemagglutinin (HA). CR6261 exhibits neutralizing activity against H1, H2, H5, H6, H8, and H9 influenza subtypes *in vitro*, as well as therapeutic and prophylactic efficacy against H1N1 and H5N1 (where N denotes neuraminidase) influenza in mice. **3.** Placebo, 5% dextrose (D-glucose) water
Intervention Description:	The human challenge virus will be administered intranasally to each participant using a nasal sprayer. A total dose of 10^7 TCID50 of virus will be administered. A single intravenous infusion of CR6261 (50mg/kg) or

placebo will be administered over a 2-hour period 24 hours after virus administration.

Primary Objective: To demonstrate that CR6261 leads to a reduction in viral shedding compared with placebo with respect to the area under the curve (AUC) as determined by quantitative PCR (qPCR) in nasopharyngeal (NP) swabs in all treated subjects

Secondary Objectives:

- To demonstrate that CR6261 leads to a reduced rate of influenza induced disease in all CR6261-treated subjects vs. those receiving placebo.
- To compare the difference in influenza clinical illness severity for CR6261-treated subjects with that in subjects given placebo
- To correlate clinical illness to quantitation of viral shedding
- To evaluate the safety of CR6261 compared with placebo
- To evaluate the pharmacokinetics (PK) of CR6261

Exploratory Objectives:

- To evaluate the difference in proportion of subjects with seroconversion to anti-hemagglutinin inhibition (HAI) antibodies between CR6261 and placebo-treated subjects
- To evaluate the incidence and severity of individual influenza symptoms in CR6261-treated subjects compared with placebo-treated subjects
- To evaluate the incidence and severity of symptoms (individual and composite) using the Flu-Pro questionnaire and physician assessment in CR6261-treated subjects compared with placebo-treated subjects
- To assess cell-mediated immunity (CMI) and RNA microarray analysis following administration of CR6261
- To assess cytokine responses in peripheral blood
- To evaluate viral infectivity following administration of CR6261 using exploratory infectivity assays

- To develop a mucosal PK assay, and if successful, to subsequently assess mucosal PK of CR6261
- To undertake mucosal cytokine assay development, and if successful, to subsequently assess mucosal cytokines
- To undertake mucosal microarray RNA assay development, and if successful, to subsequently assess mucosal microarray RNA patterns
- To investigate the development of anti-drug antibodies (ADA) after administration of CR6261
- To evaluate the development of viral resistance against CR6261 with sequencing (full length of HA) and phenotypic assays

Endpoints:

Primary:

Objective: To demonstrate that CR6261 leads to a reduction in viral shedding compared with placebo with respect to the area under the curve (AUC) as determined by quantitative PCR (qPCR) in nasopharyngeal (NP) swabs in all treated subjects

Endpoint: AUC of viral shedding will be used as a primary endpoint in all treated subjects. AUC will be measured using qRT-PCR and will be based on pg/ml of viral RNA vs. time.

Secondary:

Objective: To demonstrate that CR6261 leads to a reduced rate of influenza induced disease in all CR6261-treated subjects vs. those receiving placebo.

Endpoint: Presence of influenza-induced disease will be used as a secondary endpoint, which will be defined as viral shedding plus at least one clinical symptom as defined in this protocol. This will be used to determine if an individual was or was not protected from illness in each treatment group.

Objective: To compare the difference in influenza clinical illness severity for CR6261-treated subjects with that in subjects given placebo

Endpoint: Participant self-Assessment questionnaires will be collected daily and converted to an objective score. Mean scores in each group will be compared for a statistically significant difference in illness.

Objective: To correlate clinical illness to quantitation of viral shedding

Endpoint: Quantification of virus by qRT-PCR will be performed and timing and overall quantity of viral RNA detected will be correlated to daily clinical scores and evaluated for statistical significance in the two groups.

Objective: To evaluate the safety of CR6261 compared with placebo

Endpoint: A difference between number of AEs possibly, probably, or definitely related to the infusion will be evaluated in the treatment and placebo groups.

Objective: To evaluate the pharmacokinetics (PK) of CR6261

Endpoint: Blood draws for measurement of serum concentration of drug will be drawn at appropriate times to evaluate the kinetics of CR6261

Précis

The high morbidity and mortality associated with both pandemic and seasonal influenza and the threat of new potentially pandemic strains emerging makes influenza an important infectious disease and public health problem. Mean annual estimates of influenza deaths due to seasonal influenza alone attributes up to 36,000 deaths in the US and 250,000 to 500,000 deaths in industrialized countries to influenza.[1-3] Pandemics can have an even more devastating effect. Public health agencies must continue to be prepared by making attempts to reduce the public health impact of this important virus.

In the realm of influenza therapeutics, antiviral drugs are currently used to treat influenza infection in those who fail to be protected by current vaccines or those who do not receive a vaccine. Currently, only two classes of antivirals are FDA approved for the treatment of influenza A: neuraminidase inhibitors and matrix M2 channel blockers. Although these drugs have been shown to be effective in reducing influenza illness by 24-48 hours and reducing shedding in relatively healthy adults, as with vaccination, they have had limited effectiveness in high risk groups and those who have severe or complicated influenza infections. In addition, antiviral resistance has become very common in human influenza A viruses, as currently circulating H1N1 and H3N2 strains are resistant to the adamantane M2 channel blockers and many cases of neuraminidase inhibitor resistance have also been reported with strains of both subtypes. This resistance can develop quickly and in most cases only requires a single amino acid change. Given these significant issues with currently available treatments, novel therapies for influenza are clearly needed.[4]

Live virus challenge studies have played a pivotal role in developing influenza therapeutics in the past, and they will be instrumental in the future. No novel therapeutic or prophylactic agent has been FDA-approved since the last influenza challenge studies ceased over a decade ago. In collaboration with the Crucell Vaccine Institute (part of Crucell which is in the Janssen family of Pharmaceutical Companies of Johnson & Johnson) this protocol will evaluate mAb CR6261 for possible therapeutic value. Unlike the current antivirals that are compounds which interfere with some portion of the viral replicative cycle, this agent is a mAb that targets the stem of HA, neutralizing the virus by stabilizing the pre-fusion state and preventing the pH-dependent fusion of viral and cellular membranes.[5,6] Pre-clinical data suggest that this mAb has good cross-protective efficacy with a variety of HA subtypes unlike current vaccines, making it potentially effective in the event of an emerging influenza virus outbreak with a novel HA subtype. In addition, the conserved nature of the HA stem region suggests that amino acid changes conferring resistance are much less likely. We will effort to demonstrate that CR6261 leads to improved outcomes compared with placebo with respect to the AUC of virus shedding as determined by qPCR in NP swabs in all treated subjects.

1 Background Information and Scientific Rationale

The high morbidity and mortality associated with both pandemic and seasonal influenza, and the threat of new pandemic strains, continues to keep influenza at the forefront of infectious disease and public health research. Mean annual estimates of deaths due to seasonal influenza alone are 36,000 in the US and 250,000 to 500,000 in industrialized countries.[1,2] Pandemics can have an even more devastating effect, and we must continue to be prepared by making attempts to reduce the public health impact of this important virus.

In the realm of influenza therapeutics, antiviral drugs are currently used to treat influenza infection in those who fail to be protected by current vaccines or those who do not receive a vaccine. Currently, only two classes of antivirals are FDA approved for the treatment of influenza A: neuraminidase inhibitors and matrix M2 channel blockers. Although these drugs have been shown to be effective in reducing influenza illness by 24-48 hours and reducing shedding in relatively healthy adults, as with vaccination, they have had limited effectiveness in high-risk groups and those who have severe or complicated influenza infections. In addition, antiviral resistance has become very common in human influenza A viruses, as currently circulating H1N1 and H3N2 strains are resistant to the adamantane M2 channel blockers, and many cases of neuraminidase inhibitor resistance have also been reported with strains of both subtypes. This resistance can develop quickly and in most cases only requires a single amino acid change. Given these significant issues with currently available treatments, novel therapies for influenza are clearly needed.[4]

Live virus challenge studies have played a pivotal role in developing influenza therapeutics in the past, and they will be instrumental in the future. No novel therapeutic or prophylactic agent has been approved by the FDA since the last influenza challenge studies ceased over a decade ago. During the past year we have completed the validation of a healthy human volunteer challenge model with the Influenza A 2009 H1N1 human challenge virus. This protocol (#12-I-0077) allowed us to determine the optimal dose that induces influenza illness in >60% of individuals with an HAI titer of ≤40. In fact, at a dose of 107 $TCID_{50}$, we were able to induce mild to moderate influenza illness in 69% of participants. This is the first time a wild-type influenza challenge virus has been validated and characterized for use in future studies. This study demonstrated good safety of the challenge virus as shown in Table 1.

Table 1. Unexpected Adverse Events in Human Challenge Study Using Wild-Type Influenza Virus

Subject	Problem Description	Grade	Relationship
PD44	Bloating	1	H1N1pdm Challenge Virus - Probably Related
PD07	Creatine kinase	3	H1N1pdm Challenge Virus - Unlikely Related
PD48	Creatine kinase	3	H1N1pdm Challenge Virus - Unlikely Related
PD07	Elevated serum bilirubin (hyperbilirubinemia)	4	H1N1pdm Challenge Virus - Unlikely Related
PD47	Epistaxis	1	H1N1pdm Challenge Virus - Unlikely Related
PD47	Lightheadedness	1	H1N1pdm Challenge Virus - Probably Related
PD45	Musculoskeletal spasm	1	H1N1pdm Challenge Virus - Probably Related
PD39	Oral aphthous ulcer(s)	1	H1N1pdm Challenge Virus - Probably Related
PD47	Photophobia	1	H1N1pdm Challenge Virus - Probably Related
PD43	Presyncope	1	H1N1pdm Challenge Virus - Possibly Related
PD45	Sinus pain	1	H1N1pdm Challenge Virus - Probably Related
PD39	Sneezing	1	H1N1pdm Challenge Virus - Probably Related
PD48	Sneezing	1	H1N1pdm Challenge Virus - Probably Related
PD43	Sneezing	1	H1N1pdm Challenge Virus - Definitely Related
PD18	Sores mouth	1	H1N1pdm Challenge Virus - Definitely Related
PD29	Vomiting	1	H1N1pdm Challenge Virus - Possibly Related

Human challenge models of influenza infection have proved to be a useful alternative to field-based clinical trials to provide early evidence of efficacy of potential anti-viral agents and vaccines. Now that we have a validated H1N1 model, we believe we can apply this model to better understand the performance of novel therapeutics.

1.1 CR6261

CR6261 is a human IgG1 mAb produced in PER.C6® cells that is directed against a conserved region in the stem of influenza HA. CR6261 exhibits neutralizing activity against H1, H2, H5, H6, H8, and H9 influenza subtypes *in vitro*, as well as therapeutic and prophylactic efficacy against H1N1 and H5N1 (where N denotes neuraminidase) influenza in mice. In a placebo-controlled phase 1 study (protocol FLU-M6-C001), escalating IV doses (single doses of 2, 5, 15, 30, and 50 mg/kg) of CR6261 have been evaluated in

healthy volunteers. Dosing and follow-up in that study are now complete, and the unblinded safety data indicate that IV doses up to 50 mg/kg are well tolerated.

For the most comprehensive nonclinical and clinical information regarding CR6261, refer to the latest version of the Investigator's Brochure for CR6261.

1.1.1 Non-clinical studies

The prophylactic and therapeutic in vivo efficacy of CR6261 was demonstrated in murine lethal challenge models with H5N1 and H1N1 influenza viruses. CR6261 showed therapeutic efficacy in a lethal H5N1 influenza ferret model. In an influenza pandemic H1N1 ferret model, CR6261 protected against moderate to severe respiratory disease and reduced body weight loss and lung damage. In addition, no interference of CR6261 with oseltamivir has been observed in an in vivo experiment.

Single dose pharmacokinetic (PK) studies were performed in mice, rats, ferrets and non-human primates (NHPs) after single intravenous (IV) injection of CR6261. Depending on the dose of CR6261, in mice, the half-life in the □-phase is about 3 hours and in the □-phase it ranges from 129 to 163 hours. In rats, the half-life in the □-phase ranges from 3 to 5 hours and in the □-phase it ranges from 82 to 119 hours. In NHPs, the half-life in the □-phase ranges from 29-38 hours and the □-phase it ranges from 222 to 262 hours.

In a 30-day toxicity study in rats, no treatment-related toxic effects were observed after repeated IV administration on Days 0, 5 and 10 with CR6261, or after a 14/15 day recovery period. The no observed adverse effect level (NOAEL) for CR6261 is considered to be 100 mg/kg body weight/dose.

A non-Good Laboratory Practice (GLP) study of AlexFluor488-labelled-CR6261 using frozen sections of a panel of human and Sprague Dawley rat tissues and a GLP human tissue cross-reactivity study revealed weak and low affinity staining in many tissues. However, the staining was restricted to the cytoplasm which is considered inaccessible to mAbs in vivo and, therefore, judged to be of low toxicological concern.

1.1.1.1 Therapeutic efficacy in the H5N1 and H1N1 mouse lethal challenge models

A study was performed in female Balb/c mice to assess the therapeutic efficacy of 15 mg/kg CR6261 administered at 4 hours, 1, 2, and 3 days after lethal challenge with H5N1 A/HK/156/97 influenza virus. Animals were inoculated intranasally on Day 0 with 25 x LD_{50} of virus and followed for 21 days. At different time intervals post-infection, CR6261 or a control mAb was administered by intraperitoneal injection. Mortality, weight loss, and clinical signs were monitored daily from the day before challenge until 21 days after virus inoculation. On Day 6, four animals from each of group 2 and group 5 were euthanized, and the lungs were collected for histopathological examination. CR6261 administered at all timepoints prevented mortality in all animals except one mouse in the 4-hour treatment group. All control animals died by Day 9.

