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F. HOFFMANN-LA ROCHE LTD

CLINICAL STUDY PROTOCOL

PROTOCOL NUMBER NV20234

TAMIFLU®

(OSELTAMIVIR, RO 64-0796)

PROTOCOL APPROVAL

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Date: See last date in electronic signature manifestation below.

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Name	Reason for Signing	Date and Time (Eastern USA - New York)
[redacted]	Clinical Science Leader	14-Jun-2007 10:21:14
	Project Statistician	14-Jun-2007 11:23:12

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Confidentiality Statement

SYNOPSIS OF PROTOCOL NUMBER NV20234

TITLE	A double-blind, randomized, stratified, multi-center trial evaluating conventional and high dose oseltamivir in the treatment of immunocompromised patients with influenza
SPONSOR	F. Hoffmann-La Roche LTD CLINICAL PHASE IIIb
INDICATION	Treatment of influenza in immunocompromised patients
OBJECTIVES	Primary: To evaluate prospectively the efficacy of oseltamivir for the treatment of influenza in transplant recipients as measured by the time to resolution of influenza symptoms Secondary: To evaluate the effects of conventional and high dose oseltamivir in transplant recipients on: • The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis) • The virologic course of influenza (proportion shedding and viral loads at different time points) • Patient safety and tolerability • The development of resistant influenza virus • To characterize the population pharmacokinetics of oseltamivir (e.g. clearance, volume of distribution) in transplant recipients
TRIAL DESIGN	This is a double-blind, randomized, multi-center trial of twice daily, conventional and high dose oseltamivir for the treatment of influenza in immunocompromised patients (as represented by transplant recipients). Subjects will be stratified by age, transplant type, time since onset of flu symptoms and treatment start (≤ 24 or >24 hours) and vaccination status
NUMBER OF SUBJECTS	Approximately 250 (125 per arm)
NUMBER OF CENTERS	Approximately 140 centers in the Northern hemisphere
TARGET POPULATION	Transplant recipients (liver, kidney, liver and kidney, allogenic haematopoietic stem cell transplant), 1 year of age and older enrolled during the influenza season. The subjects will be positive for influenza by a rapid diagnostic test at baseline.
LENGTH OF STUDY	10 days of treatment, 30 days of follow up.
INVESTIGATIONAL MEDICINAL PRODUCT(S) DOSE/ ROUTE/ REGIMEN	Oseltamivir/placebo dry powder (to be reconstituted to a concentration of 12 mg/ml) and 75 mg capsules. The duration of dosing in both adults and children is 10 days. Conventional dose: Children ages 1 - 12 years: Oseltamivir syrup ≤ 15 kg 30 mg twice daily > 15 – 23 kg 45 mg twice daily > 23 – 40 kg 60 mg twice daily > 40 kg 75 mg twice daily Adults and adolescents (age ≥ 13 years): Oseltamivir capsules 75 mg twice daily Subjects randomized to the conventional dose will simultaneously

	receive matching placebo so as to blind them and the investigator from the high dose arm. **High dose**: Children ages 1 - 12 years: Oseltamivir syrup ≤ 15 kg 60 mg twice daily > 15 – 23 kg 90 mg twice daily > 23 – 40 kg 120 mg twice daily > 40 kg 150 mg twice daily Adults and adolescents (age ≥ 13 years): Oseltamivir capsules 150 mg twice daily Dose adjustments: In both treatment arms, dosing frequency will be decreased to once daily in patients with severe renal impairment (Cr Cl in adults between 10-30 ml/min and children between 10-30 ml/min/1.73M^2.).
COMPARATOR "DRUG" (or STANDARD OF CARE) DOSE/ ROUTE/ REGIMEN	Placebo (from pivotal registration trials in otherwise healthy adults)
ASSESSMENTS OF:	
- EFFICACY	Time to resolution of all influenza symptoms as recorded in the diary cards.
- SAFETY	Adverse events, physical exams, vital signs and clinical laboratory evaluations
- PHARMACOKINETICS/ PHARMACODYNAMICS	Pharmacokinetic assessments will be collected as below: Day 1 (2 'early' samples: 1 – 4 hours post dose but at least 1 hour apart) Day 6 (2 'late' samples: 4 - 12 hrs post dose, but at least 1 hour apart) Day 11 (2 'early' samples on Day 11) Pharmacodynamic assessments will be based on nose and throat swabs collected on the days specified in the schedule of assessments.

PROCEDURES (summary):

The key procedures are:
Blood draws for serum chemistry, hematology, PK and serology.
Nasal and throat swabs for viral culture and RT-PCR.

STATISTICAL ANALYSES:

Sample size calculations were performed using a two-sided t-test and are based on the comparison with a placebo control response in the pivotal registration trials in healthy adults of 112 hours for median time to alleviation of all symptoms. Assuming an expected median time to alleviation of symptoms of 78 hours for each of the two active treatment groups in this study, and a common standard deviation of 98 hours, then 111 evaluable subjects per group would give more than 90% power to detect a difference between the control and each of the active treatment groups.

The primary endpoint will be time to alleviation of all clinical influenza symptoms, as recorded in the diary cards.

For the primary analysis, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- Comparison to placebo control from pivotal registration trials

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established to match those of the current study. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

- Assessment of relative efficacy

  The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval. The methodology will be based on the use of the Hodges-Lehmann estimator in the case of censored data.

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GLOSSARY OF ABBREVIATIONS

ALT [SGPT]	alanine aminotransferase
ANC	absolute neutrophil count
ANOVA	analysis of variance
AST [SGOT]	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
b.i.d.	Bis in die (twice daily)
BP	blood pressure
CI	confidence interval
C_{max}	maximum plasma concentration
Cr Cl	Creatinine Clearance
CRF	Case Report Form[s]
DRAM	Data Reporting and Analysis Manual
ESF	eligibility screening form
hrs	Hours
HA	Hemagglutinin
HSCT	Hematopoietic stem cell transplant
ICH	International Conference on Harmonisation
ITT	Intent to treat
ITTI	Intent to treat Influenza Infected
IVRS	Interactive Voice Response System
mg	Milligram
mL	Milliliter
PD	Pharmacodynamic
PK	Pharmacokinetic

GLOSSARY OF ABBREVIATIONS

p.o.	Per os (by mouth)
PR	Pulse rate
QD	Once per day
SAE	Serious adverse event
SOT	Solid organ transplant
$TCID_{50}$	50% Tissue Culture Infectious Dose
Tmax	Time of maximum plasma concentration
t1/2	Elimination half-life
µg	Microgram

PART I: STUDY DESIGN AND CONDUCT

1. BACKGROUND AND RATIONALE

1.1 Background

Influenza is an acute respiratory infection caused by a virus of the orthomyxovirus family which occurs in three forms, influenza A, B and C. Influenza virus types A and B cause an acute febrile infection of the respiratory tract characterized by the sudden onset of fever, malaise, headaches, myalgias and cough. Influenza causes numerous deaths each year [1]. Although difficult to assess, annual influenza epidemics are thought to result in between three and five million cases of severe illness, and between 250,000 and 500,000 deaths every year around the world. [2]

The influenza viruses are segmented, negative sense, single stranded, lipid encapsulated, RNA viruses between 80 and 100 nm in size. Subtypes are defined according to glycoproteins present in the viral lipid coat. The haemagglutinin complex (HA) is the major surface protein of the virus. The neuraminidase (NA) protein is the second major surface protein in the virion and plays a role in enhancing virus penetration of the mucus layer around the target cell and in release of virus from the cell surface. The matrix proteins, M1 and M2 appear to trigger the disintegration of the M1 complex during virus fusion with the cell and may also be involved in the maturation of the HA prior to assembly of new virus particles.

1.1.1 Influenza

Influenza infection is usually a self limiting condition. However, in children, the elderly and the immunocompromised, influenza infection can be associated with substantial morbidity and mortality [1]. In patients with compromised immunity, influenza virus may cause severe lower respiratory tract involvement; unusual syndromes, including encephalitis, myocarditis, and hepatitis and death. [3] The availability of new immunosuppressive agents has enhanced the number and survival of solid organ transplant (SOT) and haematopoietic stem cell transplant (HSCT) [4] recipients making them representative of the larger immunocompromised patient population.

In transplant recipients influenza is associated with a higher rate of pulmonary complications, extrapulmonary manifestations and an increased risk of graft dysfunction and high attributable mortality. In a cohort study of influenza viral infection in SOT subjects, 30 cases of influenza viral infection were identified. Secondary bacterial pneumonia was seen in 17 % of subjects and three SOT recipients (2 liver and one kidney) had myositis, myocarditis and bronchiolitis obliterans. Biopsy of the transplanted organ was performed in 21 of 30 cases and revealed variable degrees of acute allograft rejection in 62 % of subjects. The study concluded that influenza was associated with significant morbidity in different groups of SOT recipients [5]

Among transplant recipients, subjects with HSCT are at the greatest risk for morbidity. In a prospective study from the Infectious Diseases Working Party of the European Group

for Blood and Marrow Transplantation, 1973 patients were evaluated for respiratory virus infections after stem cell transplant. The overall mortality was 23% from influenza A infection and the direct influenza A-associated mortality was 15.3% in this study. [6]

The largest study of influenza infections in transplant (allogenic, syngenic or autologous HSCT) subjects comes from the Fred Hutchinson Cancer Center, Seattle, USA. In this retrospective study, influenza virus was isolated in 62 of 4797 subjects undergoing HSCT over a 13-year period. During this period, the Center had a standardized protocol for the detection of respiratory pathogens comprising direct fluorescent antibody (DFA) staining and viral culture. Because capture of influenza infections and accurate ascertainment of all risk factors was less complete after discharge from the health care center, only infections that occurred during the first 120 days after transplantation were considered. Influenza was defined as the isolation of influenza virus by culture or as evidence of influenza antigen detected by DFA in conjunction with consistent symptomatology. Antiviral treatment with an M2 inhibitor or oseltamivir (available since 1999) was performed at the discretion of the investigator. Unlike the fixed dosing duration recommended for antivirals in healthy subjects, in this trial, treatment was continued until resolution of presenting signs and symptoms and clearance of virus from respiratory secretions. Garrett Nichols, et al, reported as many as 29 % of subjects developed pneumonia following influenza infection in this large retrospective study. Ten percent of the subjects with influenza died [7].

The increased morbidity in transplant patients is associated with increased duration of virus shedding compared to that in healthy subjects. In otherwise healthy adults, elderly subjects and children, the median duration of viral shedding in untreated subjects was 70, 96 and 118 hours respectively. [8] In contrast, in the retrospective HSCT study, influenza virus was shed in nasopharyngeal secretions (evaluated at least weekly) for a median duration of 168 hours (7 days, range 2 to 37 days) [7]. This median duration of shedding included both allogenic and autologous HSCT subjects and both treated and untreated subjects. The mean duration of shedding in treated and untreated allogenic HSCT subjects was found to be longer than in autologous HSCT subjects (11.1 versus 6.7 days).

Johny et al, evaluated the use of zanamivir in the treatment of influenza in seven allogenic bone marrow transplant subjects. As with the large retrospective HSCT study, their standard of care also was to continue zanamivir until symptoms had subsided and it was documented that viral excretion had ceased. Viral shedding was checked every 7 days. Based on this protocol, the median duration of use of zanamivir was 15 days with a range of 5 to 44 days. [9]

Currently available treatments for influenza infection are the M2 ion channel inhibitors (e.g. amantadine, rimantadine) and the neuraminidase inhibitors (e.g. oseltamivir, zanamivir). Due to the emergence of resistant virus, the Center for Disease Control (CDC) has made an interim recommendation that neither amantadine nor rimantadine be used for the treatment or prophylaxis of influenza in the United States. [10] Oseltamivir is therefore one of the main drugs available for the evaluation of the treatment of influenza in the immunocompromised population.

1.1.2 Oseltamivir

Oseltamivir (Tamiflu®, Ro 64-0796) is an ethyl ester prodrug which is rapidly absorbed from the gastrointestinal tract after oral administration and metabolized in the liver by high capacity carboxylesterases to form oseltamivir carboxylate (Ro 64-0802), a potent, stable and selective inhibitor of influenza A and B neuraminidase enzymes. The active form, oseltamivir carboxylate is excreted unchanged by the kidney via glomerular filtration and active tubular secretion by the organic anion transport system. The efficacy and safety of oseltamivir in influenza treatment and prevention has been established in an extensive series of clinical studies in man.

Oseltamivir has been approved for the treatment of influenza in Europe, the United States and most other countries around the world. In adults and adolescents, the recommended dose is 75 mg twice daily for five days. In children 1 year of age and older recommended doses are 30, 45 or 60 mg bid based on body weight. In all age groups the recommended dose is administered bid for 5 days.

The approval of oseltamivir for the treatment of influenza is based on several controlled clinical trials. In the pooled population from these clinical trials encompassing adults aged from 13-97 years, many with significant co-morbidity, 1325 subjects were treated with oseltamivir (75 mg bid) and 1056 subjects received placebo. A total of seven influenza symptoms (both respiratory and constitutional) were captured on the diary card for adults. The time to resolution of all symptoms (on the diary card) decreased by 24 hours; from 124.5 hours in the placebo arm to 100.6 hours with oseltamivir 75 mg bid ($p < 0.0001$). [8] Further, in adults and adolescents with a proven influenza illness, oseltamivir treatment reduced overall antibiotic use for any reason by 26.7 % and the incidence of influenza-related lower respiratory tract complications resulting in antibiotic therapy by 55 %. The study concluded that oseltamivir treatment of influenza reduces lower respiratory tract complications, antibiotic use and hospitalizations in healthy and 'at risk' subjects [11].

Likewise, in the influenza-infected pediatric population (1 to 12 years of age), oseltamivir treatment ($n = 217$) was compared with placebo ($n = 235$). There was a reduction in the median duration of illness (defined based on resolution of temperature, cough, coryza and return to pre-illness health and activity) of 36 hours; from 137 hours with placebo to 101 hours in the oseltamivir treatment arm ($p=<0.0001$). The Canadian Acute Respiratory Infections Scale (CARIFS), validated for use in children, was used to collect symptom data on the pediatric diary card. The CARIFS scale comprised a total of 18 symptoms which were evaluated twice daily by the parent or guardian. There was a similar reduction in the time to alleviation of all CARIFS symptoms of 36 hours; from 100 hours in the placebo group to 63 hours in the oseltamivir group ($p < 0.0001$) [12].

Thus in both adults and children, the time to resolution of all symptoms was significantly reduced in the oseltamivir treatment arm compared with placebo.

Oseltamivir was well-tolerated in clinical trials. Approximately 11,000 subjects have received oseltamivir in the development program. The most common adverse events reported by adults, the elderly, and children were nausea and vomiting. Serious adverse

events (SAEs) were reported with a low and equal frequency by subjects taking active drug and placebo. Full details are given in the Investigator Brochure. [8]

There have been no controlled studies of oseltamivir treatment or prophylaxis in immunocompromised subjects. In the study by Garrett Nichols et al, patients who did not receive antiviral therapy shed virus for longer periods (mean duration, 11.3 days) than did those who were treated with M2 inhibitors (mean duration, 9.7 days) or neuraminidase inhibitors (mean duration, 7.5 days). Therapy with oseltamivir (but not rimantadine) appeared to be associated with shorter duration of shedding after controlling for steroid dose ($p < 0.08$). [7]. The efficacy of oseltamivir in the treatment of influenza in the immunocompromised population has also been demonstrated in other studies. In another small trial of oseltamivir for the treatment of influenza infection in subjects following HSCT, only two patients (5.1 %) developed influenza-related pneumonia. [13] In this study, all 39 HSCT subjects with influenza were treated with antivirals (oseltamivir or amantadine). In as many as 43 % of subjects, the duration of illness was greater than 7 days. This is unlike data from healthy adults, suggesting the possible need to evaluate a higher dose and/or longer duration of treatment with oseltamivir for this population.

While the efficacy of antiviral agents for influenza has been demonstrated in the otherwise healthy population, it has not been tested prospectively in the immunocompromised (transplant) population. [4] There is therefore a need to confirm the efficacy of antiviral therapy for influenza in prospective controlled trials in this patient population.

1.2 Rationale for the Study

This study is designed to investigate the optimal therapy for influenza in immunocompromised transplant recipients.

The rationale for conducting this study in the immunocompromised population is based on the following:

1. Immunocompromised subjects are at the highest risk for morbidity and mortality from influenza
2. Immunocompromised subjects are at the greatest risk for contracting influenza because immunization may be ineffective in this population
3. There is no drug approved for the treatment of influenza in this population

2. OBJECTIVES

2.1 Primary Objective

The primary objective of the study is to evaluate prospectively the efficacy of oseltamivir for the treatment of influenza in transplant recipients as measured by the time to resolution of influenza symptoms.

2.2 Secondary Objectives

The secondary objectives of the study are:

To evaluate the effects of conventional and high dose oseltamivir in transplant recipients on:

- The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis)
- The virologic course of influenza (proportion shedding and viral loads at different time points)
- Patient safety and tolerability
- The development of resistant influenza virus
- To characterize the population pharmacokinetics of oseltamivir (e.g. clearance, volume of distribution) in transplant recipients

3. STUDY DESIGN

3.1 Overview of Study Design and Dosing Regimen

This is a double blind, randomized, stratified, multi-center trial evaluating the efficacy of conventional and high dose oseltamivir for the treatment of influenza in immunocompromised patients. Immunocompromised transplant recipients who develop an influenza-like illness with a positive rapid diagnostic test for influenza, will be enrolled during the influenza season. Patients will be stratified by type of transplant [SOT or HSCT], by the time between onset of symptoms and treatment start [≤ 24 hours; > 24 hours], influenza vaccination status for current flu season [Yes; No] and by age [≤12 years; >12 years]. Eligible patients will be consented and randomized to receive oseltamivir twice daily for 10 days in one of two treatment arms: conventional dose or high dose (double the conventional dose). The primary end point in the study is the time to alleviation of all symptoms.

In this study, immunocompromised subjects will be represented by the transplant population. This is justified because the availability of new immunosuppressive agents has enhanced the number and survival of SOT and HSCT recipients, making them representative of the larger immunocompromised patient population.

Heterogeneity in the transplant population could confound the assessment of safety and efficacy in this study. Consequently, the SOT population in the protocol excludes subjects with transplants other than liver, kidney or liver and kidney. However, in order to still be representative of the transplant community in particular and the immunocompromised community in general, the study specifically includes subjects at the greatest risk for morbidity and mortality from influenza - the HSCT subjects. The study also excludes subjects with comorbid conditions that might impact the metabolism and excretion of the drug. As the prodrug and active drug accumulate in the presence of hepatic and severe renal impairment respectively, subjects with hepatic or severe renal impairment are excluded from the study. Other comorbid conditions that might preclude the assessment of efficacy and safety are also excluded from the study. These are reflected in several of the inclusion and exclusion criteria.

3.1.1 Rationale for Study Design

This study incorporates several features that distinguish it from classic placebo controlled trials. At the outset, a patient population that represents the general immunocompromised population had to be defined. As a placebo control arm was considered unethical in this population, an alternative control arm had to be identified. The response to placebo in pivotal oseltamivir registration trials in the healthy adult population with influenza was chosen as a control in lieu of a placebo arm in the current trial. In order to do this, the current study design and end points were designed to be similar to that in the pivotal registration trials. The following sections provide rationale and justification for the specific aspects of the study design. They also provide information on how the results will be used to make recommendations on dose and duration of treatment.

3.1.1.1 Choice of treatment arms

The pivotal registration trials for oseltamivir in healthy adults with influenza had three treatment arms, two active (75 mg and 150 mg) and one placebo. Both treatment arms were found to be significantly better than placebo. No statistical comparisons were made between the two active treatment arms. Evaluation of the results in the two active treatment arms did not reveal any clinically meaningful difference. Based on these studies, the approved (conventional) dose for the treatment of influenza in adults is 75 mg.

A similar trial design was envisaged for this immunocompromised population. In an immunocompromised population there is a possibility of increased efficacy with a higher dose. In the trials in healthy adults, on day 4 (3 days after the start of treatment), the proportion of subjects shedding virus suggested a possible dose response relationship (36%, 28% and 23% in the placebo, 75 mg and 150 mg dose arms respectively). In healthy adults, this difference in the rate of viral shedding did not translate to any difference in clinical response in the two active treatment arms even though both active treatment arms were significantly better than the placebo arm. In the immunocompromised population too it is possible that while there might not be any difference between the two active treatment arms, one or both of these dose arms may be better than placebo. In this immunocompromised population, there is therefore an even greater need to evaluate the efficacy of a higher dose arm.

As with the trials in healthy adults, the ideal trial design for the current study would be one that compared efficacy of the conventional and high dose arms with placebo. However, as reviewed in an earlier section 1.1.1 the morbidity and mortality from influenza in the immunocompromised population is far greater than that in healthy adults. Thus in one study as many as 29 % of subjects with HSCT developed pneumonia following influenza infection. [7] In another study in SOT recipients, biopsy of the transplanted organ revealed variable degrees of acute allograft rejection in 62 % of subjects. [5] Given the extent of morbidity, a placebo control appears unethical. This is further supported by a feasibility survey conducted by the Sponsor which demonstrated that a majority of transplant sites actually used antivirals (including oseltamivir) to treat influenza in this population. It was therefore decided to compare the conventional and higher dose arms with the placebo arm from the pivotal registration trials in healthy adults with influenza. Although this approach poses limitations, the Sponsor has made

every effort to make sure that the results from such an approach are still meaningful. The following sections describe how the study design addresses these limitations.

3.1.1.2 Consistency with previous influenza treatment studies

In order to be able to compare the study findings with the response to placebo in healthy adults, this study has been intentionally designed to be consistent with previous registration trials for treatment of influenza in healthy adults. Some of the key design features consistent with previous studies include: two active treatment arms, subjects with influenza like symptoms recruited during the flu season, use of a diary card to capture symptoms, primary clinical end point based on diary card assessments, laboratory evaluation of virology and serology samples generally similar to previous trials and the primary efficacy analysis population is the Intent to treat Infected (ITTI) population.

Despite the above considerations, there are issues that can preclude the estimation of the response to placebo in an immunocompromised population comprising adults and children from that in healthy adults with influenza. To further mitigate this, the choice of endpoints and their interpretation is vital.

3.1.1.3 Interpretation of study results

Choice of primary end point and comparators

The current study includes both children and adults. This is because of the significant unmet need in both these age groups. The primary end point therefore had to be applicable to both age groups. In registration trials in adults, the primary end point was the time to resolution of all clinical symptoms (in the diary card). In registration trials in children, the primary end point was the reduction in the median duration of illness (defined based on temperature, cough, coryza and return to pre-illness health and activity). A key secondary end point in children – time to resolution of all clinical symptoms showed similar results. The primary endpoint in this study is the time to resolution (alleviation) of all symptoms.

The median of the primary end point in the active treatment groups (conventional and high dose) in this trial will be compared with the median of the time to resolution of symptoms in the placebo group from the pivotal registration trials. The high dose arm enables the detection of a possible dose response relationship in this immune compromised population. As a means to evaluate that possibility, an assessment of the relative efficacy of the two dose groups will be made in terms of median time to alleviation of symptoms. Section 8.2.5 provides details on the statistical considerations and their rationale associated with these comparisons.

Effect of study design on efficacy end points

The main limitations of using the response to placebo from previous trials to estimate the response in this trial are due to the different populations (healthy versus immunocompromised), age groups (adults versus adults and children) and the potential difference in virulence of different strains. The potential difference in virulence of the predominant influenza viruses may be a confounder whose contribution can not be estimated. However the difference in populations and age groups is likely to result in the

placebo response from pivotal registration trials underestimating the time to alleviation in the immunocompromised population. This is because the time to alleviation of symptoms is shorter in the healthy population compared to the immunocompromised population. [13] The placebo response in the pivotal trials will therefore underestimate the response in the current trial. The Sponsor is thus using a very conservative approach in comparing the active treatment arms in this study with the response to placebo in healthy subjects. If one/both of the treatment arms is statistically better than placebo, it is considered that this is a meaningful effect for this population. If both treatment arms are not different from placebo and this is seen consistently across several end points, it may be hypothesized that the risk from influenza for the immunocompromised population treated with oseltamivir is comparable to that for a healthy untreated adult with influenza.

Possible dosing recommendations based on current study

Healthy subjects who were given placebo in the previous pivotal oseltamivir treatment studies will be used as the control arm for this study. When the two active treatment arms are compared with this placebo control, several outcomes are possible. These outcomes will need to be discussed with health authorities. Some outcomes and possible interpretations of the results are presented below:

1. Conventional and high dose are both shown to be better than placebo: Both treatment arms will be compared. If no difference then the conventional dose would be the dose recommended for treatment in this population. If a difference is seen between treatment arms, then an evaluation (including benefit/risk assessment) will be made to confirm whether the higher dose should be the recommended dose.
2. One active treatment arm is superior to placebo while the other is not: In this instance the dose that is shown to be superior will be recommended unless other considerations such as safety dictate otherwise.
3. Both conventional and high dose arms are not different from placebo: If this is seen consistently across several secondary clinical end points, it may be hypothesized that oseltamivir has reduced the risk in the immunocompromised population to the more acceptable level in the untreated healthy adult population.
4. It is also possible that both conventional and high dose treatment have a more protracted clinical course than the placebo arm. In this situation, it may be difficult to make conclusions about efficacy.

Irrespective of the efficacy outcomes, the study will still provide useful information of the safety of oseltamivir in this population.

Possible recommendations for duration of treatment based on the current study

Once a dose is chosen a determination will need to be made on whether the recommended duration of treatment should be 5 or 10 days or longer. This decision will be based both on the clinical and virologic course of influenza.

The recommended duration of treatment for influenza in healthy adults is five days. In this study, the duration of dosing is ten days because viral shedding is typically longer in the immunocompromised population than in healthy adults. The general standard of care for this population has been to treat for as long as the patient continues to shed virus (even after the resolution of symptoms) [7] [9]. The duration of treatment for this population is therefore individualized based on clinical response and duration of viral shedding. It can often be more than 10 days. While it is possible that 10 or more days of treatment may be needed [7] [9], it is also possible that 5 days of treatment may still be adequate. Therefore, in this study the clinical and virologic course (proportion shedding virus at day 6 and later) will be evaluated to make recommendations on the duration of dosing. No statistical comparisons will be made.

Stratification

Stratification in this study has no bearing on any comparisons with results from previous trials. However, in order to evaluate a dose response relationship between the two active treatment arms in this trial, stratification remains essential.

Because HSCT recipients are at the greatest risk of morbidity and mortality from influenza, patients will be stratified by transplant type (SOT versus HSCT). Patients will be stratified by time between onset of symptoms and treatment start (< 24 hours or ≥ 24 hours) because previous experience in otherwise healthy adults and children has shown that patients treated early in their illness respond to treatment better than those who are treated later. Patients will also be stratified by age (≤ 12 years and > 12 years) because otherwise healthy children shed virus longer than otherwise healthy adults. It is expected that a similar difference in the duration of viral shedding would exist between immunocompromised children and adults. As vaccinated subjects may respond faster to therapy, the study is also stratified based on influenza vaccination status for the current flu season (Yes; No).

3.1.2 Rationale for Dose Selection and Adjustment

Dose Selection

The dose of oseltamivir to be used in this study is the conventional, approved dose for children and adults in the treatment of influenza. There will be a second higher dose for comparison which is two times the conventional dose. This higher dose is used based on theoretical considerations which suggest that the higher dose may be associated with improved efficacy and decreased emergence of resistance.

The anticipated pro-drug and metabolite exposures from this higher dose are not expected to exceed maximum exposures seen previously in the oseltamivir development program. The safety and tolerability of the higher dose regimen has already been demonstrated in treatment studies of immunocompetent adult subjects (n = 447). [14] In a study to

demonstrate cardiac safety, in the highest dose group treated with 450 mg b.i.d. for 5 days [n = 99], no subject had a serious adverse event, nor withdrew prematurely. In Phase I studies in adults, oseltamivir has been administered in multiple doses of up to 500 mg b.i.d. Doses of 200 mg b.i.d. and greater have been associated with increased gastrointestinal adverse effects (nausea and vomiting). [8] In adult subjects with creatinine clearances of $\leq$ 30 mL/min, doses of 100 mg b.i.d. for 6 days were well tolerated, despite steady-state oseltamivir carboxylate exposures approximately 10-fold higher than those achieved with standard dosing in renally competent individuals. [15] No other adverse effects were reported more frequently with higher doses and no serious adverse events have been reported within the volunteer studies. Co-administration of oseltamivir with food has been demonstrated to substantially reduce the frequency and severity of gastrointestinal side effects.

Thus, the rationale for the higher dose arm in this study is to explore the possibility of improved efficacy and decreased emergence of resistance without an undue increase in risk.

Drug interactions with immunosuppressive medications have also been evaluated. The pharmacokinetics of oseltamivir and oseltamivir carboxylate after administration of 75 mg oseltamivir (conventional adult dose) in subjects with a well-functioning, stable renal allograft who were being maintained on immunosuppressive therapy were studied. These were similar to those described in the literature for adults with comparable degrees of renal function. Oseltamivir was well tolerated and had no clinically relevant effect on the steady-state pharmacokinetics of cyclosporine A, tacrolimus, or mycophenolate mofetil. [8]

Duration of dosing

The duration of dosing chosen for this population (10 days) is longer than that in the healthy adult and pediatric populations (5 days). This is based on observations that the viral shedding and illness are typically longer in immunocompromised patients than it is in healthy adults. [7] [13]

Dose Adjustments

In this study, dose adjustment for subjects with an estimated Cr Cl between 10 to 30 mL/min/1.73M^2 will comprise decreasing the frequency of administration to once daily. This is based on current label recommendations for adults with renal impairment (Table 1).

Table 1 Recommended doses in healthy adults for treatment of influenza

Creatinine Clearance	Recommended dose for treatment
> 30 (ml/min)	75 mg twice daily
> 10 to ≤ 30 (ml/min)	75 mg once daily or 30 mg suspension twice daily
≤10 (ml/min)	Not recommended
dialysis patients	Not recommended

The dose adjustment recommendations are considered suitable approaches for children with renal impairment. This is based on the reasonable assumption that a similar relationship exists in both children and adults, between *changes* in creatinine clearance and resultant *changes* in exposure of oseltamivir and oseltamivir carboxylate. Doses will be adjusted for renal impairment in this protocol per the schema below. More detailed information on dosing, dose adjustments and maintenance of the blind are provided in later sections 6.1, 6.2, 6.3.

Table 2 Recommended dose modifications for NV 20234 trial subjects

Randomized Dose Arm	**Unit dose (mg)**	**Creatinine Clearance (ml/min OR ml/min/M^2)**	**Dose frequency**
Conventional	30	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	45	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	60	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	75	> 30	Twice daily
		> 10 to ≤ 30	Once daily
High	60	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	90	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	120	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	150	> 30	Twice daily
		> 10 to ≤ 30	Once daily

In general, no dose adjustment is considered necessary in subjects with mild-moderate hepatic impairment (based on pharmacokinetic findings in adults with chronic hepatic impairment) receiving usual therapeutic doses of oseltamivir. [16] However, as the

relevance of these findings to the specific patient population in this study is unclear, subjects with overt (based on physical signs and symptoms) hepatic impairment at baseline will be excluded from this study as mentioned in section 4.3.

3.1.3 End of Study

The study comprises 10 days of treatment with follow up visits approximately 5 and 30 days later as shown in the schedule of assessments. A rapid diagnostic test for influenza will be performed at the end of the 10 days of treatment. Subjects still having influenza based on the rapid diagnostic test will be treated per the local standard of care by the principal investigator.

3.2 Number of Subjects/ Assignment to Treatment Groups

A total of approximately 250 patients will be enrolled in this study (approximately 125 per arm). After screening, patients will be randomly assigned to one of the two active treatment groups.

3.3 Centers

This will be a multicenter study with approximately 140 centers in the Northern hemisphere. Centers will be activated to recruit patients during the influenza season. The centers to be included in the study are those which perform or manage SOTs, HSCTs or both.

4. STUDY POPULATION

4.1 Overview

The study population comprises immunocompromised adults (including adolescents) and children who have influenza. Additionally, the subjects must not have other medical conditions that will preclude the assessment of efficacy or safety. Influenza vaccinated and non-vaccinated subjects are eligible to participate in this study.

Under no circumstances are patients who enroll in this study permitted to be re-randomized to this study and enrolled for a second course of treatment.

