Protocol Number: IRC-004

A Randomized Double-Blind Study Comparing Oseltamivir versus Placebo for the Treatment of Influenza in Low Risk Adults

Sponsored by:
Office of Clinical Research Policy and Regulatory Operations (OCRPRO)
Division of Clinical Research (DCR)
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A Non-IND Study

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Précis

Seasonal influenza is responsible for excess hospitalizations and, despite effective antivirals, causes significant morbidity and mortality (about 24,000 deaths each year in the United States alone). Although there are four currently licensed anti-influenza medications (amantadine and rimantadine, oseltamivir, and zanamivir), previous studies have not demonstrated conclusively to what extent these medications affect influenza viral shedding. This study will evaluate whether oseltamivir modifies the viral shedding during the treatment of uncomplicated influenza in an adult population and also assess methods to detect viral replication in the upper respiratory tract.

Subjects who present with an influenza-like illness without any risk factors for severe disease will be screened for the study. Those with a confirmatory test for influenza (rapid antigen or polymerase chain reaction [PCR]) will be randomized in a 1:1 manner to receive a blinded study treatment consisting of either the oseltamivir or placebo for 5 days. Clinical, virologic, and laboratory assessments on Days 3, 7, and 28 will be used for both safety and efficacy analysis.

LIST OF ABBREVIATIONS

AE	Adverse event
BMI	Body mass index
CDC	The Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
COPD	Chronic obstructive pulmonary disease
DCR	Division of Clinical Research
DSMB	Data and Safety Monitoring Board
eCRF	electronic Case Report Form
FDA	Food and Drug Administration
GCP	Guidelines for Good Clinical Practice
H1N1	Influenza A H1N1, swine flu
HA	Hemagglutination
HAI	Hemagglutination inhibition
HIV	Human immunodeficiency virus
ICH	International Conference on Harmonisation
IDSA	Infectious Disease Society of America
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	Intention to treat
MMWR	Morbidity and Mortality Weekly Report
MOP	Manual of Operations
NA	Neuraminidase
NAI	Neuraminidase inhibitors
NIAID	National Institute of Allergy and Infectious Diseases
NP(S)	Nasopharyngeal (swab)
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OTC	Over the counter
PCR	Polymerase chain reaction
SAE	Serious adverse event
SSS	Social & Scientific Systems, Inc.
WHO	World Health Organization

TABLE OF CONTENTS

PROTOCOL SUMMARY

Full Title: A Randomized Double-Blind Study Comparing Oseltamivir versus Placebo for the Treatment of Influenza in Low Risk Adults

Short Title: IRC-004

Sponsor: Office of Clinical Research Policy and Regulatory Operations (OCRPRO), Division of Clinical Research (DCR), National Institute of Allergy and Infectious Diseases (NIAID)

Conducted by: NIAID Influenza Research Collaboration (NIRC)

Sample Size: N= 560 subjects

Accrual Ceiling: Up to 800 subjects will be screened to randomize 560 subjects

Study Population: Outpatient subjects at low risk for complications and morbidity from influenza (using US CDC criteria), and that have a diagnosis of influenza by rapid antigen or PCR.

Study Design: This randomized double-blind study will assess evaluate whether oseltamivir modifies viral shedding in an ambulatory population with uncomplicated influenza. This study is also designed to explore the relationship between virologic effects and clinical effects, effects on pro-inflammatory mediators, and to start understanding if improvements to virologic shedding correlate with improvements in clinical outcomes. This is a multicenter international study. Up to approximately 50 sites may participate in this protocol.

Subjects with an influenza-like illness will be screened for the study, and those found positive for influenza will be randomized in a 1:1 manner to receive a blinded study treatment consisting of either oseltamivir or placebo for 5 days. Subjects will be assessed on Study Day 0 (pre-dose), and on Study Days 3, 7, and 28. All subjects will undergo a series of efficacy and safety assessments during the study. Subjects will have a NP swab to test for influenza virus on Days 0, 3, 7, and blood samples will be collected on Days 0, 3, 7, and 28. Subjects will also perform self-collection of nasal swabs for the detection of influenza virus on Days 0, 1, 2, and 3.

Study Agent: Oseltamivir versus placebo.

Primary Objective:
The overall objective of the study is to evaluate the antiviral efficacy of oseltamivir compared to placebo in the treatment of subjects with confirmed influenza infection (Primary Efficacy Population) as measured by percentage of subjects with virus detectable by PCR in NP swab at Day 3.

Secondary Objectives:

1. Evaluate the antiviral efficacy of treatment with oseltamivir compared with placebo in those subjects with confirmed influenza infection (primary efficacy population) and intention to treat (ITT) population as assessed by the following parameters:

Virologic: (by PCR in NP swab)
◦ AUC of viral shedding
◦ Duration of viral shedding
◦ Percent cessation of viral shedding on Days 3 and 7
◦ Frequency of emergence of antiviral resistance

Clinical:
◦ Time to alleviation of influenza clinical symptoms
◦ Time to absence of fever
◦ Time to resolution of all symptoms AND fever
◦ Time to resumption of normal activity
◦ Proportion of subjects who require hospitalization
◦ Proportion of subjects who develop bronchitis, pneumonia, or other complications of influenza

2. Evaluate the association of viral shedding (both qualitative and quantitative) to clinical symptoms and fever.

3. Evaluate if changes in viral shedding by antiviral treatment predict changes in clinical symptoms and fever.

4. Evaluate subject self-collected nasal samples compared with study team collected samples for the determination of viral shedding.

5. Evaluate the variability in the sample collection. (pilot study only)

Endpoints:

Primary Endpoint
Percentage of subjects shedding virus by PCR in NP swab at day 3.

Secondary Endpoints
Clinical Endpoints:

- Time to alleviation of influenza clinical symptoms
- Time to absence of fever
- Time to resumption of normal activity
- Number of premature study treatment discontinuations
- Proportion of subjects who develop bronchitis, pneumonia, or other complications of influenza
- Proportion of subjects who require hospitalization
- 28-day mortality

Virologic Endpoints: (assessed by PCR in NP swab)

- Duration of viral shedding
- Change in viral shedding as a function of time
- AUC of viral shedding
- Frequency of emergence of antiviral resistance

1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Influenza Infections in Humans

1.1.1 Epidemiology

Influenza causes annual epidemics of acute respiratory illness. Most people who get influenza will recover in a few days to less than 2 weeks, but some people will develop complications (such as pneumonia) as a result of influenza, some of which can be life-threatening and result in death. Complications from influenza can happen at any age, but some people are at higher risk of developing serious influenza-related complications. People at higher risk include people 65 years and older, people of any age with certain chronic medical conditions (including asthma, diabetes, immunosuppression, and heart disease), pregnant women, and infants. Despite effective antivirals and vaccines, influenza is responsible on average for 226,000 excess hospitalizations and about 24,000 deaths each year in the United States alone. [1] The number of deaths is estimated to be over 500,000 worldwide.

1.1.2 Antiviral Therapy for Influenza

Viral glycoproteins (hemagglutinin [HA] and neuraminidase [NA]) are the main targets of the immune response to influenza. Vaccines elicit an immune response to these glycoproteins, particularly HA and are the primary means for prevention. However, influenza HA and NA antigens vary frequently as a result of antigenic drift and rarely by antigenic shift, many at-risk person do not respond adequately to immunization, influenza vaccines are under-utilized, and sometimes antigenically matched vaccines are not available in a timely manner. Antiviral drugs are the principal treatment of influenza viruses. [2, 3] There are four currently licensed anti-influenza medications: 2 oral adamantanes, (amantadine and rimantadine) and 2 neuraminidase inhibitors (NAIs) (the oral drug oseltamivir [Tamiflu®] as well as the inhaled medication zanamivir [Relenza®]).

1.1.3 Antiviral Resistance

Resistance of influenza A viruses to amantadine or rimantadine can occur spontaneously or emerge rapidly during treatment. Most (>95%) recent seasonal H3N2 isolates are resistant to these compounds [4], though still maintain sensitivity to oseltamivir and zanamivir. Most (>98%) of seasonal H1N1 isolates during 2008-9 were resistant to oseltamivir, [5] though still sensitive to amantadine, rimantadine, and zanamivir. The pandemic influenza A/H1N1 2009 isolates are resistant to amantadine and rimantadine, [6] and some isolates have additional resistance to oseltamivir and peramivir.[7]

Resistance to the M2 inhibitors is mediated by mutations in the M2 ion channel and results in cross-resistance among all drugs in the class. M2 inhibitor resistance appears to be stable and resistant variants have circulated globally in the absence of selective drug pressure. [8, 9] These features have contributed to the rapid and widespread emergence of influenza A/H3 virus resistant to M2 inhibitors which currently limits the effectiveness of this class of drugs.[10] On the other hand, NAI resistance can occur as the result of mutations in either the NA gene, HA gene, or both, although almost all oseltamivir-resistant clinical isolates have had specific mutations in NA. Resistance patterns depend on the particular mutation, virus type and subtype, and NAI. In seasonal A (H1N1) viruses, high-level resistance to oseltamivir (>300 fold reduced)

has been conferred by a H275Y mutation in NA that also diminishes susceptibility to peramivir but does not affect susceptibility to zanamivir.

1.1.4 Treatment of Influenza

The U.S. CDC has recommended the following regarding the treatment of influenza: http://www.cdc.gov/h1n1flu/recommendations.htm#3

Prompt empiric treatment is recommended for persons with suspected or confirmed influenza and:

- Illness requiring hospitalization
- Progressive, severe, or complicated illness, regardless of previous health status, and/or
- Patients at risk for severe disease

The following medical conditions have been associated with an increased risk of complications from influenza: [11]

- Asthma
- Neurological and neuro-developmental conditions [including disorders of the brain, spinal cord, peripheral nerve, and muscle such as cerebral palsy, epilepsy (seizure disorders), stroke, intellectual disability (mental retardation), moderate to severe developmental delay, muscular dystrophy, or spinal cord injury].
- Chronic lung disease (such as chronic obstructive pulmonary disease [COPD] and cystic fibrosis)
- Heart disease (such as congenital heart disease, congestive heart failure and coronary artery disease)
- Blood disorders (such as sickle cell disease)
- Endocrine disorders (such as diabetes mellitus)
- Kidney disorders
- Liver disorders
- Metabolic disorders (such as inherited metabolic disorders and mitochondrial disorders)
- Weakened immune system due to disease or medication (such as people with HIV/AIDS, or cancer, or those on chronic steroids)
- People younger than 19 years of age who are receiving long-term aspirin therapy

Additionally, for the 2009 H1N1influenza, morbid obesity (body mass index [BMI] ≥40) has been suggested as an independent risk factor. [12]

The Infectious Diseases Society of America (IDSA) guidelines for influenza recommended treatment for persons who meet the following criteria:

a. Persons with laboratory-confirmed or highly suspected influenza virus infection at high risk of developing complications within 48 hours after symptom onset.

b. Persons requiring hospitalization for laboratory-confirmed or highly suspected influenza illness, regardless of underlying illness or influenza vaccination status, if treatment can be initiated within 48 hours after onset of symptoms.