A second study was performed in female Balb/c mice to assess the therapeutic efficacy of 15 mg/kg CR6261 on Days 3, 4, 5, or 6 after lethal challenge with H5N1 A/HK/156/97

influenza virus. At different time intervals post-infection, CR6261 or a control mAb was administered by IV injection. The animals were inoculated intranasally on Day 0 with 25 x LD_{50} of virus and followed for 21 days. On Day 3, Day 4, Day 5, or Day 6 post-infection, CR6261 or a control mAb was administered by IV injection. Mortality, weight loss, and clinical signs were monitored from the day before challenge until 21 days after virus inoculation. All animals survived when CR6261 was administered 3 or 4 days after infection with 25 x LD_{50} of H5N1 strain A/HK/156/97. When CR6261 was administered 5 days after infection, 50% of the animals survived. None of the animals survived following treatment with CR6261 on Day 6 or treatment with control mAb.

A study was performed in female Balb/c mice to assess the therapeutic efficacy of 15 mg/kg CR6261 administered at 1, 2, and 3 days after lethal challenge with mouse-adapted H1N1 A/WSN/33 influenza virus. The animals were inoculated intranasally on Day 0 with 25 x LD_{50} of virus and followed for 21 days. At different time intervals post-infection, CR6261 or a control mAb was administered by IP injection. Animals in group 1 were injected with CR6261 one day prior to infection as a positive control. Mortality, weight loss, and clinical signs were monitored daily from the day before until 21 days after virus inoculation. No mortality was observed in the positive control group (Group 1) or when CR6261 was administered 1 day after infection. Administration of CR6261 on 2 and 3 days post-infection resulted in 89% and 80% survival, respectively. All of the negative control animals died or were euthanized by Day 9.

These in vivo pharmacology studies demonstrated that CR6261 exhibits potent prophylactic and therapeutic efficacy against influenza A in clinically relevant mouse models (H1N1 and H5N1) for severe disease. CR6261 reduces the viral load in lungs of infected mice.

1.1.1.2 Therapeutic Efficacy in the H5N1 Ferret Lethal Challenge Model

In a non-GLP study, the therapeutic efficacy of CR6261 was assessed in a ferret lethal challenge model with influenza A/Indonesia/5/2005 (H5N1) virus at Viroclinics.

Three groups of 10 ferrets received mAbs after challenge: CR6261 (4 or 24 hours post infection) or a control mAb (4 hours post infection) were administered by IV injection at a dose of 30 mg/kg. On Day 0, ferrets were inoculated intratracheally with approximately 105 $TCID_{50}$ of virus. Survival, body weight, temperature, virus replication in lungs and upper respiratory tract, and macro- and microscopic pathology were investigated.

Survival rate in the groups receiving CR6261 at 4 or 24 hours after challenge was 100%, whereas only 20% of the animals in the control group survived ($p<0.001$). Mean decline in body weight at the end of the experiment was 6.2% in the group of ferrets that received CR6261 at 4 hours after challenge, which was significantly less ($p=0.025$) than the 10.1% observed in control animals. Animals treated 24 hours post challenge showed a mean body weight loss of 8.4%, which was not significantly different from the control animals ($p=0.427$).

Ferrets treated with CR6261 at 4 hours post challenge did not shed infectious virus in the upper respiratory tract throughout the study. In the group treated with CR6261 at 24 hours post challenge, one ferret had a low concentration of infectious virus (2.8 log_{10} $TCID_{50}$) in the throat on Day 1, but no virus was detected on subsequent days. In contrast, all animals in the control group shed virus for at least one day. Accordingly, the mean viral loads in the lungs of ferrets treated with CR6261 at 4 and 24 hours post challenge were considerably lower than that in the control group (differences were 3.9 and 4.5 log_{10} $TCID_{50}$/g, respectively, both comparisons $p<0.001$)

The lungs of animals that received CR6261 at 4 hours post challenge showed less pulmonary lesions (alveolar edema, bronchiolitis obliterans, congestion, emphysema, bronchioloalveolar hyperplasia, and primary atypical pneumonia), or showed such lesions at a lower grade of severity, compared to the lungs of animals from the other two groups. Animals of the control group were most affected by primary atypical pneumonia. These findings were in accordance with the observation that the mean lung weights of ferrets treated with CR6261 at 4 hours post challenge were lower compared to the control group (5.7 g versus 14.9 g, $p<0.001$). Animals that received CR6261 at 24 hours post challenge showed most regenerative response (bronchioloalveolar hyperplasia) in the lungs, suggesting damage to the lung parenchyma with subsequent regenerative response. The mean lung weight in this group was significantly higher than that of the group receiving CR6261 at 4 hours post challenge (8.4 g versus 5.7 g, $p<0.001$), but lower than that of the control group (14.9 g, $p<0.001$).

1.1.1.3 Short-term sub-acute toxicity studies

A repeated-dose toxicity study was performed in Sprague Dawley rats to assess the acute toxicity of CR6261. Three doses of CR6261 were administered to rats intravenously on Days 0, 5, and 10. A total of four dosing levels (0, 8, 30, and 100 mg/kg) were evaluated. Local tolerance assessments including careful clinical and histopathologic examination of injection sites at study termination (Day 16) were performed. Recovery groups were included (scheduled necropsy on Day 30) to examine reversibility of any effects observed. The repeated intravenous treatment with CR6261 was well tolerated by the animals. There was no mortality. The clinical observations, neurobehavioral endpoints, and ophthalmoscopy were negative for CR6261-related changes. There were no CR6261-related differences in body weights or feed intake.
Hematology conducted in all rats of the main and recovery groups at necropsy did not reveal CR6261-related changes.

The following abnormalities in clinical chemistry were observed in the treatment group:

- Alkaline phosphatase activity (ALP) was significantly decreased in males in the high-dose group on Day 16 of the study.
- Albumin level was slightly, though significantly increased in the low-dose and high-dose group in both sexes on Day 16 of the study. There was no dose-response relationship.

- Cholesterol and phospholipid levels were significantly decreased in females of all test groups on Day 16 of the study, but there was no clear dose-response relationship.
- Triglycerides were significantly decreased in mid-dose females on Day 16.

No statistically significant differences in clinical chemistry values were noted among the rats of the recovery groups.

No treatment-related toxic effects were observed after repeated IV treatment of rats with CR6261, and after a 15-day recovery period no delayed toxic response was found. The toxicology data indicate that the no observed adverse effect level (NOAEL) for CR6261 is 100 mg/kg/dose IV.

1.1.1.4 Tissue cross-reactivity studies

A study was performed to evaluate the potential cross-reactivity of CR6261 with cryosections of human tissues. To detect binding, CR6261 was applied to cryosections of normal human tissues (three donors per tissue, where available) at two concentrations (5 and 1 µg/mL).

CR6261-AlexaFluor488 stained epithelial cells in several tissues, including those in the mammary glands, esophagus, small intestine, apocrine glands, thyroid glands, bladder, and uterus at weak or weak-to-moderate staining levels. Epithelial staining was generally only observed at the higher test article concentration except in one thyroid donor where CR6261-AlexaFluor488 was observed at both concentrations. In breast, urinary bladder, and uterus, there were sufficient epithelial cells stained to permit judgment of staining affinity. In these tissues, test article staining was only observed at the higher test article concentration, suggesting low-affinity staining. In the other tissues in which test article staining was observed, there were too few stained cells present to permit judgment of staining affinity.

CR6261-AlexaFluor488 also stained cytoplasm and peripheral cytoplasm in resident, migrating, infiltrating, and/or intravascular mononuclear cells in some tissues. The observed test article staining generally ranged from weak to strong and was observed in rare or very rare mononuclear cells. In salivary gland and tonsil, test article staining of mononuclear cells was observed at both test article concentrations, while in esophagus, lymph node, and thyroid, test article staining was only observed at the higher test article concentration. In all tissues in which mononuclear cell staining was observed, there were too few stained cells present to permit judgment of staining affinity.

CR6261-AlexaFluor488 staining was also observed in cytoplasm of endothelium in one placenta donor. This staining was weak and observed in rare endothelial cells at the higher test article concentration only. There were too few stained endothelial cells present to permit judgment of staining affinity.

As HA is not expected to be expressed in human tissues, the observed staining of epithelium, mononuclear cells, and endothelium likely represents non–targeted-related tissue cross-reactivity. For almost all tissues, the observed staining was weak and considered low affinity. Importantly, all observed cross-reactive staining was seen in the cytoplasm, and the cytoplasmic compartment is generally thought to be inaccessible to mAbs *in vivo*. Thus, the observed cross-reactivity was judged to be of low toxicological concern.

1.1.2 Clinical Studies

A phase 1, randomized, double-blind, placebo-controlled, dose-escalation study to evaluate CR6261 was conducted in healthy adults in the US. Five dose levels were evaluated in 40 subjects in cohorts of 8. Subjects received a single 2-hour IV infusion of CR6261 or placebo (6 CR6261:2 placebo) on Day 1. Dose allocation is shown in Table 2. After the completion of Cohort 5, a sixth cohort was enrolled. Cohort 6 was comprised of 24 subjects (randomized 5 CR6261 at 30 mg/kg : 1 placebo) who received a 2-hour IV infusion on Day 1. Dose allocation is shown in Table 2.

Table 2. Treatment Assignment in First-in-Human Trial of CR6261

Cohort	Number of doses	Dose (mg/kg)	CR6261	Placebo	Total
1	1	2	6	2	8
2	1	5	6	2	8
3	1	15	6	2	8
4	1	30	6	2	8
5	1	50	6	2	8
6	1	30	20	4	24
Total number of subjects			**50**	**14**	**64**

Subjects in each cohort were admitted to the clinic on Day -1, remained in the inpatient unit through Day 3, and were discharged upon completion of the 48-hour post-dose assessments (Day 3). Subjects returned to the clinic on Days 4, 8, 15, 29, 43, 57, and 76 for outpatient visits.

Following a single 2-hour IV infusion of CR6261 to healthy subjects, CR6261 serum concentration exhibited a bi-phasic decline typical of mAbs. Mean clearance (CL) values were 5.0, 5.4, 6.2, 6.2, and 6.6 mL/day/kg following a single IV dose of CR6261 at 2, 5, 15, 30, and 50 mg/kg, respectively. Mean Vz values were 91.7, 93.1, 120.4, 123.1, and 137.9 mL/kg for the 2, 5, 15, 30, and 50 mg/kg dose groups, respectively. Mean $T_{1/2}$ values were 13.1, 12.2, 13.4, 14.1, and 14.7 days for the 2, 5, 15, 30, and 50 mg/kg dose groups, respectively. The median T1/2 for all subjects was 13.1 days. C_{max}, $AUC_{0\text{-}7d}$ and $AUC_{0\text{-}inf}$ of serum CR6261 all increased in an approximate dose-proportional manner across the 2 to 50 mg/kg dose range, and the CL, V_z and $T_{1/2}$ values for CR6261 were similar across all dose groups, indicating linear PK for CR6261, consistent with the expectation for a mAb with no known endogenous target in healthy subjects.

Three out of the 50 CR6261-treated subjects were shown to be ADA positive, all with a low peak ADA titer of 20. No apparent PK difference was observed between the ADA-positive and ADA-negative subjects.

Safety analysis has been completed in the phase 1 trial. The more commonly reported adverse events (in >5 subjects) during follow-up of 64 subjects in Cohorts 1 to 6 include: headache (N=7), upper respiratory tract infection (N=6), red blood cells in urine (N=18), increased blood fibrinogen (N=15), and increased blood creatine phosphokinase (N=6). A small unpublished prophylactic human challenge study with a different human mAb, CR8020, directed at a more viral membrane proximal region of the influenza hemagglutin stem suggested the possibility of more viral replication and clinical symptoms in the treatment group than in the control group. The possible explanation for this finding with a different antibody is being extensively investigated.

1.2 Overall Rationale for the Study

Since neutralizing antibodies are a major component of immune protection against influenza, passive immunotherapy, such as mAbs, may be a viable option for treatment or prophylaxis of infection. Currently, there are no mAbs approved for the treatment or prophylaxis of influenza. This phase 2 study will evaluate the therapeutic efficacy of 50 mg/kg CR6261 compared with placebo administered as IV infusions to healthy volunteers following intranasal challenge with Influenza A 2009 H1N1 human challenge virus. In addition, the safety, tolerability, PK, and potential immunogenicity of CR6261 will be assessed.

2 Study Objectives

2.1 Primary Objective

To demonstrate that CR6261 leads to a reduction in viral shedding compared with placebo with respect to the area under the curve (AUC) as determined by quantitative PCR (qPCR) in nasopharyngeal (NP) swabs in all treated subjects.