4.2 Inclusion Criteria

- Age greater than or equal to 1 year
- Rapid diagnostic test positive for influenza in the 24 hours prior to first dose
- Immunocompromised subject defined as documented:
    - SOT (liver, kidney or both) recipient OR
    - Allogenic HSCT
- Receiving ongoing immunosuppression, OR, in the investigator's opinion, not immune reconstituted
- Symptoms suggestive of influenza like illness including, but not limited to fever, cough, or coryza

- Less than or equal to 48 hours between onset of influenza like illness and first dose of study drug
- Acceptable renal function defined as:
    - Most recent creatinine clearance in the 6 months prior to randomization is > 30 ml/min in adults and > 30 ml/min /1.73M^2 in children. Creatinine clearance estimated from serum creatinine (Appendix 1) measured when subject is not receiving any renal replacement therapy **OR**
    - For patients who have not had a creatinine clearance assessment in the 6 months prior to randomization, baseline creatinine clearance > 10 ml/min in adults and > 10 ml/min /1.73M^2 in children (Appendix 1) **OR**
    - For patients whose most recent creatinine clearance in the 6 months prior to randomization is < 30 ml/min in adults and < 30 ml/min /1.73M^2 in children, baseline creatinine clearance > 10 ml/min in adults and > 10 ml/min /1.73M^2 in children (Appendix 1)
- Parent/guardian willing and able to comply with study requirements and give consent. (country specific age cut off)
- Patient able to comply with study requirements and willing to give assent, as appropriate (country specific age cut off)
- For adult patients, willing and able to comprehend and give written informed consent
- Patients, in the reproductive age group, must agree to utilize an effective method of contraception throughout the study period and for one reproductive cycle following cessation of study therapy
- Females of childbearing potential must have a negative urine pregnancy test prior to start of study medication

4.3 Exclusion Criteria

- SOT within 6 months of the time of randomization
- Solid Organ Transplant other than liver, kidney or liver and kidney
- Have in the investigator's opinion experienced acute rejection in the 4 weeks prior to randomization
- HSCT patients with no evidence of engraftment (engraftment is defined as the point at which a patient can maintain a sustained absolute neutrophil count (ANC) of >500/mm^3 and sustained platelet count of ≥ 20,000/mm^3, lasting ≥ 3 consecutive days without transfusions)
- HSCT subjects not discharged from hospital after their initial hospitalization for transplantation
- Have evidence of veno-occlusive disease, acute or chronic extensive graft versus host disease at the time of randomization

- Have clinical evidence for hepatic decompensation at the time of randomization (clinical icterus, ascites, hepatic encephalopathy, coagulopathy)
- Have cirrhosis of the liver at the time of randomization
- Currently or in the six months prior to randomization using T cell depleting antibodies (example: antithymocyte globulin, antilymphocyte globulin) for management of transplant
- Have other co-morbid conditions that could affect patient survival or graft function including, but not limited to, a post-transplant lymphoproliferative disease (PTLD), autoimmune disease including inflammatory bowel disease and psoriasis, untreated thyroid disease, and significant active infection
- Patients currently receiving any form of renal replacement therapy including hemodialysis, peritoneal dialysis or hemofiltration
- Have evidence of active or uncontrolled opportunistic infections (bacterial, fungal, or viral - including cytomegalovirus [CMV] or polyoma virus [BKV]) at the time of randomization. Patients with HCV or HBV are not excluded.
- Patients with known HIV infection
- Patients who are being evaluated or treated for an active malignancy (other than the malignancy for which the SOT or HSCT may have been performed) at the time of randomization
- Patients with uncontrolled vascular, neurologic or pulmonary disease. Uncontrolled is defined as disease requiring change of therapy or hospitalization in the 4 weeks preceding randomization. Change of therapy is defined as dose increase or change of medication prior to onset of present influenza like illness.
- Patients with severe diarrhea or other gastrointestinal disorders which might interfere with their ability to absorb oral medication, including diabetic patients with previously diagnosed diabetic gastroenteropathy
- Allergy to the test medication
- Patients with hereditary fructose intolerance (for subjects who will be taking the liquid formulation)
- Influenza vaccination in the 2 weeks prior to randomization
- Antiviral treatment (example: amantadine, rimantadine, zanamivir and ribavirin) for influenza in the 2 weeks prior to randomization
- Patients taking probenecid medication
- Patients who are pregnant or breast-feeding
- Participation in a clinical trial or expanded access trial with an investigational drug in the 4 weeks prior to randomization or concomitantly with this study

4.4 Concomitant Medication and Treatment

Antiviral treatments with activity against influenza (e.g. amantadine, rimantadine, zanamivir, ribavirin, and additional oseltamivir above that specified for this study) are not allowed during the study. Concomitant use of an investigational drug during the study is also excluded. Influenza vaccination after randomization is not allowed. Live attenuated vaccines may not be advisable for this population. It is possible that concurrent use with oseltamivir may render live vaccines ineffective.

Medications required for the routine care of the patient including those required for management of the transplant (excluding lymphocyte depleting antibodies, intravenous immunoglobulins) may be continued during the study. Concomitant use of probenecid (irrespective of the indication) is not allowed during the study.

4.5 Criteria for Premature Withdrawal

The investigator should discontinue treatment if the creatinine clearance is < 10 ml/min in adults or < 10 ml/min/1.73 M^2 in children. The investigator should also discontinue treatment from all subjects with intercurrent illnesses or adverse events suggestive of hepatic decompensation. The investigator also has the right to discontinue treatment in the event of intercurrent illness, adverse events, treatment failure, protocol violations, administrative reasons or other reasons.

Subjects have the right to withdraw from the study at any time for any reason.

An excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of subjects should be avoided. Should a subject decide to withdraw, all efforts will be made to complete and report the observations prior to withdrawal as thoroughly as possible.

The investigator should contact the subject or a responsible relative either by telephone or through a personal visit to establish as completely as possible the reason for the withdrawal. A complete final evaluation at the time of the patient's withdrawal should be made with an explanation of why the subject is withdrawing from the study. If the reason for removal of a subject from the study is an Adverse Event, the principal specific event will be recorded on the CRF.

In the case that the subject decides to prematurely discontinue study treatment ["refuses treatment"], he/she should be asked if he/she can still be contacted for further information. The outcome of that discussion should be documented in both the medical records and in the CRF.

4.6 Replacement Policy [Ensuring Adequate Numbers of Evaluable Subjects]

4.6.1 For Subjects

No subject prematurely discontinued from the study for any reason will be replaced.

4.6.2 For Centers

A center may be replaced for the following administrative reasons:

- Excessively slow recruitment.
- Poor protocol adherence

5. SCHEDULE OF ASSESSMENTS AND PROCEDURES

Table 3 Schedule of Assessments

	Baseline	Treatment					Follow-up	
Study Day	**1 (pre-dose)**	**1**	**2 or 3[f]**	**6[g]**	**8[f, g]**	**11[g, h, i]**	**15[g]**	**40[g, j]**
Informed Consent/Assent	x							
Medical history	x							
Demographics	x							
Height and weight	x					x		
Pregnancy Test [a]	x					x		x
Rapid diagnostic test for influenza virus shedding	x					x		
Safety Labs [b]	x					x		
Serology for influenza antibody titer	x							x
PK sampling[c]		x		x		x		
Physical Examination	x					x		x
Vital Signs (including PR, RR, temperature, Blood pressure)	x		x	x	x	x	x	x
Nasal and throat swabs for viral shedding and viral load[d]	x		x	x	x	x	x	x
Review of diary card [e]			x	x	x	x	x	x
Drug Administration		←	—	—	—	→		
Collection of unused study medication and empty containers						x		
Previous Diseases	x							
Previous/Concomitant medications	x	←	—	—	—	—	—	→
Adverse Events/Sec Illnesses and Treatments		←	—	—	—	—	—	→
Rejection, GVHD		←	—	—	—	—	—	→

a. Urine pregnancy test for patients of child-bearing potential according to the judgment/discretion of the investigator
b. Hematology (CBC with differential) and chemistry (AST, ALT, total bilirubin, urea, Cr). Serum creatinine testing may be done at a local laboratory at any time to calculate creatinine clearance and make dose adjustments. Patient may receive his/her first dose prior to Cr Cl results being available, provided certain renal function criteria are met - see [4.2] [5.3]
c. Plasma PK samples for assessment of oseltamivir and oseltamivir carboxylate will be collected using a sparse sampling approach. [5.5.1]
d. Two nasal and one throat swab for viral culture and RT-PCR
e. Flu symptoms, temperature, date/ time of oseltamivir dose and food intake will be recorded by the patient on diary cards twice daily on days 1 – 10, and once daily thereafter.
f. A home visit may be made on day 2 or 3 (for patients who are too ill to come into the clinic) and day 8
g. Day 6, 8 visit window = + 1 day; day 11 visit window = ± 1 day. PK assessments can not be done on day 12. Day 15 and day 40 visit occur approximately 5 and 30 days after the last dose respectively. Day 15 window = ± 1 day. Day 40 window ± 2 days.
h. Only weight to be assessed. Height is not necessary at this visit. Study drug is taken on day 11, only if the first dose was taken after 4 PM on Day 1 [5.3] Subjects who are rapid diagnostic positive can be treated per standard of care. They will be required to come for their follow-up visits on day 15 and day 40.
i. Subjects who discontinue treatment prematurely will have an end of treatment (day 11) assessment at the time of treatment discontinuation or the following day. They will be required to attend their follow up visits approximately 5 and 30 days after the last dose (day 15 and day 40 assessments).
j. Subjects who discontinue during follow-up will have an end of follow-up (day 40) assessment. This visit must occur within 30 days of the last dose.

5.1 Screening Examination and Eligibility Screening Form

Trial sites will be activated during the influenza season.

All subjects must provide written informed consent (assent where applicable) before any study specific assessments or procedures are performed. Screening will take place at the baseline visit prior to first dose.

Subjects will be assessed for inclusion and exclusion criteria and those who fulfill all the inclusion and none of the exclusion criteria will be randomized into the study.

An Eligibility Screening Form [ESF] documenting the investigator's assessment of each screened subject with regard to the protocol's inclusion and exclusion criteria is to be completed by the investigator.

A screen failure log must be maintained by the investigator.

5.2 Procedures for Enrollment of Eligible Subjects

Once a subject has fulfilled the entry criteria, he/she will be randomized to one of two treatment groups. The subject randomization numbers will be generated by Roche or its designee and incorporated into double-blind labeling.

The investigator or designee will use the CRF pre-printed with the assigned subject number and enter the randomization number provided by IVRS for allocation to the treatment groups in the appropriate place on each subject's CRF.

Randomization will be stratified by transplant type (SOT or HSCT); time between onset of symptoms and treatment start (≤ 24 hrs or > 24 hrs), influenza vaccination status (Yes; No) and patient's age (≤ 12 years or > 12 years).

5.3 Clinical Assessments and Procedures

At all visits subjects will receive the routine care for their primary illness (transplant). Routine patient care (including the adjustment of doses of any of the immunosuppressive drugs) will be dictated by tests performed locally at the trial site. Dose adjustments of oseltamivir will be based on serum creatinine done at the local laboratory. All safety labs will be sent to a central laboratory. Results for these laboratory parameters will be used by the sponsor/designee to assess overall safety.

All assessments and procedures will be performed according to the Schedule of Assessments (Table 3). Additional information regarding these procedures not provided in the Schedule of Assessments is provided below.

Study Day 1

The baseline and study day 1 assessment may be performed at the same visit.

Study medication, diary cards, and thermometers will be dispensed. Patients or guardians/parents will be instructed how to complete symptom diary cards (Appendix 2) (Appendix 3), temperature recording, and treatment administration details, including time

of each oseltamivir dose and food intake. The first diary card will be completed before the first dose of study drug.

The date of the first dose of study drug is defined as study day 1. Once randomized, the first dose of study drug will be administered in the clinic. Study day 2 will begin at 12 midnight of the same calendar day. If the first dose of study drug is taken after 4 pm on day 1, the next dose of study drug will be taken in the morning of day 2. In this case, the last dose of study drug will be taken on the morning of study day 11.

If the first dose of study drug is taken prior to 4 pm on day 1, the next dose of study drug should be taken in the evening of the same day (i.e. prior to midnight on the same calendar day with a minimum of 7 hours between doses). For these patients the last dose of study drug will be taken in the evening of study day 10. More information on dosing is provided later. (6.1)

The study will utilize a central safety laboratory. However, to determine the correct dose, a local serum creatinine will be drawn at baseline in all subjects and used to calculate creatinine clearance to determine if a dose adjustment is necessary. No dose adjustments are required for subjects whose creatinine clearance is > 30 ml/min in adults (> 30 ml/min/1.73 M^2 in children). Oseltamivir dosing frequency for patients whose creatinine clearance is between 10 to 30 ml/min in adults (10 to 30 ml/min/1.73 M^2 in children) will be adjusted to once daily.

For patients who meet the following criteria in section 4.2, dosing will commence only after the baseline creatinine clearance are available and if baseline creatinine clearance > 10 ml/min in adults and > 10 ml/min/1.73M^2 in children. The dose will be adjusted to once daily if the baseline Cr Cl is between 10 to 30 ml/min in adults (10 to 30 ml/min/1.73 M^2 in children)

- most recent creatinine clearance in the 6 months prior is < 30 ml/min in adults and < 30 ml/min/1.73M^2 in children)
- patients who have not had a creatinine clearance assessment in the 6 months prior to randomization

For patients who meet the following criteria in section 4.2 dosing may commence prior to availability of baseline Cr Cl. Subjects will receive twice daily doses and dosing will be adjusted to once daily if the baseline Cr Cl is between 10 to 30 ml/min in adults (10 to 30 ml/min/1.73 M^2 in children).

- most recent creatinine clearance in the 6 months prior is > 30 ml/min in adults and > 30 ml/min /1.73M^2 in children)

Blood samples will be taken for PK analysis as mentioned in section 5.5.1.

Study Days 2 - 11

Study day 2 will begin at 12 midnight of study day 1.

It is expected the study staff will maintain close contact with patients, especially during the first few days of the study. The study staff should inquire about any adverse events, check that the patient or parent/guardian is completing the diary cards, and assess drug compliance. During the dosing period, diary card symptoms should be assessed and temperature should be recorded at the same time that the study drug is taken.

Dosage adjustment will be performed based on creatinine clearance as described in the Dose Modification section 6.1.1.

End of treatment Day 11

The end of treatment visit for all subjects is on day 11 (irrespective of whether they took one or two doses on day 1). Subjects who discontinue study medication prematurely will have all day 11 assessments completed at the time of discontinuation or the following day.

After all day 11 study assessments are completed, a rapid diagnostic test will be performed at this visit. If the rapid diagnostic test is positive, the subject may be treated per standard of care at the discretion of the investigator.

All subjects (including those who discontinue study medication prematurely and those who are positive for influenza on their rapid diagnostic test at the end of treatment visit) will be required to return for follow up approximately 5 and 30 days after the last dose (day 15 and day 40 assessments)

Study days 12 – 40

Study day 15 is an important visit as it is the first assessment of viral culture after cessation of treatment.

End of Follow-Up Day 40

All subjects must attend an end of follow-up visit on day 40. This visit is important as it is the only post-baseline visit where a serology sample for influenza antibody titers is collected.

If the patient is withdrawn after completion of treatment (after the day 11 assessment), a termination visit should be arranged. This visit should be the end of follow-up visit assessment [Day 40]. This visit must occur within 30 days of the last dose.

Patients or parents/guardians who do not return to the clinic will be contacted by telephone to ascertain their health status, or that of their child's, record information on adverse events, and to ask that they return the symptom diary card.

5.3.1 Efficacy Assessments

The primary end point in this study is the time to resolution of all influenza symptoms as recorded in the diary cards.

The clinical efficacy parameters in this study are:

1. Symptoms of influenza-like illness. These are captured in the diary card for both adults and children. (Appendix 2) (Appendix 3) Influenza symptoms are graded on a nominal scale from zero (absent/no problem) to 3 (severe or major problem). A score of zero is considered asymptomatic. The primary end point, time to alleviation of all symptoms is comprised of all the symptoms captured in the diary card.
2. Temperature. This is captured on the diary card. Temperature is used for assessment of the primary and several secondary end points.

5.3.2 Safety

Safety parameters in this study include adverse events, vital signs, and clinical laboratory evaluations.

Pre-defined symptoms of influenza captured in the adult and pediatric diary cards are not to be reported as adverse events unless they can be further qualified. Thus 'headache due to stress at work' is reported as an adverse event. However, unexplained 'headache' is considered a predefined symptom related to influenza and not an adverse event.

Adverse events such as bronchitis, pneumonia, otitis media and sinusitis are considered secondary illnesses of influenza and should be recorded as adverse events.

Other adverse events to be expected in the transplant population such as rejection and graft versus host disease (in HSCT subjects) will also be collected as adverse events.

5.4 Laboratory Assessments

The laboratory assessments include those for efficacy and safety.

Efficacy

The efficacy laboratory assessments are used for laboratory confirmation of influenza. These are:

Nasal and throat swabs. Two nasal and one throat swab will be collected as described in the Schedule of Assessments. All swabs are sent to a central laboratory for RT-PCR and viral cultures. A phenotypic assay will be performed to determine the susceptibility of the last positive viral isolate from each patient. If required, a genotypic assay to determine the contribution of both the neuraminidase and haemagglutinin genes to decreased susceptibility will be performed.

During the course of treatment, neither the investigator nor the patient will know which participants have ongoing positive viral cultures for influenza. However at the end of treatment (day 11), a rapid diagnostic test is permitted for the diagnosis of ongoing influenza.

Safety

The safety laboratory assessments in this study, including the assessment of serum chemistry and hematology will be done at the central laboratory. Serum chemistry assessments comprise AST, ALT, total bilirubin, urea and creatinine. Hematology assessments include CBC and differential count. The total volume of blood loss for laboratory assessments (other than pharmacokinetic sampling) will be approximately 20

mL for the entire duration of the study. Blood loss for pharmacokinetic sampling is explained in section 5.5.1.

Protection of patient confidentiality, section 16 will extend to any data generated from the assaying of these samples. Biological samples taken from all patients may be infectious and will be classified as "diagnostic specimens" for dispatch purposes.

The Principal Investigator may draw blood for serum creatinine to be assessed at the local laboratory at any time during the study. The Principal Investigator may use the creatinine levels to calculate creatinine clearance and adjust dose of study drug if required.

5.5 Pharmacokinetic Assessments [PK] /Pharmacodynamic [PD] Assessments

Blood samples will be collected to evaluate the pharmacokinetics of oseltamivir according to the Schedule of Assessments (Table 3) and as described below. Further details on PK/PD are to be found in sections 8.3.1 and 8.3.2.

5.5.1 Pharmacokinetic Assessments [PK]

Plasma PK samples for assessment of oseltamivir and oseltamivir carboxylate will be collected using the following sparse sampling approach:

day 1 (2 'early' samples: 1 – 4 hours post dose but at least 1 hour apart)

day 6 (2 'late' samples: 4 – 12 hours post dose but at least 1 hour apart)

day 11 (2 'early' samples on day 11)*

*Note: Subjects should attend the clinic as early as possible on the morning of Day 11. For those subjects who had their last dose on the morning of Day 11, they should have 2 samples drawn 1– 4 hours post dose but at least 1 hour apart. For those subjects who received their last dose on the evening of Day 10, they should have 2 samples drawn at least 1 hour apart, as early as possible on the morning of day 11.

For adults and adolescents, approximately 2 mL of blood will be taken at each time point, therefore the total volume blood loss for pharmacokinetic assessments will be approximately 12 mL. For pediatric subjects, not less than approximately 0.6 mL of blood will be taken at each time point, as a result the total volume blood loss for pharmacokinetic assessments will be approximately 3.6 mL.

5.5.2 Pharmacodynamic [PD] Assessments

Nasal and throat swabs will be collected from individuals on the days specified in the schedule of assessments. Specimens will be cultured at a central laboratory. The proportion of patients with viral shedding at each visit will be summarized.

6. INVESTIGATIONAL MEDICINAL PRODUCT

The Investigational Medical Product in this study is oseltamivir dry powder (to be reconstituted to a concentration of 12 mg/ml) or 75 mg capsules and matching placebo.

The Investigational Medicinal Products will be supplied, packaged individually for each subject and labeled in accordance with Roche Standard and local regulation by Roche Clinical Trial Supply, Basel, Switzerland.

6.1 Dose and Schedule of Study Drug

Oseltamivir will be given twice daily over 10 days for a total of 20 doses. The doses need to be taken at 12 hourly intervals. Under no circumstances is a subject allowed to take two doses within 7 hours of each other.

Patients will be randomized to receive either conventional or high dose of study drug.

Conventional dose:

Children ages 1 - 12 years: Oseltamivir syrup

≤ 15 kg	30 mg twice daily
> 15 – 23 kg	45 mg twice daily
> 23 – 40 kg	60 mg twice daily
> 40 kg	75 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules
75 mg twice daily

Subjects randomized to the conventional dose will simultaneously receive matching placebo so as to blind them and the investigator from the high dose arm.

High dose:

Children ages 1 - 12 years: Oseltamivir syrup

≤ 15 kg	60 mg twice daily
> 15 – 23 kg	90 mg twice daily
> 23 – 40 kg	120 mg twice daily
> 40 kg	150 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules
150 mg twice daily

6.1.1 Dose Modifications

No dose adjustments/modifications are required for subjects whose creatinine clearance is > 30 ml/min in adults (> 30 ml/min/1.73 M^2 in children).

In both treatment arms, the dosing frequency will be decreased to once daily in patients with severe renal impairment (Cr Cl in adults between 10 – 30 ml/min and children between 10 – 30 ml/min/1.73M^2). For example, the 75 mg bid frequency will be decreased to 75 mg once daily.

At any time during the study if the investigator feels that renal function is compromised, dosing may be decreased to once daily or withheld until such time creatinine clearance results are available. Dosing may then be resumed as appropriate based on the creatinine clearance. However, it is vital that dosing not be inappropriately withheld for extended

periods of time especially in the first 3 days of treatment when viral titers may be high. It is also important that dose modifications are clearly and accurately recorded for assessment of pharmacokinetic results.

For adolescents and children, the creatinine clearance will be estimated using a Schwarz equation: (Appendix 1)

For adults the method of Cockcroft-Gault will be used to estimate creatinine clearance. (Appendix 1)

6.2 Preparation and Administration of Study Drug

Oseltamivir will be provided in two forms:

1. Capsules containing 75 mg of active drug and packaging material consisting of pregelatinized starch, povidone, talc and sodium stearyl fumarate. All participants 13 years and older will receive this dosage form. *Oseltamivir capsules should be stored at 25ºC.*
2. A pediatric suspension containing 12 mg oseltamivir per ml of reconstituted solution and the following excipients: sorbitol, titanium dioxide, sodium benzoate, xanthan gum, monosodium citrate, saccharin sodium and Permaseal11900-31 Tutti Frutti (flavor). All participants 12 years and under will receive this dosage form. *Oseltamivir dry powder for suspension [pediatric syrup] should be stored at 25ºC. After reconstitution, the suspension should not be used for longer than 10 days. Store constituted suspension under refrigeration at 2º to 8ºC. Do not freeze.*

Matching placebo will be available as capsules and suspension. Subjects in the conventional dose arm will get the conventional dose and matching placebo so that they are blinded from the high dose arm.

Each subject will be dispensed a medication pack that will provide enough medication to cover 20 doses. For subjects randomized to the conventional dose arm, the medication pack will contain a bottle of oseltamivir dry powder or a blister wallet with oseltamivir capsules and matching placebo. For subjects randomized to the high dose arm, the medication pack will contain two bottles of oseltamivir dry powder or two blister wallets with oseltamivir capsules. Irrespective of the treatment group the subject is randomized to, for each dose the subject will take the same amount from both bottles or blister wallets provided in the medication pack such that the sum of the amounts from each immediate container constitutes one dose.

One dose is to be administered twice per day at approximately 12-hour intervals with a light snack or glass of milk or fruit juice. The first dose of study medication will be administered in the clinic at the time of randomization.

6.3 Blinding and Unblinding

Randomization will be administered by a central randomization center.

The Randomization List will not be available at the study center, to the study monitors, project statisticians or to the project team at Roche. Emergency codes, or another

adequate method of unblinding, will be implemented before study start, if the identity of the test medication is necessary for patient management in the case of a serious adverse event. Emergency codes should not be broken except in the case of emergency situations. Any request from the investigator for information about the treatment administered to study subjects for another purpose must be discussed with Roche/designee.

As per regulatory reporting requirement, Roche/designee will unblind the identity of the study medication for all unexpected [as per IB] serious adverse events that are considered by the investigator to be related to study drug. Details of patients who are unblinded during the study will be included in the Clinical Study Report.

All other individuals directly involved in this study will remain blinded until the final analysis of the primary parameter.

The randomization will be stratified by transplant type, duration of symptoms, influenza vaccination status and age and will be provided by the Roche randomization group for the IVRS vendor.

6.4 Assessment of Compliance

Accountability and subject compliance will be assessed by maintaining adequate "drug dispensing" and return records.

Subjects will be asked to return all used and unused drug supply containers at the end of the treatment as a measure of compliance.

A Drug Dispensing Log must be kept current and should contain the following information:

- the identification of the subject [randomization and medication numbers] to whom the study medication was dispensed
- the date[s], quantity of the study medication dispensed *to* the subject
- the date[s] and quantity of the study medication returned *by* the subject

This inventory must be available for inspection by the Monitor. All supplies, including partially used or empty containers and the dispensing logs, must be returned to the monitor at the end of the study.

6.5 Destruction of Study Drug

Local or institutional regulations may require immediate destruction of used investigational product for safety reasons. In these cases, it may be acceptable for investigational site staff to destroy dispensed investigational product before a monitoring inspection provided that source document verification is performed on the remaining inventory and reconciled against the documentation of quantity shipped, dispensed, returned and destroyed. Written authorization must be obtained from the sponsor or designee at study start up before destruction.

Written documentation of destruction must contain the following:

- Identity [batch numbers or subject numbers] of investigational product[s] destroyed
- Quantity of investigational product[s] destroyed
- Date of destruction
- Method of destruction
- Name and signature of responsible person [or company] who destroyed investigational products[s]

7. SAFETY INSTRUCTIONS AND GUIDANCE

7.1 Adverse Events (AEs) and Laboratory Abnormalities

7.1.1 Clinical AEs

Per the International Conference of Harmonization [ICH], an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign [including an abnormal laboratory finding], symptom, or disease temporally associated with the use of a medicinal [investigational] product, whether or not considered related to the medicinal [investigational] product. Pre-existing conditions which worsen during a study are to be reported as AEs. Influenza signs and symptoms reported on the diary card will be summarized as efficacy end points and need not be captured as adverse events. However, secondary illnesses due to influenza must be reported as adverse events.

7.1.1.1 Intensity

All clinical AEs encountered during the clinical study will be reported on the AE page of the CRF.

Intensity of AEs will be graded on a four -point scale [mild, moderate, severe, life-threatening] and reported in detail on the CRF.

Mild	discomfort noticed but no disruption of normal daily activity.
Moderate	discomfort sufficient to reduce or affect daily activity.
Severe	inability to work or perform normal daily activity
Life Threatening	represents an immediate threat to life

7.1.1.2 Drug - Adverse event relationship

Relationship of the AE to the treatment should always be assessed by the investigator. Description of scales can be found in Appendix 4.

7.1.1.3 Serious Adverse Events [Immediately Reportable to Roche]

A Serious Adverse Event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that at any dose fulfils at least one of the following criteria:

– is fatal; [results in death; NOTE: death is an outcome, not an event]

– is Life-Threatening [NOTE: the term "Life-Threatening" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe].

– required in-patient hospitalization or prolongation of existing hospitalization;

– results in persistent or significant disability/incapacity;

– is a congenital anomaly/birth defect;

– is medically significant or requires intervention to prevent one or other of the outcomes listed above.

The full requirements of the **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2** will be adhered to. (Appendix 5)

7.1.2 Treatment and Follow-up of AEs

AEs, especially those for which the relationship to test “drug” is not “unrelated”, should be followed up until they have returned to baseline status or stabilized. If a clear explanation is established it should be recorded on the CRF.

7.1.3 Laboratory Test Abnormalities

Laboratory test results will appear printed on laboratory reports provided to the site from the central lab.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event [SAE] should be reported as such, in addition to being recorded as an AE in the CRF.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the AE page in the CRF:

- Accompanied by clinical symptoms
- Leading to a change in study medication [e.g. dose modification, interruption or permanent discontinuation]
- Requiring a change in concomitant therapy [e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment].

7.1.4 Follow-up of Abnormal Laboratory Test Values

In the event of medically significant unexplained abnormal laboratory test values, the tests should be repeated and followed up until they have returned to the normal range

and/or an adequate explanation of the abnormality is found. If a clear explanation is established it should be recorded on the CRF.

7.2 Handling of Safety Parameters

7.2.1 Reporting of Serious Adverse Events [immediately reportable]

Any clinical AE or abnormal laboratory test value that is *serious* and which occurs during the course of the study [as defined in section 7.1.1.3 above], regardless of the treatment arm, must be reported to Roche or designee **within *one* working day** of the investigator becoming aware of the event [expedited reporting].

Related Serious Adverse Events ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed.

Unrelated Serious Adverse Events must be collected and reported during the study and up until the follow-up visit.

The definition and reporting requirements of **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2** will be adhered to. (Appendix 5)

7.2.2 Pregnancy

A female subject must be instructed to stop taking the test "drug" and immediately inform the investigator if she becomes pregnant during the study. The investigator should report all pregnancies within 24 hours to the sponsor or designee. The investigator should counsel the subject, and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy.

Pregnancy occurring in the partner of a male subject participating in the study should be reported to the investigator and the sponsor or designee after the pregnant partner's consent has been obtained. The partner should be counseled and followed as described above if acceptable, and provides informed consent.

7.3 Warnings and Precautions

Events such as convulsions and those associated with delirium have been reported during oseltamivir use in patients with influenza. In rare cases, the delirium resulted in accidental injury. The contribution of oseltamivir to those events is unknown. Such events have also been reported in patients with influenza who were not taking oseltamivir. Patients, especially children and adolescents, should be closely monitored.

Please refer to the attached Investigator's Brochure for additional warnings, precautions, and other reported adverse events.

8. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

Full details of all planned analyses will be specified in a separate Data Reporting and Analysis Manual (DRAM). The methods described below are an outline of the main planned analyses.

8.1 Primary and Secondary Study Endpoints

8.1.1 Primary Endpoints

The primary endpoint in this study is the time to alleviation of all clinical influenza symptoms (recorded in the diary card).

8.1.2 Secondary Endpoints

The following are secondary endpoints. With the exceptions of viral load and time to resolution of fever, they are defined as either the occurrence or non-occurrence of the specified events or the presence or absence of the specified symptoms,

Shedding virus by culture at day 1, 2, 6, 8, 11, 15 and 40

Shedding virus by RT-PCR at day 1, 2, 6, 8, 11, 15, and 40

Viral load by culture ($log_{10}TCID_{50}$/mL) at Day 1, 2, 6, 8, 11, 15, and 40

Viral load by quantitative RT-PCR at day 1, 2, 6, 8, 11, 15 and 40

The time (hours) from first dose of study medication until resolution of fever

Fever

Cough

Coryza

Development of secondary illnesses (otitis media, bronchitis, pneumonia, or sinusitis) at any time during the study.

Development of secondary illnesses (otitis media, bronchitis, pneumonia, or sinusitis) at any time during the study that are treated with antibiotics

Initiation of treatment with antibiotics after randomization

Hospitalization, and for those who are hospitalized, the duration of hospitalization

Development of rejection or GVHD

Baseline, post-baseline and change from baseline in antibody titers

8.1.3 Safety

Safety of the treatment will be evaluated by AEs, laboratory tests, and vital signs.

All subjects who received at least one dose of treatment and had a safety assessment performed post randomization will be included in the safety evaluation.

In addition to routine safety assessments, the proportion of subjects experiencing a rejection and/or graft versus host disease will be summarized by treatment group.

8.2 Statistical and Analytical Methods

8.2.1 Statistical Model

8.2.1.1 Primary Variables

A non-parametric model will be assumed with estimation of medians based on Kaplan-Meier methods. Subjects without alleviation of symptoms will have their time censored at the last available observation that a complete assessment was made.

For the purpose of comparing treatment groups, it will be assumed that their respective distributions for the primary endpoint differ only by a shift in location.

8.2.1.2 Secondary Variables

For the secondary endpoints defined dichotomously in terms of events or symptoms, it will be assumed that the number of patients experiencing the event or exhibiting the symptom will have a binomial distribution.

For the continuous endpoint of time to resolution of fever, the same assumptions as for the primary endpoint will be made.

For the continuous endpoints of viral load at Day 1, 2, 6, 8, 11, 15, and 40 no model will be assumed.

8.2.2 Sample Size

Sample size calculations were performed using a two-sided t-test and are based on the comparison with a placebo control response in the pivotal registration trials in healthy adults of 112 hours for median time to alleviation of all symptoms. Assuming an expected median time to alleviation of symptoms of 78 hours for each of the two active treatment groups in this study, and a common standard deviation of 98 hours, then 111 evaluable subjects per group would give more than 90% power to detect a difference between the control and each of the active treatment groups. It is assumed that as many as 90 % of subjects enrolled into the study will have influenza, and therefore 124 subjects per arm would be required in order to obtain 111 influenza positive subjects. Note that this is an approximate calculation, as the final analysis will be based on the difference in medians and the corresponding 95% confidence interval from the Kaplan-Meier curves. This is however considered as a sufficient indication for the size of the trial and the power that is expected.

8.2.3 Hypothesis Testing

Formal hypothesis testing will be not performed, instead inferences will be based on comparison of confidence intervals.

8.2.4 Analysis Populations

Three main patient populations will be used for the analysis of data from this study; the Safety Population, the Intent-to treat Population and the Intent-to-Treat Infected Population . Detailed definitions of these populations will be given in the DRAM

8.2.4.1 Intent to treat population:

All patients randomized will be included in the intent to treat population [Patients will be assigned to treatment groups as randomized for analysis purposes]

8.2.4.2 Intent to treat infected population:

All patients randomized and with laboratory confirmation of influenza infection (positive viral cultures or 4 fold or greater rise in antibody titers) will be included in the intent to treat influenza infected population [Patients will be assigned to treatment groups as randomized for analysis purposes]

The ITTI Population will be the primary population for the summary and analysis of the primary and secondary efficacy variables.

8.2.4.3 Subpopulations

In order to evaluate the potential for relapse, the following two subpopulations of the ITTI population will be evaluated:

- patients not shedding virus as assessed by culture on Day 11
- patients not shedding virus as assessed by RT-PCR on Day 11

Viral shedding and RT-PCR at days 15 and 40 will be evaluated in these subpopulations.

Based on the proportion of subjects hospitalized, an additional subpopulation may be defined to evaluate the length of hospitalization for hospitalized subjects.

Likewise, based on the proportion of children enrolled additional subpopulations of children and adults will be created to evaluate the course of influenza in children and adults.

8.2.5 Efficacy Analysis

For the primary analysis, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- Comparison to placebo control from pivotal registration trials

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established to match (in terms of efficacy evaluations, duration of observation etc.) those of the current study. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

  The median time to resolution of all influenza symptoms will be determined for each

treatment group, along with its 95% confidence interval. These confidence intervals will be compared individually to the placebo control confidence interval described above as a potential means of establishing treatment effects.

- Assessment of relative efficacy.

  The two doses groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval. The methodology will be based on the use of the Hodges-Lehmann estimator in the case of censored data.

This confidence interval will provide lower and upper limits for the treatment difference and can be used as the basis for potential inferences. For example, if it does not contain the value zero a difference will have been established. However, given the study sample size this outcome is only likely if a clinically significant difference (e.g. 30%) exists. Although this is not anticipated from the experience in pivotal registration trials in normal healthy patients, it cannot be ruled out as a possibility in immunocompromised transplant recipients.

For the dichotomous secondary endpoints, the proportion experiencing the event or symptom, and its associated 95% confidence interval will be derived for each treatment group. Where possible, the corresponding confidence intervals from the placebo control population will be presented for comparison to those derived for the active treatment groups in this trial.

The continuous secondary endpoints time to resolution of fever will be analyzed as for the primary endpoint.

For the continuous endpoints of viral load ($log_{10}TCID_{50}/mL$) at Day 1, 6, 8, 11, 15, and 40, summary statistics (including mean, median, minimum and maximum) will be derived for each treatment group.

8.2.5.1 Exclusion of Data from Analysis

No data will be excluded from the primary and secondary efficacy analyses.

8.2.5.2 Interim Analysis

No interim analyses are planned.