They further state, treatment should be considered for both adults and children with influenza virus infection who meet the following criteria:

a. Outpatients at high risk of complications (with illness that is not improving and who have a positive influenza test result from a specimen obtained >48 hours after onset of symptoms.

b. Outpatients with laboratory-confirmed or highly suspected influenza virus infection who are not at increased risk of complications, whose onset of symptoms is <48 hours before presentation, and who wish to shorten the duration of illness.

1.2 Rationale for the Study

The overall objective of the study is to evaluate the virologic efficacy of oseltamivir compared with placebo using PCR, and to determine the association between virologic improvement and clinical improvement.

1.3 Oseltamivir

The neuraminidase inhibitors currently available include zanamivir (Relenza®) and oseltamivir (Tamiflu®). Both are sialic acid analogues that potently and specifically inhibit the viral neuraminidase by competitively and reversibly interacting with the active enzyme site of influenza A and B viruses. [13] Influenza neuraminidase cleaves terminal sialic acid residues and destroys the receptors recognized by viral hemagglutinin. This action is essential for release of virus from infected cells, prevention of viral aggregates, and spread within the respiratory tract. [14]

Oral oseltamivir phosphate is cleaved by esterases in the liver, gastrointestinal (GI) tract, and blood to the active oseltamivir carboxylate with good bioavailability estimated ~80%. [15] The time to maximum plasma concentrations of the active form is about 3 to 4 hours. Administration with food may delay absorption slightly but does not decrease overall bioavailability. Following oral administration of oseltamivir, the plasma half-life ($t_{1/2}$) of oseltamivir carboxylate averages 7 to 9 hours. Both the prodrug and parent are eliminated primarily unchanged through the kidney. Distribution is not well characterized in humans, but peak bronchoalveolar lavage levels are similar to plasma levels in animals. [16] In humans, concentrations in sinus and middle ear fluid aspirates are similar to those in plasma. [17]

No clinically significant drug interactions have been recognized for either drug. Neither oseltamivir or oseltamivir carboxylate interact with human cytochrome P450 enzymes, which suggests that drug interactions as a result of competition with this pathway are unlikely. Oseltamivir elimination is inhibited by administration of probenecid, a finding that indicates that the carboxylate is excreted in part by tubular secretion. [17]

Oral oseltamivir is generally well tolerated and no serious end-organ toxicity has been found. Oral oseltamivir is associated with nausea, abdominal discomfort and, less often, emesis in a minority of influenza-infected patients. Gastrointestinal (GI) complaints are usually mild to moderate in intensity, often diminished despite continued dosing, and ameliorated by

administration with food. Additional rare side effects include severe rash, hepatic dysfunction, low platelet count, and allergic reactions/anaphylaxis.

1.3.1 Standard Oseltamivir Dosing

The standard dose (US FDA approved dose) of oseltamivir is 75 mg bid for 5 days for treatment of influenza A or B for adults and children ≥13 years old. [18]

1.3.2 Oseltamivir Therapy for Human Influenza

Prospective, placebo-controlled data supporting the use of oseltamivir in the treatment of human influenza comes from five published studies. [19-23] Three of these evaluated acute uncomplicated community influenza in ambulatory persons (2 adult trials [19, 20] and one pediatric [21]), and the other two studies evaluated experimentally induced influenza. [22, 23] No prospective, placebo controlled studies of oseltamivir treatment have been reported in hospitalized patients, those with more severe illness, or in patients with treatment initiation after 2 days from illness onset.

In the adult acute treatment trials, both studied adults who presented within 36 hours of developing fever, respiratory symptoms, and constitutional symptoms. Both trials randomized patients to placebo, oseltamivir 75 mg bid, or oseltamivir 150 mg bid. Using syndromic inclusion criteria and documentation of local influenza activity, influenza infection was documented in 58-69% of all study groups. Treatment with oseltamivir was associated with decreased duration (decreased by 29 hours at 75 mg bid and 35 hours at 150 mg bid in one study, and 31 hours and 33 hours, respectively, in the other) and severity of illness. Oseltamivir treatment resulted in decreased nose and throat swab (combined) viral titers in both studies. In only one study was this demonstrated to be statistically significant. [20] In this study, the viral shedding AUC for placebo was 130 $TCID_{50}$*h/mL, the viral shedding AUC for the oseltamivir 75 mg bid arm was 78 $TCID_{50}$*h/mL (p=0.03) and the oseltamivir 150 mg bid arm was 94 $TCID_{50}$*h/mL (p=0.003). The concern in the analysis is the difference in viral shedding AUC of 36 $TCID_{50}$*h/mL (oseltamivir 150 bid vs placebo) gave a p=0.003, while a 44% larger difference in viral shedding AUC (52 $TCID_{50}$*h/mL difference between oseltamivir 75 mg bid and placebo) gave a larger (less significant) p=0.03.

In the other adult licensing study the difference in viral shedding was not statistically significant between any of the arms, though the decrease in viral shedding was suggested (but not statistically shown) to be dose dependent. [19] Notably, the duration of upper respiratory viral shedding was reduced to a median of 0 after day 1.

The mortality was nil in all treatment groups including placebo of both studies, and the all-cause hospitalization rate very low. Of note, the tolerability of the higher oseltamivir dose was generally comparable to the currently approved one; both were associated with 10-15% frequencies of GI side effects (nausea, emesis) but no serious end-organ toxicity.

In the pediatric study of acute influenza treatment with oseltamivir, 2 mg/kg/dose bid was associated with a reduction of the duration of illness by approximately 36 hours, and decreased viral

titers in nasal washes. [21] Further analysis of the prospectively collected data from controlled studies determined that oseltamivir treatment was associated with significant reductions in lower respiratory complications of influenza, antibiotic use, and all-cause hospitalizations in the month after influenza diagnosis. [24] Subsequent retrospective studies have found similar benefits in ambulatory children and adults and in elderly nursing home residents treated early, but not later than 2 days after illness onset. [25] However retrospective studies in hospitalized patients with seasonal influenza, H5N1, and recently pandemic H1N1 infections have found evidence for reduced mortality even with treatment delayed beyond 2 days after illness onset.

In experimental models of influenza, treatment with placebo or oseltamivir 20 mg bid, 100 mg bid, 200 mg bid, or 200 mg qd was started 28 hours after inoculation with influenza A. [23] Treatment with oseltamivir (all doses) was associated with decreased symptom score, nasal viral titers, and viral shedding duration. No clear dose response was appreciated, though the number enrolled was only 16 per group. Antiviral effects were found in experimental influenza B. [22]

1.4 Risks and Benefits

1.4.1 Risks of Oseltamivir

In published data (Table 1) from the manufacturer reflecting 7642 subjects who received oseltamivir in 17 clinical protocols for the prophylaxis of influenza, the most common side effects were headache, fatigue, and nausea. [26] Upper respiratory infection was also a reported side effect but likely represented the course of influenza rather than a side effect of oseltamivir.

Table 1: Number of subjects (%) experiencing adverse events (AEs) in all oseltamivir prophylaxis studies in naturally acquired influenza (only events occurring in ≥2% of patients in placebo or oseltamivir groups are included) [26]

Adverse event	Placebo (n = 1434)	Oseltamivir 75 mg/day (n = 1480)
Nausea	62 (4.3)	118 (8.0)
Headache	251 (17.5)	298 (20.1)
Vomiting	15 (1.0)	31 (2.1)
Diarrhoea	38 (2.6)	48 (3.2)
Pain	43 (3.0)	53 (3.6)
Fatigue	107 (7.5)	117 (7.9)
Abdominal pain	23 (1.6)	30 (2.0)
Upper respiratory tract infection	115 (8.0)	120 (8.1)

Other AEs, such as rash, swelling of the face, toxic epidermal necrolysis, hepatitis, liver function test abnormalities, arrhythmias, seizures, confusion, and aggravation of diabetes have been reported during post-marketing use of oseltamivir, though causation could not be definitively established. There have been postmarketing reports (mostly from Japan) of self-injury and delirium with the use of oseltamivir in patients with influenza. The reports were primarily among pediatric patients.

There is no evidence of carcinogenesis, mutagenesis, or impaired fertility associated with oseltamivir use. A 26-week dermal carcinogenicity study in mice was negative. In an escalating

and high-dose (up to 100 × area under the curve [AUC]) fertility and embryonic development study in rats, there were no effects on fertility, or early embryonic development.

See the package insert for oseltamivir for additional details.

1.4.2 Risk of Not Treating Influenza

Most influenza in low risk ambulatory patients is not diagnosed and not treated with antivirals. It has been shown that early treatment with antiviral medications may reduce the severity and duration of symptoms and may reduce the use of outpatient services and antibiotics. However, neither the U.S. CDC nor IDSA recommend that all ambulatory patients with influenza are treated with antivirals.

The risks of not treating influenza are primarily a longer duration of illness and higher severity of symptoms.

1.4.3 Risk of Phlebotomy

The primary risks of phlebotomy include local discomfort, occasional bleeding or bruising of the skin at the site of needle puncture, and rarely hematoma, infection, or fainting. At the time of enrollment and during study visits, each subject will be asked about participation in other research studies to ensure that blood draws do not exceed the following amounts for all research protocols combined: 10.5 mL/kg or 550 mL, whichever is smaller, over any 8-week period for adults (though as mandated by restrictions within this protocol the total blood volume will be significantly less).

1.4.4 Risk of Nasal Swab

The primary risk of a nasal swab is local discomfort. Rarely, there can be local bleeding from the nasal mucosa, which is controlled with local measures such as pressure or packing with gauze.

1.4.5 Benefits

There may be no benefits to individual subjects for participation in this study, but the information may lead to generalizable knowledge about influenza. There may be earlier resolution of symptoms or fewer complications from influenza in those who are randomized to the active treatment arm, but benefit is not guaranteed.

1.4.6 Alternatives

The alternative to participation in this study is routine standard care. For uncomplicated influenza this may be no therapy or may be an antiviral (likely oseltamivir or zanamivir based largely on availability and susceptibility).

2 STUDY OBJECTIVES

2.1 Primary Objective

The overall objective of the study is to evaluate the antiviral efficacy of oseltamivir compared to placebo in the treatment of subjects with confirmed influenza infection (Primary Efficacy Population) as measured by percentage of subjects with virus detectable by PCR in NP swab at Day 3.

2.2 Secondary Objectives

1. Evaluate the antiviral efficacy of treatment with oseltamivir compared with placebo in those subjects with confirmed influenza infection (primary efficacy population) and intention to treat (ITT) population as assessed by the following parameters:

Virologic: (by PCR in NP swab)
- AUC of viral shedding
- Duration of viral shedding
- Percent cessation of viral shedding on Days 3 and 7
- Frequency of emergence of antiviral resistance

Clinical:
- Time to alleviation of influenza clinical symptoms
- Time to absence of fever
- Time to resolution of all symptoms AND fever
- Time to resumption of normal activity
- Proportion of subjects who require hospitalization
- Proportion of subjects who develop bronchitis, pneumonia, or other complications of influenza

2. Evaluate the association of viral shedding (both qualitative and quantitative) to clinical symptoms and fever.

3. Evaluate if changes in viral shedding by antiviral treatment predict changes in clinical symptoms and fever.

4. Evaluate subject self-collected nasal samples compared with study team collected samples for the determination of viral shedding.