2.2 Secondary Objectives

- To demonstrate that CR6261 leads to a reduced rate of influenza induced disease in all CR6261-treated subjects vs. those receiving placebo.
- To compare the difference in influenza clinical illness severity for CR6261-treated subjects with that in subjects given placebo
- To correlate clinical illness to quantitation of viral shedding
- To evaluate the safety of CR6261 compared with placebo
- To evaluate the pharmacokinetics (PK) of CR6261

2.3 Exploratory Objectives

- To evaluate the difference in proportion of subjects with seroconversion to anti-HAI antibodies between CR6261 and placebo-treated subjects
- To evaluate the incidence and severity of symptoms (individual and composite) using the Flu-Pro questionnaire and physician assessment in CR6261-treated subjects compared with placebo-treated subjects
- To assess CMI and RNA microarray analysis following administration of CR6261
- To assess cytokine responses in peripheral blood
- To evaluate viral infectivity following administration of CR6261 using exploratory infectivity assays
- To develop a mucosal PK assay, and if successful, to subsequently assess mucosal PK of CR6261
- To undertake mucosal cytokine assay development, and if successful, to subsequently assess mucosal cytokines
- To undertake mucosal microarray RNA assay development, and if successful, to subsequently assess mucosal microarray RNA patterns
- To investigate the development of ADA after administration of CR6261
- To evaluate the development of viral resistance against CR6261 with sequencing (full length of HA) and phenotypic assays

3 Study Design

3.1 Description of the Study Design

This is a randomized, double-blind, placebo-controlled, single-center, phase 2 study of CR6261 administered as a 2-hour IV infusion 24 hours after intranasal challenge with the Influenza A 2009 H1N1 human challenge virus. Subjects will be randomized 1:1 to either CR6261 or placebo. A placebo control will be used to establish the frequency and magnitude of changes in endpoints that may occur in the absence of active treatment. Randomization will be used to minimize bias in the assignment of participants to treatment groups, to increase the likelihood that known and unknown participant attributes (e.g., demographic and baseline characteristics) are evenly balanced across treatment groups, and to enhance the validity of statistical comparisons across treatment groups. Blinded treatment will be used to reduce potential bias during data collection and evaluation of endpoints.

3.1.1 Influenza-Induced Disease

Influenza-induced disease for the purposes of this study is defined as viral shedding detected by positive culture or any approved positive diagnostic test from a nasal wash or swab plus any 1 or more of the following symptoms or signs that are defined by the principal investigator (PI) as probably or definitely related to the challenge virus:

- Arthralgia

- Chills
- Conjunctivitis
- Coryza
- Diarrhea
- Dry Cough
- Dyspnea/Shortness of Breath
- Fatigue/Tiredness
- Fever (>38.0°C)
- Headache
- Myalgia
- Nausea
- Oxygen Saturation Decrease by ≥3% from baseline
- Productive Cough
- Rhinorrhea
- Sore Throat
- Sweats

3.2 Study Endpoints

Primary Endpoint:

Objective: To demonstrate that CR6261 leads to a reduction in viral shedding compared with placebo with respect to the area under the curve (AUC) as determined by quantitative PCR (qPCR) in nasopharyngeal (NP) swabs in all treated subjects

Endpoint: AUC of viral shedding will be used as a primary endpoint in all treated subjects. AUC will be measured using qRT-PCR and will be based on pg/ml of viral RNA vs. time.

Secondary Endpoints:

Objective: To demonstrate that CR6261 leads to a reduced rate of influenza induced disease in all CR6261-treated subjects vs. those receiving placebo.

Endpoint: Presence of influenza-induced disease will be used as a secondary endpoint, which will be defined as viral shedding plus at least one clinical symptoms as defined in this protocol. This will be used to determine if an individual was or was not protected from illness in each treatment group.

Objective: To compare the difference in influenza clinical illness severity for CR6261-treated subjects with that in subjects given placebo

Endpoint: Participant self-Assessment questionnaires will be collected daily and converted to an objective score. Mean scores in each group will be compared for a statistically significant difference in illness.

Objective: To correlate clinical illness to quantitation of viral shedding

Endpoint: Quantification of virus by qRT-PCR will be performed and timing and overall quantity of viral RNA detected will be correlated to daily clinical scores and evaluated for statistical significance in the two groups.

Objective: To evaluate the safety of CR6261 compared with placebo

Endpoint: A difference between number of AEs possibly, probably, or definitely related to the infusion will be evaluated in the treatment and placebo groups.

Objective: To evaluate the pharmacokinetics (PK) of CR6261

Endpoint: Blood draws for measurement of serum concentration of drug will be drawn at appropriate times to evaluate the kinetics of CR6261

4 Study Population

4.1 Rationale for Participant Selection

Participants will be carefully selected using the inclusion and exclusion criteria described here to select the optimum participants for completing the study objectives and minimize the risk of adverse events (AEs).

NIH employees and members of their immediate families may participate in this protocol. We will follow the Guidelines for the Inclusion of Employees in NIH Research Studies and will give each employee a copy of the “NIH information sheet on Employee Research Participation.”

For NIH employees:

- Neither participation nor refusal to participate will have an effect, either beneficial or adverse, on the participant’s employment or work situation.
- The NIH information sheet regarding NIH employee research participation will be distributed to all potential subjects who are NIH employees.
- The employee subject’s privacy and confidentiality will be preserved in accordance with NIH Clinical Center and NIAID policies, which define the scope and limitations of the protections.
- For NIH employee subjects, consent will be obtained by an individual independent of the employee’s team. Those in supervisory position to any employee and co-workers of the employee will not obtain consent.

- The importance of maintaining confidentiality when obtaining potentially sensitive and private information from co-workers or subordinates will be reviewed with the study staff at least annually and more often if warranted.

4.2 Recruitment Plan

Participants will be recruited through the screening study #11-I-0183 "Screening of Volunteers for Influenza Human Challenge and Vaccine Studies". Participants will be carefully screened and evaluated, and those who meet the study eligibility criteria will be contacted and given the opportunity to be enrolled into the study.

If a participant has completed the screening study #11-I-0183 more than 60 days prior to enrolling in this challenge study they will be asked to come to the NIH Clinical Center for another screening visit under the screening study #11-I-0183 within 60 days prior to the date of admission for this challenge study to repeat HAI and HIV testing and complete any laboratory or other testing as deemed necessary by the investigator to ensure that it remains safe for the participant to take part in this study. All eligible participants will be consented and enrolled for this study only after completion of all necessary screening studies under protocol #11-I-0183 a maximum of 60 days prior to virus administration.

4.3 Inclusion Criteria

1. ≥18 and ≤45 years of age.
2. Non-smoker.
3. Willingness to remain in isolation for the duration of viral shedding (at a minimum 10 days) and to comply with all study requirements.
4. A male subject is eligible for the study if he agrees to practicing abstinence or using a condom with spermicide plus an acceptable form of contraception (see inclusion criteria 5) being used by any female partner from 4 weeks before to 12 weeks after intranasal challenge with influenza.
5. A female participant is eligible for this study if she is not pregnant or breast feeding and 1 of the following:
    - Of nonchildbearing potential (i.e., women who have had a hysterectomy or tubal ligation or are postmenopausal, as defined by no menses in ≥1 year).
    - Of childbearing potential but agrees to practice effective contraception or abstinence for 4 weeks prior to and 8 weeks after administration of the influenza challenge virus. Acceptable methods of contraception include a male partner who is sterile and is the sole sexual partner of the female participant or a male partner who uses a condom with spermicide plus 1 or more of the following: 1) implants of levonorgestrel; 2) injectable progestogen; 3) an intrauterine device with a documented failure rate of

<1%; 4) oral contraceptives; and 5) double barrier method including diaphragm.

6. Willing to have samples stored for future research.
7. Prechallenge serum HAI titer against the challenge virus ≤1:10 within 60 days of admission for the study.
8. HIV uninfected confirmed by testing within 60 days of admission for the study.
9. Agrees to abstain from alcohol intake 24 hours before admission on Day -1, during the inpatient period of the study, and 24 hours prior to all other outpatient clinic visits.
10. Agrees to not use over-the-counter medications (including aspirin, decongestants, antihistamines, and other NSAIDs), and herbal medication (including, but not limited to, herbal tea, St. John's Wort), within 14 days prior to study drug administration through the final follow-up visit, unless approved by the investigator.

4.4 Exclusion Criteria

1. Presence of self-reported or medically documented significant medical condition including but not limited to:
    a. Chronic pulmonary disease (e.g., asthma, emphysema).
    b. Chronic cardiovascular disease (e.g., cardiomyopathy, congestive heart failure, cardiac surgery, ischemic heart disease, known anatomic defects).
    c. Chronic medical conditions requiring close medical follow-up or hospitalization during the past 5 years (e.g., insulin dependent diabetes mellitus, renal dysfunction, hemoglobinopathies).
    d. Immunosuppression or ongoing malignancy.
    e. Neurological and neurodevelopmental conditions (e.g., cerebral palsy, epilepsy, stroke, seizures).
    f. Postinfectious or postvaccine neurological sequelae.
    g. Hyperlipidemia requiring medical therapy per current American College of Cardiology (ACC) and American Heart Association (AHA) guidelines published in 2013.
2. Have close or household (i.e., share the same apartment or house) high-risk contacts including but not limited to:
    a. Persons ≥65 years of age.
    b. Children ≤5 years of age.
    c. Residents of nursing homes.
    d. Persons of any age with significant chronic medical conditions such as:

- Chronic pulmonary disease (e.g., severe asthma, COPD).
- Chronic cardiovascular disease (e.g., cardiomyopathy, congestive heart failure, cardiac surgery, ischemic heart disease, known anatomic defects).
- Contacts who required medical follow-up or hospitalization during the past 5 years because of chronic metabolic disease (e.g., insulin dependent diabetes mellitus, renal dysfunction, hemoglobinopathies).
- Immunosuppression or cancer.
- Neurological and neurodevelopmental conditions (e.g., cerebral palsy, epilepsy, stroke, seizures).
- Individuals who are receiving long-term aspirin therapy.
- Women who are pregnant or who are trying to become pregnant.

3. Positive serology for hepatitis C virus antibody or hepatitis B surface antigen.
4. Individual with body mass index (BMI) ≤18 and ≥35 or weight > 114kg.
5. Acute illness within 7 days of admission and inoculation with the challenge virus (Day -1).
6. Complete blood count (CBC) with differential outside of the NIH Department of Laboratory Medicine (DLM) normal reference range and deemed clinically significant by the PI.
7. Chemistries in the acute care, mineral, and/or hepatic panels, and/or any of the following: lactate dehydrogenase, uric acid, creatine kinase, and total protein outside of the NIH DLM normal reference range and deemed clinically significant by the PI.
8. Amylase or Lipase outside of the NIH DLM normal reference range and deemed clinically significant by the PI.
9. Urinalysis outside of the NIH DLM normal reference range and deemed clinically significant by the PI.
10. Clinically significant abnormality as deemed by the PI on electrocardiogram.
11. Clinically significant abnormality as deemed by the PI on echocardiographic (ECHO) testing.
12. Clinically significant abnormality as deemed by the PI on the Pulmonary Function Test (PFT).
13. Known allergy to treatments for influenza (including but not limited to oseltamivir, nonsteroidals).
14. Known allergy to 2 or more classes of antibiotics (e.g., penicillins, cephalosporins, fluoroquinolones, or glycopeptides).

15. Receipt of blood or blood products (including immunoglobulins) within 3 months prior to enrollment.
16. Receipt of any unlicensed drug within 3 months or 5.5 half-lives (whichever is greater) prior to enrollment.
17. Receipt of any non-influenza-related unlicensed vaccine within 6 months prior to enrollment.
18. Self-reported or known history of current alcoholism or drug abuse, or positive urine/serum test for drugs of abuse and/or ethanol (i.e., amphetamines, cocaine, benzodiazepines, opiates, or metabolites, but not tetrahydrocannabinol (THC) or metabolites).
19. Self-reported or known history of psychiatric or psychological issues deemed by the PI to be a contraindication to protocol participation.
20. Known close contact with anyone known to have influenza in the past 7 days.
21. Known or suspected hypersensitivity to CR6261 or its excipients (sucrose, L-histidine, L-histidine monohydrochloride, polysorbate 20).
22. History of a previous severe allergic reaction with generalized urticaria, angioedema, or anaphylaxis.
23. Drinks more than 1200 mL (or 5 cups of 240 mL per cup) of tea/coffee/cocoa/cola or other caffeinated beverage per day more than 1 day per week in the 2 weeks before screening.
24. Any condition or event that, in the judgment of the PI, is a contraindication to protocol participation or impairs the volunteer's ability to give informed consent.

Co-enrollment Guidelines: Participants must be co-enrolled in the screening protocol (#11-I-0183) as described in Section 4.2. Co-enrollment in other trials is restricted, but may take place with the approval of the PI and after study staff notification.

4.5 Justification for Exclusion of Pregnant Women and Children (Special Populations)

In this study, a live influenza virus and investigational drug will be administered to the participants. Therefore, children, pregnant women, and individuals at high risk of complicated influenza infection will be excluded as the risk to these individuals may be increased.

Participants younger than 18 years of age will be excluded from the study. Because there are insufficient data regarding dosing or AEs available in adults to judge the potential risk in children, the study is of "greater than minimal risk" and does not meet the criterion of 45 Code of Federal Regulations (CFR) 46, Subpart D, governing the participation of children in research.

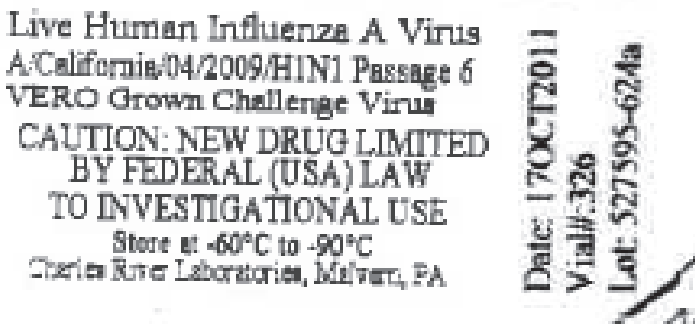
5 Study Agents/Interventions

5.1 Agent 1: Influenza Challenge Virus

5.1.1 Formulation, Packaging and Labeling

Using reverse genetics, an investigational Influenza A 2009 H1N1 human challenge virus similar to the wild-type strain A/Ca/04/2009 was manufactured in certified Vero cells under Good Manufacturing Practice (GMP) conditions by Charles River Laboratories in Malvern, PA from a seed stock produced by Dr. Matthew Memoli and his laboratory team in the Viral Pathogenesis and Evolution Section of the LID. It will be vialed at a maximum dose level of $10^{12}TCID_{50}$. This virus has been demonstrated to be sensitive to standard FDA-approved neuraminidase inhibitors such as oseltamivir and zanamivir. Similar to current seasonal H3N2 and all 2009 H1N1 pandemic viruses, it is resistant to the adamantanes.