8.2.6 Safety Data Analysis

The safety analysis population will include all subjects who receive at least one dose and had a safety assessment performed post randomization. All safety parameters will be summarized and presented in tables based on this safety population.

8.2.7 Other Analyses

The number and percentage of patients with influenza infection (defined as a positive culture from a nasal and/or throat swab or 4 fold or greater rise in antibody titers) will be summarized by treatment group for the Intent-to-treat Population.

Further exploratory analysis, (including assessments of the rapid diagnostic test, sub group analysis) will be detailed in the DRAM.

The last positive viral isolate from each patient will be tested for reduced sensitivity to oseltamivir. Clonal resistance assays will also be used to evaluate the rate of resistance to oseltamivir. These data will be summarized in a report separate from the final study report.

8.3 Pharmacokinetic and Pharmacodynamic Analysis

8.3.1 Pharmacokinetic Analysis

Nonlinear mixed effects modeling (with software NONMEM) will be used to analyze all plasma concentration-time data of oseltamivir and oseltamivir carboxylate following oseltamivir administration. Population pharmacokinetic parameters (clearance, distribution volume) will be estimated. The influence of covariates (such as age, gender, body weight and calculated creatinine) on these parameters will be investigated. Based on the population PK model individual pharmacokinetic parameters such as AUC, C_{max}, C_{min} and t_{max} may be calculated using Bayesian posthocs. If deemed appropriate and necessary, data may be pooled with data from previous studies investigating oseltamivir and oseltamivir carboxylate in order to improve model stability.

8.3.2 Pharmacodynamic Analysis

If feasible, the relationship between PK exposure of oseltamivir carboxylate and viral shedding response data will be characterized using nonlinear mixed effects modeling (using software NONMEM). Relevant population PD parameters will be derived and the influence of covariates will be investigated. If deemed appropriate and necessary, data may be pooled with data from previous studies investigating oseltamivir and oseltamivir carboxylate in order to improve the model stability.

9. DATA QUALITY ASSURANCE

The overall procedures for quality assurance of clinical study data are described in the Roche Standard Operational Procedures.

Accurate and reliable data collection will be assured by verification and cross–check of the CRFs against the investigator's records by the study monitor [source document verification], and the maintenance of a drug–dispensing log by the investigator.

The data collected will be entered into the study database from the working copy of the CRF faxed from the site.

A comprehensive validation check program will verify the data and discrepancy reports will be generated accordingly for resolution by the investigator. As patients complete the study [or prematurely withdraw] and their signed CRFs become available, a second data entry will be performed from the original, signed CRF. A comparison check will be run to identify and resolve any discrepancies between the first and second data entry.

Throughout the study the Study Management Team [SMT] will review data according to the SMT Data Review Plan as described in the Data Quality Plan.

9.1 Assignment of Preferred Terms and Original Terminology

For classification purposes, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using the current version of MedDRA (Medical Dictionary for Regulatory Activities terminology) for adverse events and diseases and the INN (International Non-Proprietary name) drug terms and procedures dictionary for treatments and surgical and medical procedures.

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PART II: ETHICS AND GENERAL STUDY ADMINISTRATION

11. ETHICAL ASPECTS

11.1 Local Regulations/Declaration of Helsinki

The investigator will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study must fully adhere to the principles outlined in "Guideline for Good Clinical Practice" ICH Tripartite Guideline [January 1997] or with local law if it affords greater protection to the subject. For studies conducted in the EU/EEA countries, the investigator will ensure compliance with the EU Clinical Trial Directive [2001/20/EC]. For studies conducted in the USA or under US IND, the investigator will additionally ensure that the basic principles of "Good Clinical Practice" as outlined in the current version of 21 CFR, subchapter D, part 312, "Responsibilities of Sponsors and Investigators", part 50, "Protection of Human Subjects", and part 56, "Institutional Review Boards", are adhered to.

In other countries where "Guideline for Good Clinical Practice" exist Roche and the investigators will strictly ensure adherence to the stated provisions.

11.2 Informed Consent

It is the responsibility of the investigator, or a person designated by the investigator [if acceptable by local regulations], to obtain written informed consent from each subject participating in this study, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. For subjects not qualified or incapable of giving legal consent, written consent must be obtained from the legally acceptable representative. In the case where both the subject and his/her legally acceptable representative are unable to read, an impartial witness should be present during the entire informed consent discussion. After the subject and representative have orally consented to participation in the trial, the witness' signature on the form will attest that the information in the consent form was accurately explained and understood. The investigator or designee must also explain that the subjects are completely free to refuse to enter the study or to withdraw from it at any time, for any reason. The Case Report Forms [CRFs] for this study contain a section for documenting informed subject consent, and this must be completed appropriately. If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All subjects [including those already being treated] should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

If children are old enough to understand the risks and benefits of the study, they should also be informed and should also provide their written consent.

11.3 Independent Ethics Committees/Institutional Review Board

Independent Ethics Committees [non-US]: This protocol and any accompanying material provided to the subject [such as subject information sheets or descriptions of the study used to obtain informed consent] as well as any advertising or compensation given to the patient, will be submitted by the investigator to an Independent Ethics Committee. Approval from the committee must be obtained before starting the study, and should be documented in a letter to the investigator specifying the date on which the committee met and granted the approval.

Any modifications made to the protocol after receipt of the Independent Ethics Committee approval must also be submitted by the investigator to the Committee in accordance with local procedures and regulatory requirements.

When no local review board exists, the investigator is expected to submit the protocol to a regional committee. If no regional committee exists, Roche will assist the investigator in submitting the protocol to the European Ethics Review Committee.

Institutional Review Board [US]: It is the understanding of the sponsor that this protocol [and any modifications] as well as appropriate consent procedures, will be reviewed and approved by an Institutional Review Board. This board must operate in accordance with the current Federal Regulations. A letter or certificate of approval will be sent by the investigator to the sponsor or designee prior to initiation of the study, and also whenever subsequent modifications to the protocol are made.

12. CONDITIONS FOR MODIFYING THE PROTOCOL

Protocol modifications to ongoing studies must be made only after consultation between an appropriate representative of the sponsor and the investigator [investigator representative[s] in the case of a multicenter trial]. Protocol modifications must be prepared by a representative of the sponsor and initially reviewed and approved by the Clinical Science Leader/Clinical Pharmacologist and Biostatistician.

All protocol modifications must be submitted to the appropriate Independent Ethics Committee or Institutional Review Board for information and approval in accordance with local requirements, and to Regulatory Agencies if required. Approval must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial subjects, or when the change[s] involves only logistical or administrative aspects of the trial [e.g. change in monitor[s], change of telephone number[s].

13. CONDITIONS FOR TERMINATING THE STUDY

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange the procedures on an individual study basis after review and consultation. In terminating the study, Roche and the investigator will assure that adequate consideration is given to the protection of the patient's interests.

14. STUDY DOCUMENTATION, CRFS AND RECORD KEEPING

14.1 Investigator's Files / Retention of Documents

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two different separate categories [1] Investigator's Study File, and [2] subject clinical source documents.

The Investigator's Study File will contain the protocol/amendments, Case Report and Query Forms, Independent Ethics Committee/Institutional Review Board and governmental approval with correspondence, sample informed consent, drug records, staff curriculum vitae and authorization forms and other appropriate documents/correspondence etc.

Subject clinical source documents [usually defined by the project in advance to record key efficacy/safety parameters independent of the CRFs] would include patient hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, EEG, X-ray, pathology and special assessment reports, signed informed consent forms, consultant letters, and subject screening and enrollment logs. The Investigator must keep these two categories of documents on file for at least 15 years after completion or discontinuation of the study. After that period of time the documents may be destroyed, subject to local regulations.

Should the Investigator wish to assign the study records to another party or move them to another location, Roche must be notified in advance.

If the Investigator can not guarantee this archiving requirement at the investigational site for any or all of the documents, special arrangements must be made between the Investigator and Roche to store these in a sealed container[s] outside of the site so that they can be returned sealed to the Investigator in case of a regulatory audit. Where source documents are required for the continued care of the patient, appropriate copies should be made for storing outside of the site.

14.2 Source Documents and Background Data

The investigator shall supply the sponsor or designee on request with any required background data from the study documentation or clinic records. This is particularly important when CRFs are illegible or when errors in data transcription are suspected. In case of special problems and/or governmental queries or requests for audit inspections, it is also necessary to have access to the complete study records, provided that patient confidentiality is protected.

14.3 Audits and Inspections

The investigator should understand that source documents for this trial should be made available to appropriately qualified personnel from the Roche Pharma Development Quality Assurance Unit or its designees, or to health authority inspectors after appropriate notification. The verification of the CRF data must be by direct inspection of source documents.

14.4 Case Report Forms

For each patient enrolled, a CRF must be completed and signed by the principal investigator or authorized delegate from the study staff. This also applies to records for those patients who fail to complete the study [even during a pre-randomization screening period if a CRF was initiated]. If a patient withdraws from the study, the reason must be noted on the CRF. If a patient is withdrawn from the study because of a treatment-limiting AE, thorough efforts should be made to clearly document the outcome.

All forms should be typed or filled out using indelible ink, and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or his/her authorized delegate. The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor or designee in the CRFs and in all required reports.

15. MONITORING THE STUDY

It is understood that the responsible Roche monitor [or designee] will contact and visit the investigator regularly and will be allowed, on request, to inspect the various records of the trial [CRFs and other pertinent data] provided that patient confidentiality is maintained in accord with local requirements.

It will be the monitor's responsibility to inspect the CRFs at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitor should have access to laboratory test reports and other patient records needed to verify the entries on the CRF. The investigator [or his/her deputy] agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

16. CONFIDENTIALITY OF TRIAL DOCUMENTS AND SUBJECT RECORDS

The investigator must assure that subjects' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents submitted to the sponsor or designee, subjects should not be identified by their names, but by an identification code. The investigator should keep a subject enrollment log showing codes, names and addresses. The investigator should maintain documents not for submission to Roche, e.g., subjects' written consent forms, in strict confidence.

17. PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to Roche prior to submission. This allows the sponsor or designee to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the investigator.

In accord with standard editorial and ethical practice, Roche will generally support publication of multicenter trials only in their entirety and not as individual center data. In this case, a coordinating investigator will be designated by mutual agreement.

Any formal publication of the study in which input of Roche personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate Roche personnel. Authorship will be determined by mutual agreement.

Appendix 1 Calculation of Creatinine Clearance and Grading of Chronic Extensive GVHD

Calculation of Creatinine Clearance

Conversion Factor for Serum Creatinine:

Conventional Units (mg/dL) = SI Units (μmol/L) ÷ 88.4

Age = number of years (12 years 11 months = 12 years)

Estimated Creatinine Clearance according to Cockcroft-Gault [17]

(for patients ≥ 18 years):

- **Males**

Creatinine

Clearance (mL/min) = [(140 – age) X Body Weight (kg)] ÷ [72 X **Serum Creatinine** (mg/dL)]

- **Females**

Creatinine Clearance = above equation X 0.85

Estimated Creatinine Clearance according to Schwartz equation [18]

(for patients < 18 years):

Creatinine Clearance
(mL/min/1.73 M^2) = k X Height (cms) ÷ **Serum Creatinine** (mg/dL)

Value for k:

k	Age (years)
0.45	< 2
0.55	≥ 2 to < 13
0.7	≥ 13 to < 18 (males)

Appendix 1 Calculation of Creatinine Clearance and Grading of Chronic Extensive GVHD (Cont.)

Grading of Chronic GVHD [19]

Type of Disease	Extent of Disease
Limited	Localized skin involvement, liver dysfunction or both
Extensive	Generalized skin involvement
	Localized skin involvement or liver dysfunction plus any one of the following: 1. Chronic aggressive hepatitis, bridging necrosis or cirrhosis 2. Eye involvement (Schirmer's test, < 5 mm) 3. Involvement of mucosalivary glands 4. Mucosal involvement (on lip biopsy) 5. Involvement of other target organs

Appendix 2 Adult Patient Diary Card and Symptom Record

The purpose of the diary card is for all adults (13 years and older) to record any symptoms of influenza-like illness for the duration of the study. Temperature, date and time of drug administration, intake of food will also be recorded on the patient diary card.

Scoring of Symptoms

Please answer All of the questions yourself by checking one box for each row.

The information you provide is very important and will remain strictly confidential.

	absent 0	mild 1	moderate 2	severe 3
1. Nasal Congestion	☐	☐	☐	☐
2. Sore Throat	☐	☐	☐	☐
3. Cough	☐	☐	☐	☐
4. Aches and Pains	☐	☐	☐	☐
5. Fatigue(Tiredness)	☐	☐	☐	☐
6. Headache	☐	☐	☐	☐
7. Chills/sweats (feverish)	☐	☐	☐	☐

Appendix 3 Diary Card for Children

Date and time of intake of food will also be recorded on the patient diary card.

Date of Assessment ☐☐☐☐☐☐ (dd mm yy) Time of Assessment ☐☐☐☐ (h min)

Temperature: ☐☐☐.☐ ºC/F

Symptoms of influenza-like illness

Please mark one box only per question

Item	No Problem 0	Minor Problem 1	Moderate Problem 2	Major Problem 3	Don't Know or not Applicable
1. Poor appetite	☐	☐	☐	☐	☐
2. Not sleeping well	☐	☐	☐	☐	☐
3. Irritable, cranky, fussy	☐	☐	☐	☐	☐
4. Feels unwell	☐	☐	☐	☐	☐
5. Low energy, tired	☐	☐	☐	☐	☐
6. Not playing well	☐	☐	☐	☐	☐
7. Crying more than usual	☐	☐	☐	☐	☐
8. Needing extra care	☐	☐	☐	☐	☐
9. Clinginess	☐	☐	☐	☐	☐
10. Headache	☐	☐	☐	☐	☐
11. Sore throat	☐	☐	☐	☐	☐
12. Muscle aches or pains	☐	☐	☐	☐	☐
13. Fever	☐	☐	☐	☐	☐
14. Cough	☐	☐	☐	☐	☐
15. Nasal congestion, runny nose	☐	☐	☐	☐	☐
16. Vomiting	☐	☐	☐	☐	☐
17. Not interested in what's going on	☐	☐	☐	☐	☐
18. Unable to get to get out of bed	☐	☐	☐	☐	☐

This form was filled out by:

1. ☐ Parent
2. ☐ Other relative
3. ☐ Nanny
4. ☐ Subject
5. ☐ Other *specify*_____

Appendix 4 Adverse Event [AEs] Categories for Determining Relationship to Test Drug

PROBABLE [must have first three]

This category applies to those AEs which are considered, with a high degree of certainty, to be related to the test drug. An AE may be considered probable, if:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It cannot be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
3. It disappears or decreases on cessation or reduction in dose. [There are important exceptions when an AE does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists; e.g., [1] bone marrow depression, [2] tardive dyskinesias.]
4. It follows a known pattern of response to the suspected drug.
5. It reappears upon rechallenge.

POSSIBLE [must have first two]

1. This category applies to those AEs in which the connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possible if, or when:
2. It follows a reasonable temporal sequence from administration of the drug.
3. It may have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
4. It follows a known pattern of response to the suspected drug.

REMOTE [must have first two]

1. In general, this category is applicable to an AE which meets the following criteria:
2. It does not follow a reasonable temporal sequence from administration of the drug.
3. It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
4. It does not follow a known pattern of response to the suspected drug.
5. It does not reappear or worsen when the drug is readministered.

UNRELATED

This category is applicable to those AEs which are judged to be clearly and incontrovertibly due only to extraneous causes [disease, environment, etc.] and do not meet the criteria for drug relationship listed under remote, possible, or probable.

Appendix 4 Adverse Event [AEs] Categories for Determining Relationship to Test Drug (Cont.)

	Probable	Possible	Remote	Unrelated
Clearly due to extraneous causes	–	–	–	+
Reasonable temporal association with drug administration	+	+	–	–
May be produced by subject clinical state, etc.	–	+	+	+
Known response pattern to suspected drug	+	+	–	–
Disappears or decreases on cessation or reduction in dose	+	–	–	–
Reappears on rechallenge	+	–	–	–

Appendix 5 ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that at any dose fulfills at least one of the following criteria:

- is fatal; [results in death] [NOTE: death is an outcome, not an event]
- is Life-Threatening [NOTE: the term "Life-Threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe].
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above

Medical and scientific judgment should be exercised in deciding whether expedited reporting to the sponsor or designee is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definitions above. These situations should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

An unexpected AE is one, the nature or severity of which is not consistent with the applicable product information.

Causality is initially assessed by the investigator. For Serious Adverse Events, possible causes of the event is indicated by selecting one or more options. (Check all that apply)

- Pre-existing/Underlying disease - specify
- Study treatment – specify the drug(s) related to the event
- Other treatment (concomitant or previous) – specify
- Protocol-related procedure
- Other (e.g. accident, new or intercurrent illness) - specify

The term severe is a measure of intensity, thus a severe AE is not necessarily serious. For example, nausea of several hours' duration may be rated as severe, but may not be clinically serious.

Appendix 5 ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 [Cont.]

A serious adverse event occurring during the study or which comes to the attention of the investigator within 15 days after stopping the treatment or during the protocol-defined follow-up period, if this is longer, whether considered treatment-related or not, must be reported. In addition, a serious adverse event that occurs after this time, if considered related to test "drug", should be reported.

Such preliminary reports will be followed by detailed descriptions later which will include copies of hospital case reports, autopsy reports and other documents when requested and applicable.

For serious adverse events, the following must be assessed and recorded on the AEs page of the CRF: intensity, relationship to test substance, action taken, and outcome to date.

The investigator must notify the Ethics Review Committee/Institutional Review Board of a serious adverse event in writing as soon as is practical and in accordance with international and local laws and regulations.

ROCHE LOCAL COUNTRY CONTACT for SAEs: Local Monitor

ROCHE HEADQUARTERS CONTACT for SAEs: Clinical Operations/Clinical Science

Within the US, weekends, holidays and after 5:00 pm, call [redacted] and ask for the physician on call. Outside the US, call the local emergency contact number provided by the Monitor.

AMENDMENT HISTORY FOR PROTOCOL NV20234B

1. SUBJECT: ADDITION OF EUDRACT AND IND NUMBERS TO COVER PAGE

Reason for change:

The EudraCT and IND numbers were inadvertently left off of the cover page.

Section: Cover page

New text:

IND Number: 53,093

EudraCT Number: 2006-002468-24

Old text:

None

2. SUBJECT: ADDITION OF A MORE TIMELY ASSESSMENT OF BASELINE NASAL AND THROAT SWAB SAMPLES FOR OSELTAMIVIR-RESISTANT VIRUS

Reason for change:

A significant increase in oseltamivir-resistant viruses circulating within many countries was identified by routine influenza virus surveillance during the 2007/2008 influenza season. These viruses have been characterized as influenza A H1N1 and they carry the H274Y mutation. This mutation confers a high level of resistance to oseltamivir, but not to amantadine, rimantadine, or zanamivir. Because oseltamivir–resistant viruses may continue to circulate during future influenza seasons, baseline nasal and throat swab samples will be assessed for the presence of the H274Y-mutated influenza virus using a real-time PCR assay. Results will be reported promptly to clinical sites to assist in determining the most appropriate treatment options for individual patients.

Section 3.1.1 Rationale for Study Design

New text:

This study incorporates several features that distinguish it from classic placebo controlled trials. At the outset, a patient population that represents the general immunocompromised population had to be defined. As a placebo control arm was considered unethical in this population, an alternative control arm had to be identified. The response to placebo in pivotal oseltamivir registration trials in the healthy adult population with influenza was chosen as a control in lieu of a placebo arm in the current trial. In order to do this, the

current study design and end points were designed to be similar to that in the pivotal registration trials.

Routine influenza surveillance during the 2007/2008 influenza season showed a significant increase in the global circulation of an oseltamivir-resistant influenza virus (A H1N1 with H274Y mutation). Therefore, baseline nasal and throat swab samples will be assessed for the presence of the H274Y-mutated influenza virus and results will be reported to clinical sites to assist in determining the most appropriate treatment options for individual patients.

The following sections provide rationale and justification for the specific aspects of the study design. They also provide information on how the results will be used to make recommendations on dose and duration of treatment.

Old text:

This study incorporates several features that distinguish it from classic placebo controlled trials. At the outset, a patient population that represents the general immunocompromised population had to be defined. As a placebo control arm was considered unethical in this population, an alternative control arm had to be identified. The response to placebo in pivotal oseltamivir registration trials in the healthy adult population with influenza was chosen as a control in lieu of a placebo arm in the current trial. In order to do this, the current study design and end points were designed to be similar to that in the pivotal registration trials. The following sections provide rationale and justification for the specific aspects of the study design. They also provide information on how the results will be used to make recommendations on dose and duration of treatment.

Section 5 Schedule of Assessments and Procedures

New text:

Table 3 Schedule of Assessments

	Baseline	Treatment					Follow-up	
Study Day	**1 (pre-dose)**	**1**	**2 or 3[f]**	**6[g]**	**8[f, g]**	**11[g, h, i]**	**15[g]**	**40[g, j]**
Nasal and throat swabs for viral shedding and viral load[c, d]	**x**		x	x	x	x	x	x

c. **Baseline swab samples will be assessed for the presence of influenza A H1N1 with H274Y mutation.**

d. Two nasal and one throat swab for viral culture and RT-PCR.

Old text:

Table 3 Schedule of Assessments

	Baseline	Treatment					Follow-up	
Study Day	**1 (pre-dose)**	**1**	**2 or 3[f]**	**6[g]**	**8[f, g]**	**11[g, h, i]**	**15[g]**	**40[g, j]**
Nasal and throat swabs for viral shedding and viral load [d]	**x**		x	x	x	x	x	x

c. . Plasma PK samples for assessment of oseltamivir and oseltamivir carboxylate will be collected using a sparse sampling approach. [5.5.1]
d. Two nasal and one throat swab for viral culture and RT-PCR

Section 5.3 Clinical Assessments and Procedures

Study Day 1

New text:

Baseline nasal and throat swab samples will be assessed for the presence of oseltamivir-resistant influenza A H1N1 with H274Y mutation. As soon as they are available, results will be reported to the sites to assist in patient management.

Old text:

None

Section 5.4 Laboratory Assessments

New text:

Nasal and throat swabs. Two nasal and one throat swab will be collected as described in the Schedule of Assessments. All swabs are sent to a central laboratory for RT-PCR and viral cultures. **Baseline samples will be assessed for the presence of oseltamivir-resistant influenza A H1N1 with the H274Y resistance mutation using a real-time PCR assay.** A phenotypic assay will be performed to determine the susceptibility of the last positive viral isolate from each patient. If required, a genotypic assay to determine the contribution of both the neuraminidase and haemagglutinin genes to decreased susceptibility will be performed.

During the course of treatment, neither the investigator nor the patient will know which participants have ongoing positive viral cultures for influenza. **However, in order to assist in patient management, the results of the assay to detect influenza A H1N1 H274Y at baseline will be reported to the sites as soon as they are available.** At the end of treatment (day 11), a rapid diagnostic test is permitted for the diagnosis of ongoing influenza.

Old text:

Nasal and throat swabs. Two nasal and one throat swab will be collected as described in the Schedule of Assessments. All swabs are sent to a central laboratory for RT-PCR and

viral cultures. A phenotypic assay will be performed to determine the susceptibility of the last positive viral isolate from each patient. If required, a genotypic assay to determine the contribution of both the neuraminidase and haemagglutinin genes to decreased susceptibility will be performed.

During the course of treatment, neither the investigator nor the patient will know which participants have ongoing positive viral cultures for influenza. However at the end of treatment (day 11), a rapid diagnostic test is permitted for the diagnosis of ongoing influenza.

3. SUBJECT: ADDITION OF NEW CRITERION FOR PREMATURE WITHDRAWAL OF PATIENTS FROM STUDY TREATMENT IF ADDITIONAL ANTIVIRALS ARE ADDED TO THE PATIENT'S TREATMENT REGIMEN

Reason for change:

Because of the addition of testing for oseltamivir-resistant influenza A H1N1 H274Y at baseline, it is envisioned that some investigators may decide that it is in the best interest of the patient to treat with another influenza antiviral. In these situations, the patient must be discontinued from study drug to avoid confounding the safety profile and efficacy analyses of oseltamivir in this study.

Section 4.5 Criteria for Premature Withdrawal

New text:

The investigator must discontinue treatment with study drug if the patient is to be treated concomitantly with another antiviral for influenza, for example, amantadine, rimantadine, or zanamavir. However, all patients, including those who discontinue study drug prematurely and/or are treated with another antiviral, will be required to return for follow up approximately 5 and 30 days after the last dose of study medication (day 15 and day 40 assessments).

Old text:

None.

4. SUBJECT: REPLACEMENT OF PATIENTS WHO ARE IDENTIFIED AS HAVING OSELTAMIVIR-RESISTANT INFLUENZA VIRUS AT BASELINE

Reason for change:

The number of patients enrolled into this study with influenza A H1N1 H274Y at baseline cannot be predicted. Therefore, a priori assumptions on how many additional patients need to be enrolled to maintain the required number of patients who are evaluable for efficacy cannot be made. To ensure that the number of evaluable patients

enrolled is sufficient for the primary efficacy analysis, unevaluable patients infected with the oseltamivir-resistant influenza virus at baseline will be replaced by enrolling additional patients on a rolling basis.

Section Synopsis: Number of Subjects

New text:

A minimum of 250 (125 per arm)

Old text:

Approximately 250 (125 per arm)

Section 3.2 Number of Subjects/Assignment to Treatment Groups

New text:

A **minimum** of 250 patients will be enrolled in this study (approximately 125 per arm).

Old text:

A total of approximately 250 patients will be enrolled in this study (approximately 125 per arm).

Section 4.6.1 Replacement Policy [Ensuring Adequate Numbers of Evaluable Patients] For Subjects

New text:

In order to maintain the required number of patients who are evaluable for efficacy, patients who are identified as being infected with influenza A H1N1 H274Y at baseline will be replaced by enrolling additional patients on a rolling basis. Replacement patients will be stratified and randomized to treatment in the same manner as original patients. Replacement patients will not be "selected" by dose group or strata (age, vaccination status, transplant type, time between symptom onset and treatment) to match the patients whom they replace.

Old text:

No subject prematurely discontinued from the study for any reason will be replaced.

Section 8.2.2 Sample Size

New text:

Sample size calculations were performed using a two-sided t-test and are based on the comparison with a placebo control response in the pivotal registration trials in healthy adults of 112 hours for median time to alleviation of all symptoms. Assuming an expected median time to alleviation of symptoms of 78 hours for each of the two active treatment groups in this study, and a common standard deviation of 98 hours, then 111 evaluable subjects per group would give more than 90% power to detect a difference between the control and each of the active treatment groups. It is assumed that as many as 90 % of subjects enrolled into the study **who are identified as not being infected with**

influenza A H1N1 H274Y will have influenza, and therefore 124 **such** subjects per arm would be required in order to obtain 111 influenza positive subjects. Note that this is an approximate calculation, as the final analysis will be based on the difference in medians and the corresponding 95% confidence interval from the Kaplan-Meier curves.

This is however considered as a sufficient indication for the **number of required subjects** and the power that is expected. **The maximum number of subjects enrolled in the trial will depend upon the number of subjects identified with the oseltamivir resistant virus who will be replaced.**

Old text:

Sample size calculations were performed using a two-sided t-test and are based on the comparison with a placebo control response in the pivotal registration trials in healthy adults of 112 hours for median time to alleviation of all symptoms. Assuming an expected median time to alleviation of symptoms of 78 hours for each of the two active treatment groups in this study, and a common standard deviation of 98 hours, then 111 evaluable subjects per group would give more than 90% power to detect a difference between the control and each of the active treatment groups. It is assumed that as many as 90 % of subjects enrolled into the study will have influenza, and therefore 124 subjects per arm would be required in order to obtain 111 influenza positive subjects. Note that this is an approximate calculation, as the final analysis will be based on the difference in medians and the corresponding 95% confidence interval from the Kaplan-Meier curves. This is however considered as a sufficient indication for the size of the trial and the power that is expected.

5. SUBJECT: CLARIFICATION OF ALLOWED USE OF OTHER ANTIVIRALS

Reason for change:

In some cases, the investigator may decide that it is in the best interest of the patient to treat his/her influenza with another antiviral, for example if oseltamivir-resistant virus is identified at baseline or if the patient is positive for influenza by rapid diagnostic test on day 11. In these cases, treatment with other antivirals will be allowed, as long as treatment with study oseltamivir is discontinued.

Section 4.4 Concomitant Medication and Treatment

New text:

Antiviral treatments with activity against influenza (e.g. amantadine, rimantadine, zanamivir, ribavirin, and additional oseltamivir above that specified for this study) are not allowed during **the 10-day treatment phase of** the study. Concomitant use of an investigational drug during the study is also excluded.

Old text:

Antiviral treatments with activity against influenza (e.g. amantadine, rimantadine, zanamivir, ribavirin, and additional oseltamivir above that specified for this study) are not

allowed during the study. Concomitant use of an investigational drug during the study is also excluded.

6. SUBJECT: EXCLUSION OF SPECIFIC DATA FROM EFFICACY ANALYSES

Reason for change:

Patients who are treated with other antivirals after discontinuing oseltamivir treatment will be required to return for follow-up approximately 5 and 30 days after the last dose of study medication. Efficacy data collected from such patients after other antiviral treatment commences will be excluded from the primary and secondary efficacy analyses so as not to confound them. Such patients will be considered failures to oseltamivir treatment.

Section 8.2.5.1 Exclusion of Data from Analysis

New Text:

Efficacy data collected from any patient who begins treatment with another antiviral for influenza (for example, amantadine, rimantadine, or zanamavir), after the other antiviral treatment commences, will be excluded from the primary and secondary efficacy analyses. **Such patients will be considered failures to oseltamivir treatment in the analyses. Further details will be provided in the DRAM.**

Old Text:

No data will be excluded from the primary and secondary efficacy analyses.

7. SUBJECT: REVISION OF DEFINITION OF ITTI POPULATION

Reason for change:

For the primary efficacy analysis of this study, the time to resolution of all influenza symptoms in both treatment groups will be compared to that of the placebo control from the pivotal oseltamivir registration trials. Oseltamivir-resistant viruses were never identified in baseline samples from patients participating in the registration trials. In order to maintain consistency with the control population and in an effort to conduct a fair comparison, patients identified as infected with oseltamivir-resistant influenza A H1N1 H274Y at baseline will be excluded from the ITTI primary efficacy analysis population.

Section 8.2.4.2 Intent to treat infected population

All patients randomized and with laboratory confirmation of influenza infection (positive viral cultures or 4 fold or greater rise in antibody titers**), excluding patients infected with oseltamivir-resistant influenza A H1N1 H274Y at baseline,** will be included in

the intent to treat influenza infected population [Patients will be assigned to treatment groups as randomized for analysis purposes]

The ITTI Population will be the primary population for the summary and analysis of the primary and secondary efficacy variables.

Old text:

All patients randomized and with laboratory confirmation of influenza infection (positive viral cultures or 4 fold or greater rise in antibody titers) will be included in the intent to treat influenza infected population [Patients will be assigned to treatment groups as randomized for analysis purposes]

The ITTI Population will be the primary population for the summary and analysis of the primary and secondary efficacy variables.

8. SUBJECT: DELETION OF SAMPLING FOR PHARMACOKINETICS ANALYSIS

Reason for change:

During the first influenza season in which this study was conducted, mandatory sampling for pharmacokinetics analysis was identified as being inconvenient for patients and detrimental to participation in the study. Revision of the mandatory sampling to optional sampling was considered, but this would decrease the robustness of the analysis due to the estimated decrease in the number of evaluable samples collected. The value of the pharmacokinetics/pharmacodynamics analyses was therefore reassessed and it was decided that it would be in the best interest of the patients and the study to delete pharmacokinetics/pharmacodynamics from the protocol.

Section Synopsis: Objectives

New text:

None

Old text:

To characterize the population pharmacokinetics of oseltamivir (e.g. clearance, volume of distribution) in transplant recipients

Section Synopsis: Assessments of Pharmacokinetics / Pharmacodynamics

New text:

None

Old text:

Pharmacokinetic assessments will be collected as below:

Day 1 (2 ‘early’ samples: 1 – 4 hours post dose but at least 1 hour apart)

Day 6 (2 'late' samples: 4 - 12 hrs post dose, but at least 1 hour apart)
Day 11 (2 'early' samples on Day 11)

Pharmacodynamic assessments will be based on nose and throat swabs collected on the days specified in the schedule of assessments.

Section Synopsis: Procedures

New text:

The key procedures are:
Blood draws for serum chemistry, hematology, and serology

Old text:

The key procedures are:
Blood draws for serum chemistry, hematology, **PK** and serology

Section 5 Schedule of Assessments and Procedures

New text:

None.

Old text:

5 SCHEDULE OF ASSESSMENTS AND PROCEDURES

Table 1 Schedule of Assessments

	Baseline	Treatment					Follow-up	
Study Day	**1 (pre-dose)**	**1**	**2 or 3[f]**	**6[g]**	**8[f, g]**	**11[g, h, i]**	**15[g]**	**40[g, j]**
PK sampling[c]		x		x		x		

c. Plasma PK samples for assessment of oseltamivir and oseltamivir carboxylate will be collected using a sparse sampling approach. [5.5.1]
g. …PK assessments cannot be done on day 12.

Section 5.3 Clinical Assessments and Procedures

New text:

None.

Old text:

Blood samples will be taken for PK analysis as mentioned in section 5.5.1.

Section 5.4 Laboratory Assessments

New text:

The total volume of blood loss for laboratory assessments will be approximately 20 mL for the entire duration of the study.

Old text:

The total volume of blood loss for laboratory assessments **(other than pharmacokinetic sampling)** will be approximately 20 mL for the entire duration of the study. **Blood loss for pharmacokinetic sampling is explained in section 5.5.1**.

Section 5.5 Pharmacokinetic Assessments [PK]/Pharmacodynamic Assessments [PD]

New text:

None

Old text:

5.5 Pharmacokinetic Assessments [PK] /Pharmacodynamic [PD] Assessments

Blood samples will be collected to evaluate the pharmacokinetics of oseltamivir according to the Schedule of Assessments (Table 3) and as described below. Further details on PK/PD are to be found in sections 8.3.1 and 8.3.2.

5.5.1 Pharmacokinetic Assessments [PK]

Plasma PK samples for assessment of oseltamivir and oseltamivir carboxylate will be collected using the following sparse sampling approach:

day 1 (2 'early' samples: 1 – 4 hours post dose but at least 1 hour apart)

day 6 (2 'late' samples: 4 – 12 hours post dose but at least 1 hour apart)

day 11 (2 'early' samples on day 11)*

*Note: Subjects should attend the clinic as early as possible on the morning of Day 11. For those subjects who had their last dose on the morning of Day 11, they should have 2 samples drawn 1– 4 hours post dose but at least 1 hour apart. For those subjects who received their last dose on the evening of Day 10, they should have 2 samples drawn at least 1 hour apart, as early as possible on the morning of day 11.