5. Evaluate the variability in the sample collection. (pilot study only)

6. Evaluate the association of viral shedding as assessed by $TCID_{50}$ to viral shedding as assessed by qualitative PCR. (pilot study only)

3 INVESTIGATIONAL PLAN

3.1 Overview of Study Design

The purpose of this randomized blinded study is to evaluate whether oseltamivir modifies viral shedding in an ambulatory population with uncomplicated influenza. This study is also designed to explore the relationship between virologic effects and clinical effects, effects on pro-inflammatory mediators, and to start understanding if improvements to virologic shedding correlate with improvements in clinical outcomes. For this reason, the study is powered to show an anticipated difference in virologic shedding. This is a multicenter international study. Up to approximately 50 sites may participate in this protocol.

It is anticipated that about 800 subjects may be enrolled to allow 560 eligible subjects to be randomized in this study. The anticipated study duration is 7 years. The original sample size of the study was powered to show a difference in virologic shedding as detected by $TCID_{50}$ (as detailed in Section 9.2). With new virologic techniques such as PCR, it is anticipated that the objectives of this study may be achievable with smaller numbers of subjects; therefore the sample size has been reduced.

Subjects with an influenza-like illness will be screened for the study, and those found positive for influenza will be randomized in a 1:1 manner to receive a blinded study treatment consisting of either oseltamivir or placebo for 5 days. The study arms are as follows (Table 2):

Table 2: Treatment Allocation

Arm	Drug	Allocation Ratio	Capsule
1	**Oseltamivir**	**1**	**Oseltamivir**
2	**Oseltamivir placebo**	**1**	**Placebo**

Subjects will be assessed on Study Day 0 (pre-dose), and on Study Days 3, 7, and 28. All subjects will undergo a series of efficacy and safety assessments during the study. Blood samples will be collected on Days 0, 3, 7, and 28.

Table 4 in Section 6 presents the schedule of assessments. A detailed presentation of assessments to be conducted at each visit is presented in Section 6.3.

The first 50 subjects randomized were part of a pilot study to help determine the optimal virologic endpoint to be used in the efficacy analysis. These subjects had duplicate virologic samples on Day 0 to determine reproducibility. Additionally, all virologic samples from these subjects were tested by both PCR and culture ($TCID_{50}$), and the results were analyzed by several different methods as outlined in a separate virology sample analysis plan. This allowed determination of the reproducibility of the virologic assays, and measure the distribution, variance, and presence and impact of missing data (see Section 9.5 for more details on the statistical considerations of the pilot study).

From this pilot study the primary endpoint was determined to be the percentage of subjects with virus detectable by PCR in NP swab at Day 3.

The data collected in this study are essentially identical as for IRC-003. Therefore, the cumulative database will encompass the spectrum of ambulatory influenza (those at risk and those not at risk, as well as placebo, monotherapy, and combination therapy treated).

Additionally, this study will explore the utility of subject self-collected samples. If self-collected samples provide the same quantitative information as study team-collected samples, self-collected samples could be utilized in future studies for more frequent assessment of viral shedding with fewer clinic visits. For this study, a subject self-collected sample will be obtained once daily on Study Days 0, 1, 2, and 3.

3.2 Definitions for the Purpose of this Study

Enrolled

For the purpose of collecting data and samples, and reporting AEs, a subject will be considered enrolled beginning from the time the informed consent form is signed until the subject is considered "screen failure," "discontinued," or "completed."

Randomized

Subjects are considered randomized when they meet all of the following criteria:

- Confirmation that the inclusion and exclusion criteria are met
- Consent is given and signed by the subject for the study
- Randomization number is assigned

Screen Failures

Subjects are considered screen failures when they meet one of the following criteria after signing consent:

- Screening tests reveal that the subject is ineligible
- Subject withdraws consent before being randomized

Discontinued

Subjects are considered discontinued when they meet one or more of the following criteria:

- Subject withdraws consent after being randomized (see Section 4.6)
- Subject is withdrawn after enrollment by investigator (see Section 4.8) including lost to follow-up

Completed

Subjects are considered completed when they are followed through Study Day 28 and complete the final study follow-up visit (Study Day 28) or die prior to this study visit.

3.3 Study Endpoints

3.3.1 Primary Endpoint

Percentage of subjects shedding virus by PCR in NP swab at day 3.

3.3.2 Secondary Endpoints

Clinical Endpoints:

- Time to alleviation of influenza clinical symptoms
- Time to absence of fever
- Time to resumption of normal activity
- Number of premature study treatment discontinuations
- Proportion of subjects who develop bronchitis, pneumonia, or other complications of influenza
- Proportion of subjects who require hospitalization
- 28-day mortality

Virologic Endpoints: (assessed by PCR in NP swab)

- Duration of viral shedding
- Change in viral shedding as a function of time
- AUC of viral shedding
- Frequency of emergence of antiviral resistance

3.3.3 Endpoint Definitions

- Duration of viral shedding as assessed by PCR is defined as time to reduction of qPCR to below limit of detection, at the first of two successive measurements.
- Development of resistance is defined as the percentage of subjects who develop a resistant strain population by day 7, as measured by RNA sequencing of NA for recognized oseltamivir resistance mutations (e.g., H275Y or N295S in N1, R292K or E119V in N2).
- Duration of clinical symptoms is defined as the time to alleviation of all clinical symptoms to 0 (absent) or 1(mild) on two successive measurements in absence of symptom relief medications.
- Absence of fever (temperature <38.0ºC/100.4ºF) for 24 hours without antipyretic.
- Time to resumption of normal activity is defined as time to the time to the first “yes” answer to the global assessment question “Are you functioning as well as you were before you had the flu?” (Section 7.3.1.4).

4 STUDY POPULATION

4.1 Rationale for Research Subject Selection

The subject population includes those at low risk for complications and morbidity from influenza (as defined by the U.S. CDC), and have a diagnosis by rapid antigen or PCR of influenza. Exclusions are primarily to increase subject safety.

4.1.1 Justification of Exclusions of Pregnant Women and Children

Pregnant women with influenza are recommended to be treated with oseltamivir or zanamivir, therefore, randomization of a pregnant woman to possibly receive placebo could not be justified. The protocol does not provide access to any treatment that children cannot currently receive, and therefore children are excluded from participation.

4.2 Recruitment

It is anticipated that subjects will primarily be recruited through emergency rooms, medical clinics, or other health care settings (e.g., university health clinics) that would be first contact of subjects with influenza. Information about this study will be disseminated to health care providers in these settings. Additionally, direct-to-subject recruitment (using posters and/or other informational items) may occur in these settings.

Any direct-to-subject recruitment will be submitted to the reviewing Institutional Review Board (IRB)/Independent Ethics Committee (IEC) for approval. Informational items about the study that will be circulated to health care staff only are not required to be reviewed by the IRB/IEC.

4.3 Inclusion Criteria for Enrollment (Screening)

1. Signed informed consent prior to initiation of any study procedures
2. Age ≥18 and < 65 years of age
3. One or more respiratory symptoms (cough, sore throat, or nasal symptoms)
4. Onset of respiratory symptoms no more than 48 hours before enrollment (screening)
5. Willingness to have samples stored

4.4 Inclusion Criteria for Randomization

Positive test for influenza (either rapid antigen or PCR)

- Randomization may proceed in cases of discrepant results (one positive and one negative)

NOTE: Results from laboratory tests obtained for clinical indications within 24 hours before screening/enrollment may be used if available.

4.5 Exclusion Criteria (for Enrollment or Randomization)

1. Hospitalization at the time of enrollment
2. Presence of a medical condition(s) that has been associated with increased risk of complications from influenza
   - Age 65 years of age or older
   - Asthma
   - Neurological and neuro-developmental conditions (including disorders of the brain, spinal cord, peripheral nerve, and muscle such as cerebral palsy, epilepsy [seizure disorders], stroke, moderate to severe developmental delay, muscular dystrophy, or spinal cord injury)
   - Chronic lung disease (such as COPD and cystic fibrosis)
   - Heart disease (such as congenital heart disease, congestive heart failure, and coronary artery disease). Hypertension or mild arrhythmia alone would not be considered exclusionary
   - Blood disorders
   - Endocrine disorders (such as diabetes mellitus)
   - Kidney disorders
   - Liver disorders

- Metabolic disorders (such as inherited metabolic disorders and mitochondrial disorders)
- Weakened immune system due to disease or medication (such as people with HIV/AIDS, or cancer, chronic steroids or other medications causing immune suppression)
- Pregnant or 4 weeks postpartum
- BMI ≥40

3. Breastfeeding
4. Inability to take oral medication or a history of GI malabsorption which would preclude the use of oral medication.
5. Received more than one dose of any antiviral influenza medications since onset of influenza symptoms
6. Known end stage renal dysfunction or creatinine clearance less than 60 mL/min
7. Known hypersensitivity to oseltamivir, peramivir, or zanamivir
8. Received live attenuated influenza virus vaccine within 3 weeks prior to study entry
9. Use of any investigational drug within 30 days or 5 half-lives (whichever is longer) prior to study entry
10. Participation in other research protocols that require more than 100 mL of blood to be drawn in any 4-week period that overlaps with this study.

4.6 Subject Withdrawal

Subjects (or their legal surrogates if subjects become unable to make informed decisions) can terminate study participation at any time without prejudice. If a subject terminates participation before completing the study, the reason for this decision will be recorded in the electronic case report form (eCRF).

Subjects who indicate interest in withdrawing from the study should also be asked permission to be contacted at Day 28 by telephone for vital status (any AEs that occurred during the study). This is not considered full withdrawal of consent, but the modified consent for limited follow-up should be noted in the medical record.

Subjects who fully withdraw consent will not be contacted further.

Subjects who withdraw from the study will not be replaced.

4.7 Discontinuation of Study Medication

The investigator will instruct subjects to stop study medication for any of the following reasons:

- The investigator becomes aware that the subject has renal insufficiency
- Any other AE for which the investigator believes that continuation of the study medication would be detrimental to the subject
- Subject is found to be pregnant
- Subject is hospitalized within 5 days of enrollment

The reason for discontinuation of the study agent is to be recorded in the source documentation and on the eCRF. All subjects who discontinue study drug will be encouraged to comply with the

study visit schedule including clinical and virologic assessment. In any event, AEs that lead to stopping study medication will be followed until resolution or stabilization if possible.