Each container will be individually labeled with the agent name, description, and caution label. An example label, shown below, is affixed to 2-mL cryogenic vials prior to fill.



5.1.2 Study Agent Storage and Stability

The Influenza A 2009 H1N1 human challenge virus will be stored at -60°C to -90°C in single-use vials. Multiple doses of virus may be prepared from the same vial after it is opened. Once opened, each vial will be discarded appropriately (see Section 5.1.4) after use. Preparation and dosing of the agent must take place within 3 hours of thawing of the vial.

5.1.3 Preparation

Dilution with sterile phosphate buffered saline or normal saline may be necessary to prepare the agent for administration, depending on the dose to be administered. This will be performed immediately after thawing the agent by trained personnel in the pharmacy before distribution for administration. The virus will be diluted to the appropriate concentration and prepared in a standard 1-mL syringe. The syringe will then be fitted with a MADTM Nasal sprayer device (Wolfe-Tory Medical, Inc., Salt Lake City, UT). An aliquot of this dilution will be made and transferred to the research team for future laboratory analysis to validate the dilutions.

The virus will be prepared and placed on wet ice after preparation. It will then be transferred to the study unit for administration. The total length of time from removal of the virus from the freezer to administration will be minimized and will not exceed 3 hours.

5.1.4 Disposal of used, partially used, and unused influenza virus

Each vial will be used to prepare doses only once and any unused agent will be discarded after use. Unused vials that have been thawed will also be discarded. Vials will be discarded by incineration following the standard operating procedures of the NIH Central Pharmacy.

5.1.5 Dosing and Administration

This study agent will be handled by the investigators and/or trained personnel and administered only in the NIH Clinical Center Clinical Center Patient Unit (CCPU). Orders for dosing and administration will be entered in CRIS and documented appropriately. All doses will be administered within 3 hours from the time the vial of virus is removed from the freezer in the pharmacy. Participants will be asked to lie down with their heads in a neutral to slightly tilted back position. The sprays will be delivered intranasally at a 10^7 $TCID_{50}$ dose and will be distributed equally between 6 sprays. The maximum total dose volume in each spray will be 0.75 mL. Doses will be administered by study staff or personnel trained to administer the study agent. Participants will be asked to remain with their heads in the neutral position for a minimum of 15 seconds after administration. Used syringes and sprayers will be disposed of in CCPU biohazard trash, which is handled according to NIH Clinical Center biohazard trash guidelines.

5.2 Agent 2: CR6261 and Placebo

5.2.1 Formulation, Packaging and Labeling

CR6261 is supplied as sterile lyophilized cakes (400 mg/vial) for intravenous infusion after reconstitution and does not contain any preservative. Each drug product is filled aseptically in a glass vial, lyophilized, sealed, and stored at 2-8 °C, protected from light. Each drug product is reconstituted with 8.0 mL sterile water for Injection.

CR6261 is manufactured and provided by Crucell. Refer to the Investigator's Brochure for a list of excipients.

Placebo is 5% dextrose (D-glucose) water, in commercially available 250 mL infusion bags. Placebo can be supplied by the site or centrally sourced.

CR6261 will be packed in open-label boxes. Study drug labels will contain information to meet the applicable regulatory requirements. Following the recommendations of the Data and Safety Monitoring Board (DSMB) in August 2017, only lot number 138398 will be used for future CR6261 infusions.

5.2.2 Study Agent Storage and Stability

CR6261 vials will be stored at 2-8 °C in the NIH Clinical Center Research Pharmacy with no access for blinded personnel. Storage temperature will be monitored daily and a log of the monitored temperature will be maintained. The study refrigerator will be equipped with a continuous temperature monitor and alarm. Study refrigerators will be equipped with back-up power systems. Complete storage instructions are provided in the Investigational Product (IP) Management Manual. In the event that study drug is out of temperature range, all relevant data will be sent to Crucell to determine if the affected study drug will be used or replaced. Dosing will be halted until further instruction from the Crucell; during this period, the affected IP will be quarantined.

Placebo will be stored according to manufacturer recommendations.

5.2.3 Preparation

Doses of CR6261 and placebo will be prepared by an unblinded pharmacist according to participant treatment assignment and kit number assignment. All other clinical staff, investigators, and participants will remain blinded to the treatment administered. Following reconstitution, CR6261 will be mixed with 250 mL of commercially available dextrose (D-glucose) 5% water. Placebo will consist of 250 mL of commercially available dextrose (D-glucose) 5% water. Final infusion solutions of both investigational product and placebo will be clear/colorless and not distinguishable from one another. Infusion bag labels will not identify whether investigational product or placebo will be administered or contain any other potentially unblinding information. Refer to the IP Management Manual (provided as separate document) for full details of investigational product preparation, labeling, and blinding. All vials used in the preparation process will be documented in the Accountability Form (see IP Management Manual for details). Treatment assignment, dose calculation, preparation, and labeling will be double checked by another pharmacy staff member for all doses prior to dispensing the dose. An independent (unblinded) drug monitor will monitor pharmacy activities.

Participants randomized to CR6261 and weighing up to 114 kg will receive 50 mg/kg CR6261 as a single-dose infusion. Participants weighing more than 114kg will not be enrolled. Participants randomized to placebo will receive a single-dose placebo infusion. Approximately 2 hours will be allowed for the CR6261 reconstitution and dose preparation. The infusion will be started within 4 hours of product preparation, i.e., from the time that reconstituted drug product is added to the infusion bag.

5.3 Disposal and Drug Accountability of CR6261

The investigator is responsible for ensuring that all study drug received at the site is inventoried and accounted for throughout the study. The study drug administered to the subject will be documented on the drug accountability form. All study drug will be stored and disposed of according to Crucell's instructions. Study-site personnel will not combine contents of the study drug containers.

Study drug will be handled in strict accordance with the protocol and the container label, and will be stored at the study site in a limited-access area or in a locked cabinet under appropriate environmental conditions. Unused study drug will be available for verification during on-site monitoring visits. The return to Crucell of unused study drug will be documented on the drug return form. If the study site is an authorized destruction unit and study drug supplies are destroyed on-site, this will also be documented on the drug return form.

5.3.1 Dosing and Administration

The study treatments will be administered in a double-blind manner as a single intravenous infusion over a 2-hour period. A window of 15 minutes is permitted to allow for infusion of the entire IV bag volume (including any overfill allowed in commercial IV bags) and to allow for purging of the IV set lines. The IV set lines will be purged with a sufficient volume of 5% dextrose (D-glucose) water following emptying of the IV bag to ensure that the full dose is administered. Details of each administration will be recorded in CRIS (including date, start and stop times of the IV infusion, start and stop times of any infusion interruptions, and volume infused).

5.4 Concomitant Medications and Procedures

All concomitant prescription medications, over-the-counter medications, or herbal remedies taken during study participation must be approved by the PI and will be recorded in the participant's file via the Clinical Research Information Management System of the NIAID (CRIMSON)/Clinical Research Information system (CRIS)/source documents. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Symptomatic treatment (antipyretics, antitussives, decongestants, etc.) in individuals not requiring an increased level of care as described in Section 7.1 will be administered at the discretion of the PI and recorded carefully in the medical records via CRIMSON/CRIS.

5.5 Antiviral Medications

Since unnecessary treatment with antivirals could interfere with our ability to meet our study objectives, treatment with any antiviral, including but not limited to oseltamivir, zanamivir, amantadine, and rimantadine, will not be permitted unless discussed with and approved by the PI or if administered by the medical care team for treatment of a serious medical condition that arises during the study.

6 Study Schedule

This study will take place at the NIH Clinical Center. All aspects of the protocol will be carried out in accordance with NIH guidelines involving human-participant research.

6.1 Screening

Screening will be performed under a separate but related NIAID protocol #11-I-0183 ("Screening of Volunteers for Human Challenge and Vaccine Studies"). The data collected

in that study will be used to determine if volunteers meet the inclusion criteria for this study. If eligible, volunteers will be contacted by phone and offered the opportunity to participate in this study. Any changes to their medical history since their enrollment in the screening protocol will be reviewed to determine if they remain eligible for this study. If necessary as described in Section 4.2, some testing may need to be repeated under protocol #11-I-0183 prior to enrollment in this study.

6.2 Enrollment

Enrollment will take place within the 60 days prior to virus administration. The participants will sign the informed consent document prior to all study procedures. The research team will thoroughly discuss the consent with the volunteer. A maximum of 20 participants will be enrolled at one time.

6.3 Randomization and Blinding

Randomization will occur as explained in Section 6.6. Under normal circumstances, the blind should not be broken until all participants have completed the study and the database is finalized. Otherwise, the blind should be broken only if specific emergency treatment/course of action would be dictated by knowing the treatment status of the participant. In such cases, the investigator may in an emergency determine the identity of the treatment by opening the sealed code. It is recommended that the investigator contact the sponsor or its designee if possible to discuss the particular situation, before breaking the blind. Telephone contact with the sponsor or its designee will be available 24 hours per day, 7 days per week. In the event the blind is broken, the sponsor must be informed as soon as possible. Accidental unblinding (e.g. if the Investigator sees the IP administration logs) must be reported within 1 working day to the Sponsor, who will advise on the corrective steps to be taken. The date and reason for the unblinding must be documented in the source document.

In general, randomization codes will be disclosed fully only if the study is completed and the clinical database is closed. However, for the interim analysis planned for this study unblinding will occur when the interim database is locked. This unblinding is limited to the study statistician and Data Safety Monitoring Board (DSMB).

6.4 Baseline/Admission (Day -1)

The following assessments and testing will be performed after admission to the SCSU or CCPU and before challenge virus administration:

- Review of medical history and addition of any new information
- Physician assessment and physical exam
- Vital signs
- Echocardiography (ECHO)
- Electrocardiogram (ECG)
- Blood chemistry testing including acute care, mineral, and hepatic panels, plus total protein, lactate dehydrogenase, uric acid, and creatine kinase
- CBC with differential

- Amylase and Lipase
- Serum pregnancy test (females only)
- Nasal wash and swabs
- Urinalysis and urine toxicology screen for illicit drugs
- Bedside spirometry and/or PFT
- A self-assessment questionnaire
- Urine alcohol screening
- Any other clinical tests that are medically indicated or appropriate to assure safety for an individual participant

As determined by the PI, results of standard procedures performed in the process of admission to the NIH may be used to determine eligibility, including but not limited to vital signs, height, weight, etc.

6.5 Virus Inoculation (Day 0)

All baseline procedures and tests will be performed prior to challenge virus administration. Once it has been determined that the participant still meets eligibility criteria for the study and that he/she is comfortable in the unit, administration of the challenge virus will proceed at a dose of 10^7 $TCID_{50}$ on Day 0. Once the influenza challenge virus has been administered, participants will remain hospitalized in isolation in the SCSU (or CCPU) for a minimum of 8 additional days and will be discharged only after they meet the discharge criteria described below.

6.6 CR6261 Administration (Day 1)

Pre-dose procedures will occur as detailed in Appendix A: Schedule of Procedures/Evaluations. CR6261 or placebo will be administered on Day 1, 24 to 28 hours after virus inoculation. Participants will first be randomized 1:1 to CR6261 or placebo based on a computer-generated randomization schedule prepared before the study by Crucell under the supervision of the sponsor. The unblinded pharmacist with primary responsibility for study treatment preparation will maintain the randomization code and complete the assignment of subjects according to the randomization allocation. The pharmacist will be provided with a sealed randomization code for each participant, containing coded details of the treatment in the double-blind phase. All randomization codes, whether opened or sealed, will be stored by pharmacist in a locked cabinet after each of the groups conclude study participation. Following randomization, subjects will have an ECG and a blood draw. An IV will be placed for administration of CR6261 or placebo and will proceed as a 2-hour infusion.

6.7 Day 2 through 8

While admitted to the NIH CC, participants will remain in isolation on their unit. They will not be isolated from one another. Participants will undergo vital signs, ECGs, medical history review, self-assessment questionnaires, physical assessments and physical exams, as well as nasal washes/swabs, urine collection, and blood draws as indicated in

Appendix A. Participants will be discharged when they meet the following discharge criteria: 2 consecutive negative nasal washes/swabs (that occur on 2 different but consecutive days) for influenza A by viral cultures or by other approved diagnostic tests performed by the clinical microbiology laboratory, are afebrile, show no signs of significant influenza symptoms, and are clinically and hemodynamically stable. It is expected that participants will shed virus for 3 to 5 days post inoculation; therefore, we expect most participants to have 2 consecutive negative nasal washes/swabs by Day 8 of the study.

The questionnaires in Appendix C titled "Influenza Symptom Questionnaire" and "Additional Daily Self-Assessment Questionnaire" will be completed either in paper form or electronically.

6.8 Follow-up

All participants will be followed for a minimum of 8 weeks after completing the inpatient portion of the study. Any participant who experiences complications due to challenge virus administration or study drug will be followed until resolution of such complications or appropriate referral to the necessary medical care has been made.