For adults and adolescents, approximately 2 mL of blood will be taken at each time point, therefore the total volume blood loss for pharmacokinetic assessments will be approximately 12 mL. For pediatric subjects, not less than approximately 0.6 mL of blood will be taken at each time point, as a result the total volume blood loss for pharmacokinetic assessments will be approximately 3.6 mL.

5.5.2 Pharmacodynamic [PD] Assessments

Nasal and throat swabs will be collected from individuals on the days specified in the schedule of assessments. Specimens will be cultured at a central laboratory. The proportion of patients with viral shedding at each visit will be summarized.

Section 8.3 Pharmacokinetic and Pharmacodynamic Analysis

New text:

None.

Old text:

8.3 Pharmacokinetic and Pharmacodynamic Analysis

8.3.1 Pharmacokinetic Analysis

Nonlinear mixed effects modeling (with software NONMEM) will be used to analyze all plasma concentration-time data of oseltamivir and oseltamivir carboxylate following oseltamivir administration. Population pharmacokinetic parameters (clearance, distribution volume) will be estimated. The influence of covariates (such as age, gender, body weight and calculated creatinine) on these parameters will be investigated. Based on the population PK model individual pharmacokinetic parameters such as AUC, C_{max}, C_{min} and t_{max} may be calculated using Bayesian posthocs. If deemed appropriate and necessary, data may be pooled with data from previous studies investigating oseltamivir and oseltamivir carboxylate in order to improve model stability.

8.3.2 Pharmacodynamic Analysis

If feasible, the relationship between PK exposure of oseltamivir carboxylate and viral shedding response data will be characterized using nonlinear mixed effects modeling (using software NONMEM). Relevant population PD parameters will be derived and the influence of covariates will be investigated. If deemed appropriate and necessary, data may be pooled with data from previous studies investigating oseltamivir and oseltamivir carboxylate in order to improve the model stability.

9. SUBJECT: DELETION OF FOOD INTAKE QUESTION FROM DIARY QUESTIONS

Reason for change:

Information regarding intake of food in relationship to time of oseltamivir dosing was important for pharmacokinetics analyses. Because pharmacokinetics analyses have been deleted from the protocol, this information is no longer necessary.

Section 5. Schedule of Assessments and Procedures

New text:

e. Flu symptoms, temperature**, and** date/ time of oseltamivir dose will be recorded by the patient on diary cards twice daily on days 1 – 10, and once daily thereafter

Old text:

e. Flu symptoms, temperature, date/ time of oseltamivir dose **and food intake** will be recorded by the patient on diary cards twice daily on days 1 – 10, and once daily thereafter.

Section 5.3 Clinical Assessments and Procedures

New text:

Study Day 1

Patients or guardians/parents will be instructed how to complete symptom diary cards (Appendix 2) (Appendix 3), temperature recording, and treatment administration details, including time of each oseltamivir dose.

Old text:

Study Day 1

Patients or guardians/parents will be instructed how to complete symptom diary cards (Appendix 2) (Appendix 3), temperature recording, and treatment administration details, including time of each oseltamivir dose **and food intake**.

Section Appendix 2 Adult Patient Diary Card and Symptom Record

New text:

Appendix 2 Adult Patient Diary Card and Symptom Record
Temperature **and** date and time of drug administration will also be recorded on the patient diary card.

Old text:

Appendix 2 Adult Patient Diary Card and Symptom Record
Temperature, date and time of drug administration, **intake of food** will also be recorded on the patient diary card.

Section Appendix 3 Diary Card for Children

New text:

Appendix 3 Diary Card for Children

Temperature and date and time of **drug administration** will also be recorded on the patient diary card

Old text:

Appendix 3 Diary Card for Children

Date and time of **intake of food** will also be recorded on the patient diary card.

10. SUBJECT: REVISION OF EXPLANATION OF LABORATORY ASSESSMENTS TO INCLUDE VIRAL SHEDDING AND SEROLOGY

Reason for change:

Viral shedding and serology were inadvertently left out of the explanation of laboratory assessments.

Section 5.4 Laboratory Assessments

New text:

Nasal and throat swabs. Two nasal and one throat swab will be collected as described in the Schedule of Assessments. All swabs are sent to a central laboratory for RT-PCR and viral cultures. **Influenza virus shedding will be assessed.**

Serology. Blood samples for influenza antibody titer will be collected according to the Schedule of Assessments.

Old text:

Nasal and throat swabs. Two nasal and one throat swab will be collected as described in the Schedule of Assessments. All swabs are sent to a central laboratory for RT-PCR and viral cultures.

11. SUBJECT: REPLACEMENT OF PAPER PATIENT DIARIES WITH ELECTRONIC PATIENT DIARIES

Reason for change:

In order to improve data quality, paper patient diaries (used in the first influenza season during which this study was conducted) will be replaced with electronic patient diaries. Electronic patient diaries will capture all of the information recorded on paper patient diaries with the added benefit of automatically controlling for simple but frequent recording errors, such as impossible dates and doses. Diary data previously collected on paper diaries by patients participating in season 1 will be used along with diary data collected in the electronic diaries for the efficacy analyses in this study.

Section Synopsis: Assessments of Efficacy

New text:

Time to resolution of all influenza symptoms as recorded in the **patient** diary.

Old text:

Time to resolution of all influenza symptoms as recorded in the diary **cards**.

Section 3.1.1.2 Consistency with previous influenza treatment studies

New text:

Some of the key design features consistent with previous studies include: two active treatment arms, subjects with influenza like symptoms recruited during the flu season, use of a **patient** diary to capture symptoms, primary clinical end point based on diary assessments, laboratory evaluation of virology and serology samples generally similar to previous trials and the primary efficacy analysis population is the Intent to treat Infected (ITTI) population.

Old text:

Some of the key design features consistent with previous studies include: two active treatment arms, subjects with influenza like symptoms recruited during the flu season, use of a diary **card** to capture symptoms, primary clinical end point based on diary **card** assessments, laboratory evaluation of virology and serology samples generally similar to previous trials and the primary efficacy analysis population is the Intent to treat Infected (ITTI) population.

Section 5. Schedule of Assessments and Procedures

New text:

Table 3 Schedule of Assessments

	Baseline	Treatment					Follow-up	
Study Day	**1 (pre-dose)**	**1**	**2 or 3[f]**	**6[g]**	**8[f, g]**	**11[g, h, i]**	**15[g]**	**40[g, j]**
Review of **electronic** diary **data** [e]			←	—	—	—	—	→

e. Flu symptoms, temperature, and date/ time of oseltamivir dose will be recorded by the patient **in electronic patient** diar**ies** twice daily on days 1 – 10, and once daily thereafter.

Old text:

Table 3 Schedule of Assessments

	Baseline	Treatment					Follow-up	
Study Day	**1 (pre-dose)**	**1**	**2 or 3[f]**	**6[g]**	**8[f, g]**	**11[g, h, i]**	**15[g]**	**40[g, j]**
Review of diary card [e]			x	x	x	x	x	x

e. Flu symptoms, temperature, date/ time of oseltamivir dose and food intake will be recorded by the patient on diary cards twice daily on days 1 – 10, and once daily thereafter.

Section 5.3 Clinical Assessments and Procedures

New text:

Study Day 1

Study medication, **electronic** diar**ies**, and thermometers will be dispensed. Patients or guardians/parents will be instructed how to complete **electronic** symptom diar**ies** (Appendix 2) (Appendix 3), temperature recording, and treatment administration details, including time of each oseltamivir dose. The first diary **entries** will be **made at the site** before the first dose of study drug.

Study Days 2-11

It is expected the study staff will maintain close contact with patients, especially during the first few days of the study. The study staff should inquire about any adverse events, check that the patient or parent/guardian **is entering data into the electronic** diary **properly**, and assess drug compliance. During the dosing period, diary symptoms should

be assessed and temperature should be recorded at the same time that the study drug is taken.

End of Follow-Up Day 40

Patients or parents/guardians who do not return to the clinic will be contacted by telephone to ascertain their health status, or that of their child's, record information on adverse events, and to ask that they return the **electronic** diary.

Old text:

Study Day 1

Study medication, diary **cards**, and thermometers will be dispensed. Patients or guardians/parents will be instructed how to complete symptom diary **cards** (Appendix 2) (Appendix 3), temperature recording, and treatment administration details, including time of each oseltamivir dose and food intake. The first diary **card** will be completed before the first dose of study drug.

Study Days 2-11

It is expected the study staff will maintain close contact with patients, especially during the first few days of the study. The study staff should inquire about any adverse events, check that the patient or parent/guardian is completing the diary **cards**, and assess drug compliance. During the dosing period, diary **card** symptoms should be assessed and temperature should be recorded at the same time that the study drug is taken.

End of Follow-Up Day 40

Patients or parents/guardians who do not return to the clinic will be contacted by telephone to ascertain their health status, or that of their child's, record information on adverse events, and to ask that they return the symptom diary **card**.

Section 5.3.1 Efficacy Assessments

New text:

The primary end point in this study is the time to resolution of all influenza symptoms as recorded in the **patient** diary.

The clinical efficacy parameters in this study are:

1. Symptoms of influenza-like illness. These are captured in the diary for both adults and children. (Apendix 2) (Appendix 3). Influenza symptoms are graded on a nominal scale from zero (absent/no problem) to 3 (severe or major problem). A score of zero is considered asymptomatic. The primary end point, time to alleviation of all symptoms is comprised of all the symptoms captured in the diary.
2. Temperature. This is captured **in** the diary. Temperature is used for assessment of the primary and several secondary end points.

Old text:

The primary end point in this study is the time to resolution of all influenza symptoms as recorded in the diary **cards**.

The clinical efficacy parameters in this study are:

1. Symptoms of influenza-like illness. These are captured in the diary **card** for both adults and children. (Appendix 2) (Appendix 3). Influenza symptoms are graded on a nominal scale from zero (absent/no problem) to 3 (severe or major problem). A score of zero is considered asymptomatic. The primary end point, time to alleviation of all symptoms is comprised of all the symptoms captured in the diary **card**.
2. Temperature. This is captured on the diary **card**. Temperature is used for assessment of the primary and several secondary end points.

Section 5.3.2 Safety

New text:

Pre-defined symptoms of influenza captured in the adult and pediatric diar**ies** are not to be reported as adverse events unless they can be further qualified. Thus 'headache due to stress at work' is reported as an adverse event. However, unexplained 'headache' is considered a predefined symptom related to influenza and not an adverse event.

Old text:

Pre-defined symptoms of influenza captured in the adult and pediatric diary cards are not to be reported as adverse events unless they can be further qualified. Thus 'headache due to stress at work' is reported as an adverse event. However, unexplained 'headache' is considered a predefined symptom related to influenza and not an adverse event.

Section 7.1.1 Clinical AEs

New text:

Influenza signs and symptoms reported on the **patient** diary will be summarized as efficacy end points and need not be captured as adverse events.

Old text:

Influenza signs and symptoms reported on the diary card will be summarized as efficacy end points and need not be captured as adverse events.

Section 8.1.1 Primary Endpoints

New text:

The primary endpoint in this study is the time to alleviation of all clinical influenza symptoms (recorded in the **patient** diary).

Old text:

The primary endpoint in this study is the time to alleviation of all clinical influenza symptoms (recorded in the diary card).

Section Appendix 2 Adult Patient Diary Card and Symptom Record

New text:

Appendix 2 Adult Patient Diary Data and Symptom Record

The purpose of the **electronic** diary is for all adults (13 years and older) to record any symptoms of influenza-like illness for the duration of the study. Temperature and date and time of drug administration will also be recorded on the **electronic** patient diary.

Old text:

Appendix 2 Adult Patient Diary Card and Symptom Record

The purpose of the diary card is for all adults (13 years and older) to record any symptoms of influenza-like illness for the duration of the study. Temperature, date and time of drug administration, intake of food will also be recorded on the patient diary card.

Section Appendix 3 Diary Card for Children

New text:

Appendix 3 Diary Data for Children

Temperature and date and time of drug administration will also be recorded on the **electronic** patient diary.

Old text:

Appendix 3 Diary Card for Children

Date and time of intake of food will also be recorded on the patient diary card.

12. SUBJECT: INCREASE IN TIME ALLOWED FOR SCREENING

Reason for change:

Although screening and baseline assessments and procedures are usually completed on the same day, it may be more convenient for some patients to be screened prior to the baseline visit, randomization, and first dose.

Section 5.1 Screening Examination and Eligibility Screening Form

New text:

All subjects must provide written informed consent (assent where applicable) before any study specific assessments or procedures are performed. Screening will take place at the baseline visit prior to first dose. **However, if necessary, screening may take place the day before the baseline visit, as long as the patient is randomized and receives his/her first dose of study medication within 48 hours of influenza symptom onset**.

Old text:

All subjects must provide written informed consent (assent where applicable) before any study specific assessments or procedures are performed. Screening will take place at the baseline visit prior to first dose.

13. SUBJECT: INCREASE IN WINDOWS AROUND DAY 2/3 AND DAY 6 VISITS

Reason for change:

Windows around the day 2/3 and Day 6 visits will be increased to make more convenient for patients.

Section 5. Schedule of Assessments and Procedures

New text:

Table 3 Schedule of Assessments

	Baseline	Treatment					Follow-up	
Study Day	**1 (pre-dose)**	**1**	**2 or 3[f,g]**	**6[g]**	**8[f, g]**	**11[g, h, i]**	**15[g]**	**40[g, j]**

g. **Day 2/3 visit window = + 1 day**. Day 6 visit window = **+ /- 1 day**. Day 8 visit window = +1 day….

Old text:

Table 3 Schedule of Assessments

	Baseline	Treatment					Follow-up	
Study Day	**1 (pre-dose)**	**1**	**2 or 3[f]**	**6[g]**	**8[f, g]**	**11[g, h, i]**	**15[g]**	**40[g, j]**

g. Day 6, 8 visit window = + 1 day….

14. SUBJECT: CLARIFICATION REGARDING WITHDRAWAL FROM STUDY MEDICATION FOR LOW CREATININE CLEARANCE AND HEPATIC DECOMPENSATION

Reason for change:

Criteria for premature withdrawal have been revised to more clearly emphasize that patients with low creatinine clearance or hepatic decompensation must be withdrawn from study medication.

Section 4.5 Criteria for Premature Withdrawal

New text:

The investigator **must** discontinue treatment if the creatinine clearance is < 10 ml/min in adults or < 10 ml/min/1.73 M^2 in children. The investigator **must** also discontinue treatment from all subjects with intercurrent illnesses or adverse events suggestive of hepatic decompensation.

Old text:

The investigator should discontinue treatment if the creatinine clearance is < 10 ml/min in adults or < 10 ml/min/1.73 M^2 in children. The investigator should also discontinue treatment from all subjects with intercurrent illnesses or adverse events suggestive of hepatic decompensation.

15. SUBJECT: REVISION OF WARNINGS AND PRECAUTIONS

Reason for change:

Warnings and Precautions section has been revised to provide additional information and clarification.

Section 7.3 Warnings and Precautions

New text:

Events such as convulsions and delirium **(including symptoms such as altered level of consciousness, confusion, abnormal behavior, delusions, hallucinations, agitation, anxiety, nightmares)** have been reported during oseltamivir use in patients with influenza, **predominately in children and adolescents**. In rare cases, **these events** resulted in accidental injury. The contribution of oseltamivir to those events is unknown. Such events have also been reported in patients with influenza who were not taking oseltamivir. Patients, especially children and adolescents, should be closely monitored **for signs of abnormal behavior.**

Old text:

Events such as convulsions and **those associated with** delirium have been reported during oseltamivir use in patients with influenza. In rare cases, the **delirium** resulted in accidental injury. The contribution of oseltamivir to those events is unknown. Such events have also been reported in patients with influenza who were not taking oseltamivir. Patients, especially children and adolescents, should be closely monitored.

16. SUBJECT: REVISION OF CALCULATION OF ESTIMATED CREATININE CLEARANCE TO INCLUDE FEMALES ≥ 13 TO < 18 YEARS OLD

Reason for change:

"k" value for Schwartz equation for females ≥ 13 to < 18 years old was inadvertently left out.

Section Appendix 1 Calculation of Creatinine Clearance and Grading of Chronic Extensive GVHD

New text:

Value for k:

k	Age (years)

0.45	< 2
0.55	≥ 2 to < 13
0.7	≥ 13 to < 18 (males)
0.55	**≥ 13 to < 18 (females)**

Old text:

Value for k:

k	Age (years)
0.45	< 2
0.55	≥ 2 to < 13
0.7	≥ 13 to < 18 (males)

F. HOFFMANN-LA ROCHE LTD
CLINICAL STUDY PROTOCOL
PROTOCOL NUMBER NV20234B
RO 64-0796
TAMIFLU® (OSELTAMIVIR)
IND NUMBER 53,093
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PROTOCOL APPROVAL

Protocol Number / Version: NV20234 / B

Date: See last date in electronic signature manifestation below.

Protocol approved by: See electronic signature manifestation below.

Name	Reason for Signing	Date and Time (Eastern USA - New York)
[redacted]	Clinical Science Leader	31-Jul-2008 15:37:08
	Project Statistician	31-Jul-2008 16:51:33

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SYNOPSIS OF PROTOCOL NUMBER NV20234B

TITLE	A double-blind, randomized, stratified, multi-center trial evaluating conventional and high dose oseltamivir in the treatment of immunocompromised patients with influenza
SPONSOR	F. Hoffmann-La Roche LTD CLINICAL PHASE IIIb
INDICATION	Treatment of influenza in immunocompromised patients
OBJECTIVES	Primary: To evaluate prospectively the efficacy of oseltamivir for the treatment of influenza in transplant recipients as measured by the time to resolution of influenza symptoms Secondary: To evaluate the effects of conventional and high dose oseltamivir in transplant recipients on: • The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis) • The virologic course of influenza (proportion shedding and viral loads at different time points) • Patient safety and tolerability • The development of resistant influenza virus
TRIAL DESIGN	This is a double-blind, randomized, multi-center trial of twice daily, conventional and high dose oseltamivir for the treatment of influenza in immunocompromised patients (as represented by transplant recipients). Subjects will be stratified by age, transplant type, time since onset of flu symptoms and treatment start (≤ 24 or >24 hours) and vaccination status
NUMBER OF SUBJECTS	**A minimum of** 250 (125 per arm)
NUMBER OF CENTERS	Approximately 140 centers in the Northern hemisphere
TARGET POPULATION	Transplant recipients (liver, kidney, liver and kidney, allogenic haematopoietic stem cell transplant), 1 year of age and older enrolled during the influenza season. The subjects will be positive for influenza by a rapid diagnostic test at baseline.
LENGTH OF STUDY	10 days of treatment, 30 days of follow up.
INVESTIGATIONAL MEDICINAL PRODUCT(S) DOSE/ ROUTE/ REGIMEN	Oseltamivir/placebo dry powder (to be reconstituted to a concentration of 12 mg/ml) and 75 mg capsules. The duration of dosing in both adults and children is 10 days. Conventional dose: Children ages 1 - 12 years: Oseltamivir syrup ≤ 15 kg 30 mg twice daily > 15 – 23 kg 45 mg twice daily > 23 – 40 kg 60 mg twice daily > 40 kg 75 mg twice daily Adults and adolescents (age ≥ 13 years): Oseltamivir capsules 75 mg twice daily Subjects randomized to the conventional dose will simultaneously receive matching placebo so as to blind them and the investigator from the high dose arm.

	High dose: Children ages 1 - 12 years: Oseltamivir syrup ≤ 15 kg 60 mg twice daily > 15 – 23 kg 90 mg twice daily > 23 – 40 kg 120 mg twice daily > 40 kg 150 mg twice daily Adults and adolescents (age ≥ 13 years): Oseltamivir capsules 150 mg twice daily Dose adjustments: In both treatment arms, dosing frequency will be decreased to once daily in patients with severe renal impairment (Cr Cl in adults between 10-30 ml/min and children between 10-30 ml/min/1.73M^2.).
COMPARATOR "DRUG" (or STANDARD OF CARE) DOSE/ ROUTE/ REGIMEN	Placebo (from pivotal registration trials in otherwise healthy adults)
ASSESSMENTS OF:	
- EFFICACY	Time to resolution of all influenza symptoms as recorded in the **patient** diary.
- SAFETY	Adverse events, physical exams, vital signs and clinical laboratory evaluations

PROCEDURES (summary):

The key procedures are:
Blood draws for serum chemistry, hematology, and serology.
Nasal and throat swabs for viral culture and RT-PCR.

STATISTICAL ANALYSES:

Sample size calculations were performed using a two-sided t-test and are based on the comparison with a placebo control response in the pivotal registration trials in healthy adults of 112 hours for median time to alleviation of all symptoms. Assuming an expected median time to alleviation of symptoms of 78 hours for each of the two active treatment groups in this study, and a common standard deviation of 98 hours, then 111 evaluable subjects per group would give more than 90% power to detect a difference between the control and each of the active treatment groups.

The primary endpoint will be time to alleviation of all clinical influenza symptoms, as recorded in the diary cards.

For the primary analysis, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- Comparison to placebo control from pivotal registration trials

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established to match those of the current study. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

- Assessment of relative efficacy

  The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval. The methodology will be based on the use of the Hodges-Lehmann estimator in the case of censored data.

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GLOSSARY OF ABBREVIATIONS

ALT [SGPT]	alanine aminotransferase
ANC	absolute neutrophil count
ANOVA	analysis of variance
AST [SGOT]	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
b.i.d.	Bis in die (twice daily)
BP	blood pressure
CI	confidence interval
C_{max}	maximum plasma concentration
Cr Cl	Creatinine Clearance
CRF	Case Report Form[s]
DRAM	Data Reporting and Analysis Manual
ESF	eligibility screening form
hrs	Hours
HA	Hemagglutinin
HSCT	Hematopoietic stem cell transplant
ICH	International Conference on Harmonisation
ITT	Intent to treat
ITTI	Intent to treat Influenza Infected
IVRS	Interactive Voice Response System
mg	Milligram
mL	Milliliter
PD	Pharmacodynamic
PK	Pharmacokinetic

GLOSSARY OF ABBREVIATIONS

p.o.	Per os (by mouth)
PR	Pulse rate
QD	Once per day
SAE	Serious adverse event
SOT	Solid organ transplant
$TCID_{50}$	50% Tissue Culture Infectious Dose
Tmax	Time of maximum plasma concentration
t1/2	Elimination half-life
µg	Microgram

PART I: STUDY DESIGN AND CONDUCT

1. BACKGROUND AND RATIONALE

1.1 Background

Influenza is an acute respiratory infection caused by a virus of the orthomyxovirus family which occurs in three forms, influenza A, B and C. Influenza virus types A and B cause an acute febrile infection of the respiratory tract characterized by the sudden onset of fever, malaise, headaches, myalgias and cough. Influenza causes numerous deaths each year [1]. Although difficult to assess, annual influenza epidemics are thought to result in between three and five million cases of severe illness, and between 250,000 and 500,000 deaths every year around the world. [2]

The influenza viruses are segmented, negative sense, single stranded, lipid encapsulated, RNA viruses between 80 and 100 nm in size. Subtypes are defined according to glycoproteins present in the viral lipid coat. The haemagglutinin complex (HA) is the major surface protein of the virus. The neuraminidase (NA) protein is the second major surface protein in the virion and plays a role in enhancing virus penetration of the mucus layer around the target cell and in release of virus from the cell surface. The matrix proteins, M1 and M2 appear to trigger the disintegration of the M1 complex during virus fusion with the cell and may also be involved in the maturation of the HA prior to assembly of new virus particles.

1.1.1 Influenza

Influenza infection is usually a self limiting condition. However, in children, the elderly and the immunocompromised, influenza infection can be associated with substantial morbidity and mortality [1]. In patients with compromised immunity, influenza virus may cause severe lower respiratory tract involvement; unusual syndromes, including encephalitis, myocarditis, and hepatitis and death. [3] The availability of new immunosuppressive agents has enhanced the number and survival of solid organ transplant (SOT) and haematopoietic stem cell transplant (HSCT) [4] recipients making them representative of the larger immunocompromised patient population.

In transplant recipients influenza is associated with a higher rate of pulmonary complications, extrapulmonary manifestations and an increased risk of graft dysfunction and high attributable mortality. In a cohort study of influenza viral infection in SOT subjects, 30 cases of influenza viral infection were identified. Secondary bacterial pneumonia was seen in 17 % of subjects and three SOT recipients (2 liver and one kidney) had myositis, myocarditis and bronchiolitis obliterans. Biopsy of the transplanted organ was performed in 21 of 30 cases and revealed variable degrees of acute allograft rejection in 62 % of subjects. The study concluded that influenza was associated with significant morbidity in different groups of SOT recipients [5]

Among transplant recipients, subjects with HSCT are at the greatest risk for morbidity. In a prospective study from the Infectious Diseases Working Party of the European Group

for Blood and Marrow Transplantation, 1973 patients were evaluated for respiratory virus infections after stem cell transplant. The overall mortality was 23% from influenza A infection and the direct influenza A-associated mortality was 15.3% in this study. [6]

The largest study of influenza infections in transplant (allogenic, syngenic or autologous HSCT) subjects comes from the Fred Hutchinson Cancer Center, Seattle, USA. In this retrospective study, influenza virus was isolated in 62 of 4797 subjects undergoing HSCT over a 13-year period. During this period, the Center had a standardized protocol for the detection of respiratory pathogens comprising direct fluorescent antibody (DFA) staining and viral culture. Because capture of influenza infections and accurate ascertainment of all risk factors was less complete after discharge from the health care center, only infections that occurred during the first 120 days after transplantation were considered. Influenza was defined as the isolation of influenza virus by culture or as evidence of influenza antigen detected by DFA in conjunction with consistent symptomatology. Antiviral treatment with an M2 inhibitor or oseltamivir (available since 1999) was performed at the discretion of the investigator. Unlike the fixed dosing duration recommended for antivirals in healthy subjects, in this trial, treatment was continued until resolution of presenting signs and symptoms and clearance of virus from respiratory secretions. Garrett Nichols, et al, reported as many as 29 % of subjects developed pneumonia following influenza infection in this large retrospective study. Ten percent of the subjects with influenza died [7].

The increased morbidity in transplant patients is associated with increased duration of virus shedding compared to that in healthy subjects. In otherwise healthy adults, elderly subjects and children, the median duration of viral shedding in untreated subjects was 70, 96 and 118 hours respectively. [8] In contrast, in the retrospective HSCT study, influenza virus was shed in nasopharyngeal secretions (evaluated at least weekly) for a median duration of 168 hours (7 days, range 2 to 37 days) [7]. This median duration of shedding included both allogenic and autologous HSCT subjects and both treated and untreated subjects. The mean duration of shedding in treated and untreated allogenic HSCT subjects was found to be longer than in autologous HSCT subjects (11.1 versus 6.7 days).

Johny et al, evaluated the use of zanamivir in the treatment of influenza in seven allogenic bone marrow transplant subjects. As with the large retrospective HSCT study, their standard of care also was to continue zanamivir until symptoms had subsided and it was documented that viral excretion had ceased. Viral shedding was checked every 7 days. Based on this protocol, the median duration of use of zanamivir was 15 days with a range of 5 to 44 days. [9]

Currently available treatments for influenza infection are the M2 ion channel inhibitors (e.g. amantadine, rimantadine) and the neuraminidase inhibitors (e.g. oseltamivir, zanamivir). Due to the emergence of resistant virus, the Center for Disease Control (CDC) has made an interim recommendation that neither amantadine nor rimantadine be used for the treatment or prophylaxis of influenza in the United States. [10] Oseltamivir is therefore one of the main drugs available for the evaluation of the treatment of influenza in the immunocompromised population.

1.1.2 Oseltamivir

Oseltamivir (Tamiflu®, Ro 64-0796) is an ethyl ester prodrug which is rapidly absorbed from the gastrointestinal tract after oral administration and metabolized in the liver by high capacity carboxylesterases to form oseltamivir carboxylate (Ro 64-0802), a potent, stable and selective inhibitor of influenza A and B neuraminidase enzymes. The active form, oseltamivir carboxylate is excreted unchanged by the kidney via glomerular filtration and active tubular secretion by the organic anion transport system. The efficacy and safety of oseltamivir in influenza treatment and prevention has been established in an extensive series of clinical studies in man.

Oseltamivir has been approved for the treatment of influenza in Europe, the United States and most other countries around the world. In adults and adolescents, the recommended dose is 75 mg twice daily for five days. In children 1 year of age and older recommended doses are 30, 45 or 60 mg bid based on body weight. In all age groups the recommended dose is administered bid for 5 days.

The approval of oseltamivir for the treatment of influenza is based on several controlled clinical trials. In the pooled population from these clinical trials encompassing adults aged from 13-97 years, many with significant co-morbidity, 1325 subjects were treated with oseltamivir (75 mg bid) and 1056 subjects received placebo. A total of seven influenza symptoms (both respiratory and constitutional) were captured on the diary card for adults. The time to resolution of all symptoms (on the diary card) decreased by 24 hours; from 124.5 hours in the placebo arm to 100.6 hours with oseltamivir 75 mg bid ($p < 0.0001$). [8] Further, in adults and adolescents with a proven influenza illness, oseltamivir treatment reduced overall antibiotic use for any reason by 26.7 % and the incidence of influenza-related lower respiratory tract complications resulting in antibiotic therapy by 55 %. The study concluded that oseltamivir treatment of influenza reduces lower respiratory tract complications, antibiotic use and hospitalizations in healthy and 'at risk' subjects [11].

Likewise, in the influenza-infected pediatric population (1 to 12 years of age), oseltamivir treatment ($n = 217$) was compared with placebo ($n = 235$). There was a reduction in the median duration of illness (defined based on resolution of temperature, cough, coryza and return to pre-illness health and activity) of 36 hours; from 137 hours with placebo to 101 hours in the oseltamivir treatment arm ($p=<0.0001$). The Canadian Acute Respiratory Infections Scale (CARIFS), validated for use in children, was used to collect symptom data on the pediatric diary card. The CARIFS scale comprised a total of 18 symptoms which were evaluated twice daily by the parent or guardian. There was a similar reduction in the time to alleviation of all CARIFS symptoms of 36 hours; from 100 hours in the placebo group to 63 hours in the oseltamivir group ($p < 0.0001$) [12].

Thus in both adults and children, the time to resolution of all symptoms was significantly reduced in the oseltamivir treatment arm compared with placebo.

Oseltamivir was well-tolerated in clinical trials. Approximately 11,000 subjects have received oseltamivir in the development program. The most common adverse events reported by adults, the elderly, and children were nausea and vomiting. Serious adverse

events (SAEs) were reported with a low and equal frequency by subjects taking active drug and placebo. Full details are given in the Investigator Brochure. [8]

There have been no controlled studies of oseltamivir treatment or prophylaxis in immunocompromised subjects. In the study by Garrett Nichols et al, patients who did not receive antiviral therapy shed virus for longer periods (mean duration, 11.3 days) than did those who were treated with M2 inhibitors (mean duration, 9.7 days) or neuraminidase inhibitors (mean duration, 7.5 days). Therapy with oseltamivir (but not rimantadine) appeared to be associated with shorter duration of shedding after controlling for steroid dose ($p < 0.08$). [7]. The efficacy of oseltamivir in the treatment of influenza in the immunocompromised population has also been demonstrated in other studies. In another small trial of oseltamivir for the treatment of influenza infection in subjects following HSCT, only two patients (5.1 %) developed influenza-related pneumonia. [13] In this study, all 39 HSCT subjects with influenza were treated with antivirals (oseltamivir or amantadine). In as many as 43 % of subjects, the duration of illness was greater than 7 days. This is unlike data from healthy adults, suggesting the possible need to evaluate a higher dose and/or longer duration of treatment with oseltamivir for this population.

While the efficacy of antiviral agents for influenza has been demonstrated in the otherwise healthy population, it has not been tested prospectively in the immunocompromised (transplant) population. [4] There is therefore a need to confirm the efficacy of antiviral therapy for influenza in prospective controlled trials in this patient population.

1.2 Rationale for the Study

This study is designed to investigate the optimal therapy for influenza in immunocompromised transplant recipients.

The rationale for conducting this study in the immunocompromised population is based on the following:

1. Immunocompromised subjects are at the highest risk for morbidity and mortality from influenza
2. Immunocompromised subjects are at the greatest risk for contracting influenza because immunization may be ineffective in this population
3. There is no drug approved for the treatment of influenza in this population

2. OBJECTIVES

2.1 Primary Objective

The primary objective of the study is to evaluate prospectively the efficacy of oseltamivir for the treatment of influenza in transplant recipients as measured by the time to resolution of influenza symptoms.

2.2 Secondary Objectives

The secondary objectives of the study are:

To evaluate the effects of conventional and high dose oseltamivir in transplant recipients on:

- The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis)
- The virologic course of influenza (proportion shedding and viral loads at different time points)
- Patient safety and tolerability
- The development of resistant influenza virus
- To characterize the population pharmacokinetics of oseltamivir (e.g. clearance, volume of distribution) in transplant recipients

3. STUDY DESIGN

3.1 Overview of Study Design and Dosing Regimen

This is a double blind, randomized, stratified, multi-center trial evaluating the efficacy of conventional and high dose oseltamivir for the treatment of influenza in immunocompromised patients. Immunocompromised transplant recipients who develop an influenza-like illness with a positive rapid diagnostic test for influenza, will be enrolled during the influenza season. Patients will be stratified by type of transplant [SOT or HSCT], by the time between onset of symptoms and treatment start [≤ 24 hours; > 24 hours], influenza vaccination status for current flu season [Yes; No] and by age [≤12 years; >12 years]. Eligible patients will be consented and randomized to receive oseltamivir twice daily for 10 days in one of two treatment arms: conventional dose or high dose (double the conventional dose). The primary end point in the study is the time to alleviation of all symptoms.

In this study, immunocompromised subjects will be represented by the transplant population. This is justified because the availability of new immunosuppressive agents has enhanced the number and survival of SOT and HSCT recipients, making them representative of the larger immunocompromised patient population.