4.8 Discontinuation of Subject by Investigator

The investigator has the right to withdraw subjects from the study. Subjects may be withdrawn from the study for any of the following reasons:

- The subject is lost to follow-up
- The subject experiences an AE, and the investigator believes that continuation in the study would be detrimental to the subject (in contrast to discontinuing the study medication and continuing follow-up as discussed in Section 4.7)
- Subject is hospitalized within 5 days of enrollment

The reason for withdrawal from the study is to be recorded on the eCRF. If a non-serious AE is unresolved at the time of discontinuation, efforts should be made to follow-up until the event resolves or stabilizes, the subject is lost to follow-up, or there is some other resolution of the event. The investigator is to make every attempt to follow all serious adverse events (SAEs) to resolution.

Subjects who miss a study visit should be contacted to reschedule. If subjects cannot be contacted immediately, periodic attempts should continue until study completion (Study Day 28 $\pm$3). Lost to follow-up is defined as unsuccessful contact after at least 3 documented telephone calls.

Any subject withdrawn from the study will not be replaced.

4.9 Discontinuation of Study

The National Institute of Allergy and Infectious Diseases (NIAID), as the study sponsor, has the right to terminate this study at one or all sites at any time.

The reviewing IRB/IEC and any reviewing national regulatory agencies (if applicable) have the right to terminate the study at the sites it is responsible for at any time. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of an AE in this or other studies indicates a potential health hazard to subjects
- Subject enrollment is unsatisfactory
- Data recording is inaccurate or incomplete
- Investigators do not adhere to the protocol or applicable regulatory guidelines in conducting the study

4.10 Participation in Other Protocols

Subjects in this protocol may participate in other research protocols, as long as the other research protocol(s) does not require more than 100 mL of blood to be given in any 4-week period of time that overlaps with this study, and does not administer any unlicensed drug, unlicensed vaccine,

or any investigational treatment for influenza within the 30 days or 5 half-lives (whichever is longer) prior to enrollment or during the duration of this study.

Note: The above may not represent the maximum amount of blood permitted to be collected by most IRBs/IECs. However, each site should ensure that these collection amounts are within the site's institutional and local limits.

4.11 Emergency Unblinding

Any pregnancy occurring on study will be immediately unblinded (regardless of known AE or known fetal toxicities).

Any hospitalization occurring during treatment (Days 0-5 of this study) due to influenza symptoms will be unblinded, as knowing treatment may influence the subjects subsequent care (including choice of antivirals).

If AEs occur and investigators are concerned about the treatment allocation, the treatment can be discontinued as discussed in Section 4.8. However, if all of the following three criteria are met, the subject's randomization may be unblinded:

1. The AE must be a SAE as also defined in Section 8.2
2. The AE must be thought to be probably or definitely related to the study drug.
3. The treating clinician states that knowledge of the treatment arm may change the therapy provided to the patient.

The procedure for unblinding will be detailed further in the Manual of Operations (MOP).

5 TREATMENT

Subjects will be randomized to one of the following two arms (Table 3):

Table 3: Treatment Allocation

Arm	Drug	Allocation Ratio	Capsule
1	**Oseltamivir**	**1**	**Oseltamivir**
2	**Oseltamivir placebo**	**1**	**Placebo**

5.1 Method of Assignment to Treatment

Subjects who meet all criteria for enrollment will be randomized using an interactive web-based system. A central randomization scheme will be prepared by the sponsor or designee.

Additionally randomization will be stratified based on duration of symptoms prior to randomization: (0–24 hours and 25–48 hours).

5.2 Study Regimens and Administration

Each randomized subject will receive a study drug kit consisting of 5 days of study capsules. Subjects take one capsule twice daily, and each dose will consist of the following:

- One of capsule A (Oseltamivir 75 mg)
  Total dose: 150 mg/day for 5 days

OR

- One of capsule B (matching placebo)
  Total dose: 2 placebo capsules/day for 5 days

The Division of Clinical Research, NIAID, NIH (the sponsor), will supply the oseltamivir and matching placebo for the study regimen.

The active capsule consists of commercial oseltamivir capsules over encapsulated with an opaque capsule. The placebo capsule consists of the identical opaque capsule filled with microcrystalline cellulose. Assembled treatment kits will be supplied to the participating sites.

Subjects should be instructed to take the study agent with food to avoid possible GI upset.

5.3 Justification of Dose

Oseltamivir: 75 mg bid is the standard licensed dose for the treatment of influenza.

5.4 Surveillance

During the conduct of this study the potential exists for the emergence of viruses resistant to oseltamivir. The protocol team will monitor the emergence of resistance to oseltamivir and current treatment recommendations. Should such a change occur where oseltamivir is no longer active for the majority of circulating strains (thus losing the ability to compare the two arms in the study), the study may be held or stopped.

5.5 Study Drug Acquisition

Study drug will be stored in a central repository. Sites will be given an initial supply of study drug after the local IRB/IEC has approved the study, all applicable national regulatory agencies have granted the study safe to proceed, the sponsor has approved shipment of study agent, and after all import licenses have been obtained, as applicable.

5.6 Study Drug Accountability

The investigator or his/her designee is required to maintain accurate drug accountability records. A binder containing instructions and the required accountability documentation will be provided to the investigator or his/her designee. When the study is completed, copies of the study drug accountability records will be maintained at the study site. Copies of the drug accountability records must be maintained with the rest of the documentation for the study. All unused study drug must be disposed of upon authorization by NIAID or its designee. All records regarding the disposition of study drug must be available for inspection by the study monitors and regulatory authorities.

5.7 Concomitant Medications

Subjects will be monitored throughout the study for use of concomitant medications. Any prescription medications, over-the-counter (OTC) preparations, herbal remedies, and/or nutritional supplements taken during the study period must be recorded on the source document and in the eCRF.

5.8 Prohibited Medications

Subjects may not receive investigational medications (other than the study drug provided) any time during the study. Their use would not be considered standard care, and therefore, withholding these compounds would still be consistent with best medical practice. This includes licensed drugs at non-approved doses, or drugs available under an Emergency Use Authorization (EUA).

5.9 Treatment Compliance

Treatment compliance will be assessed by examination of subject's remaining study product on each study visit. Subjects should be asked to bring the study kit to each study visit and returned to the study team on Day 7.

6 STUDY PROCEDURES

The following table (Table 4) outlines all of the assessments to be conducted during the study. A detailed presentation of assessments immediately follows the table. Total volumes of blood drawn are noted in the text for each study day. Details regarding the research samples (tubes, swabs, etc) will be detailed in the Manual of Procedures. The day when the subject is enrolled and randomized is denoted as Study Day 0, the first day after randomization is Study Day 1, etc.

Table 4: Schedule of Assessments

	Screen	*Baseline*					
Day +/- Window	**−1 to 0**	**0**	**1 Home**	**2 Home**	**3**	**7 ± 1**	**28 ± 3**
Evaluation/Procedure							
ELIGIBILTY							
Informed consent	X						
Demographics	X						
Medical history	X						
Site influenza testing (PCR or rapid Ag, *See Section 7.2.2)*	X						
RANDOMIZATION/ STUDY DRUG							
Randomize subject		X					
Dispense Study Treatment Kit		X					
STUDY PROCEDURES							
Review and collect diary card *(see Section 7.3.1)*		X[1]			X	X	X
Assess for complications of influenza *(see Section 7.3.2)*		X			X	X	X
Vital signs, including SaO2 *(see Section 7.3.3)*	X	X			X	X	X
Assessment of study drug compliance *(see Section 7.5)*					X		
Review of concomitant medications					X	X	X
Physical exam[2] *(see Section 7.1.2)*	X				X	X	X
Adverse events					X	X	X
SAFETY LABORATORY							
Urine βHCG (for females of childbearing potential)	X						
REFERENCE PROCEDURE							
Nasopharyngeal swab for virus isolation		X			X	X	
Subject sample self-collection *(see Section 6.3.3.2)*		X	X	X	X		
Stored Serum		X			X	X	X

Notes: *1. On Day 0, subject will be instructed on how to complete the diary card, and baseline assessment will be collected.*

2. Brief physical examinations will be conducted at screening to ensure there are not medical conditions (either chronic or acute) that would increase a subject's risk for participation in this study. Symptom-targeted physical examinations will be conducted at all other visits as needed to evaluate new complaints and possible adverse events.

6.1 Personnel and Location for Study Procedures

All study assessments should be performed by members of the investigative team that are specifically designated to perform such activities (according to site practices, local law and as designated on the appropriate study documents).

The initial screening evaluation and baseline visits should be performed within a health care facility (emergency rooms, medical clinics, inpatient units, or other health care settings). Subsequent visits may occur in a health care facility or at home according to site practices and local law. Any sites performing home visits should have:

- IRB approval for home visits
- Written study specific procedures including obtaining samples with the storage condition and timeframe of this protocol that are approved by the study team.
- Documentation of institutional permission or policies permitting home visits including liability concerns
- Adequate staff and resources to perform home visits (e.g., portable pulse oximeter for SaO2 measurements)

6.2 Hospitalization

Hospitalized subjects are excluded from this study. Subjects who initially are outpatients and are then hospitalized for influenza related symptoms should be unblinded to know the treatment obtained during this study and should receive antivirals as clinically indicated.

6.3 Detailed Description of Assessments

6.3.1 Study Day −1 to 0: Screening

Screening is defined as the period of evaluation after signing the informed consent that is used to determine if a subject is eligible for randomization. Up to 24 hours is permitted between enrollment (signing consent) and the first dose of study medication. However, efforts should be made to minimize the time between enrollment and the first dose of study to the extent possible.

6.3.1.1 Informed Consent

The site investigator or designee is to review informed consent with the subject.

6.3.1.2 Demographics

The following information should be recorded:

- Age
- Sex
- Ethnicity
- Race

6.3.1.3 Medical History

The investigator (or designee) will take a medical history at the time of consent and conduct a complete physical examination. The following information should be recorded:

- Day and time of onset of influenza symptoms
- Influenza vaccination history
- History of chronic medical conditions
- Current use of prescription and OTC medications within the last 7 days
- History of allergies
- Participation in any recent research protocols (within the last 12 months)
- Smoking history

Medical conditions noted at the time of consent (including influenza symptoms) should be recorded in the eCRF as part of the medical history (Pre-Existing Medical History eCRF). Any conditions that develop after consent should be recorded on the AE page of the eCRF if they are determined to be a recordable AE as outlined in section 8.3.1 .

6.3.1.4 Physical Exam

A brief physical exam including vital signs and SaO2, and major organ systems should be performed and will be used to ensure there are not medical conditions (either chronic or acute) that would increase a subject's risk for participation in this study.

6.3.1.5 Laboratory Testing

Subjects should have the following laboratory evaluations performed at screening:

- Urine pregnancy test (serum pregnancy test may be substituted as needed) for females of childbearing potential.
- Diagnostic test for influenza (PCR or rapid antigen). If more than one influenza diagnostic test is performed, study teams can await subsequent diagnostic test prior to deciding about randomization. Randomization may proceed in cases of discrepant results (one positive and one negative).

No other laboratory testing is required for enrollment. This is consistent with the standard clinical use of oseltamivir where laboratory testing is often not performed.

Results from laboratory tests obtained for clinical indications within 24 hours before screening/enrollment may be used if available, however, lab test results obtained nearest to randomization will be used for eligibility requirements.