Follow-up visits will take place on Day 29 (+/-4 days) and Day 66 (+/-5 days), or more often if deemed medically necessary; the procedures/evaluations to be conducted at these visits are shown in Appendix A. Day 66 will be the final study visit unless the participant requires additional follow-up of study-related complications.

Participants will be asked to report to the study team if any close contacts become ill with an influenza-like illness. If a close contact becomes ill with an influenza-like illness during the follow-up period, the participant will be asked to contact that individual and have them contact the NIH study team. The possibly infected person will be asked to come to the NIH Clinical Center for an assessment. The study team may then request that this individual enroll into the NIAID Protocol number 07-I-0229 ("Influenza in the Non-immunocompromised and Immunocompromised Host") to determine if their illness is caused by an influenza virus and identify the particular influenza virus strain. All efforts will be made to confirm and report any transmission of the challenge virus that may have occurred after discharge.

7 Study Procedures/ Evaluations

7.1 Clinical and Laboratory Evaluations

See Appendix A for clinical and laboratory procedures performed during the inpatient and follow-up portions of the study. See Appendix B for Blood Volumes for Specimen Collection. Many of these tests are being performed to monitor safety and assure that participants are healthy enough to undergo challenge, including PFT, ECHO, and ECG. If a participant has completed the pre-challenge PFT, and/or ECHO as part of a previous admission to this study (or as part of the screening study 11-I-0183) but does not qualify to be inoculated at that time (e.g. due to a positive nasal wash), these tests do not need

to be repeated pre-challenge if the participant is brought back in for an admission within 90 days.

7.1.1 Laboratory Testing of Collected Samples for Efficacy/Endpoints

The primary endpoint, AUC of viral shedding, will be the assessment of virus shedding by qPCR in NP swabs collected a maximum of three times daily from the day after influenza challenge up to Day 8, as assessed through the $\log_{10}$ of the AUC.

The secondary endpoint, rate of influenza-induced disease, will be determined by evaluating the number of patients with at least one clinical symptom plus a positive test for influenza from either a nasal wash or swab.

7.1.2 Pharmacokinetic and Immunogenicity Evaluations CR6261

7.1.2.1 Pharmacokinetics

Serum samples will be analyzed to determine concentrations of CR6261 using a validated, specific, and sensitive method at Biologics Clinical Pharmacology Department, Janssen R&D LLC, under the supervision of Crucell.

7.1.2.2 Immunogenicity (ADA)

The detection and characterization of serum anti-CR6261 antibodies (ADAs) will be performed using a validated assay method at Biologics Clinical Pharmacology Department, Janssen R&D LLC, under the supervision of Crucell. All samples collected for detection of antibodies to CR6261 will also be evaluated for CR6261 serum concentration to enable interpretation of the antibody data. Samples collected may additionally be used for further characterization of immunogenicity, such as the ability of ADAs to neutralize the effect of CR6261, or evaluation of safety or efficacy aspects that address concerns arising during or after the study period.

7.1.2.3 Hemagglutinin Inhibition Antibodies

This study will include an assessment of seroconversion to HAI in serum samples.

7.1.2.4 Pharmacokinetic Parameters

Serum concentrations of CR6261 will be summarized for all subjects with evaluable data. Based on the individual serum concentration-time data, using the actual dose taken and the actual sampling times, PK parameters such as C_{max}, $AUC_{0\text{-}8d,}$ $AUC_{0\text{-}15d}$, etc., will be calculated by a suitable method.

A population PK model will be explored to identify and quantify significant covariates affecting PK, such as gender, age, body weight, etc. If deemed necessary, data may be combined with data from other studies when a population pharmacokinetic model is built. Population PK modeling results will be presented in a separate report.

7.1.3 Laboratory Testing of Collected Samples for Secondary/Exploratory Objectives

Nasal washes/swabs, whole blood, and serum specimens will be analyzed using a variety of real-time reverse transcriptase polymerase chain reaction (RT-PCR), Luminex, Elispot, flow cytometry, microarray, and serologic assays to measure the mucosal and systemic innate and adaptive immune response to influenza. Nasal wash or swab specimens will also be processed using traditional quantitative virology techniques (plaque assay, $TCID_{50}$, hemagglutination inhibition assay) and real-time RT-PCR to measure viral replication and shedding. Viruses isolated from these samples may be grown, characterized, and sequenced. These viruses may be used in a variety of experiments including, but not limited to, both in vitro and in vivo animal studies of pathogenesis and viral fitness.

7.2 Escalation of Care Plan

7.2.1 Extended shedding beyond expected time period

Participants who continue to shed virus on Day 8 may be treated with standard-of-care influenza antivirals and will remain as an inpatient in the Clinical Center until they have had 2 consecutive negative clinical diagnostic tests for influenza performed on 2 separate but consecutive days. A prolonged stay in the Clinical Center required due to extended shedding or expected Grade 1 or 2 events related to influenza infection as defined in Section 11.2 will not be considered an SAE.

7.2.2 Increased severity of clinical illness

Any change in clinical status deemed significant by the examining physician in consultation with the Principal Investigator will initiate an escalation of care for that individual. Indicators for increased care may include the following:

1. Temperature ≥39°C.
2. New fever >38.0°C with rigors/shaking/chills after resolution of initial symptoms or before the expected window for fever.
3. Increase or decrease in heart rate from baseline beyond that expected, such as HR ≥110 bpm or ≤50 bpm at rest, or in conjunction with a change in the participant's overall hemodynamic status.
4. Change in blood pressure from baseline beyond that expected, mean blood pressure <65 mmHg or systolic pressure >145 mmHg, or in conjunction with a change in the participant's overall hemodynamic status.
5. Any evidence of hypoxia or decrease in room air oxygen saturation from baseline by 5% or more.
6. Any significant change in respiratory exam including tachypnea, significant wheezing, or any signs of respiratory distress.
7. Any significant change in cardiac exam or ECG, including significant tachycardia, congestive heart failure, new murmurs, rubs, or arrythmias.

8. Any other significant change noted in the physician's exam or symptoms/signs deemed significant by the responsible medical and/or study staff in conjunction with the PI.

The following will be absolute indicators for escalation of care:

1. Temperature ≥40.5 °C.
2. Symptomatic hypertension or a systolic pressure that is ≥180 mmHg or a diastolic pressure that is ≥120 mmHg.
3. Respiratory distress that occurs at rest and causes an inability to perform usual social and functional activities.
4. Any life threatening condition, cardiac, respiratory, or otherwise.

Escalation of care will be individualized to address the specific needs of the participant and immediate steps will be taken. If deemed appropriate, FDA approved antiviral and/or nonsteroidal treatment will be started immediately as deemed necessary by the PI. This treatment can be changed to other approved antiviral medications or different duration/dose at the discretion of the treating physician. Appropriate laboratory, radiographic, and microbiological diagnostic testing will be performed to evaluate the participant and diagnose primary viral pneumonia, secondary bacterial infections, cardiac arrhythmia, or any other complicating illness.

Criteria for placement on telemetry for continuous monitoring of heart rate/rhythm, oxygen saturation, and blood pressure are as follows: heart rate >110 bpm at rest and a mean blood pressure <65 mm Hg, clinically significant abnormality on ECG, or other evidence as judged by the investigative or clinical team indicating need for increased clinical monitoring. Individuals placed on telemetry will have vital signs assessed and documented every 4 hours. Criteria for admission to the NIH Clinical Center intensive care unit (ICU) are as follows: progressive hypoxia with oxygen saturation <93% and/or PaO_2 <60 mmHg despite administration of inhaled oxygen by nasal cannula or face mask, progressive hypotension with mean blood pressure of <60 mmHg despite intravenous administration of 1-2 liters of crystalloid (0.9% normal saline or lactated ringers), or other evidence as judged by the investigative or clinical team indicating need for ICU level of care.

8 Potential Risks and Benefits

8.1 Potential Risks

8.1.1 Risks of Influenza Challenge

Complications or severe signs or symptoms of influenza infection, which are unlikely to occur in healthy volunteers who participate in this study, include:

- Pneumonia
- Dehydration
- Severe bronchitis

- Myocarditis
- Pericarditis
- Guillain Barre Syndrome
- Transverse myelitis
- Encephalitis
- Sinus infections
- Ear infections
- Worsening of chronic health conditions
- Hypoxemia/respiratory failure
- Arrhythmia/cardiac arrest
- Death

The expected events of virus inoculation are listed in Section 11.2.

8.1.2 Risks of CR6261

Non-clinical studies indicate that CR6261 is a nontoxic and effective mAb for the treatment of influenza A infection. The safety and tolerability results from the phase I dose-escalation study indicate that CR6261 is generally well tolerated. However, the safety profile of CR6261 is not fully established; therefore, subjects may be placing themselves at an increased risk of unexpected events by participating in this study.

Infusion reactions or allergic reactions have been observed with administration of other mAbs. Some examples of potential non-serious infusion reactions include flushing, pruritus, back pain, vasovagal reactions, chills, nausea, headache, diaphoresis, lightheadedness, or myalgias.

Examples of potential serious infusion reactions include asymptomatic/symptomatic hypotension, symptomatic hypertension, urticaria, rash, chest pain, vomiting, peripheral edema of extremities, fever, rigors, somnolence, bronchospasm with wheezing, laryngeal/tracheal edema, laryngospasm, congestive heart failure, circulatory failure/cardiogenic shock, angioedema, acute respiratory distress syndrome (e.g. new and rapid onset hypoxia, pulmonary infiltrates/edema, dyspnea requiring ventilatory support), or brady- or tachyarrhythmias.

Serious allergic reactions (e.g. anaphylactic shock) may occur at any time during the administration of study drug.

Serum sickness-like reactions have been observed with other mAbs 1 to 14 days after treatment. Symptoms associated with these reactions include fever, rash, headache, sore throat, myalgias, polyarthralgias, hand and facial edema, and/or dysphagia.

8.1.3 Risks of Blood Draw and IV Placement

The hazards of blood drawing and IV placement include local discomfort, bruising, and rarely fainting or infection. The blood volumes anticipated for specimen collection are listed in Appendix B.

The amount of blood drawn will be within the limits allowed for adult subjects by the NIH Clinical Center (Medical Administrative Policy 95-9: Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://cc-internal.cc.nih.gov/policies/PDF/M95-9.pdf. Exceptions to this limit will be submitted to the Institutional Review Board (IRB) for prior approval.

8.1.4 Risks of Spirometry/Pulmonary Function Testing

There are minimal risks associated with bedside spirometry and PFTs for healthy volunteers. It may cause mild shortness of breath and fatigue, but is otherwise noninvasive and safe.

8.1.5 Risks of ECG and ECHO

The electrodes of an ECG may feel cold when applied; in rare cases, a rash or skin irritation develops where the patches are placed. This type of irritation usually resolves by itself, but topical medication is occasionally required. ECHO is a safe technique that has no complications.

8.2 Potential Benefits

There is no direct benefit to the participant.

9 Research Use of Stored Human Samples, Specimens, or Data

Intended Use: Samples and data collected under this protocol may be used to study aspects of influenza infection and disease related to influenza infection. Genetic testing will not be performed.

Storage: Access to stored samples will be limited using a locked freezer in a locked laboratory. Samples and data will be stored using codes assigned by the investigators. Data will be kept in password-protected computers. Only investigators will have access to the samples and data.

Tracking: Samples will initially be stored in Building 33 in the Laboratory of Infectious Diseases, Viral Pathogenesis and Evolution Section, as well as in the laboratories of associate investigators. Samples will be tracked using a database located on a password-protected computer, which will be maintained by the investigators and their designees. Only investigators and their designees will have access to this database.

Disposition at the Completion of the Protocol:

- In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. Before any sharing of samples, data, or clinical information, either IRB approval must be obtained or the NIH Office of Human Subjects Research Protections (OHSRP) must determine that the research is

exempt from IRB oversight. OHSRP can make this determination for some research where the samples or data have no personal identifying information about the study participant, and the researcher is not able to ascertain it.

- At the time of protocol termination, samples will either be destroyed, or, after IRB approval, transferred to another existing protocol. Data will be archived by the study team in compliance with requirements for retention of research records; alternatively, after IRB and study sponsor approval, the data may be either destroyed or transferred to another repository.

Reporting the Loss or Destruction of Samples/Specimens/Data to the IRB:

- Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that meets the definition of protocol deviation, unanticipated problem (UP), and/or compromises the scientific integrity of the data collected for the study will be reported to the IRB. The PI will also notify the IRB if the decision is made to destroy the samples.
- Additionally, subjects may decide at any point not to have their samples stored. In this case, the principal investigator will destroy all known remaining samples and report what was done to both the participant and to the IRB. This decision will not affect the subject's participation in this protocol or any other protocols at NIH.

10 Remuneration Plan

Participants will be compensated according to Table 3.

Table 3. Participant remuneration

Day -1/Admission	$220
Day 0	$225
Day 1	$400
Day 2	$225
Day 3	$225
Day 4	$275
Day 5	$275
Day 6	$300
Day 7	$325
Day 8	$350
Expected Inpatient Total:	***$2820***
Day 29 +/- 3 days	$350
Day 66 +/- 3 days	$400
Expected Follow-Up Total:	***$750***
Expected total for completion of ALL study visits	$3570

Additional necessary inpatient days:	$350
Study-requested interim visits:	$100

Follow-up visits will be compensated according to the number of visits the participant completes regardless of weeks past discharge. Participants will only be reimbursed for the protocol visits and interim visits requested by the investigators if medically necessary. Remuneration will not be provided for interim visits requested by the participant. Remuneration checks will be mailed to the address requested by the participant in two intervals; once they have completed the inpatient portion of the study and after completion of the outpatient portion of the study.