Heterogeneity in the transplant population could confound the assessment of safety and efficacy in this study. Consequently, the SOT population in the protocol excludes subjects with transplants other than liver, kidney or liver and kidney. However, in order to still be representative of the transplant community in particular and the immunocompromised community in general, the study specifically includes subjects at the greatest risk for morbidity and mortality from influenza - the HSCT subjects. The study also excludes subjects with comorbid conditions that might impact the metabolism and excretion of the drug. As the prodrug and active drug accumulate in the presence of hepatic and severe renal impairment respectively, subjects with hepatic or severe renal impairment are excluded from the study. Other comorbid conditions that might preclude the assessment of efficacy and safety are also excluded from the study. These are reflected in several of the inclusion and exclusion criteria.

3.1.1 Rationale for Study Design

This study incorporates several features that distinguish it from classic placebo controlled trials. At the outset, a patient population that represents the general immunocompromised population had to be defined. As a placebo control arm was considered unethical in this population, an alternative control arm had to be identified. The response to placebo in pivotal oseltamivir registration trials in the healthy adult population with influenza was chosen as a control in lieu of a placebo arm in the current trial. In order to do this, the current study design and end points were designed to be similar to that in the pivotal registration trials.

Routine influenza surveillance during the 2007/2008 influenza season showed a significant increase in the global circulation of an oseltamivir-resistant influenza virus (A H1N1 with H274Y mutation). Therefore, baseline nasal and throat swab samples will be assessed for the presence of the H274Y-mutated influenza virus and results will be reported to clinical sites to assist in determining the most appropriate treatment options for individual patients.

The following sections provide rationale and justification for the specific aspects of the study design. They also provide information on how the results will be used to make recommendations on dose and duration of treatment.

3.1.1.1 Choice of treatment arms

The pivotal registration trials for oseltamivir in healthy adults with influenza had three treatment arms, two active (75 mg and 150 mg) and one placebo. Both treatment arms were found to be significantly better than placebo. No statistical comparisons were made between the two active treatment arms. Evaluation of the results in the two active treatment arms did not reveal any clinically meaningful difference. Based on these studies, the approved (conventional) dose for the treatment of influenza in adults is 75 mg.

A similar trial design was envisaged for this immunocompromised population. In an immunocompromised population there is a possibility of increased efficacy with a higher dose. In the trials in healthy adults, on day 4 (3 days after the start of treatment), the proportion of subjects shedding virus suggested a possible dose response relationship (36%, 28% and 23% in the placebo, 75 mg and 150 mg dose arms respectively). In healthy adults, this difference in the rate of viral shedding did not translate to any difference in clinical response in the two active treatment arms even though both active treatment arms were significantly better than the placebo arm. In the immunocompromised population too it is possible that while there might not be any difference between the two active treatment arms, one or both of these dose arms may be better than placebo. In this immunocompromised population, there is therefore an even greater need to evaluate the efficacy of a higher dose arm.

As with the trials in healthy adults, the ideal trial design for the current study would be one that compared efficacy of the conventional and high dose arms with placebo. However, as reviewed in an earlier section 1.1.1 the morbidity and mortality from influenza in the immunocompromised population is far greater than that in healthy adults.

Thus in one study as many as 29 % of subjects with HSCT developed pneumonia following influenza infection. [7] In another study in SOT recipients, biopsy of the transplanted organ revealed variable degrees of acute allograft rejection in 62 % of subjects. [5] Given the extent of morbidity, a placebo control appears unethical. This is further supported by a feasibility survey conducted by the Sponsor which demonstrated that a majority of transplant sites actually used antivirals (including oseltamivir) to treat influenza in this population. It was therefore decided to compare the conventional and higher dose arms with the placebo arm from the pivotal registration trials in healthy adults with influenza. Although this approach poses limitations, the Sponsor has made every effort to make sure that the results from such an approach are still meaningful. The following sections describe how the study design addresses these limitations.

3.1.1.2 Consistency with previous influenza treatment studies

In order to be able to compare the study findings with the response to placebo in healthy adults, this study has been intentionally designed to be consistent with previous registration trials for treatment of influenza in healthy adults. Some of the key design features consistent with previous studies include: two active treatment arms, subjects with influenza like symptoms recruited during the flu season, use of a **patient** diary to capture symptoms, primary clinical end point based on diary assessments, laboratory evaluation of virology and serology samples generally similar to previous trials and the primary efficacy analysis population is the Intent to treat Infected (ITTI) population.

Despite the above considerations, there are issues that can preclude the estimation of the response to placebo in an immunocompromised population comprising adults and children from that in healthy adults with influenza. To further mitigate this, the choice of endpoints and their interpretation is vital.

3.1.1.3 Interpretation of study results

Choice of primary end point and comparators

The current study includes both children and adults. This is because of the significant unmet need in both these age groups. The primary end point therefore had to be applicable to both age groups. In registration trials in adults, the primary end point was the time to resolution of all clinical symptoms (in the diary card). In registration trials in children, the primary end point was the reduction in the median duration of illness (defined based on temperature, cough, coryza and return to pre-illness health and activity). A key secondary end point in children – time to resolution of all clinical symptoms showed similar results. The primary endpoint in this study is the time to resolution (alleviation) of all symptoms.

The median of the primary end point in the active treatment groups (conventional and high dose) in this trial will be compared with the median of the time to resolution of symptoms in the placebo group from the pivotal registration trials. The high dose arm enables the detection of a possible dose response relationship in this immune compromised population. As a means to evaluate that possibility, an assessment of the relative efficacy of the two dose groups will be made in terms of median time to

alleviation of symptoms. Section 8.2.5 provides details on the statistical considerations and their rationale associated with these comparisons.

Effect of study design on efficacy end points

The main limitations of using the response to placebo from previous trials to estimate the response in this trial are due to the different populations (healthy versus immunocompromised), age groups (adults versus adults and children) and the potential difference in virulence of different strains. The potential difference in virulence of the predominant influenza viruses may be a confounder whose contribution can not be estimated. However the difference in populations and age groups is likely to result in the placebo response from pivotal registration trials underestimating the time to alleviation in the immunocompromised population. This is because the time to alleviation of symptoms is shorter in the healthy population compared to the immunocompromised population. [13] The placebo response in the pivotal trials will therefore underestimate the response in the current trial. The Sponsor is thus using a very conservative approach in comparing the active treatment arms in this study with the response to placebo in healthy subjects. If one/both of the treatment arms is statistically better than placebo, it is considered that this is a meaningful effect for this population. If both treatment arms are not different from placebo and this is seen consistently across several end points, it may be hypothesized that the risk from influenza for the immunocompromised population treated with oseltamivir is comparable to that for a healthy untreated adult with influenza.

Possible dosing recommendations based on current study

Healthy subjects who were given placebo in the previous pivotal oseltamivir treatment studies will be used as the control arm for this study. When the two active treatment arms are compared with this placebo control, several outcomes are possible. These outcomes will need to be discussed with health authorities. Some outcomes and possible interpretations of the results are presented below:

1. Conventional and high dose are both shown to be better than placebo: Both treatment arms will be compared. If no difference then the conventional dose would be the dose recommended for treatment in this population. If a difference is seen between treatment arms, then an evaluation (including benefit/risk assessment) will be made to confirm whether the higher dose should be the recommended dose.
2. One active treatment arm is superior to placebo while the other is not: In this instance the dose that is shown to be superior will be recommended unless other considerations such as safety dictate otherwise.
3. Both conventional and high dose arms are not different from placebo: If this is seen consistently across several secondary clinical end points, it may be hypothesized that oseltamivir has reduced the risk in the immunocompromised population to the more acceptable level in the untreated healthy adult population.
4. It is also possible that both conventional and high dose treatment have a more protracted clinical course than the placebo arm. In this situation, it may be difficult to make conclusions about efficacy.

Irrespective of the efficacy outcomes, the study will still provide useful information of the safety of oseltamivir in this population.

Possible recommendations for duration of treatment based on the current study

Once a dose is chosen a determination will need to be made on whether the recommended duration of treatment should be 5 or 10 days or longer. This decision will be based both on the clinical and virologic course of influenza.

The recommended duration of treatment for influenza in healthy adults is five days. In this study, the duration of dosing is ten days because viral shedding is typically longer in the immunocompromised population than in healthy adults. The general standard of care for this population has been to treat for as long as the patient continues to shed virus (even after the resolution of symptoms) [7] [9]. The duration of treatment for this population is therefore individualized based on clinical response and duration of viral shedding. It can often be more than 10 days. While it is possible that 10 or more days of treatment may be needed [7] [9], it is also possible that 5 days of treatment may still be adequate. Therefore, in this study the clinical and virologic course (proportion shedding virus at day 6 and later) will be evaluated to make recommendations on the duration of dosing. No statistical comparisons will be made.

Stratification

Stratification in this study has no bearing on any comparisons with results from previous trials. However, in order to evaluate a dose response relationship between the two active treatment arms in this trial, stratification remains essential.

Because HSCT recipients are at the greatest risk of morbidity and mortality from influenza, patients will be stratified by transplant type (SOT versus HSCT). Patients will be stratified by time between onset of symptoms and treatment start (< 24 hours or ≥ 24 hours) because previous experience in otherwise healthy adults and children has shown that patients treated early in their illness respond to treatment better than those who are treated later. Patients will also be stratified by age (≤ 12 years and > 12 years) because otherwise healthy children shed virus longer than otherwise healthy adults. It is expected that a similar difference in the duration of viral shedding would exist between immunocompromised children and adults. As vaccinated subjects may respond faster to therapy, the study is also stratified based on influenza vaccination status for the current flu season (Yes; No).

3.1.2 Rationale for Dose Selection and Adjustment

Dose Selection

The dose of oseltamivir to be used in this study is the conventional, approved dose for children and adults in the treatment of influenza. There will be a second higher dose for comparison which is two times the conventional dose. This higher dose is used based on theoretical considerations which suggest that the higher dose may be associated with improved efficacy and decreased emergence of resistance.

The anticipated pro-drug and metabolite exposures from this higher dose are not expected to exceed maximum exposures seen previously in the oseltamivir development program. The safety and tolerability of the higher dose regimen has already been demonstrated in treatment studies of immunocompetent adult subjects (n = 447). [14] In a study to demonstrate cardiac safety, in the highest dose group treated with 450 mg b.i.d. for 5 days [n = 99], no subject had a serious adverse event, nor withdrew prematurely. In Phase I studies in adults, oseltamivir has been administered in multiple doses of up to 500 mg b.i.d. Doses of 200 mg b.i.d. and greater have been associated with increased gastrointestinal adverse effects (nausea and vomiting). [8] In adult subjects with creatinine clearances of $\leq$ 30 mL/min, doses of 100 mg b.i.d. for 6 days were well tolerated, despite steady-state oseltamivir carboxylate exposures approximately 10-fold higher than those achieved with standard dosing in renally competent individuals. [15] No other adverse effects were reported more frequently with higher doses and no serious adverse events have been reported within the volunteer studies. Co-administration of oseltamivir with food has been demonstrated to substantially reduce the frequency and severity of gastrointestinal side effects.

Thus, the rationale for the higher dose arm in this study is to explore the possibility of improved efficacy and decreased emergence of resistance without an undue increase in risk.

Drug interactions with immunosuppressive medications have also been evaluated. The pharmacokinetics of oseltamivir and oseltamivir carboxylate after administration of 75 mg oseltamivir (conventional adult dose) in subjects with a well-functioning, stable renal allograft who were being maintained on immunosuppressive therapy were studied. These were similar to those described in the literature for adults with comparable degrees of renal function. Oseltamivir was well tolerated and had no clinically relevant effect on the steady-state pharmacokinetics of cyclosporine A, tacrolimus, or mycophenolate mofetil. [8]

Duration of dosing

The duration of dosing chosen for this population (10 days) is longer than that in the healthy adult and pediatric populations (5 days). This is based on observations that the viral shedding and illness are typically longer in immunocompromised patients than it is in healthy adults. [7] [13]

Dose Adjustments

In this study, dose adjustment for subjects with an estimated Cr Cl between 10 to 30 mL/min/1.73M^2 will comprise decreasing the frequency of administration to once daily. This is based on current label recommendations for adults with renal impairment (Table 1).

Table 1 Recommended doses in healthy adults for treatment of influenza

Creatinine Clearance	Recommended dose for treatment
> 30 (ml/min)	75 mg twice daily
> 10 to ≤ 30 (ml/min)	75 mg once daily or 30 mg suspension twice daily
≤10 (ml/min)	Not recommended
dialysis patients	Not recommended

The dose adjustment recommendations are considered suitable approaches for children with renal impairment. This is based on the reasonable assumption that a similar relationship exists in both children and adults, between *changes* in creatinine clearance and resultant *changes* in exposure of oseltamivir and oseltamivir carboxylate. Doses will be adjusted for renal impairment in this protocol per the schema below. More detailed information on dosing, dose adjustments and maintenance of the blind are provided in later sections 6.1, 6.2, 6.3.

Table 2 Recommended dose modifications for NV 20234 trial subjects

Randomized Dose Arm	**Unit dose (mg)**	**Creatinine Clearance (ml/min OR ml/min/M^2)**	**Dose frequency**
Conventional	30	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	45	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	60	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	75	> 30	Twice daily
		> 10 to ≤ 30	Once daily
High	60	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	90	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	120	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	150	> 30	Twice daily
		> 10 to ≤ 30	Once daily

In general, no dose adjustment is considered necessary in subjects with mild-moderate hepatic impairment (based on pharmacokinetic findings in adults with chronic hepatic impairment) receiving usual therapeutic doses of oseltamivir. [16] However, as the

relevance of these findings to the specific patient population in this study is unclear, subjects with overt (based on physical signs and symptoms) hepatic impairment at baseline will be excluded from this study as mentioned in section 4.3.

3.1.3 End of Study

The study comprises 10 days of treatment with follow up visits approximately 5 and 30 days later as shown in the schedule of assessments. A rapid diagnostic test for influenza will be performed at the end of the 10 days of treatment. Subjects still having influenza based on the rapid diagnostic test will be treated per the local standard of care by the principal investigator.

3.2 Number of Subjects/ Assignment to Treatment Groups

A **minimum** of 250 patients will be enrolled in this study (approximately 125 per arm). After screening, patients will be randomly assigned to one of the two active treatment groups.

3.3 Centers

This will be a multicenter study with approximately 140 centers in the Northern hemisphere. Centers will be activated to recruit patients during the influenza season. The centers to be included in the study are those which perform or manage SOTs, HSCTs or both.

4. STUDY POPULATION

4.1 Overview

The study population comprises immunocompromised adults (including adolescents) and children who have influenza. Additionally, the subjects must not have other medical conditions that will preclude the assessment of efficacy or safety. Influenza vaccinated and non-vaccinated subjects are eligible to participate in this study.

Under no circumstances are patients who enroll in this study permitted to be re-randomized to this study and enrolled for a second course of treatment.

4.2 Inclusion Criteria

- Age greater than or equal to 1 year
- Rapid diagnostic test positive for influenza in the 24 hours prior to first dose
- Immunocompromised subject defined as documented:
    - SOT (liver, kidney or both) recipient OR
    - Allogenic HSCT
- Receiving ongoing immunosuppression, OR, in the investigator's opinion, not immune reconstituted
- Symptoms suggestive of influenza like illness including, but not limited to fever, cough, or coryza

- Less than or equal to 48 hours between onset of influenza like illness and first dose of study drug
- Acceptable renal function defined as:
    - Most recent creatinine clearance in the 6 months prior to randomization is > 30 ml/min in adults and > 30 ml/min /1.73M^2 in children. Creatinine clearance estimated from serum creatinine (Appendix 1) measured when subject is not receiving any renal replacement therapy **OR**
    - For patients who have not had a creatinine clearance assessment in the 6 months prior to randomization, baseline creatinine clearance > 10 ml/min in adults and > 10 ml/min /1.73M^2 in children (Appendix 1) **OR**
    - For patients whose most recent creatinine clearance in the 6 months prior to randomization is < 30 ml/min in adults and < 30 ml/min /1.73M^2 in children, baseline creatinine clearance > 10 ml/min in adults and > 10 ml/min /1.73M^2 in children (Appendix 1)
- Parent/guardian willing and able to comply with study requirements and give consent. (country specific age cut off)
- Patient able to comply with study requirements and willing to give assent, as appropriate (country specific age cut off)
- For adult patients, willing and able to comprehend and give written informed consent
- Patients, in the reproductive age group, must agree to utilize an effective method of contraception throughout the study period and for one reproductive cycle following cessation of study therapy
- Females of childbearing potential must have a negative urine pregnancy test prior to start of study medication

4.3 Exclusion Criteria

- SOT within 6 months of the time of randomization
- Solid Organ Transplant other than liver, kidney or liver and kidney
- Have in the investigator's opinion experienced acute rejection in the 4 weeks prior to randomization
- HSCT patients with no evidence of engraftment (engraftment is defined as the point at which a patient can maintain a sustained absolute neutrophil count (ANC) of >500/mm^3 and sustained platelet count of ≥ 20,000/mm^3, lasting ≥ 3 consecutive days without transfusions)
- HSCT subjects not discharged from hospital after their initial hospitalization for transplantation
- Have evidence of veno-occlusive disease, acute or chronic extensive graft versus host disease at the time of randomization

- Have clinical evidence for hepatic decompensation at the time of randomization (clinical icterus, ascites, hepatic encephalopathy, coagulopathy)
- Have cirrhosis of the liver at the time of randomization
- Currently or in the six months prior to randomization using T cell depleting antibodies (example: antithymocyte globulin, antilymphocyte globulin) for management of transplant
- Have other co-morbid conditions that could affect patient survival or graft function including, but not limited to, a post-transplant lymphoproliferative disease (PTLD), autoimmune disease including inflammatory bowel disease and psoriasis, untreated thyroid disease, and significant active infection
- Patients currently receiving any form of renal replacement therapy including hemodialysis, peritoneal dialysis or hemofiltration
- Have evidence of active or uncontrolled opportunistic infections (bacterial, fungal, or viral - including cytomegalovirus [CMV] or polyoma virus [BKV]) at the time of randomization. Patients with HCV or HBV are not excluded.
- Patients with known HIV infection
- Patients who are being evaluated or treated for an active malignancy (other than the malignancy for which the SOT or HSCT may have been performed) at the time of randomization
- Patients with uncontrolled vascular, neurologic or pulmonary disease. Uncontrolled is defined as disease requiring change of therapy or hospitalization in the 4 weeks preceding randomization. Change of therapy is defined as dose increase or change of medication prior to onset of present influenza like illness.
- Patients with severe diarrhea or other gastrointestinal disorders which might interfere with their ability to absorb oral medication, including diabetic patients with previously diagnosed diabetic gastroenteropathy
- Allergy to the test medication
- Patients with hereditary fructose intolerance (for subjects who will be taking the liquid formulation)
- Influenza vaccination in the 2 weeks prior to randomization
- Antiviral treatment (example: amantadine, rimantadine, zanamivir and ribavirin) for influenza in the 2 weeks prior to randomization
- Patients taking probenecid medication
- Patients who are pregnant or breast-feeding
- Participation in a clinical trial or expanded access trial with an investigational drug in the 4 weeks prior to randomization or concomitantly with this study

4.4 Concomitant Medication and Treatment

Antiviral treatments with activity against influenza (e.g. amantadine, rimantadine, zanamivir, ribavirin, and additional oseltamivir above that specified for this study) are not allowed during the **10-day treatment phase of the** study. Concomitant use of an investigational drug during the study is also excluded. Influenza vaccination after randomization is not allowed. Live attenuated vaccines may not be advisable for this population. It is possible that concurrent use with oseltamivir may render live vaccines ineffective.

Medications required for the routine care of the patient including those required for management of the transplant (excluding lymphocyte depleting antibodies, intravenous immunoglobulins) may be continued during the study. Concomitant use of probenecid (irrespective of the indication) is not allowed during the study.

4.5 Criteria for Premature Withdrawal

The investigator **must** discontinue treatment if the creatinine clearance is < 10 ml/min in adults or < 10 ml/min/1.73 M^2 in children. The investigator **must** also discontinue treatment from all subjects with intercurrent illnesses or adverse events suggestive of hepatic decompensation. The investigator also has the right to discontinue treatment in the event of intercurrent illness, adverse events, treatment failure, protocol violations, administrative reasons or other reasons.

The investigator must discontinue treatment with study drug if the patient is to be treated concomitantly with another antiviral for influenza, for example, amantadine, rimantadine, or zanamavir. However, all patients, including those who discontinue study drug prematurely and/or are treated with another antiviral, will be required to return for follow up approximately 5 and 30 days after the last dose of study medication (day 15 and day 40 assessments).

Subjects have the right to withdraw from the study at any time for any reason.

An excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of subjects should be avoided. Should a subject decide to withdraw, all efforts will be made to complete and report the observations prior to withdrawal as thoroughly as possible.

The investigator should contact the subject or a responsible relative either by telephone or through a personal visit to establish as completely as possible the reason for the withdrawal. A complete final evaluation at the time of the patient's withdrawal should be made with an explanation of why the subject is withdrawing from the study. If the reason for removal of a subject from the study is an Adverse Event, the principal specific event will be recorded on the CRF.

In the case that the subject decides to prematurely discontinue study treatment ["refuses treatment"], he/she should be asked if he/she can still be contacted for further information. The outcome of that discussion should be documented in both the medical records and in the CRF.

4.6 Replacement Policy [Ensuring Adequate Numbers of Evaluable Subjects]

4.6.1 For Subjects

In order to maintain the required number of patients who are evaluable for efficacy, patients who are identified as being infected with influenza A H1N1 H274Y at baseline will be replaced by enrolling additional patients on a rolling basis. Replacement patients will be stratified and randomized to treatment in the same manner as original patients. Replacement patients will not be "selected" by dose group or strata (age, vaccination status, transplant type, time between symptom onset and treatment) to match the patients whom they replace.

4.6.2 For Centers

A center may be replaced for the following administrative reasons:

– Excessively slow recruitment.

– Poor protocol adherence

5. SCHEDULE OF ASSESSMENTS AND PROCEDURES

Table 3 Schedule of Assessments

	Baseline	Treatment					Follow-up	
Study Day	**1 (pre-dose)**	**1**	**2 or 3[f,g]**	**6[g]**	**8[f, g]**	**11[g, h, i]**	**15[g]**	**40[g, j]**
Informed Consent/Assent	**x**							
Medical history	**x**							
Demographics	**x**							
Height and weight	**x**					x		
Pregnancy Test [a]	**x**					x		x
Rapid diagnostic test for influenza virus shedding	**x**					x		
Safety Labs [b]	**x**					x		
Serology for influenza antibody titer	**x**							x
Physical Examination	**x**					x		x
Vital Signs (including PR, RR, temperature, Blood pressure)	**x**		x	x	x	x	x	x
Nasal and throat swabs for viral shedding and viral load [c, d]	**x**		x	x	x	x	x	x
Review of **electronic** diary **data** [e]			x	x	x	x	x	x
Drug Administration		←	—	—	—	→		
Collection of unused study medication and empty containers						x		
Previous Diseases	**x**							
Previous/Concomitant medications	**x**	←	—	—	—	—	—	→
Adverse Events/Sec Illnesses and Treatments		←	—	—	—	—	—	→
Rejection, GVHD		←	—	—	—	—	—	→

a. Urine pregnancy test for patients of child-bearing potential according to the judgment/discretion of the investigator

b. Hematology (CBC with differential) and chemistry (AST, ALT, total bilirubin, urea, Cr). Serum creatinine testing may be done at a local laboratory at any time to calculate creatinine clearance and make dose adjustments. Patient may receive his/her first dose prior to Cr Cl results being available, provided certain renal function criteria are met - see [4.2] [5.3]

c **Baseline swab samples will be assessed for the presence of influenza A H1N1 with H274Y mutation.**

d. Two nasal and one throat swab for viral culture and RT-PCR

e. Flu symptoms, temperature, **and** date/ time of oseltamivir dose will be recorded by the patient **in electronic patient** diar**ies** twice daily on days 1 – 10, and once daily thereafter.

f. A home visit may be made on day 2 or 3 (for patients who are too ill to come into the clinic) and day 8

g. **Day 2/3 visit window = +1 day**. Day 6 visit window = **+/- 1 day**; Day 8 visit window = +1 day. Day 11 visit window = ± 1 day. Day 15 and day 40 visit occur approximately 5 and 30 days after the last dose respectively. Day 15 window = ± 1 day. Day 40 window ± 2 days.

h. Only weight to be assessed. Height is not necessary at this visit. Study drug is taken on day 11, only if the first dose was taken after 4 PM on Day 1 [5.3] Subjects who are rapid diagnostic positive can be treated per standard of care. They will be required to come for their follow-up visits on day 15 and day 40.

i. Subjects who discontinue treatment prematurely will have an end of treatment (day 11) assessment at the time of treatment discontinuation or the following day. They will be required to attend their follow up visits approximately 5 and 30 days after the last dose (day 15 and day 40 assessments).

j. Subjects who discontinue during follow-up will have an end of follow-up (day 40) assessment. This visit must occur within 30 days of the last dose.

5.1 Screening Examination and Eligibility Screening Form

Trial sites will be activated during the influenza season.

All subjects must provide written informed consent (assent where applicable) before any study specific assessments or procedures are performed. Screening will take place at the baseline visit prior to first dose. **However, if necessary, screening may take place the day before the baseline visit, as long as the patient is randomized and receives his/her first dose of study medication within 48 hours of influenza symptom onset**.

Subjects will be assessed for inclusion and exclusion criteria and those who fulfill all the inclusion and none of the exclusion criteria will be randomized into the study.

An Eligibility Screening Form [ESF] documenting the investigator's assessment of each screened subject with regard to the protocol's inclusion and exclusion criteria is to be completed by the investigator.

A screen failure log must be maintained by the investigator.

5.2 Procedures for Enrollment of Eligible Subjects

Once a subject has fulfilled the entry criteria, he/she will be randomized to one of two treatment groups. The subject randomization numbers will be generated by Roche or its designee and incorporated into double-blind labeling.

The investigator or designee will use the CRF pre-printed with the assigned subject number and enter the randomization number provided by IVRS for allocation to the treatment groups in the appropriate place on each subject's CRF.

Randomization will be stratified by transplant type (SOT or HSCT); time between onset of symptoms and treatment start (≤ 24 hrs or > 24 hrs), influenza vaccination status (Yes; No) and patient's age (≤ 12 years or > 12 years).

5.3 Clinical Assessments and Procedures

At all visits subjects will receive the routine care for their primary illness (transplant). Routine patient care (including the adjustment of doses of any of the immunosuppressive drugs) will be dictated by tests performed locally at the trial site. Dose adjustments of oseltamivir will be based on serum creatinine done at the local laboratory. All safety labs will be sent to a central laboratory. Results for these laboratory parameters will be used by the sponsor/designee to assess overall safety.

All assessments and procedures will be performed according to the Schedule of Assessments (Table 3). Additional information regarding these procedures not provided in the Schedule of Assessments is provided below.

Study Day 1

The baseline and study day 1 assessment may be performed at the same visit.

Study medication, **electronic** diar**ies**, and thermometers will be dispensed. Patients or guardians/parents will be instructed how to complete **electronic** symptom diar**ies**

(Appendix 2) (Appendix 3), temperature recording, and treatment administration details, including time of each oseltamivir dose. The first diary **entries** will be **made at the site** before the first dose of study drug.

Baseline nasal and throat swab samples will be assessed for the presence of oseltamivir-resistant influenza A H1N1 with H274Y mutation. As soon as they are available, results will be reported to the sites to assist in patient management.

The date of the first dose of study drug is defined as study day 1. Once randomized, the first dose of study drug will be administered in the clinic. Study day 2 will begin at 12 midnight of the same calendar day. If the first dose of study drug is taken after 4 pm on day 1, the next dose of study drug will be taken in the morning of day 2. In this case, the last dose of study drug will be taken on the morning of study day 11.

If the first dose of study drug is taken prior to 4 pm on day 1, the next dose of study drug should be taken in the evening of the same day (i.e. prior to midnight on the same calendar day with a minimum of 7 hours between doses). For these patients the last dose of study drug will be taken in the evening of study day 10. More information on dosing is provided later. 6.1

The study will utilize a central safety laboratory. However, to determine the correct dose, a local serum creatinine will be drawn at baseline in all subjects and used to calculate creatinine clearance to determine if a dose adjustment is necessary. No dose adjustments are required for subjects whose creatinine clearance is > 30 ml/min in adults (> 30 ml/min/1.73 M^2 in children). Oseltamivir dosing frequency for patients whose creatinine clearance is between 10 to 30 ml/min in adults (10 to 30 ml/min/1.73 M^2 in children) will be adjusted to once daily.

For patients who meet the following criteria in section 4.2, dosing will commence only after the baseline creatinine clearance are available and if baseline creatinine clearance > 10 ml/min in adults and > 10 ml/min/1.73M^2 in children. The dose will be adjusted to once daily if the baseline Cr Cl is between 10 to 30 ml/min in adults (10 to 30 ml/min/1.73 M^2 in children)

- most recent creatinine clearance in the 6 months prior is < 30 ml/min in adults and < 30 ml/min/1.73M^2 in children)
- patients who have not had a creatinine clearance assessment in the 6 months prior to randomization

For patients who meet the following criteria in section 4.2 dosing may commence prior to availability of baseline Cr Cl. Subjects will receive twice daily doses and dosing will be adjusted to once daily if the baseline Cr Cl is between 10 to 30 ml/min in adults (10 to 30 ml/min/1.73 M^2 in children).

- most recent creatinine clearance in the 6 months prior is > 30 ml/min in adults and > 30 ml/min /1.73M^2 in children)

Study Days 2 - 11

Study day 2 will begin at 12 midnight of study day 1.

It is expected the study staff will maintain close contact with patients, especially during the first few days of the study. The study staff should inquire about any adverse events, check that the patient or parent/guardian **is entering data into the electronic** diary **properly**, and assess drug compliance. During the dosing period, diary symptoms should be assessed and temperature should be recorded at the same time that the study drug is taken.

Dosage adjustment will be performed based on creatinine clearance as described in the Dose Modification section 6.1.1.

End of treatment Day 11

The end of treatment visit for all subjects is on day 11 (irrespective of whether they took one or two doses on day 1). Subjects who discontinue study medication prematurely will have all day 11 assessments completed at the time of discontinuation or the following day.

After all day 11 study assessments are completed, a rapid diagnostic test will be performed at this visit. If the rapid diagnostic test is positive, the subject may be treated per standard of care at the discretion of the investigator.

All subjects (including those who discontinue study medication prematurely and those who are positive for influenza on their rapid diagnostic test at the end of treatment visit) will be required to return for follow up approximately 5 and 30 days after the last dose (day 15 and day 40 assessments)

Study days 12 – 40

Study day 15 is an important visit as it is the first assessment of viral culture after cessation of treatment.

End of Follow-Up Day 40

All subjects must attend an end of follow-up visit on day 40. This visit is important as it is the only post-baseline visit where a serology sample for influenza antibody titers is collected.

If the patient is withdrawn after completion of treatment (after the day 11 assessment), a termination visit should be arranged. This visit should be the end of follow-up visit assessment [Day 40]. This visit must occur within 30 days of the last dose.

Patients or parents/guardians who do not return to the clinic will be contacted by telephone to ascertain their health status, or that of their child's, record information on adverse events, and to ask that they return the **electronic** diary.

5.3.1 Efficacy Assessments

The primary end point in this study is the time to resolution of all influenza symptoms as recorded in the **patient** diary.

The clinical efficacy parameters in this study are:

1. Symptoms of influenza-like illness. These are captured in the diary for both adults and children. (Appendix 2) (Appendix 3) Influenza symptoms are graded on a nominal scale from zero (absent/no problem) to 3 (severe or major problem). A score of zero is considered asymptomatic. The primary end point, time to alleviation of all symptoms is comprised of all the symptoms captured in the diary.
2. Temperature. This is captured **in** the diary. Temperature is used for assessment of the primary and several secondary end points.

5.3.2 Safety

Safety parameters in this study include adverse events, vital signs, and clinical laboratory evaluations.

Pre-defined symptoms of influenza captured in the adult and pediatric diar**ies** are not to be reported as adverse events unless they can be further qualified. Thus 'headache due to stress at work' is reported as an adverse event. However, unexplained 'headache' is considered a predefined symptom related to influenza and not an adverse event.

Adverse events such as bronchitis, pneumonia, otitis media and sinusitis are considered secondary illnesses of influenza and should be recorded as adverse events.

Other adverse events to be expected in the transplant population such as rejection and graft versus host disease (in HSCT subjects) will also be collected as adverse events.

5.4 Laboratory Assessments

The laboratory assessments include those for efficacy and safety.

Efficacy

The efficacy laboratory assessments are used for laboratory confirmation of influenza. These are:

Nasal and throat swabs. Two nasal and one throat swab will be collected as described in the Schedule of Assessments. All swabs are sent to a central laboratory for RT-PCR and viral cultures. **Influenza virus shedding will be assessed**. **Baseline samples will be assessed for the presence of oseltamivir-resistant influenza A H1N1 with the H274Y resistance mutation using a real-time PCR assay.** A phenotypic assay will be performed to determine the susceptibility of the last positive viral isolate from each patient. If required, a genotypic assay to determine the contribution of both the neuraminidase and haemagglutinin genes to decreased susceptibility will be performed.

During the course of treatment, neither the investigator nor the patient will know which participants have ongoing positive viral cultures for influenza. **However, in order to assist in patient management, the results of the assay to detect influenza A H1N1 H274Y at baseline will be reported to the sites as soon as they are available.** At the

end of treatment (day 11), a rapid diagnostic test is permitted for the diagnosis of ongoing influenza.

Serology. Blood samples for influenza antibody titer will be collected according to the Schedule of Assessments.

Safety

The safety laboratory assessments in this study, including the assessment of serum chemistry and hematology will be done at the central laboratory. Serum chemistry assessments comprise AST, ALT, total bilirubin, urea and creatinine. Hematology assessments include CBC and differential count. The total volume of blood loss for laboratory assessments will be approximately 20 mL for the entire duration of the study.

Protection of patient confidentiality, section 16 will extend to any data generated from the assaying of these samples. Biological samples taken from all patients may be infectious and will be classified as "diagnostic specimens" for dispatch purposes.

The Principal Investigator may draw blood for serum creatinine to be assessed at the local laboratory at any time during the study. The Principal Investigator may use the creatinine levels to calculate creatinine clearance and adjust dose of study drug if required.

6. INVESTIGATIONAL MEDICINAL PRODUCT

The Investigational Medical Product in this study is oseltamivir dry powder (to be reconstituted to a concentration of 12 mg/ml) or 75 mg capsules and matching placebo.

The Investigational Medicinal Products will be supplied, packaged individually for each subject and labeled in accordance with Roche Standard and local regulation by Roche Clinical Trial Supply, Basel, Switzerland.