6.3.2 Determination of Eligibility and Randomization

6.3.2.1 Determination of Eligibility

After the previously mentioned evaluations have been completed (i.e., medical history and laboratory tests), the investigator will review the inclusion/exclusion criteria and determine the subject's eligibility for study participation. Any clinically obtained laboratory results should be reviewed for clinically significant findings; however no additional laboratory tests are required per protocol.

6.3.2.2 Randomization

Data from eligible subjects is then entered into the randomization system, subjects are then randomized, and a specific study drug kit number is provided by the system. This study drug kit number must be provided to the pharmacist or designee.

The following information will be collected on screening failures: demographics (age, screen number, sex, birth date, ethnicity, and race) and reason for ineligibility. Subjects who are found to be ineligible will be told the reason for ineligibility and may not be re-screened for the study.

6.3.3 Study Day 0: Baseline Evaluation

Baseline evaluation must be completed prior to the first dose of study medication.

6.3.3.1 Clinical Data on Day 0

- Assessment for presence of complications of influenza (See Section 7.3.2)
- Vital signs, including SaO2 and height (See section 7.3.3)
    - If this assessment is within 6 hours from the screening assessment, the screening vitals can be used, otherwise vital sign measurements should be repeated for this assessment
- Assessment for AEs occurring after consent and prior to administration of study drug

6.3.3.2 Research Procedures on Day 0

At baseline, the following research procedures will be performed:

- Nasopharyngeal (NP) swab

All subjects will also be instructed on self-collection of nasal swabs. The subject should perform the first self-collection at this time in the research facility/ clinic with the study staff present. The self-collection procedure can be found in the Manual of Operations (MOP) and will be described in written instructions that may be provided to the subject.

See Section 7.3.4 for an overview of the planned virology assessments.

6.3.3.3 Reference Laboratory Tests on Day 0

- Up to 20 mL of blood will be taken for the stored samples (see section 7.4)

6.3.3.4 Study Day 0: Study Drug Distribution

The study drug kit will be distributed to the subject, with instruction on how to take each dose every 12 hours. Treatment should begin as soon as possible. However up to 24 hours is permitted between enrollment (signing consent) and the first dose of study medication. To ensure timely administration of the first dose of the study mediation, it may be taken in the research facility/ clinic. The time that the first dose is taken should be recorded.

6.3.3.5 Distribution of Diary Cards

A diary card booklet assessing fever, symptoms, and functional assessment, will be distributed to subjects. Collection of baseline assessments and subject recalled pre-illness condition(s) (See Section 7.3.1) will be completed by the study team and instructions will be given to the subject on the completion of the diary card.

6.3.4 Day 1

Subjects will collect nasal specimen per the self-collection procedure described in Section 6.3.3.2, and will bring the specimen to the next study visit.

6.3.5 Day 2

Subjects will collect the nasal specimen per the self-collection procedure described in Section 6.3.3.2, and will bring the specimen to the next study visit.

6.3.6 Study Day 3

6.3.6.1 Clinical Data on Day 3

- Review and collection of diary card for fever, symptoms, and functional assessment (See Section 7.3.1)
- Assessment for presence of complications of influenza (See Section 7.3.2)
- Vital signs, including SaO2 (See section 7.3.3)
- Review of study drug and assessment of compliance (see Section 7.5)

- Review of concomitant medications
- Symptom targeted physical exam (for safety assessment, see Section 7.1.2)
- Assessment for AEs

6.3.6.2 Research Procedures on Day 3

The following research procedures will be performed:

- Nasopharyngeal swab
- Subject sample self-collection as described in Section 6.3.3.2

See Section 7.3.4 for an overview of the planned virology assessments

6.3.7 Reference Laboratory Tests on Day 3

- Up to 20 mL of blood will be taken for the stored samples (see section 7.4)

6.3.8 Study Day 7 (+/- 1 Day)

6.3.8.1 Clinical Data on Day 7

- Review and collection of diary card for fever, symptoms, and functional assessment (See Section 7.3.1)
- Assessment for presence of complications of influenza (See Section 7.3.2)
- Vital signs, including SaO2 (See Section 7.3.3)
- Review of study drug and assessment of compliance and collection of study agent kit (see Section 7.5)
- Review of concomitant medications
- Symptom targeted physical exam (for safety assessment, see Section 7.1.2)
- Assessment for AEs

6.3.8.2 Research Procedures on Day 7

The following research procedures will be performed:

- Nasopharyngeal swab

See Section 7.3.4 for an overview of the planned virology assessments

6.3.8.3 Reference Laboratory Tests on Day 7

- Up to 20 mL of blood will be taken for the stored samples (see section 7.4)

6.3.9 Study Day 28 (+/- 3 Days)

6.3.9.1 Clinical Data on Day 28

- Review and collection of diary card for fever, symptoms, and functional assessment (See Section 7.3.1)
- Assessment for presence of complications of influenza (See Section 7.3.2)
- Vital signs, including SaO2 (See section 7.3.3)

- Review of concomitant medications
- Symptom targeted physical exam (for safety assessment, see Section 7.1.2)
- Assessment for AEs

6.3.9.2 Reference Laboratory Tests on Day 28

- Up to 20 mL of blood will be taken for the stored samples (see section 7.4)

7 MEASURES OF SAFETY, EFFICACY, AND COMPLIANCE

7.1 Safety Evaluations

7.1.1 Laboratory Evaluations

All laboratory evaluations (except reference endpoint assays) will be performed at the local site.

Urine (or serum) will be collected on females with childbearing potential at the time of screening for a pregnancy test.

No other clinical laboratory testing will be performed as part of this study. This would be consistent with the standard care treatment of influenza where oseltamivir is prescribed without any laboratory testing.

7.1.2 Other Safety Examinations

A brief physical examination will be conducted at screening to ensure there are no medical conditions (either chronic or acute) that would increase a subject's risk for participation in this study. Physical examinations may be completed by those authorized at a site according to local law and institutional policies. Symptom-targeted physical examinations will be conducted at all other visits as needed to evaluate new complaints and possible AEs. Due to the variability and difficulty to standardize, the physical exam will not be used as efficacy data.

7.2 Influenza A Diagnostics

7.2.1 Determination of Influenza Infection

This study will use a case definition published by the WHO for confirmed influenza virus infection. A subject will be classified as having laboratory-confirmed influenza by having one or more of the following:

- Influenza virus isolation by RT-PCR at reference lab (not site diagnostics for influenza)
- Influenza virus isolation by culture at reference lab (not site diagnostics for influenza)
- Four-fold rise in virus-specific neutralizing or HAI antibodies

7.2.2 Site Influenza Testing

Each site must have the ability to perform a diagnostic test for influenza, either rapid antigen or PCR. The methodology will be determined by the individual sites.

7.3 Efficacy Evaluations

7.3.1 Diary Card for Fever, Symptoms, and Functional Assessment

Subjects will maintain a diary card, and will be asked to make an entry twice daily (approximately 8AM and 8PM) through Day 7, and then every evening from Day 8 through 14. The subject completes the final diary card at the time of the final visit on Day 28.

On Day 0, subjects will be instructed on the diary card, and the initial (baseline) assessment will be completed by the study team and collected. Additionally, for the SF-36 physical domain, subjects will be asked to recall their status prior to enrollment.

At each subsequent study visit, the diary card for the day(s) since the last visit will be reviewed and collected. This diary will contain the information listed below (Section 7.3.1.1 – 7.3.1.4). However, any influenza symptoms listed on the diary cards will not be captured as adverse events for this study in accordance with section 8.3.1 of this protocol.

7.3.1.1 Fever

Subjects will be asked to measure temperature orally at the time of completing the diary card and at anytime for symptoms of feverishness, and will be asked to record the maximal temperature since the time of entry for the previous diary card).

7.3.1.2 Symptoms

The symptoms assessed will be those assessed in previous licensure studies with oseltamivir (denoted by the ¶ symbol), [19, 20], as well as the addition of rhinorrhea and symptoms that are frequently a component of influenza (denoted by the § symbol). Using a 4-point scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe), each symptom will be assessed for severity. Subjects will be asked to assess their symptoms twice daily for Study Days 0-7, and once daily for Study Days 8-14.

The symptoms assessed are:

- Cough ¶
- Nasal obstruction ¶
- Sore throat ¶
- Fatigue ¶
- Headache ¶
- Myalgia ¶
- Feverishness ¶
- Rhinorrhea §
- Nausea §
- Vomiting §
- Diarrhea §

Symptoms present at the time of enrollment and not worsened during treatment will not be considered AEs. Any new or worsened symptoms during treatment should be assessed for AEs in accordance with section 8.3.1.

7.3.1.3 Functional Status

The 36-item Health Survey (SF-36 v1) is a frequently used health survey used for multiple disease states. [27] This study will assess the 10-item domain of physical function (specifically, questions 3-12 of the SF-36 v1) (see Table 5), and subjects will be asked to note the response once each study day (approximately 8PM – reflecting the function during that day). These include (as originally numbered in the SF-36 v1):

Table 5: SF-36 Physical Function Domain Questions

Question	Yes, Limited a Lot	Yes, Limited a Little	No, Not limited at All
3. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	[1]	[2]	[3]
4. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	[1]	[2]	[3]
5. Lifting or carrying groceries	[1]	[2]	[3]
6. Climbing several flights of stairs	[1]	[2]	[3]
7. Climbing one flight of stairs	[1]	[2]	[3]
8. Bending, kneeling, or stooping	[1]	[2]	[3]
9. Walking more than a mile	[1]	[2]	[3]
10. Walking several blocks	[1]	[2]	[3]
11. Walking one block	[1]	[2]	[3]
12. Bathing or dressing yourself	[1]	[2]	[3]

7.3.1.4 Global Assessment

Subjects will be asked the global assessment questions:

"Are you feeling as good as you did before you had the flu?" With a yes/no response, referring to the status immediately prior to onset of influenza symptoms.

"Are you functioning as well as you were before you had the flu?" With a yes/no response, referring to the status immediately prior to onset of influenza symptoms.

7.3.2 Assessment for Presence of Complications of Influenza

Subjects will be assessed for the signs/symptoms suggestive of one of the following complications.

- Sinusitis
- Otitis Media
- Bronchitis / Bronchiolitis
- Pneumonia
- Antibiotic use for reason other than above

In the event a complication occurs, additional information to support the diagnosis of the complication should be collected (e.g., microbiologic or radiographic data). The development of any of these complications may represent an adverse event and should be assessed as such.

7.3.3 Vital Signs, Including SaO2

Vital signs assessments (BP, HR, temperature, respiration rate, and SaO2) will be taken at screening and on Study Days 0, 3, 7, and 28.

7.3.4 Virology Measures

Standardization of virologic samples is critical for successful execution of the study and meaningful virologic data. The procedures for sample collection, labeling, local lab processing, storage and shipment are described in detail in the MOP. The Virology Plan will detail the processing, testing, and reporting of the virologic studies. The virology analysis will be contained within the Statistical Analysis Plan. The differences in testing and analysis in the pilot study as compared to the main study will be discussed in these plans.