11 Assessment of Safety

11.1 Documenting, Recording, and Reporting AEs

At each contact with the participant, information regarding AEs will be elicited by appropriate questioning and examinations and will be:

- immediately documented in the participant's medical record/source document,
- recorded in CRIMSON, and
- reported as outlined below (e.g., IND Sponsor, IRB, FDA).

11.2 Definitions

Expected Events

The following mild (Grade 1) to moderate (Grade 2) signs or symptoms are induced by or associated with influenza, and any combination of these is expected to occur with virus challenge. These symptoms will not be reported as unexpected adverse events unless they are associated with administration of CR6261 and deemed possibly, probably, or definitely related to CR6261 by the PI. If deemed related to influenza by the PI they will be recorded as Expected Events of influenza infection in CRIMSON per protocol, unless deemed a risk to the participants' rights or wellbeing or as deemed appropriate by the Principal Investigator. These events will be checked by the study team on a daily basis.

- Abdominal pain
- Anxiety
- Arthralgia
- Chest pain
- Chills/shakes
- Conjunctivitis
- Coryza (nasal/sinus congestion/sneezing)
- Decreased appetite
- Depression

- Diarrhea
- Difficulty Concentrating
- Difficulty Sleeping
- Dry cough
- Dyspnea/Shortness of Breath
- Fatigue/Tiredness/Lethargy
- Fever (>38.0°C)
- Hallucination
- Headache
- Lymphopenia (600-650/mm^3)
- Minimum oxygen saturation level
- Myalgia
- Nausea
- Nightmares
- Oxygen saturation decreased by ≥3% from baseline
- Productive cough
- Rhinorrhea
- Skin rash
- Slowed thinking
- Sore throat
- Sweats

Infusion Adverse Reactions
Infusion reactions or allergic reactions have been observed with administration of mAbs. Serious allergic reactions (e.g. anaphylactic shock) may occur at any time during the administration of study drug.

All subjects must be observed carefully for signs of an infusion reaction during the infusion and for at least 60 minutes after the IV infusion of study drug has been completed. The investigator should use clinical judgment in assessing the intensity of any infusion reaction. The examples given below are for guidance only and may be considered either serious or non-serious depending on the clinical circumstance.

Some examples of potential non-serious infusion reactions include flushing, back pain, vasovagal reactions, chills, nausea, headache, diaphoresis, lightheadedness, or myalgias.

Examples of potential serious infusion reactions include asymptomatic/symptomatic hypotension, symptomatic hypertension, urticaria, rash, chest pain, vomiting, peripheral

edema of extremities, fever, rigors, somnolence, bronchospasm with wheezing, laryngeal/tracheal edema, laryngospasm, congestive heart failure, circulatory failure/cardiogenic shock, angioedema, acute respiratory distress syndrome (e.g. new and rapid onset hypoxia, pulmonary infiltrates/edema, dyspnea requiring ventilatory support), or brady- or tachyarrhythmias.

Infusion reactions should be graded in the following manner:

Grade 1	Mild transient reaction; infusion interruption not indicated; intervention not indicated
Grade 2	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g. antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for less than 24 hours
Grade 3	Prolonged (e.g. not rapidly responsive to symptomatic medication and/brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae
Grade 4	Life-threatening consequences; urgent intervention indicated
Grade 5	Death

Adverse Event
An AE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam, or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Adverse Reaction (AR)
An AE that is caused by an investigational agent (drug or biologic).

Suspected AR (SAR)
An AE for which there is a reasonable possibility that the investigational agent caused the AE. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the AE. A suspected AR implies a lesser degree of certainty about causality than AR which implies a high degree of certainty.

Serious Adverse Event (SAE)
An SAE is an AE that results in 1 or more of the following outcomes:

- death
- a life-threatening (i.e., an immediate threat to life) event
- an inpatient hospitalization or prolongation of an existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

- a congenital anomaly/birth defect
- a medically important event*

*Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the other outcomes listed above.

Unexpected AE
For this study, an AE is unexpected if it is not listed as "expected" (see Expected Events above) or is not listed at the specificity or severity that has been observed (and is described above). It is the responsibility of the IND Sponsor to make this determination.

Serious and Unexpected SAR (SUSAR)
A SUSAR is a SAR that is both serious and unexpected.

Unanticipated Problem (UP)
A UP is any event, incident, experience, or outcome that is

1. unexpected in terms of nature, severity, or frequency in relation to
    a. the research risks that are described in the IRB-approved research protocol and informed consent document 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.)

UP that is not an AE (UPnonAE)
A UP that does not fit the definition of an AE, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered a non-serious UP. For example, breaches of confidentiality, accidental destruction of study records, or unaccounted-for study agent will be reported.

Protocol Deviation
Any change, divergence, or departure from the IRB-approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as:

1. Those that occur because a member of the research team deviates from the protocol.
2. Those that are identified before they occur, but cannot be prevented.
3. Those that are discovered after they occur.

Serious Protocol Deviation
A deviation that meets the definition of an SAE or compromises the safety, welfare, or rights of subjects or others.

Non-compliance
The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human subjects. Non-compliance is further characterized as:

1. Serious: Non-compliance that:
    a. Increases risks, or causes harm, to participants
    b. Decreases potential benefits to participants
    c. Compromises the integrity of the NIH-HRPP
    d. Invalidates the study data
2. Continuing: Non-compliance that is recurring
3. Minor: Non-compliance that is neither serious nor continuing

11.3 Investigator Assessment of AEs

If a diagnosis is clinically evident (or subsequently determined), the diagnosis, rather than the individual signs and symptoms or lab abnormalities, will be recorded as the AE.

All AEs occurring from the time the informed consent is signed through the 8-week follow-up period will be documented and recorded.

The Investigator will evaluate all AEs with respect to **Seriousness** (criteria listed above), **Severity** (intensity or grade), and **Causality** (relationship to study agent and relationship to research) according to the following guidelines.

11.3.1 Severity

The investigator will grade the severity of each AE according to the FDA "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" September 2007, which can be found at: http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm074775.htm

Microscopic hematuria will be graded using the above toxicity table except for the Grade 1 values which are being adjusted to be consistent with the CC Department of Laboratory Medicine normal laboratory values. Grade 1 will be 6-10 RBC/HPF instead of 1-10 RBC/HPF listed in the FDA toxicity table.

Grade 1 and 2 lab and vital sign abnormalities found prior to administration of CR6261 will be documented as baseline abnormalities, but not as an AE. After administration of CR6261, all new gradable abnormalities not found at baseline, regardless of

expectedness, will be reported as AEs. If a participant has a grade 1 or 2 baseline-abnormality prior to administration of CR6261 then the gradable abnormality will only be reported as an AE if there is a change in severity from baseline, if the AE resolves and then recurs, or if the PI deems it clinically significant.

Severity grading for clinical events that are not found in the FDA Healthy Volunteer Toxicity Table will be graded according to the following grading scale:

- Grade 1 (Mild)
  Events causing no or minimal interference with daily activity

- Grade 2 (Moderate)
  Events causing greater than minimal interference with daily activity but not requiring medical intervention

- Grade 3 (Severe)
  Events causing inability to perform daily activity and requiring medical intervention

- Grade 4 (Potentially Life-Threatening)*
  Events causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death

*** Note:** A severity assessment of “potentially life-threatening” is not necessarily the same as life-threatening as an "SAE" criterion. The latter means that the event is an immediate threat to life as opposed to a potential threat to life.

11.3.2 Causality

Causality (likelihood that the event is/is not related to the study agent) will be assessed considering the factors listed under the following categories:

Definitely Related
- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related
- reasonable temporal relationship
- follows a suspected response pattern (based on similar products)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related
- does not have a reasonable temporal relationship
OR
- good evidence for a more likely alternative etiology

Not Related
- does not have a temporal relationship
OR
- definitely due to an alternative etiology

Note: Other factors may also be considered for each causality category when appropriate. 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.

11.4 Investigator Reporting Responsibilities to the Sponsor

11.4.1 AEs

Line listings, frequency tables, and other summary AE data will be submitted to the IND Sponsor when needed for periodic safety reviews, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

11.4.2 SAEs

All SAEs (regardless of relationship and whether or not they are also UPs) must be reported on the Safety Expedited Report Form (SERF) and sent to the Sponsor Clinical Safety Office (CSO) by fax or e-mail attachment. 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.

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

11.4.3 Unanticipated Problems

UPs that are also AEs must be reported to the CSO and sent by fax or e-mail attachment no later than 7 calendar days of site awareness of the event. UPs that are not AEs are not reported to the Sponsor CSO.

Report all UPs that are also adverse events to the CSO on the NIH Problem Report Form.

11.4.4 Pregnancy

Pregnancy itself is not an AE. However, complications of pregnancies are AEs and may be SAEs. Pertinent obstetrical information for all pregnancies will be reported to the CSO via fax or email within 3 business days from site awareness of the pregnancy.

Pregnancy outcome data (e.g., delivery outcome, spontaneous, or elective termination of the pregnancy) will be reported to the CSO within 3 business days of the site's awareness of the outcome on a protocol-specified form.

Participants who become pregnant during the study will be asked to report this to the study team immediately. The participants will be referred for appropriate obstetric care and, if they have not received the influenza challenge, they will be discontinued from the study immediately. If the participant has received the influenza challenge, the pregnancy will be followed to determine the outcome.

11.5 Investigator Reporting Responsibilities to the NIAID IRB

11.5.1 Special Reporting Situations

Safety events of interest on a sponsor study drug that may require expedited reporting and/or safety evaluation include, but are not limited to:

- Overdose of a sponsor study drug
- Suspected abuse/misuse of a sponsor study drug
- Inadvertent or accidental exposure to a sponsor study drug
- Medication error involving a sponsor product (with or without subject/patient exposure to the sponsor study drug, e.g., name confusion)

Special reporting situations should be recorded in the CRIMSON. Any special reporting situation that meets the criteria of a serious adverse event should be recorded as a serious adverse event in CRIMSON and reported as described in Section 11.4.2.

11.5.2 Expedited Reporting to the NIAID IRB

Serious and non-serious UPs, deaths, serious deviations, and serious or continuing non-compliance will be reported within 7 calendar days of investigator awareness. SAEs that are possibly, probably, or definitely related to the research will be reported to the NIAID IRB within 7 days of site awareness, regardless of expectedness.

11.5.3 Waiver of Reporting Anticipated Protocol Deviations, Expected UPnonAEs and Deaths to the NIAID IRB

Anticipated deviations in the conduct of the protocol will not be reported to the IRB unless they occur at a rate greater than anticipated by the study team. Expected AEs will not be reported to the IRB unless they occur at a rate greater than that known to occur in

influenza. If the rate of these events exceeds the rate expected by the study team, the events will be classified and reported as though they are UPs.

11.5.4 Annual Reporting to the NIAID IRB

The following items will be reported to the NIAID IRB in summary at the time of Continuing Review:

- Serious and non-serious UPs
- SAEs that are possibly, probably, or definitely related to the research
- SAEs that are not related to the research
- All AEs, except expected AEs granted a waiver of reporting
- Serious and non-serious protocol deviations
- Serious, continuing, and minor non-compliance
- Any trends or events which in the opinion of the investigator should be reported
- A summary of accumulated safety data

11.6 Follow-Up of AEs and SAEs

AEs that occur following enrollment of the participant (by signing the informed consent) will be followed until the final outcome is known or until the end of the 8-week study follow-up period. AEs that have not resolved by the end of the study follow-up period will be recorded in CRIMSON as “ongoing.” Any participant who experiences complications due to challenge virus administration will be followed until resolution of such complications or appropriate referral to the necessary medical care has been made.

SAEs that have not resolved by the end of the follow-up period will be followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the participant is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator in CRIMSON on the SERF.

SAEs that occur after the 8-week study follow-up period that are reported to and are assessed by the Investigator to be possibly, probably, or definitely related will be reported to the CSO, as described above.

11.7 Sponsor’s Reporting Responsibilities

SUSARs as defined in 21 CFR 312.32 and determined by the IND Sponsor will be reported to FDA as IND Safety Reports.

The IND Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

11.8 Halting Rules for the Protocol

Halting the study requires discontinuation of study agent administration for all study participants and suspension of enrollment. Participants currently enrolled who have been administered the challenge virus and/or the study drug prior to the halting will continue on

in the study to ensure the safety of the participant; procedures will be limited as described in Section 11.11 until a decision is made whether or not to continue study agent administration.

The halting criteria for this study include:

- one or more participants experience an SAE that is unexpected (in consultation with the sponsor) that is possibly, probably, or definitely related to the study agent(s); **OR**
- two or more of the same AE in different participants that are equal to or greater than grade 3 and are possibly, probably, or definitely related to the study agent(s) NOTE: in the case that the adverse event is elevated amylase or lipase values, the event must be associated with symptoms consistent with pancreatitis and deemed clinically significant by the PI and/or Medical Monitor; **OR**
- any significant safety issue that the PI determines should halt the trial.

11.8.1 Reporting of Study Halting

If any of the above halting requirements are met, a description of the event(s) or safely issue will be reported by the PI within 1 business day to the OCRPRO CSO by fax or email. In addition, the PI will inform the IRB, the DSMB and the FDA that a halting rule has been met.

11.8.2 Resumption of a Halted Study

The OCRPRO CSO, in collaboration with the PI and the DSMB, will determine if it is safe to resume the study. If study halting is triggered in the 24-hour period after challenge virus administration but prior to CR6261 administration, the Sponsor Medical Monitor and PI may make the decision to proceed with CR6261 administration in the absence of a response from the DSMB. The PI will notify the IRB of the decision to resume the study.