6.1 Dose and Schedule of Study Drug

Oseltamivir will be given twice daily over 10 days for a total of 20 doses. The doses need to be taken at 12 hourly intervals. Under no circumstances is a subject allowed to take two doses within 7 hours of each other.

Patients will be randomized to receive either conventional or high dose of study drug.

Conventional dose:

Children ages 1 - 12 years: Oseltamivir syrup

≤ 15 kg	30 mg twice daily
> 15 – 23 kg	45 mg twice daily
> 23 – 40 kg	60 mg twice daily
> 40 kg	75 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules
75 mg twice daily

Subjects randomized to the conventional dose will simultaneously receive matching placebo so as to blind them and the investigator from the high dose arm.

High dose:

Children ages 1 - 12 years: Oseltamivir syrup

≤ 15 kg	60 mg twice daily
> 15 – 23 kg	90 mg twice daily
> 23 – 40 kg	120 mg twice daily
> 40 kg	150 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules
150 mg twice daily

6.1.1 Dose Modifications

No dose adjustments/modifications are required for subjects whose creatinine clearance is > 30 ml/min in adults (> 30 ml/min/1.73 M^2 in children).

In both treatment arms, the dosing frequency will be decreased to once daily in patients with severe renal impairment (Cr Cl in adults between 10 – 30 ml/min and children between 10 – 30 ml/min/1.73M^2). For example, the 75 mg bid frequency will be decreased to 75 mg once daily.

At any time during the study if the investigator feels that renal function is compromised, dosing may be decreased to once daily or withheld until such time creatinine clearance results are available. Dosing may then be resumed as appropriate based on the creatinine clearance. However, it is vital that dosing not be inappropriately withheld for extended periods of time especially in the first 3 days of treatment when viral titers may be high. It is also important that dose modifications are clearly and accurately recorded for assessment of pharmacokinetic results.

For adolescents and children, the creatinine clearance will be estimated using a Schwarz equation: (Appendix 1)

For adults the method of Cockcroft-Gault will be used to estimate creatinine clearance. (Appendix 1)

6.2 Preparation and Administration of Study Drug

Oseltamivir will be provided in two forms:

1. Capsules containing 75 mg of active drug and packaging material consisting of pregelatinized starch, povidone, talc and sodium stearyl fumarate. All participants 13 years and older will receive this dosage form. *Oseltamivir capsules should be stored at 25ºC.*
2. A pediatric suspension containing 12 mg oseltamivir per ml of reconstituted solution and the following excipients: sorbitol, titanium dioxide, sodium benzoate, xanthan gum, monosodium citrate, saccharin sodium and Permaseal11900-31 Tutti Frutti (flavor). All participants 12 years and under will receive this dosage form. *Oseltamivir dry powder for suspension [pediatric syrup] should be stored at 25ºC.*

After reconstitution, the suspension should not be used for longer than 10 days. Store constituted suspension under refrigeration at 2º to 8ºC. Do not freeze.

Matching placebo will be available as capsules and suspension. Subjects in the conventional dose arm will get the conventional dose and matching placebo so that they are blinded from the high dose arm.

Each subject will be dispensed a medication pack that will provide enough medication to cover 20 doses. For subjects randomized to the conventional dose arm, the medication pack will contain a bottle of oseltamivir dry powder or a blister wallet with oseltamivir capsules and matching placebo. For subjects randomized to the high dose arm, the medication pack will contain two bottles of oseltamivir dry powder or two blister wallets with oseltamivir capsules. Irrespective of the treatment group the subject is randomized to, for each dose the subject will take the same amount from both bottles or blister wallets provided in the medication pack such that the sum of the amounts from each immediate container constitutes one dose.

One dose is to be administered twice per day at approximately 12-hour intervals with a light snack or glass of milk or fruit juice. The first dose of study medication will be administered in the clinic at the time of randomization.

6.3 Blinding and Unblinding

Randomization will be administered by a central randomization center.

The Randomization List will not be available at the study center, to the study monitors, project statisticians or to the project team at Roche. Emergency codes, or another adequate method of unblinding, will be implemented before study start, if the identity of the test medication is necessary for patient management in the case of a serious adverse event. Emergency codes should not be broken except in the case of emergency situations. Any request from the investigator for information about the treatment administered to study subjects for another purpose must be discussed with Roche/designee.

As per regulatory reporting requirement, Roche/designee will unblind the identity of the study medication for all unexpected [as per IB] serious adverse events that are considered by the investigator to be related to study drug. Details of patients who are unblinded during the study will be included in the Clinical Study Report.

All other individuals directly involved in this study will remain blinded until the final analysis of the primary parameter.

The randomization will be stratified by transplant type, duration of symptoms, influenza vaccination status and age and will be provided by the Roche randomization group for the IVRS vendor.

6.4 Assessment of Compliance

Accountability and subject compliance will be assessed by maintaining adequate “drug dispensing” and return records.

Subjects will be asked to return all used and unused drug supply containers at the end of the treatment as a measure of compliance.

A Drug Dispensing Log must be kept current and should contain the following information:

- the identification of the subject [randomization and medication numbers] to whom the study medication was dispensed
- the date[s], quantity of the study medication dispensed *to* the subject
- the date[s] and quantity of the study medication returned *by* the subject

This inventory must be available for inspection by the Monitor. All supplies, including partially used or empty containers and the dispensing logs, must be returned to the monitor at the end of the study.

6.5 Destruction of Study Drug

Local or institutional regulations may require immediate destruction of used investigational product for safety reasons. In these cases, it may be acceptable for investigational site staff to destroy dispensed investigational product before a monitoring inspection provided that source document verification is performed on the remaining inventory and reconciled against the documentation of quantity shipped, dispensed, returned and destroyed. Written authorization must be obtained from the sponsor or designee at study start up before destruction.

Written documentation of destruction must contain the following:

– Identity [batch numbers or subject numbers] of investigational product[s] destroyed

– Quantity of investigational product[s] destroyed

– Date of destruction

– Method of destruction

– Name and signature of responsible person [or company] who destroyed investigational products[s]

7. SAFETY INSTRUCTIONS AND GUIDANCE

7.1 Adverse Events (AEs) and Laboratory Abnormalities

7.1.1 Clinical AEs

Per the International Conference of Harmonization [ICH], an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign [including an abnormal laboratory finding], symptom, or disease temporally associated with the use of a medicinal [investigational] product, whether or not considered related to the medicinal [investigational] product. Pre-existing conditions which worsen during a study are to be reported as AEs. Influenza signs and symptoms reported on the **patient** diary

will be summarized as efficacy end points and need not be captured as adverse events. However, secondary illnesses due to influenza must be reported as adverse events.

7.1.1.1 Intensity

All clinical AEs encountered during the clinical study will be reported on the AE page of the CRF.

Intensity of AEs will be graded on a four -point scale [mild, moderate, severe, life-threatening] and reported in detail on the CRF.

Mild	discomfort noticed but no disruption of normal daily activity.
Moderate	discomfort sufficient to reduce or affect daily activity.
Severe	inability to work or perform normal daily activity
Life Threatening	represents an immediate threat to life

7.1.1.2 Drug - Adverse event relationship

Relationship of the AE to the treatment should always be assessed by the investigator. Description of scales can be found in Appendix 4.

7.1.1.3 Serious Adverse Events [Immediately Reportable to Roche]

A Serious Adverse Event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that at any dose fulfils at least one of the following criteria:

- is fatal; [results in death; NOTE: death is an outcome, not an event]
- is Life-Threatening [NOTE: the term "Life-Threatening" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe].
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above.

The full requirements of the **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2** will be adhered to. (Appendix 5)

7.1.2 Treatment and Follow-up of AEs

AEs, especially those for which the relationship to test “drug” is not “unrelated”, should be followed up until they have returned to baseline status or stabilized. If a clear explanation is established it should be recorded on the CRF.

7.1.3 Laboratory Test Abnormalities

Laboratory test results will appear printed on laboratory reports provided to the site from the central lab.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event [SAE] should be reported as such, in addition to being recorded as an AE in the CRF.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the AE page in the CRF:

- Accompanied by clinical symptoms
- Leading to a change in study medication [e.g. dose modification, interruption or permanent discontinuation]
- Requiring a change in concomitant therapy [e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment].

7.1.4 Follow-up of Abnormal Laboratory Test Values

In the event of medically significant unexplained abnormal laboratory test values, the tests should be repeated and followed up until they have returned to the normal range and/or an adequate explanation of the abnormality is found. If a clear explanation is established it should be recorded on the CRF.

7.2 Handling of Safety Parameters

7.2.1 Reporting of Serious Adverse Events [immediately reportable]

Any clinical AE or abnormal laboratory test value that is *serious* and which occurs during the course of the study [as defined in section 7.1.1.3 above], regardless of the treatment arm, must be reported to Roche or designee **within *one* working day** of the investigator becoming aware of the event [expedited reporting].

Related Serious Adverse Events ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed.

Unrelated Serious Adverse Events must be collected and reported during the study and up until the follow-up visit.

The definition and reporting requirements of **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2** will be adhered to. (Appendix 5)

7.2.2 Pregnancy

A female subject must be instructed to stop taking the test "drug" and immediately inform the investigator if she becomes pregnant during the study. The investigator should report all pregnancies within 24 hours to the sponsor or designee. The investigator should counsel the subject, and discuss the risks of continuing with the pregnancy and the

possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy.

Pregnancy occurring in the partner of a male subject participating in the study should be reported to the investigator and the sponsor or designee after the pregnant partner's consent has been obtained. The partner should be counseled and followed as described above if acceptable, and provides informed consent.

7.3 Warnings and Precautions

Events such as convulsions and delirium **(including symptoms such as altered level of consciousness, confusion, abnormal behavior, delusions, hallucinations, agitation, anxiety, nightmares)** have been reported during oseltamivir use in patients with influenza, **predominately in children and adolescents**. In rare cases, **these events** resulted in accidental injury. The contribution of oseltamivir to those events is unknown. Such events have also been reported in patients with influenza who were not taking oseltamivir. Patients, especially children and adolescents, should be closely monitored **for signs of abnormal behavior.**

Please refer to the attached Investigator's Brochure for additional warnings, precautions, and other reported adverse events.

8. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

Full details of all planned analyses will be specified in a separate Data Reporting and Analysis Manual (DRAM). The methods described below are an outline of the main planned analyses.

8.1 Primary and Secondary Study Endpoints

8.1.1 Primary Endpoints

The primary endpoint in this study is the time to alleviation of all clinical influenza symptoms (recorded in the **patient** diary).

8.1.2 Secondary Endpoints

The following are secondary endpoints. With the exceptions of viral load and time to resolution of fever, they are defined as either the occurrence or non-occurrence of the specified events or the presence or absence of the specified symptoms,

Shedding virus by culture at day 1, 2, 6, 8, 11, 15 and 40

Shedding virus by RT-PCR at day 1, 2, 6, 8, 11, 15, and 40

Viral load by culture ($\log_{10}TCID_{50}$/mL) at Day 1, 2, 6, 8, 11, 15, and 40

Viral load by quantitative RT-PCR at day 1, 2, 6, 8, 11, 15 and 40

The time (hours) from first dose of study medication until resolution of fever

Fever

Cough

Coryza

Development of secondary illnesses (otitis media, bronchitis, pneumonia, or sinusitis) at any time during the study.

Development of secondary illnesses (otitis media, bronchitis, pneumonia, or sinusitis) at any time during the study that are treated with antibiotics

Initiation of treatment with antibiotics after randomization

Hospitalization, and for those who are hospitalized, the duration of hospitalization

Development of rejection or GVHD

Baseline, post-baseline and change from baseline in antibody titers

8.1.3 Safety

Safety of the treatment will be evaluated by AEs, laboratory tests, and vital signs.

All subjects who received at least one dose of treatment and had a safety assessment performed post randomization will be included in the safety evaluation.

In addition to routine safety assessments, the proportion of subjects experiencing a rejection and/or graft versus host disease will be summarized by treatment group.

8.2 Statistical and Analytical Methods

8.2.1 Statistical Model

8.2.1.1 Primary Variables

A non-parametric model will be assumed with estimation of medians based on Kaplan-Meier methods. Subjects without alleviation of symptoms will have their time censored at the last available observation that a complete assessment was made.

For the purpose of comparing treatment groups, it will be assumed that their respective distributions for the primary endpoint differ only by a shift in location.

8.2.1.2 Secondary Variables

For the secondary endpoints defined dichotomously in terms of events or symptoms, it will be assumed that the number of patients experiencing the event or exhibiting the symptom will have a binomial distribution.

For the continuous endpoint of time to resolution of fever, the same assumptions as for the primary endpoint will be made.

For the continuous endpoints of viral load at Day 1, 2, 6, 8, 11, 15, and 40 no model will be assumed.

8.2.2 Sample Size

Sample size calculations were performed using a two-sided t-test and are based on the comparison with a placebo control response in the pivotal registration trials in healthy adults of 112 hours for median time to alleviation of all symptoms. Assuming an expected median time to alleviation of symptoms of 78 hours for each of the two active treatment groups in this study, and a common standard deviation of 98 hours, then 111 evaluable subjects per group would give more than 90% power to detect a difference between the control and each of the active treatment groups. It is assumed that as many as 90 % of subjects enrolled into the study **who are identified as not being infected with influenza A H1N1 H274Y** will have influenza, and therefore 124 **such** subjects per arm would be required in order to obtain 111 influenza positive subjects. Note that this is an approximate calculation, as the final analysis will be based on the difference in medians and the corresponding 95% confidence interval from the Kaplan-Meier curves. This is however considered as a sufficient indication for the **number of required subjects** and the power that is expected. **The maximum number of subjects enrolled in the trial will depend upon the number of subjects identified with the oseltamivir resistant virus who will be replaced.**

8.2.3 Hypothesis Testing

Formal hypothesis testing will be not performed, instead inferences will be based on comparison of confidence intervals.

8.2.4 Analysis Populations

Three main patient populations will be used for the analysis of data from this study; the Safety Population, the Intent-to treat Population and the Intent-to-Treat Infected Population . Detailed definitions of these populations will be given in the DRAM

8.2.4.1 Intent to treat population:

All patients randomized will be included in the intent to treat population [Patients will be assigned to treatment groups as randomized for analysis purposes]

8.2.4.2 Intent to treat infected population:

All patients randomized and with laboratory confirmation of influenza infection (positive viral cultures or 4 fold or greater rise in antibody titers), **excluding patients infected with oseltamivir-resistant influenza A H1N1 H274Y at baseline,** will be included in the intent to treat influenza infected population [Patients will be assigned to treatment groups as randomized for analysis purposes]

The ITTI Population will be the primary population for the summary and analysis of the primary and secondary efficacy variables.

8.2.4.3 Subpopulations

In order to evaluate the potential for relapse, the following two subpopulations of the ITTI population will be evaluated:

- patients not shedding virus as assessed by culture on Day 11
- patients not shedding virus as assessed by RT-PCR on Day 11

Viral shedding and RT-PCR at days 15 and 40 will be evaluated in these subpopulations.

Based on the proportion of subjects hospitalized, an additional subpopulation may be defined to evaluate the length of hospitalization for hospitalized subjects.

Likewise, based on the proportion of children enrolled additional subpopulations of children and adults will be created to evaluate the course of influenza in children and adults.

8.2.5 Efficacy Analysis

For the primary analysis, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- Comparison to placebo control from pivotal registration trials

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established to match (in terms of efficacy evaluations, duration of observation etc.) those of the current study. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

  The median time to resolution of all influenza symptoms will be determined for each treatment group, along with its 95% confidence interval. These confidence intervals will be compared individually to the placebo control confidence interval described above as a potential means of establishing treatment effects.

- Assessment of relative efficacy

  The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval. The methodology will be based on the use of the Hodges-Lehmann estimator in the case of censored data.

This confidence interval will provide lower and upper limits for the treatment difference and can be used as the basis for potential inferences. For example, if it does not contain the value zero a difference will have been established. However, given the study sample size this outcome is only likely if a clinically significant difference (e.g. 30%) exists. Although this is not anticipated from the experience in pivotal registration trials in normal healthy patients, it cannot be ruled out as a possibility in immunocompromised transplant recipients.

For the dichotomous secondary endpoints, the proportion experiencing the event or symptom, and its associated 95% confidence interval will be derived for each treatment group. Where possible, the corresponding confidence intervals from the placebo control population will be presented for comparison to those derived for the active treatment groups in this trial.

The continuous secondary endpoints time to resolution of fever will be analyzed as for the primary endpoint.

For the continuous endpoints of viral load ($log_{10}TCID_{50}$/mL) at Day 1, 6, 8, 11, 15, and 40, summary statistics (including mean, median, minimum and maximum) will be derived for each treatment group.

8.2.5.1 Exclusion of Data from Analysis

Efficacy data collected from any patient who begins treatment with another antiviral for influenza (for example, amantadine, rimantadine, or zanamavir), after the other antiviral treatment commences, will be excluded from the primary and secondary efficacy analyses. **Such patients will be considered failures to oseltamivir treatment in the analyses. Further details will be provided in the DRAM.**

8.2.5.2 Interim Analysis

No interim analyses are planned.

8.2.6 Safety Data Analysis

The safety analysis population will include all subjects who receive at least one dose and had a safety assessment performed post randomization. All safety parameters will be summarized and presented in tables based on this safety population.

8.2.7 Other Analyses

The number and percentage of patients with influenza infection (defined as a positive culture from a nasal and/or throat swab or 4 fold or greater rise in antibody titers) will be summarized by treatment group for the Intent-to-treat Population.

Further exploratory analysis, (including assessments of the rapid diagnostic test, sub group analysis) will be detailed in the DRAM.

The last positive viral isolate from each patient will be tested for reduced sensitivity to oseltamivir. Clonal resistance assays will also be used to evaluate the rate of resistance to oseltamivir. These data will be summarized in a report separate from the final study report.

9. DATA QUALITY ASSURANCE

The overall procedures for quality assurance of clinical study data are described in the Roche Standard Operational Procedures.

Accurate and reliable data collection will be assured by verification and cross–check of the CRFs against the investigator's records by the study monitor [source document verification], and the maintenance of a drug–dispensing log by the investigator.

The data collected will be entered into the study database from the working copy of the CRF faxed from the site.

A comprehensive validation check program will verify the data and discrepancy reports will be generated accordingly for resolution by the investigator. As patients complete the study [or prematurely withdraw] and their signed CRFs become available, a second data entry will be performed from the original, signed CRF. A comparison check will be run to identify and resolve any discrepancies between the first and second data entry.

Throughout the study the Study Management Team [SMT] will review data according to the SMT Data Review Plan as described in the Data Quality Plan.

9.1 Assignment of Preferred Terms and Original Terminology

For classification purposes, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using the current version of MedDRA (Medical Dictionary for Regulatory Activities terminology) for adverse events and diseases and the INN (International Non-Proprietary name) drug terms and procedures dictionary for treatments and surgical and medical procedures.

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PART II: ETHICS AND GENERAL STUDY ADMINISTRATION

11. ETHICAL ASPECTS

11.1 Local Regulations/Declaration of Helsinki

The investigator will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study must fully adhere to the principles outlined in "Guideline for Good Clinical Practice" ICH Tripartite Guideline [January 1997] or with local law if it affords greater protection to the subject. For studies conducted in the EU/EEA countries, the investigator will ensure compliance with the EU Clinical Trial Directive [2001/20/EC]. For studies conducted in the USA or under US IND, the investigator will additionally ensure that the basic principles of "Good Clinical Practice" as outlined in the current version of 21 CFR, subchapter D, part 312, "Responsibilities of Sponsors and Investigators", part 50, "Protection of Human Subjects", and part 56, "Institutional Review Boards", are adhered to.

In other countries where "Guideline for Good Clinical Practice" exist Roche and the investigators will strictly ensure adherence to the stated provisions.

11.2 Informed Consent

It is the responsibility of the investigator, or a person designated by the investigator [if acceptable by local regulations], to obtain written informed consent from each subject participating in this study, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. For subjects not qualified or incapable of giving legal consent, written consent must be obtained from the legally acceptable representative. In the case where both the subject and his/her legally acceptable representative are unable to read, an impartial witness should be present during the entire informed consent discussion. After the subject and representative have orally consented to participation in the trial, the witness' signature on the form will attest that the information in the consent form was accurately explained and understood. The investigator or designee must also explain that the subjects are completely free to refuse to enter the study or to withdraw from it at any time, for any reason. The Case Report Forms [CRFs] for this study contain a section for documenting informed subject consent, and this must be completed appropriately. If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All subjects [including those already being treated] should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

If children are old enough to understand the risks and benefits of the study, they should also be informed and should also provide their written consent.

11.3 Independent Ethics Committees/Institutional Review Board

Independent Ethics Committees [non-US]: This protocol and any accompanying material provided to the subject [such as subject information sheets or descriptions of the study used to obtain informed consent] as well as any advertising or compensation given to the patient, will be submitted by the investigator to an Independent Ethics Committee. Approval from the committee must be obtained before starting the study, and should be documented in a letter to the investigator specifying the date on which the committee met and granted the approval.

Any modifications made to the protocol after receipt of the Independent Ethics Committee approval must also be submitted by the investigator to the Committee in accordance with local procedures and regulatory requirements.

When no local review board exists, the investigator is expected to submit the protocol to a regional committee. If no regional committee exists, Roche will assist the investigator in submitting the protocol to the European Ethics Review Committee.

Institutional Review Board [US]: It is the understanding of the sponsor that this protocol [and any modifications] as well as appropriate consent procedures, will be reviewed and approved by an Institutional Review Board. This board must operate in accordance with the current Federal Regulations. A letter or certificate of approval will be sent by the investigator to the sponsor or designee prior to initiation of the study, and also whenever subsequent modifications to the protocol are made.

12. CONDITIONS FOR MODIFYING THE PROTOCOL

Protocol modifications to ongoing studies must be made only after consultation between an appropriate representative of the sponsor and the investigator [investigator representative[s] in the case of a multicenter trial]. Protocol modifications must be prepared by a representative of the sponsor and initially reviewed and approved by the Clinical Science Leader/Clinical Pharmacologist and Biostatistician.

All protocol modifications must be submitted to the appropriate Independent Ethics Committee or Institutional Review Board for information and approval in accordance with local requirements, and to Regulatory Agencies if required. Approval must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial subjects, or when the change[s] involves only logistical or administrative aspects of the trial [e.g. change in monitor[s], change of telephone number[s].

13. CONDITIONS FOR TERMINATING THE STUDY

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange the procedures on an individual study basis after review and consultation. In terminating the study, Roche and the investigator will assure that adequate consideration is given to the protection of the patient's interests.

14. STUDY DOCUMENTATION, CRFS AND RECORD KEEPING

14.1 Investigator's Files / Retention of Documents

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two different separate categories [1] Investigator's Study File, and [2] subject clinical source documents.

The Investigator's Study File will contain the protocol/amendments, Case Report and Query Forms, Independent Ethics Committee/Institutional Review Board and governmental approval with correspondence, sample informed consent, drug records, staff curriculum vitae and authorization forms and other appropriate documents/correspondence etc.

Subject clinical source documents [usually defined by the project in advance to record key efficacy/safety parameters independent of the CRFs] would include patient hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, EEG, X-ray, pathology and special assessment reports, signed informed consent forms, consultant letters, and subject screening and enrollment logs. The Investigator must keep these two categories of documents on file for at least 15 years after completion or discontinuation of the study. After that period of time the documents may be destroyed, subject to local regulations.

Should the Investigator wish to assign the study records to another party or move them to another location, Roche must be notified in advance.

If the Investigator can not guarantee this archiving requirement at the investigational site for any or all of the documents, special arrangements must be made between the Investigator and Roche to store these in a sealed container[s] outside of the site so that they can be returned sealed to the Investigator in case of a regulatory audit. Where source documents are required for the continued care of the patient, appropriate copies should be made for storing outside of the site.

14.2 Source Documents and Background Data

The investigator shall supply the sponsor or designee on request with any required background data from the study documentation or clinic records. This is particularly important when CRFs are illegible or when errors in data transcription are suspected. In case of special problems and/or governmental queries or requests for audit inspections, it is also necessary to have access to the complete study records, provided that patient confidentiality is protected.

14.3 Audits and Inspections

The investigator should understand that source documents for this trial should be made available to appropriately qualified personnel from the Roche Pharma Development Quality Assurance Unit or its designees, or to health authority inspectors after appropriate notification. The verification of the CRF data must be by direct inspection of source documents.

14.4 Case Report Forms

For each patient enrolled, a CRF must be completed and signed by the principal investigator or authorized delegate from the study staff. This also applies to records for those patients who fail to complete the study [even during a pre-randomization screening period if a CRF was initiated]. If a patient withdraws from the study, the reason must be noted on the CRF. If a patient is withdrawn from the study because of a treatment-limiting AE, thorough efforts should be made to clearly document the outcome.

All forms should be typed or filled out using indelible ink, and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or his/her authorized delegate. The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor or designee in the CRFs and in all required reports.

15. MONITORING THE STUDY

It is understood that the responsible Roche monitor [or designee] will contact and visit the investigator regularly and will be allowed, on request, to inspect the various records of the trial [CRFs and other pertinent data] provided that patient confidentiality is maintained in accord with local requirements.

It will be the monitor's responsibility to inspect the CRFs at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitor should have access to laboratory test reports and other patient records needed to verify the entries on the CRF. The investigator [or his/her deputy] agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

16. CONFIDENTIALITY OF TRIAL DOCUMENTS AND SUBJECT RECORDS

The investigator must assure that subjects' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents submitted to the sponsor or designee, subjects should not be identified by their names, but by an identification code. The investigator should keep a subject enrollment log showing codes, names and addresses. The investigator should maintain documents not for submission to Roche, e.g., subjects' written consent forms, in strict confidence.

17. PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to Roche prior to submission. This allows the sponsor or designee to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the investigator.

In accord with standard editorial and ethical practice, Roche will generally support publication of multicenter trials only in their entirety and not as individual center data. In this case, a coordinating investigator will be designated by mutual agreement.

Any formal publication of the study in which input of Roche personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate Roche personnel. Authorship will be determined by mutual agreement.

Appendix 1 Calculation of Creatinine Clearance and Grading of Chronic Extensive GVHD

Calculation of Creatinine Clearance

Conversion Factor for Serum Creatinine:

Conventional Units (mg/dL) = SI Units (μmol/L) ÷ 88.4

Age = number of years (12 years 11 months = 12 years)

Estimated Creatinine Clearance according to Cockcroft-Gault [17]

(for patients ≥ 18 years):

- **Males**

Creatinine

Clearance (mL/min) = [(140 – age) X Body Weight (kg)] ÷ [72 X **Serum Creatinine** (mg/dL)]

- **Females**

Creatinine Clearance = above equation X 0.85

Estimated Creatinine Clearance according to Schwartz equation [18]

(for patients < 18 years):

Creatinine Clearance (mL/min/1.73 M^2) = k X Height (cms) ÷ **Serum Creatinine** (mg/dL)

Value for k:

k	Age (years)
0.45	< 2
0.55	≥ 2 to < 13
0.7	≥ 13 to < 18 (males)
0.55	**≥ 13 to < 18 (females)**

Appendix 1 Calculation of Creatinine Clearance and Grading of Chronic Extensive GVHD (Cont.)

Grading of Chronic GVHD [19]

Type of Disease	Extent of Disease
Limited	Localized skin involvement, liver dysfunction or both
Extensive	Generalized skin involvement
	Localized skin involvement or liver dysfunction plus any one of the following: 1. Chronic aggressive hepatitis, bridging necrosis or cirrhosis 2. Eye involvement (Schirmer's test, < 5 mm) 3. Involvement of mucosalivary glands 4. Mucosal involvement (on lip biopsy) 5. Involvement of other target organs

Appendix 2 Adult Patient Diary Data and Symptom Record

The purpose of the **electronic** diary is for all adults (13 years and older) to record any symptoms of influenza-like illness for the duration of the study. Temperature **and** date and time of drug administration will also be recorded on the **electronic** patient diary.

Scoring of Symptoms

Please answer All of the questions yourself by checking one box for each row.

The information you provide is very important and will remain strictly confidential.

	absent 0	mild 1	moderate 2	severe 3
1. Nasal Congestion	☐	☐	☐	☐
2. Sore Throat	☐	☐	☐	☐
3. Cough	☐	☐	☐	☐
4. Aches and Pains	☐	☐	☐	☐
5. Fatigue(Tiredness)	☐	☐	☐	☐
6. Headache	☐	☐	☐	☐
7. Chills/sweats (feverish)	☐	☐	☐	☐

Appendix 3 Diary Data for Children

Temperature and date and time of **drug administration** will also be recorded on the **electronic** patient diary.

Date of Assessment ☐☐☐☐☐☐ (dd mm yy) Time of Assessment ☐☐☐☐ (h min)

Temperature: ☐☐☐.☐ ºC/F

Symptoms of influenza-like illness

Please mark one box only per question

Item	No Problem 0	Minor Problem 1	Moderate Problem 2	Major Problem 3	Don't Know or not Applicable
1. Poor appetite	☐	☐	☐	☐	☐
2. Not sleeping well	☐	☐	☐	☐	☐
3. Irritable, cranky, fussy	☐	☐	☐	☐	☐
4. Feels unwell	☐	☐	☐	☐	☐
5. Low energy, tired	☐	☐	☐	☐	☐
6. Not playing well	☐	☐	☐	☐	☐
7. Crying more than usual	☐	☐	☐	☐	☐
8. Needing extra care	☐	☐	☐	☐	☐
9. Clinginess	☐	☐	☐	☐	☐
10. Headache	☐	☐	☐	☐	☐
11. Sore throat	☐	☐	☐	☐	☐
12. Muscle aches or pains	☐	☐	☐	☐	☐
13. Fever	☐	☐	☐	☐	☐
14. Cough	☐	☐	☐	☐	☐
15. Nasal congestion, runny nose	☐	☐	☐	☐	☐
16. Vomiting	☐	☐	☐	☐	☐
17. Not interested in what's going on	☐	☐	☐	☐	☐
18. Unable to get to get out of bed	☐	☐	☐	☐	☐

This form was filled out by:

1. ☐ Parent
2. ☐ Other relative
3. ☐ Nanny
4. ☐ Subject
5. ☐ Other *specify*_____

Appendix 4 Adverse Event [AEs] Categories for Determining Relationship to Test Drug

PROBABLE [must have first three]

This category applies to those AEs which are considered, with a high degree of certainty, to be related to the test drug. An AE may be considered probable, if:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It cannot be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
3. It disappears or decreases on cessation or reduction in dose. [There are important exceptions when an AE does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists; e.g., [1] bone marrow depression, [2] tardive dyskinesias.]
4. It follows a known pattern of response to the suspected drug.
5. It reappears upon rechallenge.

POSSIBLE [must have first two]

1. This category applies to those AEs in which the connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possible if, or when:
2. It follows a reasonable temporal sequence from administration of the drug.
3. It may have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
4. It follows a known pattern of response to the suspected drug.

REMOTE [must have first two]

1. In general, this category is applicable to an AE which meets the following criteria:
2. It does <u>not</u> follow a reasonable temporal sequence from administration of the drug.
3. It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
4. It does not follow a known pattern of response to the suspected drug.
5. It does not reappear or worsen when the drug is readministered.

UNRELATED

This category is applicable to those AEs which are judged to be clearly and incontrovertibly due only to extraneous causes [disease, environment, etc.] and do not meet the criteria for drug relationship listed under remote, possible, or probable.

Appendix 4 Adverse Event [AEs] Categories for Determining Relationship to Test Drug (Cont.)

	Probable	Possible	Remote	Unrelated
Clearly due to extraneous causes	–	–	–	+
Reasonable temporal association with drug administration	+	+	–	–
May be produced by subject clinical state, etc.	–	+	+	+
Known response pattern to suspected drug	+	+	–	–
Disappears or decreases on cessation or reduction in dose	+	–	–	–
Reappears on rechallenge	+	–	–	–

Appendix 5 ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that at any dose fulfills at least one of the following criteria:

– is fatal; [results in death] [NOTE: death is an outcome, not an event]

– is Life-Threatening [NOTE: the term "Life-Threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe].

– required in-patient hospitalization or prolongation of existing hospitalization;

– results in persistent or significant disability/incapacity;

– is a congenital anomaly/birth defect;

– is medically significant or requires intervention to prevent one or other of the outcomes listed above

Medical and scientific judgment should be exercised in deciding whether expedited reporting to the sponsor or designee is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definitions above. These situations should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

An unexpected AE is one, the nature or severity of which is not consistent with the applicable product information.

Causality is initially assessed by the investigator. For Serious Adverse Events, possible causes of the event is indicated by selecting one or more options. (Check all that apply)

– Pre-existing/Underlying disease - specify

– Study treatment – specify the drug(s) related to the event

– Other treatment (concomitant or previous) – specify

– Protocol-related procedure

– Other (e.g. accident, new or intercurrent illness) - specify

The term severe is a measure of intensity, thus a severe AE is not necessarily serious. For example, nausea of several hours' duration may be rated as severe, but may not be clinically serious.

Appendix 5 ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 [Cont.]

A serious adverse event occurring during the study or which comes to the attention of the investigator within 15 days after stopping the treatment or during the protocol-defined follow-up period, if this is longer, whether considered treatment-related or not, must be reported. In addition, a serious adverse event that occurs after this time, if considered related to test "drug", should be reported.

Such preliminary reports will be followed by detailed descriptions later which will include copies of hospital case reports, autopsy reports and other documents when requested and applicable.

For serious adverse events, the following must be assessed and recorded on the AEs page of the CRF: intensity, relationship to test substance, action taken, and outcome to date.

The investigator must notify the Ethics Review Committee/Institutional Review Board of a serious adverse event in writing as soon as is practical and in accordance with international and local laws and regulations.

ROCHE LOCAL COUNTRY CONTACT for SAEs: Local Monitor

ROCHE HEADQUARTERS CONTACT for SAEs: Clinical Operations/Clinical Science

Within the US, weekends, holidays and after 5:00 pm, call [redacted] and ask for the physician on call. Outside the US, call the local emergency contact number provided by the Monitor.

AMENDMENT HISTORY FOR PROTOCOL NV20234C

1. SUBJECT: MODIFICATION OF INCLUSION CRITERION TO BROADEN THE TIME BETWEEN ONSET OF INFLUENZA LIKE ILLNESS AND FIRST DOSE OF STUDY DRUG TO WITHIN 96 HOURS

Reason for change:

Over the last several seasons, the majority of subjects were ineligible to be screened as they had been symptomatic for > 48 hours when they presented at the study site.