The clinical virology plan to support this clinical study will be conducted in two tiers, each with a specific objective (see Table 6). The analyses in Tier 1 will determine the virus subtype (via HA subtyping), and viral shedding as a function of time. The objective of Tier 2 is to determine whether resistance is the cause of prolonged viral shedding.

Table 6. Clinical Virology Plan

Sampling Tier	Objective	Subject Population	Analytical Method
Tier 1 – pilot study	- Determine HA subtype - Quantify viral shedding by $TCID_{50}$ - Quantify viral shedding by qPCR	All subjects in pilot study (viral shedding at all time points, subtyping at baseline)	- PCR of HA gene - $TCID_{50}$ - qPCR of M1 gene
Tier 1 – main study	- Determine HA subtype - Quantify viral shedding by either $TCID_{50}$ or qPCR (as determined in pilot study)	All subjects (viral shedding at all time points, subtyping at baseline)	- PCR of HA gene - $TCID_{50}$ or qPCR of M1 gene
Tier 2	- Quantify if resistance emerged	All subjects with virus isolated at Day 7, all time points	- DNA Sequencing (Sanger or pyro) of M2, NA and HA

7.3.4.1 Pilot Study - Tier 1 Analyses - HA Subtyping and Quantification of Virus

Nasopharyngeal and throat swabs were obtained from the first 50 subjects at the specified time points according to the Schedule of Assessments (Table 4). Analyses involved HA subtyping of all patients at baseline and viral shedding quantitation of all time points by two methods, $TCID_{50}$ and qPCR.

HA subtyping has been performed on baseline samples by means of a multiplex real-time PCR, and the virus subtype will be reported as H1, H3, or H1 2009, or B. The viral shedding will be determined for all subjects at all study visits by quantitative viral culture and will be reported as log_{10} $TCID_{50}$/mL, and by quantitative RT-PCR (qRT-PCR) and will be reported as log_{10} RNA copies/mL.

Data from $TCID_{50}$ and qPCR has been used in the pilot study analysis. This analysis assessed the variability of these sample-assay combinations in order to determine the analytical methodology for the remainder of the study. The variability at baseline between two swabs taken from the left and right sides (NPS x 2 versus OPS x 2) will be characterized using both PCR and $TCID_{50}$, as described in the Virology Plan.

7.3.4.2 Main Study - Tier 1 Analyses - HA Subtyping and Quantification of Virus

Nasopharyngeal will be obtained from all subjects at the specified time points according to the Schedule of Assessments (Section 6, Table 4). Analyses will involve HA subtyping of all patients at baseline (BL) and viral shedding quantitation of all time points by the method determined in the pilot study.

HA subtyping will be performed on baseline samples by means of a multiplex real-time PCR and the virus subtype will be reported as H1, H3, or B. The viral shedding will be determined for all subjects at all study visits by quantitative RT-PCR (qRT-PCR) and will be reported as log_{10} RNA copies/mL. Data from qPCR will be used to assess primary and secondary endpoints (e.g., efficacy of treatment with combination antivirals as compared with oseltamivir alone in those subjects with confirmed influenza infection).

7.3.4.3 Tier 2 Analyses: Resistance Typing of Known Regions via RNA Sequencing

Virus from subjects who are actively shedding virus on Day 7 will be tested for resistance. The baseline samples and samples from Day 7 will be sequenced by the Sanger method at the M2, NA and HA genes. Resistance to amantadine will be reported as amino acid changes at codons known to confer resistance in M2. Resistance to oseltamivir will be reported as amino acid changes at codons known to confer resistance in NA. Other changes in amino acid sequence from baseline that are not known to confer resistance will be reported.

7.3.4.4 Exploratory Virology

If a mutation from Tier 2 is detected which has not previously been characterized, and if it is associated with a lack of response to therapy, phenotypic assays may be performed to confirm resistance to amantadine, and oseltamivir. The shift in EC_{50} compared with the baseline sample will be measured and reported. Further phenotypic analyses may be conducted using purified viruses, in which virus is amplified in the presence of drugs to maintain phenotype. Additional testing may examine other virus genotypic changes, replication kinetics, or other parameters to explore resistance in this population.

Determination of subpopulations of viruses present during viral shedding may be performed by deep sequencing. Other studies of the viruses may occur.

7.3.4.5 Self-Collected Samples

This study will explore the utility of subject self-collected samples. If self-collected samples provide the same quantitative information as study team-collected samples, self-collected samples could be utilized in future studies for more frequent assessment of viral shedding with fewer clinic visits. For this study, a subject self-collected sample will be obtained once daily on Study Days 0, 1, and 2, and 3.

7.4 Serologic Analysis

Serologic determination of influenza infection will be used those subjects that don't have the influenza virus detected in the baseline (or any analyzed) virologic sample. The serologic determination of influenza infection will be performed either by the hemagglutinin inhibition assay (HAI) and/or microneutralization (MN), and using samples on Day 0 and Day 28. The serology assay will be performed for contemporary strains from the season the subject was enrolled.

7.5 Compliance Measures

Treatment compliance will be assessed by examination of subject's remaining study product at each study visit, and by asking subjects if they have missed doses. No pharmacokinetics or other drug exposure testing is planned for this study.

7.6 Research Assays

At visits noted in Section 6, blood will be obtained for other research laboratory tests that help characterize subject's immune response to influenza. This could include antibody response to influenza and other biomarkers (including cytokines) of severity of disease. No genetic testing is done on these samples. Any use of these specimens beyond the above stated purposes will require review of the planned research by the IRB/IEC.

8 SAFETY MONITORING AND REPORTING

8.1 Documenting, Recording, and Reporting Adverse Events

At each contact with the subject, information regarding adverse events will be elicited by appropriate questioning and examinations and documented, recorded and reported as specified in Section 8.3.1.

8.2 Definitions

Adverse Event (AE)

An adverse event is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., 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 adverse event that is caused by an investigational agent (drug or biologic).

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

Serious Adverse Event (SAE)
A Serious Adverse Event is an AE that results in one 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 they may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Unexpected Adverse Event
An AE is unexpected if it is not listed in the Investigator's Brochure or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. It is the responsibility of the Sponsor to make this determination.

Serious and Unexpected Suspected Adverse Reaction (SUSAR)
A SUSAR is a Suspected Adverse Reaction that is both Serious and Unexpected.

Unanticipated Problem (UP)
An Unanticipated Problem 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; Investigator's Brochure or other study documents; and
   b. the characteristics of the subject population being studied; and
2. possibly, probably, or definitely related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. (Per the Sponsor, an AE with a serious outcome will be considered increased risk.)

Unanticipated Problem that is not an Adverse Event (UPnonAE)
An incident, experience or outcome that is not associated with an adverse event which meets the 3 criteria of a UP. Examples include occurrences of breaches of confidentiality, accidental destruction of study records, and unaccounted-for study drug.

Protocol Violation
A Protocol Violation is any change, divergence, or departure from the study design or procedures in an IRB-approved research protocol that has a major impact on the subject's rights, safety, or well-being and/or the completeness, accuracy or reliability of the study data.

Protocol Deviation
A Protocol Deviation is any change, divergence, or departure from the study design or procedures of an IRB-approved research protocol that does not have a major impact on the subject's rights, safety or well-being, and/or the completeness, accuracy and reliability of the study data.

8.3 Investigator Assessment of Adverse Events

8.3.1 Determination of Adverse Event

Oseltamivir was first licensed in 1999, and is currently licensed in most countries (including all countries participating in this study). Cumulative safety and tolerability data for oseltamivir from over 4000 subjects in randomized controlled trials, as well as significant postmarketing surveillance for significant side effects has been published. [28] The most common side effects of oseltamivir include nausea with or without vomiting and headache. It is unlikely that this study will significantly contribute to what is known about oseltamivir safety and tolerability.

Symptoms associated with influenza include: fever, headache, eye irritation, nasal congestion or discharge, sneezing, sore throat, cough, abdominal pain, diarrhea, dyspepsia, nausea, vomiting, chills, fatigue, arthralgia, myalgia, neck pain, malaise, ear congestion or discomfort. Physical findings include the patient appearing flushed, oropharyngeal hyperemia, cervical lymphadenopathy, wheezing, and tachypnea. Recording influenza symptoms as adverse events is neither an appropriate way to capture this data nor is this study likely to significantly contribute to what is known about the signs and symptoms of influenza.

Symptoms captured on the diary cards either in the structured assessment and/or as free text should be assessed to determine if it fits with known presentations of influenza or known side effects of oseltamivir.
Therefore, this study will not capture in the source nor eCRF grade 1-3 adverse events that are known side effects of oseltamivir (as defined in the package insert) nor known signs and symptoms associated with influenza (as noted, or similar to what is noted above).

However, the following still need to be recorded in the source records and the eCRF:

- Adverse events that do not follow side effects of oseltamivir or known signs and symptoms associated with influenza (e.g. visual changes)
- Complications of influenza (e.g. pneumonia, sinusitis, or myocarditis)

- Any adverse events that are judged by the site investigator to be clinically significant (e.g. new onset seizures, acute allergic reactions)
- All Grade 4 (life-threatening) or Grade 5 (death) AEs regardless of association with influenza or oseltamivir
- Serious Adverse Event (SAE) as defined in section 8.2
- Unanticipated Problem that is not an Adverse Event as defined in section 8.2

8.3.2 Period of Assessment

AEs that should be recorded (as defined above) occurring from the time the informed consent is signed through Day 28 will be documented, recorded, and reported. Additionally, after Day 28 AE/SAE related to the pregnancy or the developing fetus will be reported. After the end of the protocol-defined SAE reporting period, sites must report to the safety office serious, unexpected, clinically suspected adverse drug reactions if the study site staff becomes aware of the event on a passive basis.

8.3.3 Assessment of Adverse Event

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

8.3.4 Severity

The Investigator will grade the severity of recorded AEs according to the "Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events" Version 1.0, December, 2004: (Clarification August 2009) which can be found at:
http://rsc.tech-res.com/Document/safetyandpharmacovigilance/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Events.pdf

8.3.5 Causality of Adverse Event to the Intervention

The Investigator will assess recorded AEs for causality. A causal relationship means that the study intervention/procedure caused (or is reasonably likely to have caused) the AE. This usually implies a relationship in time between the intervention and the AE (e.g. the AE occurred shortly after the subject received the study intervention/procedure).

The clinician who examines and evaluates the subject will determine the event's causality. Acceptance that an AE is causally related to the intervention usually requires a plausible mechanism of action (i.e., a believable sequence of events by which the intervention brought about the AE).

The best estimate of the Investigator at the time of reporting of the causal relationship between the experimental intervention and an adverse event and the degree of certainty about causality will be graded as follows:

Unrelated
Adverse event is clearly due to extraneous causes (e.g., underlying disease, environment). For an "unrelated" designation of causality, there should be a clear and plausible cause that excludes the possibility that the adverse event could be caused by the study treatments.