11.9 Study Discontinuation

OCRPRO, the study sponsor, the Institutional Review Board (IRB), and the FDA have the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

1. The incidence or severity of AEs in this study indicates a potential health hazard to participants
2. Participant enrollment is unsatisfactory
3. Data recording is inaccurate or incomplete
4. Investigators do not adhere to the protocol, or applicable regulatory guidelines in conducting the study

The IRB, the NIAID, the FDA, or other government agencies, as part of their duties to ensure that research participants are protected may discontinue the study at any time. Subsequent review of serious, unexpected and related adverse events by the IRB, the DSMB, the sponsor(s), the FDA, and other regulatory authorities may also result in

suspension of further trial interventions/administration of study agent at a site. The FDA, other regulatory authorities, and the study sponsor(s) retain the authority to suspend additional enrollment and Study Agent(s)/Intervention(s) administration for the entire study as applicable.

11.10 Premature Withdrawal of a Participant

A study participant will be withdrawn from the study by the PI prior to receipt of the influenza challenge for:

1. Any clinical AE, laboratory abnormality, intercurrent illness, or other medical condition or situation such that continued participation in the study would not be in the best interest of the participant.
2. Development of any exclusion criteria may be cause for discontinuation.

Participants may withdraw prior to inoculation with the challenge virus and no further testing or follow up will be performed. Participants will be strongly discouraged from withdrawing from the study after inoculation. If the participant would like to withdraw after inoculation, no further research testing will be performed, but nasal washes/swabs and clinical laboratory tests for safety purposes will continue. The participant will also be treated medically as deemed appropriate by the PI, which may include the administration of antiviral treatment. The participant will be asked to remain isolated until virus is not detectable in 2 consecutive diagnostic tests (obtained on 2 separate days). For the purposes of safety testing, the participant will also be asked to come for follow-up visits. If the participant refuses to stay in isolation after inoculation the PI and study team will take appropriate steps to ensure the participant's and public safety and encourage the participant to return for the follow-up visits. If the participant does not return for scheduled follow-up visits the study staff will make every reasonable effort to contact the participant by phone, mail, or email, or a combination of the latter and reiterate that follow-up visits are strongly encouraged for safety reasons.

11.11 Replacement of a Participant

If a participant withdraws or appears ineligible to continue the study before viral challenge, he/she will be removed and no data will be used in analysis or publication of the study. The participant may be replaced in the accrual with a new volunteer who qualifies and consents to the study as discussed in Section 5.1.5. Safety data from all participants that have withdrawn will be used and included in the safety analysis.

If, after inoculation, a participant is diagnosed with or has a significant exposure to any other infection or illness that is unrelated to the challenge virus but could interfere with the integrity of the study (such as a viral infection that has gone undetected during an incubation period but manifests and is discovered after inoculation), the investigator may use his discretion to remove the participant from the analysis. Safety data from all participants will still be used and included in the safety analysis and all reporting. This participant will still undergo all study procedures, complete all study related visits, and be accounted for in total study participant accrual.

11.12 Safety Oversight

11.12.1 Safety Review and Communications Plan (SRCP)

A Safety Review and Communication Plan (SRCP) has been developed for the protocol. The SRCP is an internal communications document between the PI and the IND Sponsor 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.

11.12.2 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 routine safety surveillance assessments of study data reported to the Clinical Safety Office by the PI as described in a Safety Review and Communications Plan (SRCP).

11.12.3 Data and Safety Monitoring Board (DSMB)

The NIAID Intramural DSMB will review the study prior to initiation and after each group has been enrolled. The Board may convene additional reviews as necessary. The next group will not be enrolled until the review is complete. The Board will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study. All SAEs, all UPs, and all IND Safety Reports will be reported by the PI to the DSMB at the same time they are submitted to the IRB or IND Sponsor. 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 halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written DSMB summary reports with recommendations to the IRB(s).

12 Compliance With NIH Guidelines for Research Involving Products Containing Recombinant DNA

Because this study involves products containing recombinant DNA, it must comply with regulations set forth in the NIH's *Guidelines for Research Involving Recombinant DNA Molecules*. Information about the study must be submitted to site Institutional Biosafety Committees (IBC) and must be approved before participants are enrolled in the study. IBC review and approval must be documented by the investigator and submitted as part of protocol registration for this trial.

13 Clinical Monitoring Structure

13.1 Site Monitoring Plan

As per International Conference on Harmonization (ICH) Good Clinical Practice (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 process for each monitored participant; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare CRIMSON data abstracts with individual participants' records and source documents (participants' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original participant 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 (FDA and Office for Human Research Protections [OHRP]) 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, CRIMSON data abstracts, and pertinent hospital or clinical records) readily available for inspection by the FDA, IRB, site monitors, and NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the Principal Investigator 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.

14 Statistical Considerations

14.1 Study Hypothesis

The primary hypothesis proposed is that administration of CR6261 24 hours post successful influenza challenge does not alter the course of the infection compared to placebo administration with respect to the area under the curve (AUC) of virus shedding as determined by quantitative PCR (qPCR) in nasopharyngeal (NP) swabs. The secondary hypothesis is that the same treatment does not alter the rate of laboratory-confirmed influenza disease compared with placebo as manifest by the occurrence of viral shedding and at least one of the protocol defined clinical symptoms.

14.2 Sample Size Justification and Analysis Plan

The sample size calculation is based on the primary objective: to demonstrate a positive effect of CR6261 compared with placebo with respect to the AUC of log base 10 viral shedding assessed by qPCR on NP swabs obtained thrice daily over study days 1 through 8. The primary objective will be addressed by the comparison of this AUC between treated subjects in the CR6261 group and the placebo group. The statistical hypothesis is: H_0: The median influenza AUC of log qPCR following CR6261 administration is equal to that following placebo administration versus H_A: the median influenza AUC of log qPCR following CR6261 administration differs from that following placebo administration (i.e. a 2-sided test situation). The primary objective could be established if at the trial's conclusion that median AUC were lower for the CR6261 group than for the placebo group

and the two-sided Wilcoxon rank-sum test comparing the two groups were significant at the 0.05 level. However, we propose to conduct an interim test of this hypothesis and thus even the final testing procedure will be altered; please see separate Section 14.4 below. Note that throughout the rest of the statistical section, all raw, measured qPCR flu viral counts are increased by 1 prior to being logged, since counts that are zero (none detected) could not otherwise be logged. This has no effect on the ranks of the outcome values and thus no effect on the Wilcoxon test.

We used data from a recent previous study using the same challenge model and the same population (subset with prior HAI antibody titer ≤1:10) and set the number of subjects who had no detectable shedding to 20%, which reflects our experience. The resulting reference data included n=15 subjects who had overall Day 1 to 8 mean AUC of log of shed virus 8.8 and standard deviation 7.0. (Specifically, 12 of those subjects had detectable shedding, i.e. non-zero AUC, and 3 had no shedding; the mean AUC of 8.8 included both the zero and non-zero values.) Setting type II error to 0.10 (i,e, power of 0.90), for the Wilcoxon test we find that sample sizes of 58 per group or 116 total are required to detect a decrease in mean AUC of 50% (i.e. from 8.8 to 4.4) assuming that the standard deviation is the same in the treated group as in the placebo group and that asymptotic formulas suffice. However, these sample sizes require adjustment for our plan to conduct interim analysis at both 33% an 67% of the final sample size (see Section 14.4) as well as to allow for 5% possible loss from final analysis. After making those adjustments, the total final sample size (not counting potential replacements for certain subjects who withdraw) was found to number 122. (The calculations were performed using PASS 2008 and SAS 9.3.)

Due to a recommendation by the DSMB in August 2017 to only use lot number 13898, this will end accrual after 95 participants have been enrolled due to limited supply of CR6261. The same 1:1 randomization plan will be used for the remaining participants (Section 6.6). The power of the study will be reduced. With 95 total participants and the assumptions from above (mean between group AUC difference of 4.4 and standard deviation of 7.0) the study will have 83% power. If the true between group difference is 4.9 the study will have 90% power. With the previous sample size of n=122, an interim analysis for futility was to be performed at 75% enrollment (approximately 91). Given the change in sample size, no additional interim analyses will be performed.

The power of this planned sample size for assessing the main secondary study objective was also assessed. The secondary objective is to demonstrate that treatment with CR6261 leads to a reduced rate of influenza induced disease compared to use of placebo. For this objective we compute power for tests using the full sample size and for half of that, since the trial could end early based on interim analysis of the primary outcome. The endpoint for each group will consist of the proportion of subjects who experienced any of the defined clinical symptoms and had a confirmed infection with the challenge virus. The test will be performed using Fisher’s exact test.

For an analysis at the initially planned sample size of 122, there would be 87-88% power for detecting a difference in proportion of 0.30 having the secondary endpoint of influenza disease if the rate in placebo recipients is either 0.65 or 0.75. At the time of the first interim analysis the corresponding power for influenza disease would only be 37-38%, but at the second interim analysis that power would be 68-69%.

Since the estimated standard deviation of the primary outcome plays a crucial role in determining the sample size, we plan to use interim data to re-estimate the standard deviation and thus the required sample size. We will do this after 50% of the planned sample size has completed the primary outcome in a blinded fashion, i.e. based on the data from all participants lumped together. If the standard deviation is higher than originally assumed, the required sample size will be adjusted upward (as long as the new size remains feasible). [Note that this would merely maintain the desired power of 0.90 for the primary endpoint of viral shedding. However, it would actually increase the power for the secondary, clinical endpoint.] If however the estimated sample size actually *decreases*, the trial will continue per the originally planned size. This approach is recommended for example by Proschan et al (2006).[4]

14.3 Analysis Plan

Descriptive analysis will include frequencies and percentages for categorical baseline and outcome characteristics by study groups. Variables that are continuous in nature or can be treated as such will be summarized by mean, standard deviation, median, maximum and minimum for the raw data (and/or logged data, where appropriate). For selected study data graphical displays will supplement the tabulated data. Where appropriate, 95% confidence intervals for the observed data will be included.

Safety analyses will report the number of participants who experience treatment-emergent reactions. Additional analyses will report the number of subjects with AEs that were assessed as related to (definitely, probably, or possibly) CR6261. Details concerning those reactions will be tabulated and listed.

For flu outcome variables the following descriptive analyses will be performed:

The primary outcome variable is the AUCs for the challenge flu strain. The distribution of those AUC values will be summarized via tabulations and graphs.

The frequency of occurrence of the secondary outcome of clinical flu illness will be reported. In addition, the distribution of flu illness severity and the distribution of individual flu symptoms will be reported

The primary efficacy analysis will entail the comparison of subjects' AUCs of qPCR flu count across days 1 through 8. It will be conducted on the population of all participants who received the challenge and provided follow-up nasal swab specimens. While the

protocol calls for three such measurements to be performed at approximately equal intervals on each of those 8 days, we believe that the presence of a few missing values will not tend to affect the measurement much. Therefore, we will include subjects in the analysis if the number of missing values averages at most one per day and the maximum time between assessed measurement is no more than 16 hours.

Because the analyses will be entail multiple looks at the data, they are described in the Interim Analysis section below.

14.4 Interim Analysis

The protocol is designed to conduct an efficacy analysis of the primary outcome twice (after one-third and two-thirds of the final planned study size have data on the primary outcome). This is accomplished using the Haybittle-Peto approach.[5] We will follow its traditional approach of conducting the interim tests at the 0.001 level of significance. The test will be two-tailed, and we plan to stop the trial early if it identifies a significant interim effect, whether that reflects benefit or harm of CR6261.The final test can then be performed at the 0.0488 level. The provision for interim testing typically requires a small increase in sample size compared to performing only a single analysis at the end of the trial, but it is not noticeable in this case (total n=116 analyzable in either case). It provides for rejection of the null hypothesis of no effect of CR6261 on flu qPCR AUC if either the interim efficacy analysis or the third (final) potential analysis rejects the null hypothesis, i.e. if the test finds that the AUCs for the CR6261 group differ “significantly” from those for the placebo group. Significance here is determined by the two-sided Wilcoxon test having p-value < 0.001 at one of the interim analyses (which would be sufficient for ending the trial at that interim point) or having p-value < 0.0488 at the planned final analysis. If an interim analysis does not support early rejection of the null hypothesis, then it calls for the trial to continue until the next analysis. The overall procedure then has a Type I error rate of 0.05.

The interim analysis described above addresses the possibility of identifying either benefit or harm early during the trial. In addition, futility analyses will be performed to find early evidence that *no* significant difference is apt to be found by the end of the trial. A traditional conditional power approach is proposed for this. Because it would be very unlikely to identify futility early in the trial, it is proposed to be performed at approximately 50% of planned enrollment. It is suggested that conditional power of 10% or less (at either look) serve as grounds to terminate the trial due to futility. The DSMB will be asked for its concurrence with the futility plan.

The interim efficacy data and analyses, including for futility, will be presented only to the DSMB for evaluation. The study team will not see any of the interim data unless the trial is stopped according to the interim efficacy criterion (or if for any other reason the trial is permanently stopped). Note: if the sample size is increased (see Section 14.2), the second interim analysis will be rescheduled to occur at 67% of the new total accrual instead of at 67% of the original number.

15 Ethics/Protection of Human Participants

15.1 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research participant. It is an ongoing conversation between the human research participant and the researchers, which begins before consent is given and continues until the end of the participant's involvement in the research. Discussions about the research will provide essential information about the study and include: purpose, duration, experimental procedures, alternatives, risks, and benefits. Participants will be given the opportunity to ask questions and have them answered.