2. SUBJECT: DIAGNOSIS OF INFLUENZA BY PCR AND CULTURE IN ADDITION TO A RAPID DIAGNOSTIC

Reason for change:

Among patients who were screened there was a high screen failure rate possibly due to the low sensitivity of rapid diagnostic tests. Therefore, influenza confirmation by PCR or culture at baseline will be allowed.

3. SUBJECT: BROADENING OF THE TARGET IMMUNOCOMPROMISED STUDY POPULATION

Reason for change:

Transplant guidelines recommend the use of anti-virals for treatment of influenza. The availability of anti-virals outside the scope of a clinical trial precludes the need for this population to participate in a clinical trial.

4. SUBJECT: MODIFICATION OF THE STUDY OBJECTIVES WHICH EVALUATE EFFICACY AND SAFETY, AND STATISTICAL ANALYSES

Reason for change:

A placebo arm was always (versions A and B) considered unethical for this population. In order to make comparisons, the efficacy data for the oseltamivir arms from this study were to be compared to the placebo arm from pivotal registration trials in healthy subjects. Consequently version A and B of the protocol were designed to be similar to pivotal registration trials, ie only patients with influenza symptoms for < 48 h were to be enrolled. There were several limitations to this original approach, the main being that it is known that healthy children, adults and the elderly clear the influenza virus faster than the immunocompromised subject.

The need to enroll patients within 48 hours of the onset of influenza resulted in several patients being ineligible for screening. This required modification of the inclusion criteria to allow patients who had been symptomatic with influenza for more than 48 hours to be enrolled in the study. This imposed an even greater limitation on the ability to compare data between healthy subjects on placebo enrolled within 48 hours from pivotal registration trials with immunocompromised subjects who received oseltamivir within 96 hours.

Furthermore, since the original study design, and particularly following the pandemic, there has been increasing evidence for efficacy in the immunocompromised population. National guidelines now recommend the use of anti-virals for the treatment of influenza in the transplant population. In immunocompromised patients, however, there remains the risk for resistance. It was therefore decided to revise the primary objective of this study to the descriptive characterization of safety, tolerability and resistance.

5. SUBJECT: MODIFICATION OF SAMPLE SIZE

Reason for change:

The original sample size was determined to provide an adequate number of patients for analysis with a primary objective of efficacy. The sample size has been revised to reflect the amended primary objective of safety/tolerability and the development of resistant influenza virus, whilst still allowing for an adequate number of patients with influenza A.

6. SUBJECT: RENAL IMPAIRMENT

Reason for change:

While the dosing recommended for patients with renal failure in version B of the protocol continues to be safe, it is anticipated that Roche will develop more specific dosing guidelines for patients with renal failure. It was therefore decided to discontinue study medication for all patients with renal failure ($CrCl < 60mL/min/1.73M^2$).

7. SUBJECT: DELETING SPECIFIC RESISTANCE MUTATIONS

Reason for change:

Due to the circulation of a wild type oseltamivir resistant H274Y strain of influenza A H1N1 in the 2007/8 and 2008/9 Northern hemisphere influenza seasons, baseline PCR to detect the strain was introduced in version B of the protocol. With resistance being one of the primary objectives of the study, PCR testing for several more strains is planned. As the processing and handling of these tests will take time, test results will not be available in a timely manner and therefore will not be reported to the clinical trial site.

8. SUBJECT: PARTICIPATING CENTERS

Reason for change:

The number of estimated participating centers was adjusted to be more accurate.

9. SUBJECT: CORRECTIONS TO PREVIOUS PROTOCOL VERSION B

Reason for change:

Correction of administrative errors that were discovered after publication of Amendment B.

Section Synopsis Objectives

New text:

Primary:

To evaluate prospectively the **safety and tolerability** of oseltamivir for the treatment of influenza in **immunocompromised patients and characterize the effects of oseltamivir in immunocompromised patients on the development of resistant influenza virus.**

Secondary:

To evaluate the effects of conventional and high dose oseltamivir in **immunocompromised patients** on:

- **The time to resolution of influenza symptoms**
- The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis)
- The virologic course of influenza (proportion shedding and viral loads at different time points)

Old text:

Primary:

To evaluate prospectively the efficacy of oseltamivir for the treatment of influenza in transplant recipients as measured by the time to resolution of influenza symptoms.

Secondary:

To evaluate the effects of conventional and high dose oseltamivir in transplant recipients on:

- The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis)
- The virologic course of influenza (proportion shedding and viral loads at different time points)
- Patient safety and tolerability
- The development of resistant influenza virus

Section Synopsis Trial Design

New text:

This is a double-blind, randomized, multi-center trial of twice daily, conventional and high dose oseltamivir for the treatment of influenza in immunocompromised patients. Subjects will be stratified by age **(≤ 12, > 12 years), transplant status** (yes, no), time since onset of influenza symptoms and treatment start **(up to 96 hours)** (≤ **48** or > **48** hours) and vaccination status **(yes, no).**

Old text:

This is a double-blind, randomized, multi-center trial of twice daily, conventional and high dose oseltamivir for the treatment of influenza in immunocompromised patients (as represented by transplant recipients). Subjects will be stratified by age, transplant type, time since onset of flu symptoms and treatment start (≤ 24 or >24 hours) and vaccination status.

Section Synopsis Number of Subjects

New text:

A minimum of **166** (**83** per arm) to **allow an adequate number of influenza A patients per arm; including 50 transplant recipients.**

Old text:

A minimum of 250 (125 per arm).

Section Synopsis Number of Centers

New text:

Approximately **100 - 110** centers in the Northern hemisphere.

Old text:

Approximately 140 centers in the Northern hemisphere.

Section Synopsis Target Population

New text:

Patients immunocompromised due to a primary or secondary immunodeficiency, 1 year of age and older. The subjects will be positive for influenza by a rapid diagnostic test, **PCR or virus culture** at baseline.

Old text:

Transplant recipients (liver, kidney, liver and kidney, allogenic haematopoietic stem cell transplant), 1 year of age and older enrolled during the influenza season.

Section Synopsis Investigational Medicinal Product(s) Dose/Route/Regimen

New text:

Dose adjustments: In both treatment arms, patients **whose CrCl decreases to < 60ml/min/1.73M² will discontinue study medication**.

Old text:

Dose adjustments: In both treatment arms, dosing frequency will be decreased to once daily in patients with severe renal impairment (CrCl in adults between 10-30 ml/min and children between 10-30 ml/min/1.73M^2).

Section Synopsis Assessments of:

New text:

ASSESSMENTS OF:	
- **SAFETY**	**Adverse events, physical exams, vital signs, clinical laboratory evaluations**
- **RESISTANCE**	**Development of resistance**
- **EFFICACY**	**Time to resolution of all influenza symptoms as recorded in the patient diary.**

Old text:

ASSESSMENTS OF:	
- EFFICACY	Time to resolution of all influenza symptoms as recorded in the patient diary.
- SAFETY	Adverse events, physical exams, vital signs and clinical laboratory evaluations

Section Synopsis Statistical Analyses

New text:

The sample size has been chosen to provide an adequate number of patients to estimate the development of resistance with reasonable precision. Assuming that 90% of enrolled patients will have laboratory-confirmed influenza, there will be 75 patients in each treatment arm in the population evaluable for the development of resistance. The following table shows the 95% Pearson-Clopper confidence intervals that would result with a sample size of 75 patients in a treatment arm if certain event rates are observed in the study.

During the study the number of influenza A virus infected patients and the rate of development of resistance will be monitored in a blinded fashion, in order to ensure a reasonable precision for the estimation is maintained. Additional patients may be enrolled if necessary.

Observed Rate (%)	95% Confidence Interval
0.0	0% - 4.8%
1.3	0.0% - 7.2%
2.7	0.3% - 9.3%
5.3	1.5% - 13.1%
10.7	4.7%-19.9%

A total of 83 patients will be enrolled per arm and will be evaluable for the assessment of safety. This number of patients would provide estimates of adverse event rates with similar precision.

For the primary objective of evaluating the safety of oseltamivir conventional and high dose treatments, AEs, laboratory tests, and vital signs will be summarized and compared with the known safety profile of the drug. For the summary of the development of resistance for each treatment arm, 95% confidence intervals will be provided with the estimated rates.

For the secondary objective of evaluating the efficacy of oseltamivir as measured by the time to resolution of influenza symptoms, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- **Comparison to placebo control from pivotal registration trials**

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established that is comparable to patients in the current study. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

- **Assessment of relative efficacy**

  The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval.

Old text:

Sample size calculations were performed using a two-sided t-test and are based on the comparison with a placebo control response in the pivotal registration trials in healthy adults of 112 hours for median time to alleviation of all symptoms. Assuming an expected median time to alleviation of symptoms of 78 hours for each of the two active treatment groups in this study, and a common standard deviation of 98 hours, then 111 evaluable subjects per group would give more than 90% power to detect a difference between the control and each of the active treatment groups.

The primary endpoint will be time to alleviation of all clinical influenza symptoms, as recorded in the diary cards.

For the primary analysis, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- Comparison to placebo control from pivotal registration trials

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established to match those of the current study. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

- Assessment of relative efficacy

  The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval. The methodology will be based on the use of the Hodges-Lehmann estimator in the case of censored data.

Section GLOSSARY OF ABBREVIATIONS

New text:

None. Text deleted.

Old text:

DRAM	Data Reporting and Analysis Manual

Section GLOSSARY OF ABBREVIATIONS

New text:

AIDS	**Acquired immunodeficiency syndrome**
ALL	**Acute lymphocytic leukemia**
AML	**Acute myeloid leukemia**
CDC	**Centers for Disease Control and Prevention**
CML	**Chronic myeloid leukemia**
HIV	**Human immunodeficiency virus**
SAP	**Statistical Analysis Plan**
SCID	**Severe combined immunodeficiency**

Old text:

None. Text added.

Section 1.1.1 Influenza in the Immunodeficient Population

New text:

Influenza infection is usually a self limiting condition. However, in children, the elderly and the immunocompromised, influenza infection can be associated with substantial morbidity and mortality [1]. In patients with compromised immunity, influenza virus may cause severe lower respiratory tract involvement; unusual syndromes, including encephalitis, myocarditis, and hepatitis and death [3].

Conditions that compromise immunity may be classified based on etiology into primary (genetic) and secondary (acquired) immunodeficiency. Of the immunodeficient conditions, the ones that affect cell mediated immunity are likely to have adverse outcomes following viral infections [20].

<u>Primary immunodeficiency</u>

Primary immunodeficiencies are relatively common, may be either congenital or manifest later in life and are classified according to whether the genetic defect affects T or B cells or both [20]. There are four groups of disorders: severe combined immunodeficiency (SCID), primary T cell deficiency (e.g. CD8 deficiency,

DiGeorge syndrome), predominantly antibody deficiency (e.g. common variable immunodeficiency, selective IgA deficiency) and other well-defined immunodeficiency syndromes (e.g. Wiskott Aldrich syndrome) [20].

Of the primary immunodeficiencies, antibody deficiencies are the most frequent. However some of the more common antibody deficiency conditions (isolated IgA deficiency, IgG subclass deficiency and common variable immunodeficiency) have intact cell-mediated immunity and therefore the clinical course of viral infections (unless complicated by bacterial infections) does not differ significantly from that in the normal host [20]. A list of primary immunodeficiency disorders at risk for viral infections is provided in Appendix 6A. The incidence of some of these conditions has been estimated. The incidence of SCID is 1 in 100,000 to 1 in 1,000,000 [20]. The more severe forms of primary immunodeficiency are relatively rare, have their onset early in life, and all too frequently result in death during childhood [20].

Secondary Immunodeficiency

Secondary immunodeficiencies are not caused by intrinsic abnormalities in development of T and B cells [20]. Secondary immunodeficiency may result from diseases (human immunodeficiency virus [HIV], hematologic malignancy) or immunosuppressive and cytotoxic drugs (such as those used for treatment of transplant recipients, collagen vascular disease, malignancies).

Secondary immunodeficiency due to disease

HIV-infected individuals with a CD4+ T cell count of < 200/μL are highly susceptible to opportunistic disease [20]. Studies in HIV/acquired immunodeficiency syndrome (AIDS) subjects have shown an increased risk for heart and lung-related hospitalizations during the influenza season compared to other times of the year, prolonged duration of influenza symptoms, increased risk for influenza-related complications and a higher risk of influenza-related death [21].

Several hematologic malignancies affect the immune system (Appendix 7). Several authors have reported influenza in children and adults with hematologic malignancies [22, 23, 24, 25].

Secondary immunodeficiency due to drugs (e.g. transplant recipients, collagen vascular disease, malignancies)

The enhanced survival of the transplant population following the availability of newer immunosuppressive drugs has made them representative of the immunocompromised population in general; and secondary immunodeficiency due to drugs, in particular.

In transplant recipients influenza is associated with a higher rate of pulmonary complications, extrapulmonary manifestations and an increased risk of graft dysfunction and high attributable mortality. In a cohort study of influenza viral infection in **solid organ transplant (SOT)** subjects, 30 cases of influenza viral infection were identified. Secondary bacterial pneumonia was seen in 17% of subjects and three SOT recipients (2 liver and one kidney) had myositis, myocarditis and bronchiolitis obliterans. Biopsy

of the transplanted organ was performed in 21 of 30 cases and revealed variable degrees of acute allograft rejection in 62% of subjects. The study concluded that influenza was associated with significant morbidity in different groups of SOT recipients [5].

Among transplant recipients, subjects with **hematopoetic stem cell transplant (HSCT)** are at the greatest risk for morbidity. In a prospective study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation, 1973 patients were evaluated for respiratory virus infections after stem cell transplant. The overall mortality was 23% from influenza A infection and the direct influenza A-associated mortality was 15.3% in this study [6]. **A large retrospective study (4797 subjects undergoing HSCT over a 13-year period) identified 62 patients with influenza of whom as many as 29% (18 of 62 patients) developed pneumonia. Ten percent of the subjects with influenza died [7].**

The increased morbidity in transplant patients is associated with increased duration of virus shedding compared to that in healthy subjects. In otherwise healthy adults, elderly subjects and children, the median duration of viral shedding in untreated subjects was 70, 96 and 118 hours respectively [8]. In contrast, in the retrospective HSCT study, influenza virus was shed in nasopharyngeal secretions (evaluated at least weekly) for a median duration of 168 hours (7 days, range 2 to 37 days) [7].

Currently available treatments for influenza infection are the M2 ion channel inhibitors (e.g. amantadine, rimantadine) and the neuraminidase inhibitors (e.g. oseltamivir, zanamivir).

Old text:

Influenza infection is usually a self limiting condition. However, in children, the elderly and the immunocompromised, influenza infection can be associated with substantial morbidity and mortality [1]. In patients with compromised immunity, influenza virus may cause severe lower respiratory tract involvement; unusual syndromes, including encephalitis, myocarditis, and hepatitis and death. [3] The availability of new immunosuppressive agents has enhanced the number and survival of solid organ transplant (SOT) and haematopoietic stem cell transplant (HSCT) [4] recipients making them representative of the larger immunocompromised patient population.

In transplant recipients influenza is associated with a higher rate of pulmonary complications, extrapulmonary manifestations and an increased risk of graft dysfunction and high attributable mortality. In a cohort study of influenza viral infection in SOT subjects, 30 cases of influenza viral infection were identified. Secondary bacterial pneumonia was seen in 17 % of subjects and three SOT recipients (2 liver and one kidney) had myositis, myocarditis and bronchiolitis obliterans. Biopsy of the transplanted organ was performed in 21 of 30 cases and revealed variable degrees of acute allograft rejection in 62 % of subjects. The study concluded that influenza was associated with significant morbidity in different groups of SOT recipients [5].

Among transplant recipients, subjects with HSCT are at the greatest risk for morbidity. In a prospective study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation, 1973 patients were evaluated for respiratory virus

infections after stem cell transplant. The overall mortality was 23% from influenza A infection and the direct influenza A-associated mortality was 15.3% in this study [6].

The largest study of influenza infections in transplant (allogenic, syngenic or autologous HSCT) subjects comes from the Fred Hutchinson Cancer Center, Seattle, USA. In this retrospective study, influenza virus was isolated in 62 of 4797 subjects undergoing HSCT over a 13-year period. During this period, the Center had a standardized protocol for the detection of respiratory pathogens comprising direct fluorescent antibody (DFA) staining and viral culture. Because capture of influenza infections and accurate ascertainment of all risk factors was less complete after discharge from the health care center, only infections that occurred during the first 120 days after transplantation were considered. Influenza was defined as the isolation of influenza virus by culture or as evidence of influenza antigen detected by DFA in conjunction with consistent symptomatology. Antiviral treatment with an M2 inhibitor or oseltamivir (available since 1999) was performed at the discretion of the investigator. Unlike the fixed dosing duration recommended for antivirals in healthy subjects, in this trial, treatment was continued until resolution of presenting signs and symptoms and clearance of virus from respiratory secretions. Garrett Nichols, et al, reported as many as 29 % of subjects developed pneumonia following influenza infection in this large retrospective study. Ten percent of the subjects with influenza died [7].

The increased morbidity in transplant patients is associated with increased duration of virus shedding compared to that in healthy subjects. In otherwise healthy adults, elderly subjects and children, the median duration of viral shedding in untreated subjects was 70, 96 and 118 hours respectively. [8] In contrast, in the retrospective HSCT study, influenza virus was shed in nasopharyngeal secretions (evaluated at least weekly) for a median duration of 168 hours (7 days, range 2 to 37 days) [7]. This median duration of shedding included both allogenic and autologous HSCT subjects and both treated and untreated subjects. The mean duration of shedding in treated and untreated allogenic HSCT subjects was found to be longer than in autologous HSCT subjects (11.1 versus 6.7 days).

Johny et al, evaluated the use of zanamivir in the treatment of influenza in seven allogenic bone marrow transplant subjects. As with the large retrospective HSCT study, their standard of care also was to continue zanamivir until symptoms had subsided and it was documented that viral excretion had ceased. Viral shedding was checked every 7 days. Based on this protocol, the median duration of use of zanamivir was 15 days with a range of 5 to 44 days [9].

Currently available treatments for influenza infection are the M2 ion channel inhibitors (e.g. amantadine, rimantadine) and the neuraminidase inhibitors (e.g. oseltamivir, zanamivir). Due to the emergence of resistant virus, the Center for Disease Control (CDC) has made an interim recommendation that neither amantadine nor rimantadine be used for the treatment or prophylaxis of influenza in the United States. [10] Oseltamivir is therefore one of the main drugs available for the evaluation of the treatment of influenza in the immunocompromised population.

Section 1.1.2 Oseltamivir

New text:

The safety profile of oseltamivir has been well characterized for the prophylaxis indication in a prospective randomized placebo controlled trial conducted in the adult and pediatric immunocompromised (HSCT and SOT) population. In the oseltamivir group, the indications for transplant included hematologic malignancies (acute and chronic leukemias, multiple myeloma and myelodysplastic syndrome), lymphoid malignancies (Hodgkins or non-Hodgkins lymphomas), primary immunodeficiencies (Wiskott-Aldrich syndrome, X-linked lymphoproliferative disease and severe combined immunodeficiency). Other rare indications included bone marrow aplasia, paroxysmal nocturnal haemoglobinuria, myelofibrosis and multiple sclerosis. In the safety population, there were 239 subjects randomized to the conventional dose of oseltamivir and 238 to placebo. The total number of adverse events reported in the placebo group (361 events) was generally similar to that in the oseltamivir group (323 events). Diarrhea was the most frequently reported adverse event (placebo, 8%; oseltamivir, 6%). There were no deaths in the oseltamivir group. Oseltamivir was found to be safe in immunosuppressed transplant recipients [26].

Limited safety and/or efficacy of oseltamivir for the treatment indication is available from several case reports in children and adolescents. Oseltamivir was shown to be safe and/or effective in HIV infected children (n=10) [27], children (age 3 to 12 years) with acute lymphocytic leukemia (ALL) (n=10) [25], in a nosocomial H1N1 outbreak in a pediatric (children aged 10 months to 13 years) oncology ward (n=8) [24], in children and adolescents (age 2 to 19 years) with malignancies (ALL, neuroblastoma, brain tumor, non-Hodgkin's lymphoma, osteosarcoma, Ewing sarcoma, myelodysplasia, acute myeloid leukemia (AML), Wilms tumor, aplastic anemia, chronic myeloid leukemia (CML) and acute promyelocytic leukemia) (n=51) [22], in children aged 4 to 14 years on immunosuppressive drugs (n=5) [28] and in children (aged 5 months to 5 years) with bone marrow transplant (n=3) [29].

Oseltamivir has also been shown to be safe and/or effective in mixed populations of children and adults (62 patients) with HSCT [7], and in a large epidemiologic study (n=221) with SOT [30].

Finally, oseltamivir has been shown to be safe and/or effective in immunocompromised adults with HSCT [13] and adults with lung transplant [31, 32, 33].

The dose of oseltamivir was the same as the conventional dose in a majority of these reports. In one report as many as 25 adult patients received twice the conventional dose [30] while in another report, three of nine adult patients received twice the conventional dose [33]. Treatment with oseltamivir generally ranged from 5 to 10 days [32, 33, 30] and occasionally until the patient was symptom free [32, 33]. In exceptional cases treatment was given for as long as 20 days [22] or for as long as the patient was positive by RT-PCR [25].

There is some concern about the development of resistance in the immunocompromised population. During the pandemic influenza season, more than 23,000 clinical isolates of novel H1N1 pandemic virus were tested for resistance in the 6 international WHO regions. A total of 225 isolates (from 225 subjects) were resistant (H275Y mutation in the neuraminidase coding sequence) to oseltamivir for an approximate incidence of 1%. Information on immune status was available for 142 of 225 patients. Among the 142 patients, 56 (40%) were immunocompromised [34]. Prolonged viral replication and lack of immune-mediated virus clearance in immunosuppressed patients treated with antivirals can result in a higher incidence of selection of drug-resistant viruses, a phenomenon that has been documented previously [35, 36].

Old text:

There have been no controlled studies of oseltamivir treatment or prophylaxis in immunocompromised subjects. In the study by Garrett Nichols et al, patients who did not receive antiviral therapy shed virus for longer periods (mean duration, 11.3 days) than did those who were treated with M2 inhibitors (mean duration, 9.7 days) or neuraminidase inhibitors (mean duration, 7.5 days). Therapy with oseltamivir (but not rimantadine) appeared to be associated with shorter duration of shedding after controlling for steroid dose ($p < 0.08$). [7]. The efficacy of oseltamivir in the treatment of influenza in the immunocompromised population has also been demonstrated in other studies. In another small trial of oseltamivir for the treatment of influenza infection in subjects following HSCT, only two patients (5.1 %) developed influenza-related pneumonia. [13] In this study, all 39 HSCT subjects with influenza were treated with antivirals (oseltamivir or amantadine). In as many as 43 % of subjects, the duration of illness was greater than 7 days. This is unlike data from healthy adults, suggesting the possible need to evaluate a higher dose and/or longer duration of treatment with oseltamivir for this population.

While the efficacy of antiviral agents for influenza has been demonstrated in the otherwise healthy population, it has not been tested prospectively in the immunocompromised (transplant) population. [4] There is therefore a need to confirm the efficacy of antiviral therapy for influenza in prospective controlled trials in this patient population.

Section 1.2 Rationale for the Study

New text:

Because of the increasing body of evidence (Section 1.1.2), oseltamivir is now recommended in national guidelines as an option for the treatment of influenza in the transplant population [40]. As the transplant population is considered representative of the immunocompromised population, the primary objective of this study is to evaluate safety and resistance, while evaluating efficacy as a secondary objective.

Old text:

This study is designed to investigate the optimal therapy for influenza in immunocompromised transplant recipients.

The rationale for conducting this study in the immunocompromised population is based on the following:

1. Immunocompromised subjects are at the highest risk for morbidity and mortality from influenza
2. Immunocompromised subjects are at the greatest risk for contracting influenza because immunization may be ineffective in this population
3. There is no drug approved for the treatment of influenza in this population

Section 2.1 Primary Objective

New text:

The primary objective of the study is to evaluate prospectively the safety and tolerability of oseltamivir for the treatment of influenza in immunocompromised patients and characterize the effects of oseltamivir in immunocompromised patients on the development of resistant influenza virus.

Old text:

The primary objective of the study is to evaluate prospectively the efficacy of oseltamivir for the treatment of influenza in transplant recipients as measured by the time to resolution of influenza symptoms.

Section 2.2 Secondary Objectives

New text:

To evaluate the effects of conventional and high dose oseltamivir in **immunocompromised patients** on:

- **The time to resolution of influenza symptoms.**
- The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis).
- The virologic course of influenza (proportion shedding and viral loads at different time points).

Old text:

To evaluate the effects of conventional and high dose oseltamivir in transplant recipients on:

- The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis)
- The virologic course of influenza (proportion shedding and viral loads at different time points)
- Patient safety and tolerability
- The development of resistant influenza virus
- To characterize the population pharmacokinetics of oseltamivir (e.g. clearance, volume of distribution) in transplant recipients

Section 3.1 Overview of Study Design and Dosing Regimen

New text:

This is a double blind, randomized, stratified, multi-center trial of conventional and high dose oseltamivir for the treatment of influenza in immunocompromised patients. Immunocompromised **patients** who develop an influenza-like illness with a positive rapid diagnostic test, **PCR, or viral culture** for influenza, will be enrolled during the influenza season. Patients will be stratified by transplant **status** [**yes, no**], the time **between** onset of **influenza** symptoms and treatment start **(up to 96 hours)** [≤ **48** hours; > **48** hours], influenza vaccination status for current influenza season [Yes; No] and by age [≤ 12 years; > 12 years]. Eligible patients will be consented and randomized to receive oseltamivir twice daily for 10 days in one of two treatment arms: conventional dose or high dose (double the conventional dose).

Old text:

This is a double blind, randomized, stratified, multi-center trial evaluating the efficacy of conventional and high dose oseltamivir for the treatment of influenza in immunocompromised patients. Immunocompromised transplant recipients who develop an influenza-like illness with a positive rapid diagnostic test for influenza, will be enrolled during the influenza season. Patients will be stratified by type of transplant [SOT or HSCT], by the time between onset of symptoms and treatment start [≤ 24 hours; > 24 hours], influenza vaccination status for current flu season [Yes; No] and by age [≤12 years; >12 years]. Eligible patients will be consented and randomized to receive oseltamivir twice daily for 10 days in one of two treatment arms: conventional dose or high dose (double the conventional dose). The primary end point in the study is the time to alleviation of all symptoms.

In this study, immunocompromised subjects will be represented by the transplant population. This is justified because the availability of new immunosuppressive agents has enhanced the number and survival of SOT and HSCT recipients, making them representative of the larger immunocompromised patient population.

Heterogeneity in the transplant population could confound the assessment of safety and efficacy in this study. Consequently, the SOT population in the protocol excludes subjects with transplants other than liver, kidney or liver and kidney. However, in order to still be representative of the transplant community in particular and the immunocompromised community in general, the study specifically includes subjects at the greatest risk for morbidity and mortality from influenza - the HSCT subjects. The study also excludes subjects with comorbid conditions that might impact the metabolism and excretion of the drug. As the prodrug and active drug accumulate in the presence of hepatic and severe renal impairment respectively, subjects with hepatic or severe renal impairment are excluded from the study. Other comorbid conditions that might preclude the assessment of efficacy and safety are also excluded from the study. These are reflected in several of the inclusion and exclusion criteria.

Section 3.1.1 Rationale for Study Design

New text:

This study incorporates several features that distinguish it from classic placebo controlled trials.

There is no placebo control arm in this study as it was considered unethical for this high risk population. The development of resistance following treatment with oseltamivir (one of the primary objectives of the study) is an objective assessment (determined by laboratory tests) and is unlikely to be impacted by the absence of a placebo arm.

As a placebo control arm was considered unethical in this population, an alternative control arm had to be identified **for efficacy end points**. The response to placebo in pivotal oseltamivir registration trials in the healthy adult population with influenza was chosen as a control in lieu of a placebo arm in the current trial. In order to do this, the end points were designed to be similar to that in the pivotal registration trials.

The following sections provide rationale and justification for specific aspects of the study design **which differ from the currently approved dosing for influenza or from previous pivotal registration trials**. They also provide information on how the results will be used to make recommendations on dose and duration of treatment.

Old text:

This study incorporates several features that distinguish it from classic placebo controlled trials. At the outset, a patient population that represents the general immunocompromised population had to be defined. As a placebo control arm was considered unethical in this population, an alternative control arm had to be identified. The response to placebo in pivotal oseltamivir registration trials in the healthy adult population with influenza was chosen as a control in lieu of a placebo arm in the current trial. In order to do this, the current study design and end points were designed to be similar to that in the pivotal registration trials.

Routine influenza surveillance during the 2007/2008 influenza season showed a significant increase in the global circulation of an oseltamivir-resistant influenza virus (A H1N1 with H274Y mutation). Therefore, baseline nasal and throat swab samples will be assessed for the presence of the H274Y-mutated influenza virus and results will be reported to clinical sites to assist in determining the most appropriate treatment options for individual patients.

The following sections provide rationale and justification for the specific aspects of the study design. They also provide information on how the results will be used to make recommendations on dose and duration of treatment.

Section 3.1.1.1 Choice of Treatment Arms and Duration of Treatment

New text:

The currently approved dose of oseltamivir for the treatment indication is the conventional dose with a duration of five days. In this study a conventional and

high dose (twice the conventional dose) are being evaluated. The pivotal registration trials for oseltamivir in healthy adults with influenza had three treatment arms, two active (75 mg and 150 mg) and one placebo. Both treatment arms were found to be significantly better than placebo. No statistical comparisons were made between the two active treatment arms. Evaluation of the results in the two active treatment arms did not reveal any clinically meaningful difference. **However, the proportion of subjects shedding virus on day 4 (3 days after the start of treatment), suggested a possible dose response relationship (36%, 28% and 23% in the placebo, 75 mg and 150 mg dose arms respectively). Because defective immune-mediated virus clearance in immunosuppressed patients treated with antivirals can result in a higher incidence of selection of drug-resistant viruses, it was decided to use both the conventional and high dose arm for this study. A longer duration of treatment was chosen because a large retrospective study has shown that the median duration of viral shedding of 7 days [7] was greater for HSCT recipients than that seen in the healthy children, adult and elderly population with influenza (Section 1.1.1) [8].**

Old text:

The pivotal registration trials for oseltamivir in healthy adults with influenza had three treatment arms, two active (75 mg and 150 mg) and one placebo. Both treatment arms were found to be significantly better than placebo. No statistical comparisons were made between the two active treatment arms. Evaluation of the results in the two active treatment arms did not reveal any clinically meaningful difference. Based on these studies, the approved (conventional) dose for the treatment of influenza in adults is 75 mg.

A similar trial design was envisaged for this immunocompromised population. In an immunocompromised population there is a possibility of increased efficacy with a higher dose. In the trials in healthy adults, on day 4 (3 days after the start of treatment), the proportion of subjects shedding virus suggested a possible dose response relationship (36%, 28% and 23% in the placebo, 75 mg and 150 mg dose arms respectively). In healthy adults, this difference in the rate of viral shedding did not translate to any difference in clinical response in the two active treatment arms even though both active treatment arms were significantly better than the placebo arm. In the immunocompromised population too it is possible that while there might not be any difference between the two active treatment arms, one or both of these dose arms may be better than placebo. In this immunocompromised population, there is therefore an even greater need to evaluate the efficacy of a higher dose arm.

As with the trials in healthy adults, the ideal trial design for the current study would be one that compared efficacy of the conventional and high dose arms with placebo. However, as reviewed in an earlier section 1.1.1 the morbidity and mortality from influenza in the immunocompromised population is far greater than that in healthy adults. Thus in one study as many as 29 % of subjects with HSCT developed pneumonia following influenza infection. [7] In another study in SOT recipients, biopsy of the transplanted organ revealed variable degrees of acute allograft rejection in 62 % of subjects. [5] Given the extent of morbidity, a placebo control appears unethical. This is further supported by a feasibility survey conducted by the Sponsor which demonstrated

that a majority of transplant sites actually used antivirals (including oseltamivir) to treat influenza in this population. It was therefore decided to compare the conventional and higher dose arms with the placebo arm from the pivotal registration trials in healthy adults with influenza. Although this approach poses limitations, the Sponsor has made every effort to make sure that the results from such an approach are still meaningful. The following sections describe how the study design addresses these limitations.

Section 3.1.1.2 Inclusion of Patients Symptomatic up to 96 Hours (new section-previous section replaced)

New text:

In immunocompromised patients, time from onset of symptoms to seeking medical attention (presentation at the clinic) of > 48 hours has been shown in several case reports [37, 31, 32, 33] including two instances of nosocomial outbreaks in children [29, 24]. This notwithstanding, oseltamivir has been shown to be effective in immunocompromised populations that included patients treated with oseltamivir beyond 48 hours of presentation; patients with lung transplant (median time to presentation 3 days) [31], bone marrow transplant (treatment started more than 48 hours after onset of symptoms in all three patients) [29], organ transplant (62 out of 221 patients started treatment after 96 hours) [30] and children with ALL (one child presented after 3 days and two children after 5 days of symptoms) [25].

In a prospective, observational study involving adults hospitalized with influenza, the study authors concluded that 'Weakened host defenses slow viral clearance, whereas antivirals started within the first four days of illness enhance viral clearance' [38].

Additionally, in November 2009, in a communication for clinicians on antiviral treatments for H1N1, the US Centers for Disease Control and Prevention (CDC) stated that while antiviral treatment is most effective when started early; both outpatients with risk factors and hospitalized patients still benefit when treatment with oseltamivir is started more than 48 hours after illness onset [39].

Old text:

Replaced Section 3.1.1.2 Consistency with Previous Influenza Treatment Studies

In order to be able to compare the study findings with the response to placebo in healthy adults, this study has been intentionally designed to be consistent with previous registration trials for treatment of influenza in healthy adults. Some of the key design features consistent with previous studies include: two active treatment arms, subjects with influenza like symptoms recruited during the flu season, use of a patient diary to capture symptoms, primary clinical end point based on diary assessments, laboratory evaluation of virology and serology samples generally similar to previous trials and the primary efficacy analysis population is the Intent to treat Infected (ITTI) population.

Despite the above considerations, there are issues that can preclude the estimation of the response to placebo in an immunocompromised population comprising adults and children from that in healthy adults with influenza. To further mitigate this, the choice of endpoints and their interpretation is vital.

Section 3.1.1.3 Interpretation of Study Results

New text:

Safety and tolerability and the development of resistance are the primary objectives of this study. These will be characterized descriptively. For the secondary objective of efficacy, the subset of the population enrolled in the first 48 hours will be compared with placebo patients from pivotal registration trials (where patients were enrolled in the first two days of illness). Sections 8.2.5, 8.2.6, and 8.2.7 provide details on the statistical considerations and their rationale associated with these comparisons.