Unlikely (must have 2)
Adverse event:
1. does not have temporal relationship to intervention
2. could readily have been produced by the subject's clinical state
3. could have been due to environmental or other interventions
4. does not follow known pattern of response to intervention
5. does not reappear or worsen with reintroduction of intervention

Possible (must have 2)
Adverse event:
1. has a reasonable temporal relationship to intervention
2. could not readily have been produced by the subject's clinical state
3. could not readily have been due to environmental or other interventions
4. follows a known pattern of response to intervention

Probable (must have 3)
Adverse event:
1. has a reasonable temporal relationship to intervention
2. could not readily have been produced by the subject's clinical state or have been due to environmental or other interventions
3. follows a known pattern of response to intervention
4. disappears or decreases with reduction in dose or cessation of intervention

Definite (must have all 4)
Adverse event:
1. has a reasonable temporal relationship to intervention
2. could not readily have been produced by the subject's clinical state or have been due to environmental or other interventions
3. follows a known pattern of response to intervention
4. disappears or decreases with reduction in dose or cessation of intervention and recurs with re-exposure

8.4 Investigator Reporting Responsibilities to the Sponsor

8.4.1 Adverse Events

AEs that are to be recorded for this study (as noted above) will be fully and completely documented on the AE eCRF and in the subject's clinical chart.

In the event that the subject is withdrawn from the study due to an AE, it must be recorded on the eCRF as such. The subject should be followed and treated by the Investigator until the adverse event has resolved or stabilized, even if this extends past the 28 day follow up period. It is up to the clinician to determine that the AE/SAE is resolved or has reached a stable state, after which no further follow-up is necessary. There should be documentation to support that determination.

Once an AE or SAE is known, research team members at the study site should ensure that subjects receive appropriate care. All actions taken by the Investigator after observing an AE or SAE should be documented, including increased monitoring of the subject, suspension of the treatment, etc.

Assessment of safety will include clinical observations and monitoring of hematological, blood chemistry, and immunologic parameters. The investigator must document all directly observed AEs and all spontaneously reported AEs. AEs will be documented on the AE eCRF in appropriate medical terminology. The severity and the relationship to the study intervention will be determined and reported on the eCRF.

Any intermittent or as needed use of any medication (specifically, any newly prescribed medication) during the course of the study may indicate the occurrence of an AE that may need to be recorded on both the AE eCRF and the concomitant medication form.

8.4.2 Serious Adverse Events

SAEs (whether or not they are also UPs) must be reported on the SAE/UP Report Form and sent to the Sponsor Clinical Safety Office (CSO) by fax or e-mail attachment. Deaths and immediately life threatening SAEs must be reported within 1 business day after the site becomes aware of the event. All other SAEs must be reported within 3 business days of site awareness.

SPONSOR CLINICAL SAFETY OFFICE CONTACT INFORMATION:

OCRPRO Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704

Phone 301-846-5301
Fax 301-846-6224
E-mail: rchspsafety@mail.nih.gov

Non-US sites may have country specific SAE reporting requirements – refer to the MOPs for details.

8.4.3 Unanticipated Problems

Non-Serious AEs that are UPs must also be reported on the SAE/UP Report Form and sent to the CSO by fax or e-mail attachment no later than 7 calendar days of site awareness of the event. The UPs that are not AEs are not reported to the Sponsor CSO.

8.4.4 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 on a protocol-specified form.

In the event of a pregnancy, the subject's treatment should be unblinded per the unblinding section of the protocol.

Pregnancy Outcomes should be reported to the Clinical Safety Office. However, if the Investigator learns at any time after a subject has completed the Day 28, End-of- Study visit, of an untoward medical occurrence that would have qualified as an SAE, and such event is reasonably related to previous study drug exposure, the Investigator should promptly notify the Sponsor and the IRB/IEC.

8.5 Investigator Reporting Responsibilities to the Site IRB/EC

8.5.1 Safety reports

Investigators are responsible for submitting Safety Reports and UP summaries that are received from the Sponsor to their local IRB/Ethics Committee. Investigators must also comply with all local IRB/Ethics Committee reporting requirements.

8.5.2 Expedited Reporting to the Site IRB/EC

Unanticipated problems that are either AEs or non-AEs, (as defined above) and protocol violations which do not meet the definition of a UP will be reported within 7 calendar days of investigator awareness (or as required by the site IRB/EC).

8.5.3 Annual Reporting to the IRB/EC

The following items will be reported to the IRB/EC in summary at the time of Continuing Review (or more frequently as required by the site IRB/ECs):

- All unanticipated problems
- All protocol deviations which in the opinion of the investigator should be reported
- Summaries of AEs recorded for this study

8.6 Follow-up of Adverse Events and Serious Adverse Events

AEs that occur following enrollment of the subject (by signing the informed consent) are followed until the final outcome is known or until the end of the study follow-up period (Study Day 28).

SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to

follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE CRF (if the CRF is still open) and the SAE/UP Report Form.

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

8.7 Safety Monitoring

8.7.1 Study Monitoring

The trial will be conducted in compliance with this protocol, International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP) and any applicable regulatory requirement(s). This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID will visit the clinical research site to monitor all aspects of the study in accordance with the appropriate regulations. The objectives of a monitoring visit will be:

1. To verify the prompt reporting of all data points, including reporting SAEs, and also check the availability of signed informed consent.
2. To compare individual subjects records and the source documents (supporting data, laboratory specimen records and medical records to include physician progress notes, nurse' notes, subjects' hospital charts).
3. To ensure compliance with the protocol, and accuracy and completeness of records.

The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (national requirements, US FDA) 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 and electronic case report forms (eCRFs)) and pertinent hospital or clinical records readily available for inspection by the local IRB/IEC, the local and national regulatory authorities, the US FDA, the site monitors, and the NIAID staff for confirmation of the study data.

8.7.2 Investigator Safety Monitoring

Investigators participating in this clinical trial are responsible for:

- Protecting the safety and welfare of subjects
- Evaluating subject safety, including physician assessment of AEs for seriousness, severity, and causality
- Notifying the sponsor of SAEs and immediately-reportable events (per 8.3)
- Providing detailed written reports, including confirmatory tests promptly following immediate initial reports
- Informing the IRB/IEC of SAEs as required by applicable regulatory requirements

8.7.3 Protocol Team Monitoring

The protocol team will review the enrollment and study-related safety data (AEs and SAEs) from all sites periodically during the study. These reviews will occur at a minimum twice monthly

during active enrollment and follow-up. During periods of low influenza activity when no enrollment is occurring, these reviews do not need to occur.

8.7.4 Medical Monitor

A medical monitor representing the sponsor (OCRPRO) will be responsible for compliance with all safety requirements. In this capacity, the medical monitor will: 1) review all documented AEs on a regular basis (as determined by the medical monitor) throughout the study, 2) review all SAEs, 3) be available to advise the investigators on study-related medical questions or problems, and 4) evaluate cumulative subject safety data and make recommendations regarding the safe continuation of the study.

8.7.5 Data and Safety Monitoring Plan

A DSMB will review the safety of this protocol including enrollment and outcomes. The DSMB will review the study to monitor the conduct of the protocol and to evaluate the scientific validity and integrity of the data. All serious adverse events and all unanticipated problems will be reported to the DSMB at the same time as other oversight parties receive them. The PI will notify the DSMB of any cases of intentional or unintentional unblinding as soon as possible. The site PI will submit the written DSMB summary reports with recommendations to the IRB(s).

The DSMB will review and approve a detailed monitoring plan before the study commences.

The DSMB will review study-related data at the following intervals:

- Before enrollment
- Periodic safety review during the study (details below)
- At least annually while the study is open for enrollment

No interim efficacy analysis is planned.

8.7.5.1 Before Enrollment

Before any subjects are enrolled, the DSMB reviewed the protocol design, the informed consent form (ICF), the package insert, the safety monitoring plan, and the plans for data to be provided for the periodic safety reviews.

8.7.5.2 Periodic Safety Review

The DSMB members will receive safety data for periodic review at intervals requested by the DSMB. In addition, as long as subjects are enrolled into the study, the Board will review the study at least annually.

9 STATISTICAL CONSIDERATIONS

This section briefly describes the statistical analyses to be used for the study. A Statistical Analysis Plan will provide further details.

9.1 General Considerations

All statistical inferences will be based on two-sided tests with an α-level of 0.05. Categorical data will be summarized by the number and percent of subjects falling within each category. Continuous variables will be summarized by descriptive statistics, including mean, standard deviation, minimum, maximum, median, and relevant percentiles. Time-to-event endpoints will be summarized by median durations, hazard ratios, and corresponding 95% confidence intervals. Study Day 0 will be defined as the day of randomization.

The Statistical Analysis Plan will provide the procedure for accounting for missing, unused, and spurious data.

9.2 Sample Size and Power Calculations

The original sample size was estimated using the AUC of the $\log_{10}$ $TCID_{50}$ × h/mL reported in the Phase 3 oseltamivir study. [20] Because the values of the standard deviations of AUC were not given in the paper, we can only estimate the standard deviations based on the two reported p-values. An additional constraint on the standard deviations of the three groups is required because there are three unknown parameters to be estimated in two equations. A common practice is to assume that the three groups have a common standard deviation of AUC. The last column of the following table (Table 7) gives the estimated value of the common standard deviation. This calculation is based on an assumed t-test between the treatments and control and assuming that the mean AUC is similar to the medians provided by Nicholson. Since Nicholson used the Wilcoxon rank sum test, our calculation is probably conservative.

Table 7: AUC Results from Previous Oseltamivir Studies

	N	Median AUC ($\log_{10}$ $TCID_{50}$ ×h/mL)	P-value	Common SD
Placebo	**117**	**130.8**		**140**
Oseltamivir 75mg	**117**	**78.2**	**0.03**	
Oseltamivir 150mg	**117**	**94.4**	**0.003**	

Note: in this paper, a total of 350 subjects with viral shedding data were used in the analysis. We assume they are equally split among the three arms. Additionally, the 75 mg group had a greater treatment effect but a larger (less significant) p-value when compared with the 150 mg group. All calculations were based on the data as reported. If the p-values reported were reported incorrectly (inadvertently swapped), this would decrease the sample size by only 15-20%.

Our design is for a two-arm study with an oseltamivir group (O) and a placebo group (P). The effect of P versus O in the previous study is approximately 131-(78+94)/2, or about 45. For sample size calculation, we assume that the standard deviation of the AUC is 140 $\log_{10}$ $TCID_{50}$ ×h/mL. The estimated sample sizes are summarized in Table 8 for a two-sided alpha=.05 t-test assuming equal variances and treatment effects of 100%, 75%, and 50% of the estimated AUC difference in the previous study.