The participants will sign the informed consent document prior to undergoing any procedures. The participant may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The researcher will document the signing of the consent form in the participant's medical record. The rights and welfare of the participant will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

15.2 Non–English-Speaking Participants

If a non–English-speaking participant is unexpectedly eligible for enrollment, the participant will be provided with the CC Short Written Consent Form for Non–English-Speaking Research Participants in the participant's native language and a verbal explanation of the purpose, procedures and risks of the study as described in MAS Policy M77-2, NIH HRPP SOP 12, and 45 CFR 46.117(b)(2). The IRB-approved English consent form will serve as basis for the verbal explanation of the study. The investigator will obtain an interpreter unless the investigator is fluent in the prospective participant's language. Preferably, the interpreter will be someone who is independent of the participant (i.e., not a family member). Interpreters provided by the CC will be used whenever possible. The interpreters will translate the IRB-approved English consent form verbatim and facilitate discussion between the participant and investigator.

The IRB-approved English consent form will be signed by the investigator obtaining consent and a witness to the oral presentation. The CC Short Written Consent Form will be signed by the participant and a witness who observed the presentation of information. The interpreter may sign the consent document as the witness and, in this case, will note "Interpreter" under the signature line. A copy of both signed forms will be provided to the participant to take home.

The investigator obtaining consent will document the consent process in the participant's medical record (CRIMSON), including the name of the interpreter. Further, all instances

of use of the CC Short Written Consent Form will be reported to the IRB at the time of annual review. If the CC Short Written Consent Form is used three times or more for the same language within an IRB approval period, this will be reported to the IRB immediately.

15.3 Participant Confidentiality

All records will be kept confidential to the extent provided by federal, state, and local law. The study monitors and other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records. Records will be kept in secure electronic systems (CRIMSON and CRIS). Clinical information will not be released without written permission of the participant, except as necessary for monitoring by IRB, the FDA, the NIAID, the OHRP, or the sponsor's designee.

16 Data Handling and Record Keeping

16.1 Data Capture and Management

Study data will be collected and maintained in CRIMSON and CRIS and collected directly from participants during study visits and telephone calls. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry into CRIMSON and CRIS will be performed by authorized individuals. The Investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

Data that may potentially unblind the treatment assignment (e.g., study drug serum concentrations, antibodies to study drug, study drug preparation/accountability data, and treatment allocation) will be handled with special care to ensure that the integrity of the blind is maintained and the potential for bias is minimized. This can include making special provisions, such as segregating the data in question from view by the investigators, sponsor clinical team, or others as appropriate until the time of database lock and unblinding.

16.2 Record Retention

The investigator is responsible for retaining all essential documents listed in the ICH GCP Guideline. Study records will be maintained by the PI for a minimum of 3 years and in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever is longest. All stored records will be kept confidential to the extent required by federal, state, and local law.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. Destruction or relocation of research records will not proceed without written permission from NIAID/OCRPRO.

SCIENTIFIC REFERENCES

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4. Proschan MA. Statistical methods for monitoring clinical trials. *Journal of biopharmaceutical statistics.* Nov 1999;9(4):599-615.
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Appendix A: Schedule of Procedures/Evaluations

Study Phase >	Admit	Inpatient											Follow-up
Study Day >	-1‖	0	1			2	3	4	5	6	7	8*	29 ± 4	66 ± 5
		Chall	Pre	Dose	Post									
Inpatient Hospitalization	X	X	X	X	X	X	X	X	X	X	X	Discharge		
Outpatient Visit													X	X
Written Consent	X													
Medical/Medication History	X	X	X			X	X	X	X	X	X	X	X	X
Physician Assessment and PE	X	X	X			X	X	X	X	X	X	X	X	X
Self-administered Flu-Pro questionnaire§	X	X			X	X	X	X	X	X	X	X	X	
Vital signs †	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum Pregnancy test‡	X												X	X
Urine toxicology screening	X													
Urinalysis	X		X			X	X	X				X	X	X
Ethanol Testing	X													
Nasal wash & nasal swab samples Δ	X		X		X	X	X	X	X	X	X	X		
PFT and/or Spirometry	X													
ECG	X		X		X≈			X		X		X	X	X
Echocardiogram ξ	X													
Randomization			X											
IMP administration				X										
Challenge virus inoculation		X												
CBC + diff	X					X	X	X		X		X	X	X
D-dimer		X				X		X		X		X		
PT/PTT	X					X	X	X		X		X		
Amylase/Lipase	X					X	X	X		X		X	X	X
Acute Care, Mineral, and Hepatic Panels	X					X	X	X		X		X	X	X
LDH, Uric Acid, Creatine Kinase, and Total Protein	X					X	X	X		X		X	X	X

Study Phase >	Admit	Inpatient											Follow-up
Study Day >	-1‖	0	1			2	3	4	5	6	7	8*	29 ± 4	66 ± 5
		Chall	Pre	Dose	Post									
Serum/Whole Blood Collection		X	X			X		X		X		X	X	X
PK samples⋈			X		X	X	X		X			X	X	X

*If continued viral shedding is detected on Day 8, the participant(s) will remain in the CC as an inpatient and will continue to have nasal washes (/swabs) and physician assessment and exams. Any other labs, self-assessment questionnaire(s), ECGs etc. will be performed if deemed necessary by the study physician.

†Vital Signs and ECG's: participants must be supine for a minimum of 5 minutes prior to these procedures being performed; vital signs include blood pressure, mean blood pressure, heart rate, respiratory rate, temperature, weight, pulse oximetry; height will be taken at Day -1 only. Both Vital Signs and ECG's are to be taken +/- 15 minutes.

† Day 1 Vital signs will also take place within 10 minutes prior to dose and every 30 minutes while dosing as well as at 2, 4, 8 and 16 hours post dose (+/- 15 minutes)

‡Females only.

§Self-Assessment questionnaire(s) will be filled out daily and may also be used at home between follow up visits during the outpatient portion of the study.

‖PFTs, Echocardiography, ECG, Nasal washes, and laboratory tests may be completed any time after admission and prior to inoculation on day 0.

ξ If a participant has completed the PFT, and/or Echocardiography as part of a previous admission to this study (or as part of the screening study 11-I-0183) but does not qualify to be inoculated at that time (e.g. due to a positive nasal wash), these tests do not need to be repeated if the participant is brought back in for an admission within 90 days.

≈ECG at 4 hours post dose +/- 15 minutes.

⋈ PK samples will be drawn pre-dose on Day 1, 15 minutes (± 15 minutes) post dose, on Day 2 at 24 hours (± 30 minutes), Day 3 at 48 hours (± 1 hour) Day 5 at 96 hours (± 1 hour), Day 8 at 168 hours (± 2 hours), Day 29 (± 1 day) and at Day 66 (± 3 days). ADA samples will be collected on Day 1 pre-dose, and at Days 29 and 66.

∆ NP nasal sampling will take place 4 times a day as scheduled by the study team with a +/- 15 minute window. (3 nasal swabs, and one wash per day)

Appendix B: Blood Volumes for Specimen Collection

Study Schedule/Procedures		Day										2 follow-up visits (Day 29 +/- 4 days and 66 +/- 5 days)
		-1	0	1	2	3	4	5	6	7	8*	
b-hCG, Pregnancy	4mL per blood draw	4mL										4mL
CBC + Diff	3mL per blood draw	3mL			3 mL		3mL		3mL		3mL	3mL
D-dimer	2.7mL per blood draw		2.7mL		2.7mL		2.7mL		2.7mL		2.7mL	
Acute Care, Mineral, and Hepatic Panels	4mL per blood draw	4mL			4 mL		4mL		4mL		4mL	4mL
Serum/Whole Blood Collection	8mL X 2 for serum, 10mL X 4 for whole blood, and 2.5mL for RNA per blood draw		58.5mL	18.5mL **(No whole blood)**	58.5mL		58.5mL		58.5mL		58.5mL	58.5mL
Amylase/Lipase	3mL per blood draw	3mL			3 mL		3mL		3 mL		3mL	3mL
PT-aPTT Order Set	4.5mL per blood draw	4.5mL			4.5mL		4.5mL		4.5mL		4.5mL	4.5mL
PK/ADA samples	8 mL per blood draw			16mL	8mL	8mL		8mL			8mL	8mL
Daily Volume (mL)		**18.5**	**61.2**	**34.5**	**83.7**	**8**	**75.7**	**8**	**75.7**		**83.7**	**85mL x 2 visits = 170mL**
Cumulative Volume		**18.5**	**79.7**	**114.2**	**197.9**	**205.9**	**281.6**	**289.6**	**365.3**		**449**	**619mL**

Appendix C: Participant Self-Assessment Questionnaire

Participant ID: _____ Participant Initials: _____ Date: ____/_____/_____

Influenza Symptom Questionnaire

People experience the flu in different ways. We would like to know about the symptoms you have been experiencing during the past 24 hours. For each symptom, please mark one box ☐ under the response that best matches your experience. Mark the "Not at all" box, if you did not have that symptom in the past 24 hours.

What time is it? _____ AM / PM (please circle)

Please rate the extent to which you had each symptom during the past 24 hours.

	Not at all	A little bit	Somewhat	Quite a bit	Very much
Runny or dripping nose	☐	☐	☐	☐	☐
Congested or stuffy nose	☐	☐	☐	☐	☐
Scratchy or itchy throat	☐	☐	☐	☐	☐
Sore or painful throat	☐	☐	☐	☐	☐
Swollen throat	☐	☐	☐	☐	☐
Difficulty swallowing	☐	☐	☐	☐	☐
Teary or watery eyes	☐	☐	☐	☐	☐
Sore or painful eyes	☐	☐	☐	☐	☐
Eyes sensitive to light	☐	☐	☐	☐	☐
Trouble breathing	☐	☐	☐	☐	☐
Chest congestion	☐	☐	☐	☐	☐
Chest tightness	☐	☐	☐	☐	☐
Dry or hacking cough	☐	☐	☐	☐	☐
Wet or loose cough	☐	☐	☐	☐	☐
Headache	☐	☐	☐	☐	☐
Head congestion	☐	☐	☐	☐	☐
Sinus pressure	☐	☐	☐	☐	☐
Felt dizzy	☐	☐	☐	☐	☐
Felt lightheaded	☐	☐	☐	☐	☐

Participant ID: _____ **Participant Initials:** _____ **Date:** ____/_____/_____

Please rate the extent to which you had each symptom during the past 24 hours.

	Not at all	A little bit	Somewhat	Quite a bit	Very much
Lack of appetite (did not feel like eating)	☐	☐	☐	☐	☐
Felt nauseous (feeling like you wanted to throw-up)	☐	☐	☐	☐	☐
Stomach ache	☐	☐	☐	☐	☐
Sleeping more than usual	☐	☐	☐	☐	☐
Difficulty staying asleep	☐	☐	☐	☐	☐
Difficulty falling asleep	☐	☐	☐	☐	☐
Body aches or pains	☐	☐	☐	☐	☐
Weak or tired	☐	☐	☐	☐	☐
Chills or shivering	☐	☐	☐	☐	☐
Felt cold	☐	☐	☐	☐	☐
Felt hot	☐	☐	☐	☐	☐
Sweating	☐	☐	☐	☐	☐
Felt uncomfortable (general discomfort)	☐	☐	☐	☐	☐

In the past 24 hours, how often have you had any of the following symptoms?

	0 times	1 time	2 times	3 times	4 or more times
How many times did you vomit?	☐	☐	☐	☐	☐
How many times did you have diarrhea?	☐	☐	☐	☐	☐

	Never	Rarely	Sometimes	Often	Always
Sneezing	☐	☐	☐	☐	☐
Coughing	☐	☐	☐	☐	☐
Coughed up mucus or phlegm	☐	☐	☐	☐	☐

ADDITIONAL DAILY DIARY ITEMS FOR FLU-PRO VALIDATION STUDY

Items to be asked in the daily diary through to Day 14 along with the FLU-PRO items.

1. Did you take any medication for your flu symptoms today? (Please select one response only)
   - ☐1 Yes
   - ☐0 No

2. Do you have asthma, COPD (chronic obstructive pulmonary disease) or both?
   - ☐1 Yes
   - ☐0 No

3. [Only asked if answer to the question above is "yes"]. Did you use any rescue medication today for your asthma or COPD? (Please select one response only)
   - ☐1 Yes
   - ☐0 No

4. Overall, how severe were your flu symptoms today? (Please select one response only)
   - ☐0 No flu symptoms today
   - ☐1 Mild
   - ☐2 Moderate
   - ☐3 Severe
   - ☐4 Very severe

5. Overall, how were your flu symptoms today compared to yesterday? (Please select one response only)
   - ☐1 Much better
   - ☐2 Somewhat better
   - ☐3 A little better
   - ☐4 About the same
   - ☐5 A little worse
   - ☐6 Somewhat worse
   - ☐7 Much worse

6. How much did your flu symptoms interfere with your usual activities today? (Please select one response only)
   - ☐1 Not at all
   - ☐2 A little bit
   - ☐3 Somewhat
   - ☐4 Quite a bit
   - ☐5 Very much

7. Have you returned to your usual activities today?
   - ☐1 Yes

☐$_0$ No

8. In general, how would you rate your physical health today? (Please select one response only)
   ☐$_5$ Excellent
   ☐$_4$ Very Good
   ☐$_3$ Good
   ☐$_2$ Fair
   ☐$_1$ Poor

9. Have you returned to your usual health today?
   ☐$_1$ Yes
   ☐$_0$ No