Table 8: Sample Size Estimates Under Different Treatment Effects

AUC difference (O vs. P)	Fraction of O vs. P in the previous study	Power	Allocation ratio (O:C)	Total cases with viral shedding data	Total cases[¶]	Total N[§]
45	1.0	0.80	1:1	306	340	378
		0.90	1:1	410	456	507
33.75	0.75	0.80	1:1	544	604	671
		0.90	1:1	726	807	897
22.5	0.5	0.80	1:1	1218	1353	1503
		0.90	1:1	1630	1811	2012

[¶] *Total flu cases is calculated by assuming that 90% of enrolled subjects have viral shedding data.*
[§] *Total enrollment is calculated by a 10% loss to follow-up rate among influenza patients.*

We see that if the O vs. P mean AUC difference is 45 $\log_{10}$ $TCID_{50}$, or 100% of the effect seen in previous studies, a total sample size of 507 is required to achieve 90% power.

The addition of 50 subjects randomized for the pilot study brought the initial sample size for this study to 560 subjects.

9.2.1 Sample Size Re-estimation

After analysis of the pilot study data, the primary endpoint was chosen as the percentage of subjects shedding virus by PCR in NP swab at Day 3.

Assumptions:

- Pooled percentage of subjects with virus detectable by PCR at Day 3 combining both arms is 50% (based on pilot study data)
- Require 90% power to detect difference between arms using two-sided Fisher's Exact Test with Type I error rate of 0.05.
- Proportion of participants with missing qPCR at Day 3 is 10% (due to losses to follow-up, sample handling and laboratory assay problems).

Magnitude of difference in proportions between arms (proportions in the two randomized arms)	Sample size (combined across both arms) without adjustment for missing values	Sample size (combined across both arms) with adjustment for 10% with missing values & not accounting for the 50 subjects in the pilot study)
25% (37.5% vs 62.5%)	174	194
20% (40% vs 60%)	280	312
15% (42.5% vs 57.5%)	490	546

Table 9:Re-estimated Sample Size Under Different Treatment Effects

This sample size has been chosen to give approximately 90% power to detect an absolute difference of 15% in the proportion of subjects with undetectable virus shedding at Day 3 in NP swabs (e.g., a reduction of 15% from 57.5% in the oseltamivir arm to 42.5% in the placebo arm). Less than a 15% absolute difference in viral shedding is unlikely to be viewed as clinically meaningful. Therefore, the revised sample size calculations confirm keeping the sample size 560 subjects, including 50 in the pilot study and 510 in the main study.

This power calculation is based on the fact that approximately 50% of subjects in the pilot study had undetectable virus in NP swabs at Day 3 (pooled over randomized arms) and included allowance for 10% of subjects to have unavailable viral shedding results at Day 3. The calculation assumed use of a t-test to compare proportions using a two-sided type I error rate of 0.05.

9.3 Subject Populations

Safety Population: The Safety Population will consist of all participants who are randomized and received at least one dose of the study drug. For interim reviews, this will be limited to data entered into the electronic database up to the cut-off date.

Intention to Treat (ITT) Population: The ITT will be the same as the Safety Population.

Primary Efficacy Population: The Primary Efficacy Population will consist of all randomized (as defined in Section 3.2) subjects with confirmed influenza (as defined in Section 7.3.4, not site diagnostics for influenza). Primary efficacy analyses will be based on this subject population.

9.4 Statistical Analysis

Details of the planned analysis are presented in the Statistical Analysis Plan.

9.5 Pilot Study (completed)

Due to the lack of reliable data concerning the AUC virologic endpoint**,** an "external" pilot study was conducted on the first 50 patients randomized to identify a primary endpoint and method of analysis, and to possibly modify the sample size. To ensure no effect on the type I error rate, data from these 50 patients will be excluded from the primary and secondary efficacy analyses but will be used in other analyses of secondary objectives and regression analyses between clinical and virologic endpoints. The study continues to enroll into the main study while the results of the pilot study are being analyzed. The pilot study will not change what data and samples are collected, but rather how the collected data and samples are analyzed.

Data in the pilot study included duplicate samples for both throat and nose locations and for both methods of measuring virus. Duplicate sampling was only done at baseline. Bland-Altman plots, regression, and correlation were used to assess the reproducibility and variability of both methods of counting virions. Additionally, post randomization viral and clinical endpoints were assessed by study arm to examine the distribution and possibly treatment effect of different clinical and virologic endpoints. This analysis was conducted by a statistician who conveyed the results blinded to treatment allocation to the Pilot Study Analysis Group who recommended a method to the protocol team for a final decision. The analyzing statistician was not part of the

group making decisions on which endpoint to use in the main study. Further details for analysis of the Pilot Study will be presented in the Statistical Analysis Plan.

10 DATA MANAGEMENT AND MONITORING

10.1 Source Documents

The primary source document for this study will be the subject's medical record. If the investigators maintain separate research records, both the medical record and the research records will be considered the source documents for the purposes of auditing the study. The investigator will retain a copy of source documents. The investigator will permit monitoring and auditing of the data, and will allow NIAID, Social & Scientific Systems, Inc. (SSS) and other NIAID contractors, IRB/IEC, and regulatory authorities access to the original source documents, regardless of media.

The investigator is responsible for ensuring that the data collected is complete, accurate, and recorded in a timely manner. Source documentation (the point of initial recording of information) should support the data collected on the eCRF. All eCRFs should be reviewed by the investigator and signed as required with written or electronic signature, as appropriate. Data for eCRFs will be collected directly from subjects, clinical assessments, and tests during study visits or will be abstracted from subjects' medical records. The subjects' medical records must record their participation in the clinical trial, including obtaining informed consent, concomitant medications (with doses and frequency), medical interventions or treatments that were administered, and adverse reactions experienced during the trial.

10.2 Data Management Plan

Study data will be collected at the study sites and maintained on eCRFs. These forms are to be completed on an ongoing basis during the study. Corrections on any study related source record must be made by striking through the incorrect entry with a single line (taking care not to obliterate or render the original entry illegible) and entering the correct information adjacent to the incorrect entry. Corrections to paper source documents must be initialed and dated by the person making the correction.

10.3 Data Capture Methods

Clinical data will be entered into an Internet Data Entry System (IDES) (US 21 CFR 11-compliant). The data system includes password protection and internal quality checks, to identify data that appear inconsistent, incomplete, or inaccurate.

10.4 Study Record Retention

The investigator is responsible for retaining all essential documents listed in the ICH Good Clinical Practice Guideline. Since this is a non-IND study, NIH policy requires that study records be retained for at least 3 years after completion of the research. These records are also to be

maintained in compliance with IRB/IEC, state, and federal medical records retention requirements, whichever is longest.

All stored records are to be kept confidential to the extent provided by national and local law. It is the investigator's responsibility to retain copies of source documents until receipt of written notification to the contrary from OCRPRO/DCR/NIAID. No study document should be destroyed without prior written agreement from OCRPRO/DCR/NIAID. Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator must provide written notification of such intent to OCRPRO/DCR/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location.

OCRPRO/DCR/NIAID must be notified in writing and written OCRPRO/DCR/NIAID permission must be received by the site prior to destruction or relocation of research records.

11 HUMAN SUBJECTS

11.1 IRB/IEC Approval

This protocol, informed consent document, diary cards, relevant supporting information, and all types of patient recruitment or advertisement information must be submitted to the IRB/IEC for review and must be approved before the study is initiated.

Any amendments must also be approved by the IRB/IEC prior to implementing changes in the study.

The investigator is responsible for keeping the IRB/IEC apprised of the progress of the study as deemed appropriate, but in any case at least once a year. The investigator must also keep the IRB/IEC informed of any significant AEs.

11.2 Compliance with Good Clinical Practices (GCP)

This study will be conducted in compliance with the conditions stipulated by NIAID and local IRB/IEC, informed consent regulations, local regulatory agencies, and supported by ICH/GCP guidelines. In addition, all local regulatory requirements will be adhered to, in particular those which afford greater protection to the safety of the trial subjects.

11.3 Informed Consent Process

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

The subjects will sign the informed consent document prior to any procedures being done specifically for the study. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The researcher will document the signing of the consent form in the subject's medical record. The rights and welfare of the subjects 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.

In the case of illiterate subjects, the consent can be read to the subjects. IRB/IEC approval for use of the oral consent process will be obtained if required by the policy at the individual site. As warranted, translated copies of the informed consent documents (into Spanish or other languages as dictated by a given sites usual demographics) may be provided. The translated ICF will be approved by the site's IRB/IEC prior to use.

11.4 Anonymity and Confidentiality

As each subject is consented and then enrolled, he or she will be allocated a unique study ID number. To ensure participant confidentiality, these numeric codes will substitute for personal identifiers on all paper documents, computer records, and blood sample vials. The information obtained during the conduct of this clinical study is confidential, and disclosure to third parties other than those noted is prohibited. The results of the research study may be published, but subjects' names or identities will not be revealed. Records will remain confidential. To maintain confidentiality, the principal investigator will keep records in locked cabinets, and the results of tests will be coded to prevent association with subjects' names. It is expected that these data will be reported in scientific journals and scientific meetings. Confidentiality of subjects will be maintained in all forms of reporting. Subjects will be informed in general terms of the results as soon as possible.

11.5 Conflict of Interest

There is no real or apparent conflict of interest.

11.6 Compensation

Each site is responsible for generating a compensation scheme as applicable (accounting for travel cost and time lost from work for outpatient visits), and that fits the local legal and regulatory requirements as well as the cultural norm. Any compensation must be reviewed by the local IRB/IEC. The site may use a site specific "Appendix A", or other method to convey this information to the IRB/IEC without changing the protocol.

12 RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS, AND DATA

12.1 Intended Use of the Samples/Specimens/Data

Samples and data collected under this protocol may be used to study the clinical features, immunology, and virology of human influenza.

12.2 Storage of Samples/Specimens/Data

As part of this protocol, samples will be stored for further research. Any future research (aside from the scope of research listed within this protocol and performed at a future date) will require approval from the IRB/IEC. Samples and data will be stored using codes assigned by the sponsor or its designee. Data will be kept in password-protected computers, which are located in locked rooms. Samples will be stored in locked facilities.

Stored samples are coded and linked to a specific patient ID (PID). However, neither the stored samples nor data in the database can be linked to a particular subject. Only the site will have the ability to link the subject ID to the actual person.

12.3 Storage of Genetic Sample

No samples are being stored for genetic testing on the subjects (genetic testing of influenza viruses may occur).

12.4 Tracking Samples/Specimens/Data

Samples will be tracked by a commercial software program.

12.5 Use of Samples/Specimens/Data at the Completion of the Protocol

Samples will be maintained for further laboratory testing for up to 5 years after completion (or termination) of the protocol. This length of time for storing samples may be extended only after permission of the IRB/IEC.

In the future, other investigators besides those listed in this protocol may wish to study these samples and/or data. In that case, IRB/IEC approval will be sought prior to any sharing of samples. Any clinical information shared about the sample with or without subject identifiers would similarly require prior IRB/IEC approval.

12.6 Reporting Loss or Destruction of Samples/Specimens/Data

Any loss or unanticipated destruction of locally maintained samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) will be reported to the corresponding IRB/IECs.

Any loss or unanticipated destruction of centrally maintained samples or data will be reported to all IRB/IECs.

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