Stratification

Stratification in this study has no bearing on any comparisons with results from previous trials. However, in order to evaluate a dose response relationship between the two active treatment arms in this trial, stratification remains essential.

Patients will be stratified by transplant status (yes, no) because transplant patients form a relatively large homogenous group in this study and might influence outcome.

Patients will be stratified by time between onset of symptoms and treatment start (< **48** hours or ≥ **48** hours) because previous experience in otherwise healthy adults and children has shown that patients treated early in their illness respond to treatment better than those who are treated later. Patients will also be stratified by age (≤ 12 years and > 12 years) because otherwise healthy children shed virus longer than otherwise healthy adults. It is expected that a similar difference in the duration of viral shedding would exist between immunocompromised children and adults. As vaccinated subjects may respond faster to therapy, the study is also stratified based on influenza vaccination status for the current influenza season (Yes; No).

Old text:

Choice of primary end point and comparators

The current study includes both children and adults. This is because of the significant unmet need in both these age groups. The primary end point therefore had to be applicable to both age groups. In registration trials in adults, the primary end point was the time to resolution of all clinical symptoms (in the diary card). In registration trials in children, the primary end point was the reduction in the median duration of illness (defined based on temperature, cough, coryza and return to pre-illness health and activity). A key secondary end point in children – time to resolution of all clinical symptoms showed similar results. The primary endpoint in this study is the time to resolution (alleviation) of all symptoms.

The median of the primary end point in the active treatment groups (conventional and high dose) in this trial will be compared with the median of the time to resolution of symptoms in the placebo group from the pivotal registration trials. The high dose arm enables the detection of a possible dose response relationship in this immune compromised population. As a means to evaluate that possibility, an assessment of the

relative efficacy of the two dose groups will be made in terms of median time to alleviation of symptoms. Section 8.2.5 provides details on the statistical considerations and their rationale associated with these comparisons.

Effect of study design on efficacy end points

The main limitations of using the response to placebo from previous trials to estimate the response in this trial are due to the different populations (healthy versus immunocompromised), age groups (adults versus adults and children) and the potential difference in virulence of different strains. The potential difference in virulence of the predominant influenza viruses may be a confounder whose contribution can not be estimated. However the difference in populations and age groups is likely to result in the placebo response from pivotal registration trials underestimating the time to alleviation in the immunocompromised population. This is because the time to alleviation of symptoms is shorter in the healthy population compared to the immunocompromised population. [13] The placebo response in the pivotal trials will therefore underestimate the response in the current trial. The Sponsor is thus using a very conservative approach in comparing the active treatment arms in this study with the response to placebo in healthy subjects. If one/both of the treatment arms is statistically better than placebo, it is considered that this is a meaningful effect for this population. If both treatment arms are not different from placebo and this is seen consistently across several end points, it may be hypothesized that the risk from influenza for the immunocompromised population treated with oseltamivir is comparable to that for a healthy untreated adult with influenza.

Possible dosing recommendations based on current study

Healthy subjects who were given placebo in the previous pivotal oseltamivir treatment studies will be used as the control arm for this study. When the two active treatment arms are compared with this placebo control, several outcomes are possible. These outcomes will need to be discussed with health authorities. Some outcomes and possible interpretations of the results are presented below:

1. Conventional and high dose are both shown to be better than placebo: Both treatment arms will be compared. If no difference then the conventional dose would be the dose recommended for treatment in this population. If a difference is seen between treatment arms, then an evaluation (including benefit/risk assessment) will be made to confirm whether the higher dose should be the recommended dose.
2. One active treatment arm is superior to placebo while the other is not: In this instance the dose that is shown to be superior will be recommended unless other considerations such as safety dictate otherwise.
3. Both conventional and high dose arms are not different from placebo: If this is seen consistently across several secondary clinical end points, it may be hypothesized that oseltamivir has reduced the risk in the immunocompromised population to the more acceptable level in the untreated healthy adult population.
4. It is also possible that both conventional and high dose treatment have a more protracted clinical course than the placebo arm. In this situation, it may be difficult to make conclusions about efficacy.

Irrespective of the efficacy outcomes, the study will still provide useful information of the safety of oseltamivir in this population.

Possible recommendations for duration of treatment based on the current study

Once a dose is chosen a determination will need to be made on whether the recommended duration of treatment should be 5 or 10 days or longer. This decision will be based both on the clinical and virologic course of influenza.

The recommended duration of treatment for influenza in healthy adults is five days. In this study, the duration of dosing is ten days because viral shedding is typically longer in the immunocompromised population than in healthy adults. The general standard of care for this population has been to treat for as long as the patient continues to shed virus (even after the resolution of symptoms) [7] [9]. The duration of treatment for this population is therefore individualized based on clinical response and duration of viral shedding. It can often be more than 10 days. While it is possible that 10 or more days of treatment may be needed [7] [9], it is also possible that 5 days of treatment may still be adequate. Therefore, in this study the clinical and virologic course (proportion shedding virus at day 6 and later) will be evaluated to make recommendations on the duration of dosing. No statistical comparisons will be made.

Stratification

Stratification in this study has no bearing on any comparisons with results from previous trials. However, in order to evaluate a dose response relationship between the two active treatment arms in this trial, stratification remains essential.

Because HSCT recipients are at the greatest risk of morbidity and mortality from influenza, patients will be stratified by transplant type (SOT versus HSCT). Patients will be stratified by time between onset of symptoms and treatment start (< 24 hours or ≥ 24 hours) because previous experience in otherwise healthy adults and children has shown that patients treated early in their illness respond to treatment better than those who are treated later. Patients will also be stratified by age (≤ 12 years and > 12 years) because otherwise healthy children shed virus longer than otherwise healthy adults. It is expected that a similar difference in the duration of viral shedding would exist between immunocompromised children and adults. As vaccinated subjects may respond faster to therapy, the study is also stratified based on influenza vaccination status for the current flu season (Yes; No).

Section 3.1.2 Rationale for Dose Selection and Adjustment

New text:

Dose Adjustments

In this study, patients whose CrCl decreases to < 60mL/min/1.73M^2 will be discontinued from treatment.

Old text:

Dose Adjustments

In this study, dose adjustment for subjects with an estimated Cr Cl between 10 to 30 mL/min/1.73M^2 will comprise decreasing the frequency of administration to once daily. This is based on current label recommendations for adults with renal impairment (Table 1).

Table 1 Recommended doses in healthy adults for treatment of influenza

Creatinine Clearance	Recommended dose for treatment
> 30 (ml/min)	75 mg twice daily
> 10 to ≤ 30 (ml/min)	75 mg once daily or 30 mg suspension twice daily
≤10 (ml/min)	Not recommended
dialysis patients	Not recommended

The dose adjustment recommendations are considered suitable approaches for children with renal impairment. This is based on the reasonable assumption that a similar relationship exists in both children and adults, between *changes* in creatinine clearance and resultant *changes* in exposure of oseltamivir and oseltamivir carboxylate. Doses will be adjusted for renal impairment in this protocol per the schema below. More detailed information on dosing, dose adjustments and maintenance of the blind are provided in later sections 6.1, 6.2, 6.3.

Table 2 Recommended dose modifications for NV 20234 trial subjects

Randomized Dose Arm	**Unit dose (mg)**	**Creatinine Clearance (ml/min OR ml/min/M^2)**	**Dose frequency**
Conventional	30	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	45	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	60	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	75	> 30	Twice daily
		> 10 to ≤ 30	Once daily
High	60	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	90	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	120	> 30	Twice daily
		> 10 to ≤ 30	Once daily
	150	> 30	Twice daily
		> 10 to ≤ 30	Once daily

In general, no dose adjustment is considered necessary in subjects with mild-moderate hepatic impairment (based on pharmacokinetic findings in adults with chronic hepatic

impairment) receiving usual therapeutic doses of oseltamivir. [16] However, as the relevance of these findings to the specific patient population in this study is unclear, subjects with overt (based on physical signs and symptoms) hepatic impairment at baseline will be excluded from this study as mentioned in section 4.3.

Section 3.2 Number of Subjects/ Assignment to Treatment Groups

New text:

A minimum of **166** patients **(approximately 83 per arm) to allow an adequate number of influenza A patients, including 50 transplant recipients** will be enrolled in this study.

Old text:

A minimum of 250 patients will be enrolled in this study (approximately 125 per arm).

Section 3.3 Centers

New text:

This will be a multicenter study with approximately **100-110** centers in the Northern hemisphere.

Old text:

This will be a multicenter study with approximately 140 centers in the Northern hemisphere. Centers will be activated to recruit patients during the influenza season. The centers to be included in the study are those which perform or manage SOTs, HSCTs or both.

Section 4.1 Overview

New text:

Principal investigators will review oseltamivir resistance patterns of strains circulating in the area and weigh the risk versus the benefit before enrolling patients with a potentially resistant strain.

Old text:

None. Text added.

Section 4.2 Inclusion Criteria

New text:

- Age greater than or equal to 1 year
- Rapid diagnostic test, **PCR, or viral culture positive** for influenza in the **96** hours prior to first dose
- Immunocompromised subject **defined as one who meets any of the following**:
    - **Primary immunodeficiency at risk for viral infections (representative examples in Appendix 6) OR**
    - **Secondary immunodeficiency**
        - SOT **with ongoing immunosuppression OR**
        - Allogenic **HSCT with ongoing immunosuppression OR**

        - **HIV with CD4 count < 200/mm^3 OR**
        - **Hematologic malignancies (representative examples in Appendix 7) OR**
        - **Systemic (eg. enteric, sc, im or iv) immunosuppressive therapy, irrespective of medical indication, started at least 12 weeks prior to, and ongoing at the time of first dose of study drug (representative examples in Appendix 8)**
- Symptoms suggestive of influenza like illness including, but not limited to fever, cough, or coryza
- **In patients with history or clinical presentation at randomization suggestive of renal failure; a CrCl > 60ml/min/1.73M^2**
- Less than or equal to **96** hours between onset of influenza like illness and first dose of study drug
- Parent/guardian willing and able to comply with study requirements and give consent, (country specific age cut off)
- Patient able to comply with study requirements and willing to give assent, as appropriate (country specific age cut off)
- For adult patients, willing and able to comprehend and give written informed consent
- Patients, in the reproductive age group, must agree to utilize an effective method of contraception throughout the study period and **for females** for one reproductive cycle following cessation of study therapy
- Females of childbearing potential must have a negative urine pregnancy test prior to start of study medication

Old text:

- Age greater than or equal to 1 year
- Rapid diagnostic test positive for influenza in the 24 hours prior to first dose
- Immunocompromised subject defined as documented:
    - SOT (liver, kidney or both) recipient OR
    - Allogenic HSCT
- Receiving ongoing immunosuppression, OR, in the investigator's opinion, not immune reconstituted
- Symptoms suggestive of influenza like illness including, but not limited to fever, cough, or coryza
- Less than or equal to 48 hours between onset of influenza like illness and first dose of study drug
- Acceptable renal function defined as:
    - Most recent creatinine clearance in the 6 months prior to randomization is > 30 ml/min in adults and > 30 ml/min /1.73M2 in children. Creatinine clearance estimated from serum creatinine (Appendix 1) measured when subject is not receiving any renal replacement therapy OR
    - For patients who have not had a creatinine clearance assessment in the 6 months prior to randomization, baseline creatinine clearance > 10 ml/min in adults and > 10 ml/min /1.73M2 in children (Appendix 1) OR

    - For patients whose most recent creatinine clearance in the 6 months prior to randomization is < 30 ml/min in adults and < 30 ml/min /1.73M2 in children, baseline creatinine clearance > 10 ml/min in adults and > 10 ml/min /1.73M2 in children (Appendix 1)
- Parent/guardian willing and able to comply with study requirements and give consent. (country specific age cut off)
- Patient able to comply with study requirements and willing to give assent, as appropriate (country specific age cut off)
- For adult patients, willing and able to comprehend and give written informed consent
- Patients, in the reproductive age group, must agree to utilize an effective method of contraception throughout the study period and for one reproductive cycle following cessation of study therapy
    - Females of childbearing potential must have a negative urine pregnancy test prior to start of study medication

Section 4.3 Exclusion Criteria

New text:

- SOT within 6 months of the time of randomization
- Have in the investigator's opinion experienced acute rejection in the 4 weeks prior to randomization
- HSCT patients with no evidence of engraftment (engraftment is defined as the point at which a patient can maintain a sustained absolute neutrophil count (ANC) of >500/mm^3 and sustained platelet count of $\geq$ 20,000/mm^3, lasting $\geq$ 3 consecutive days without transfusions)
- HSCT subjects not discharged from hospital after their initial hospitalization for transplantation
- Have clinical evidence for hepatic decompensation at the time of randomization (clinical icterus, ascites, hepatic encephalopathy, coagulopathy)
- Have cirrhosis of the liver at the time of randomization
- Patients currently receiving any form of renal replacement therapy including hemodialysis, peritoneal dialysis or hemofiltration
- Have evidence of active or uncontrolled opportunistic infections (bacterial, fungal, or viral-including cytomegalovirus [CMV] or polyoma virus [BKV]) at the time of randomization. Patients with HCV or HBV are not excluded
- Patients with **co-morbid conditions which are** uncontrolled. Uncontrolled is defined as disease requiring change of therapy or hospitalization in the 4 weeks preceding randomization. Change of therapy is defined as dose increase or change of medication prior to onset of present influenza like illness
- Patients with gastrointestinal disorders which might interfere with their ability to absorb oral medication
- Allergy to the test medication
- Patients with hereditary fructose intolerance (for subjects who will be taking the liquid formulation)

- Influenza vaccination **with live attenuated vaccine** in the 2 weeks prior to randomization
- Antiviral treatment (example: amantadine, rimantadine, **oseltamivir, laninamivir, peramivir,** zanamivir and ribavirin) for influenza in the 2 weeks prior to randomization

Old text:

- SOT within 6 months of the time of randomization
- Solid Organ Transplant other than liver, kidney or liver and kidney
- Have in the investigator's opinion experienced acute rejection in the 4 weeks prior to randomization
- HSCT patients with no evidence of engraftment (engraftment is defined as the point at which a patient can maintain a sustained absolute neutrophil count (ANC) of >500/mm^3 and sustained platelet count of ≥ 20,000/mm^3, lasting ≥ 3 consecutive days without transfusions)
- HSCT subjects not discharged from hospital after their initial hospitalization for transplantation
- Have evidence of veno-occlusive disease, acute or chronic extensive graft versus host disease at the time of randomization
- Have clinical evidence for hepatic decompensation at the time of randomization (clinical icterus, ascites, hepatic encephalopathy, coagulopathy)
- Have cirrhosis of the liver at the time of randomization
- Currently or in the six months prior to randomization using T cell depleting antibodies (example: antithymocyte globulin, antilymphocyte globulin) for management of transplant
- Have other co-morbid conditions that could affect patient survival or graft function including, but not limited to, a post-transplant lymphoproliferative disease (PTLD), autoimmune disease including inflammatory bowel disease and psoriasis, untreated thyroid disease, and significant active infection
- Patients currently receiving any form of renal replacement therapy including hemodialysis, peritoneal dialysis or hemofiltration
- Have evidence of active or uncontrolled opportunistic infections (bacterial, fungal, or viral - including cytomegalovirus [CMV] or polyoma virus [BKV]) at the time of randomization. Patients with HCV or HBV are not excluded.
- Patients with known HIV infection
- Patients who are being evaluated or treated for an active malignancy (other than the malignancy for which the SOT or HSCT may have been performed) at the time of randomization
- Patients with uncontrolled vascular, neurologic or pulmonary disease. Uncontrolled is defined as disease requiring change of therapy or hospitalization in the 4 weeks preceding randomization. Change of therapy is defined as dose increase or change of medication prior to onset of present influenza like illness
- Patients with severe diarrhea or other gastrointestinal disorders which might interfere with their ability to absorb oral medication, including diabetic patients with previously diagnosed diabetic gastroenteropathy

- Allergy to the test medication
- Patients with hereditary fructose intolerance (for subjects who will be taking the liquid formulation)
- Influenza vaccination in the 2 weeks prior to randomization
- Antiviral treatment (example: amantadine, rimantadine, zanamivir and ribavirin) for influenza in the 2 weeks prior to randomization

Section 4.4 Concomitant Medication and Treatment

New text:

Antiviral treatments with activity against influenza (e.g. amantadine, rimantadine, zanamivir, ribavirin, **laninamivir, peramivir,** and additional oseltamivir above that specified for this study) are not allowed during the 10-day treatment phase of the study.

Concomitant use of an investigational drug during the study is also excluded. Live attenuated vaccines may not be advisable for this population. It is possible that concurrent use with oseltamivir may render live vaccines ineffective.

Medications required for the routine care of the patient may be continued during the study. Concomitant use of probenecid (irrespective of the indication) is not allowed during the study.

Old text:

Antiviral treatments with activity against influenza (e.g. amantadine, rimantadine, zanamivir, ribavirin, and additional oseltamivir above that specified for this study) are not allowed during the 10-day treatment phase of the study.

Concomitant use of an investigational drug during the study is also excluded. Influenza vaccination after randomization is not allowed. Live attenuated vaccines may not be advisable for this population. It is possible that concurrent use with oseltamivir may render live vaccines ineffective.

Medications required for the routine care of the patient including those required for management of the transplant (excluding lymphocyte depleting antibodies, intravenous immunoglobulins) may be continued during the study. Concomitant use of probenecid (irrespective of the indication) is not allowed during the study.

Section 4.5 Criteria for Premature Withdrawal

New text:

The investigator must discontinue treatment if the creatinine clearance is **< 60** ml/min in adults or **< 60** ml/min/1.73 M^2 in children.

Old text:

The investigator must discontinue treatment if the creatinine clearance is < 10 ml/min in adults or < 10 ml/min/1.73 M^2 in children.

New text:

The investigator must discontinue treatment with study drug if the patient is to be treated concomitantly with another antiviral for influenza, for example, amantadine, rimantadine, **laninamivir, peramivir,** or zanamavir.

Old text:

The investigator must discontinue treatment with study drug if the patient is to be treated concomitantly with another antiviral for influenza, for example, amantadine, rimantadine, or zanamavir.

Section 4.6.1 For Subjects

New text:

No subject prematurely discontinued from the study for any reason will be replaced.

Old text:

In order to maintain the required number of patients who are evaluable for efficacy, patients who are identified as being infected with influenza A H1N1 H274Y at baseline will be replaced by enrolling additional patients on a rolling basis. Replacement patients will be stratified and randomized to treatment in the same manner as original patients. Replacement patients will not be "selected" by dose group or strata (age, vaccination status, transplant type, time between symptom onset and treatment) to match the patients whom they replace.

Section 5 Table 3 Schedule of Assessments

New text:

None. Row deleted.

Old text:

Serology for influenza antibody titer	x							x

Section 5 Table 3 Schedule of Assessments footnotes b and c

New text:

b. Hematology (CBC with differential) and chemistry (AST, ALT, total bilirubin, urea, Cr). Serum creatinine testing may be done at a local laboratory **when clinically indicated** to calculate creatinine **clearance**.
c. Baseline swab samples will be assessed for the presence of **resistance mutations.**

Old text:

b. Hematology (CBC with differential) and chemistry (AST, ALT, total bilirubin, urea, Cr). Serum creatinine testing may be done at a local laboratory at any time to calculate creatinine clearance and make dose adjustments. Patient may receive his/her first dose prior to Cr Cl results being available, provided certain renal function criteria are met - see [4.2] [5.3].

c. Baseline swab samples will be assessed for the presence of influenza A H1N1 with H274Y mutation.

Section 5.1 Screening Examination and Eligibility Screening Form

New text:

None. Text deleted.

Old text:

Trial sites will be activated during the influenza season.

New text:

However, if necessary, screening may take place the day before the baseline visit, as long as the patient is randomized and receives his/her first dose of study medication within **96** hours of influenza symptom onset.

Old text:

However, if necessary, screening may take place the day before the baseline visit, as long as the patient is randomized and receives his/her first dose of study medication within 48 hours of influenza symptom onset.

Section 5.2 Procedures for Enrollment of Eligible Subjects

New text:

Randomization will be stratified by transplant **status** (**Yes; No**); time between onset of symptoms and treatment start (≤ **48** hrs or > **48** hrs), influenza vaccination status (Yes; No) and patient's age (≤ 12 years or > 12 years).

Old text:

Randomization will be stratified by transplant type (SOT or HSCT); time between onset of symptoms and treatment start (≤ 24 hrs or > 24 hrs), influenza vaccination status (Yes; No) and patient's age (≤ 12 years or > 12 years).

Section 5.3 Clinical Assessments and Procedures

New text: (text deleted)

At all visits subjects will receive the routine care for their primary illness. Routine patient care (including the adjustment of doses of any of the immunosuppressive drugs) will be dictated by tests performed locally at the trial site.

Old text:

At all visits subjects will receive the routine care for their primary illness (transplant). Routine patient care (including the adjustment of doses of any of the immunosuppressive drugs) will be dictated by tests performed locally at the trial site. Dose adjustments of oseltamivir will be based on serum creatinine done at the local laboratory.

Study Day 1

New text:

Baseline nasal and throat swab samples will be assessed for the presence of **oseltamivir-resistance mutations.**

Old text:

Baseline nasal and throat swab samples will be assessed for the presence of oseltamivir-resistant influenza A H1N1 with H274Y mutation. As soon as they are available, results will be reported to the sites to assist in patient management.

New text:

None. Text deleted.

Old text:

The study will utilize a central safety laboratory. However, to determine the correct dose, a local serum creatinine will be drawn at baseline in all subjects and used to calculate creatinine clearance to determine if a dose adjustment is necessary. No dose adjustments are required for subjects whose creatinine clearance is > 30 ml/min in adults (> 30 ml/min/1.73 M^2 in children). Oseltamivir dosing frequency for patients whose creatinine clearance is between 10 to 30 ml/min in adults (10 to 30 ml/min/1.73 M^2 in children) will be adjusted to once daily.

For patients who meet the following criteria in section 4.2, dosing will commence only after the baseline creatinine clearance are available and if baseline creatinine clearance > 10 ml/min in adults and > 10 ml/min/1.73M^2 in children. The dose will be adjusted to once daily if the baseline Cr Cl is between 10 to 30 ml/min in adults (10 to 30 ml/min/1.73 M^2 in children).

- most recent creatinine clearance in the 6 months prior is < 30 ml/min in adults and < 30 ml/min/1.73M^2 in children).
- patients who have not had a creatinine clearance assessment in the 6 months prior to randomization.

For patients who meet the following criteria in section 4.2, dosing may commence prior to availability of baseline Cr Cl. Subjects will receive twice daily doses and dosing will be adjusted to once daily if the baseline Cr Cl is between 10 to 30 ml/min in adults (10 to 30 ml/min/1.73 M^2 in children).

- most recent creatinine clearance in the 6 months prior is > 30 ml/min in adults and > 30 ml/min /1.73M^2 in children)

New text:

None. Text deleted.

Old text:

Dosage adjustment will be performed based on creatinine clearance as described in the Dose Modification section 6.1.1.

New text: (text deleted)
End of Follow-Up Day 40

All subjects must attend an end of follow-up visit on day 40.

Old text:
End of Follow-Up Day 40

All subjects must attend an end of follow-up visit on day 40. This visit is important as it is the only post-baseline visit where a serology sample for influenza antibody titers is collected.

Section 5.3.1 Efficacy Assessments

New text:

The primary **efficacy** end point in this study is the time to resolution of all influenza symptoms as recorded in the patient diary.

The clinical efficacy parameters in this study are:

1. Symptoms of influenza-like illness. These are captured in the diary for both adults and children. (Appendix 2) (Appendix 3) Influenza symptoms are graded on a nominal scale from zero (absent/no problem) to 3 (severe or major problem). A score of zero is considered asymptomatic. The primary **efficacy** end point, time to alleviation of all symptoms is comprised of all the symptoms captured in the diary.
2. Temperature. This is captured in the diary. Temperature is used for assessment of the primary and several secondary **efficacy** end points.

Old text:

The primary end point in this study is the time to resolution of all influenza symptoms as recorded in the patient diary.

The clinical efficacy parameters in this study are:

1. Symptoms of influenza-like illness. These are captured in the diary for both adults and children. (Appendix 2) (Appendix 3) Influenza symptoms are graded on a nominal scale from zero (absent/no problem) to 3 (severe or major problem). A score of zero is considered asymptomatic. The primary end point, time to alleviation of all symptoms is comprised of all the symptoms captured in the diary.
2. Temperature. This is captured in the diary. Temperature is used for assessment of the primary and several secondary end points.

Section 5.4 Laboratory Assessments

Efficacy

New text:

Baseline samples will be assessed for the presence of oseltamivir-**resistance mutations**.

Old text:

Baseline samples will be assessed for the presence of oseltamivir-resistant influenza A H1N1 with the H274Y resistance mutation using a real-time PCR assay.

New text:

None. Text deleted.

Old text:

Serology. Blood samples for influenza antibody titer will be collected according to the Schedule of Assessments.

Section 5.4 Laboratory Assessments

Safety

New text:

None. Text deleted.

Old text:

However, in order to assist in patient management, the results of the assay to detect influenza A H1N1 H274Y at baseline will be reported to the sites as soon as they are available.

New text:

The Principal Investigator may draw blood for serum creatinine to be assessed at the local laboratory **when clinically indicated** during the study **to calculate creatinine clearance**.

Old text:

The Principal Investigator may draw blood for serum creatinine to be assessed at the local laboratory at any time during the study. The Principal Investigator may use the creatinine levels to calculate creatinine clearance and adjust dose of study drug if required.

Section 6.1.1 Dose Modifications

New text:

No dose modifications will be allowed on study.

Old text:

No dose adjustments/modifications are required for subjects whose creatinine clearance is > 30 ml/min in adults (> 30 ml/min/1.73 M^2 in children).

In both treatment arms, the dosing frequency will be decreased to once daily in patients with severe renal impairment (Cr Cl in adults between 10 – 30 ml/min and children between 10 – 30 ml/min/1.73M^2). For example, the 75 mg bid frequency will be decreased to 75 mg once daily.

At any time during the study if the investigator feels that renal function is compromised, dosing may be decreased to once daily or withheld until such time creatinine clearance results are available. Dosing may then be resumed as appropriate based on the creatinine clearance. However, it is vital that dosing not be inappropriately withheld for extended

periods of time especially in the first 3 days of treatment when viral titers may be high. It is also important that dose modifications are clearly and accurately recorded for assessment of pharmacokinetic results.

For adolescents and children, the creatinine clearance will be estimated using a Schwarz equation: (Appendix 1).

For adults the method of Cockcroft-Gault will be used to estimate creatinine clearance. (Appendix 1).

Section 6.3 Blinding and Unblinding

New text:

The randomization will be stratified by transplant **status**, duration of symptoms, influenza vaccination status and age and will be provided by the Roche randomization group for the IVRS vendor.

Old text:

The randomization will be stratified by transplant type, duration of symptoms, influenza vaccination status and age and will be provided by the Roche randomization group for the IVRS vendor.

Section 7.2.2 Pregnancy

New text:

A female subject must be instructed to stop taking the test "drug" and immediately inform the investigator if she becomes pregnant during the study. The investigator should report all pregnancies within 24 hours to the sponsor or designee. The investigator should counsel the subject, and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. **Pregnancies occurring within 28 days of treatment completion should be reported to Roche.**

Old text:

A female subject must be instructed to stop taking the test "drug" and immediately inform the investigator if she becomes pregnant during the study. The investigator should report all pregnancies within 24 hours to the sponsor or designee. The investigator should counsel the subject, and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy.

Pregnancy occurring in the partner of a male subject participating in the study should be reported to the investigator and the sponsor or designee after the pregnant partner's consent has been obtained. The partner should be counseled and followed as described above if acceptable, and provides informed consent.

Section 8 Statistical Considerations and Analytical Plan

New text:

Full details of all planned analyses will be specified in a separate **Statistical Analysis Plan (SAP) for the safety and efficacy variables and in the Resistance Plan for the development of resistance.**

Old text:

Full details of all planned analyses will be specified in a separate Data Reporting and Analysis Manual (DRAM).

Section 8.1.1 Primary Endpoints

New text:

The primary endpoints for safety will be assessments of adverse events, physical exams, vital signs and clinical laboratory evaluations. The primary endpoint of development of resistance will be determined from the genotypic and phenotypic variables measured post baseline and is described further in the Resistance Plan.

Old text:

The primary endpoint in this study is the time to alleviation of all clinical influenza symptoms (recorded in the patient diary).

Section 8.1.2 Secondary Endpoints

New text:

The following are secondary endpoints **which measure efficacy, the primary of which is time to alleviation of all clinical symptoms.** With the exception **of this variable,** viral load and time to resolution of fever, they are defined as either the occurrence or non-occurrence of the specified events or the presence or absence of the specified symptoms.

> **The time (hours) to alleviation of all clinical influenza symptoms (recorded in the patient diary)**

Old text:

The following are secondary endpoints. With the exceptions of viral load and time to resolution of fever, they are defined as either the occurrence or non-occurrence of the specified events or the presence or absence of the specified symptoms.

New text:

None. Text deleted.

Old text:

Baseline, post-baseline and change from baseline in antibody titers.

Section 8.1.3 Safety

New text:

Safety of the treatment will be evaluated by AEs, **physical exams, clinical** laboratory tests, and vital signs.

Old text:

Safety of the treatment will be evaluated by AEs, laboratory tests, and vital signs.

All subjects who received at least one dose of treatment and had a safety assessment performed post randomization will be included in the safety evaluation.

Section 8.2.1.1 (Title changed)

New text: ***Time to Event Variables***

Old text: ***Primary Variables***

Section 8.2.1.2 (Title changed)

New text: ***Dichotomous Variables and Viral Load***

Old text: ***Secondary Variables***

New text:

For the endpoints defined dichotomously in terms of events or symptoms **including the development of resistance**, it will be assumed that the number of patients experiencing the event or exhibiting the symptom will have a binomial distribution.

Old text:

For the secondary endpoints defined dichotomously in terms of events or symptoms, it will be assumed that the number of patients experiencing the event or exhibiting the symptom will have a binomial distribution.

For the continuous endpoint of time to resolution of fever, the same assumptions as for the primary endpoint will be made.

Section 8.2.2 Sample Size

New text:

The sample size has been chosen to provide an adequate number of patients to estimate the development of resistance with reasonable precision. Assuming that 90% of enrolled patients will have laboratory-confirmed influenza, there will be 75 patients in each treatment arm in the population evaluable for the development of resistance. The following table shows the 95% Pearson-Clopper confidence intervals that would result with a sample size of 75 patients in a treatment arm if certain event rates are observed in the study.

During the study the number of influenza A virus infected patients and the rate of development resistance will be monitored in a blinded fashion, in order to ensure a reasonable precision for the estimation. An additional number of patients may be enrolled if necessary.

Observed Rate (%)	95% Confidence Interval
0.0	0% - 4.8%
1.3	0.0% - 7.2%
2.7	0.3% - 9.3%
5.3	1.5% - 13.1%
10.7	4.7%-19.9%

A total of 83 patients will be enrolled per arm and will be evaluable for the assessment of safety. This number of patients would provide estimates of adverse event rates with similar precision.

Old text:

Sample size calculations were performed using a two-sided t-test and are based on the comparison with a placebo control response in the pivotal registration trials in healthy adults of 112 hours for median time to alleviation of all symptoms. Assuming an expected median time to alleviation of symptoms of 78 hours for each of the two active treatment groups in this study, and a common standard deviation of 98 hours, then 111 evaluable subjects per group would give more than 90% power to detect a difference between the control and each of the active treatment groups. It is assumed that as many as 90 % of subjects enrolled into the study who are identified as not being infected with influenza A H1N1 H274Y will have influenza, and therefore 124 such subjects per arm would be required in order to obtain 111 influenza positive subjects. Note that this is an approximate calculation, as the final analysis will be based on the difference in medians and the corresponding 95% confidence interval from the Kaplan-Meier curves. This is however considered as a sufficient indication for the number of required subjects and the power that is expected. The maximum number of subjects enrolled in the trial will depend upon the number of subjects identified with the oseltamivir resistant virus who will be replaced.

Section 8.2.4 Analysis Populations

New text:

Detailed definitions of these populations will be given in the **SAP**.

Old text:

Detailed definitions of these populations will be given in the DRAM.

Section 8.2.4.2 *Intent to Treat Infected Population*

New text:

All patients randomized and with laboratory confirmation of influenza infection, excluding patients infected with oseltamivir-resistant influenza at baseline, will be included in the intent to treat influenza infected population [Patients will be assigned to treatment groups as randomized for analysis purposes].

The ITTI Population will be the primary population for the summary and analysis of the **development of resistance and the** efficacy variables.

Old text:

All patients randomized and with laboratory confirmation of influenza infection (positive viral cultures or 4 fold or greater rise in antibody titers), excluding patients infected with oseltamivir-resistant influenza A H1N1 H274Y at baseline, will be included in the intent to treat influenza infected population [Patients will be assigned to treatment groups as randomized for analysis purposes].

The ITTI Population will be the primary population for the summary and analysis of the primary and secondary efficacy variables.

Section 8.2.4.3 *Subpopulations*

New text:

A subpopulation of the ITTI population will be defined comprising patients who received their first dose of study medication within 48 hours of influenza symptom onset for the comparison of efficacy endpoints with the age appropriate placebo treated patients (pediatric or adult) from the registration trials.

Old text:

None. Text added.

Section 8.2.5 Efficacy Analysis

New text:

For the analysis **of time to alleviation of all clinical symptoms**, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- Comparison to placebo control from pivotal registration trials

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established **that is comparable to the subpopulation of patients in** the current study **whose first dose of study medication was within 48 hours of symptom onset.** For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

  The median time to resolution of all influenza symptoms will be determined for each treatment group, along with its 95% confidence interval. These confidence intervals will be compared individually to the placebo control confidence interval described above as a potential means of establishing treatment effects.

- Assessment of relative efficacy

  The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval.

This confidence interval will provide lower and upper limits for the treatment difference and can be used as the basis for potential inferences. For example, if it does not contain the value zero a difference will have been established.

For the dichotomous endpoints, the proportion experiencing the event or symptom, and its associated 95% confidence interval will be derived for each treatment group. Where possible, the corresponding confidence intervals from the placebo control population will be presented for comparison to those derived for the active treatment groups in this trial.

The continuous secondary endpoint time to resolution of fever will be analyzed as for the primary **efficacy** endpoint.

For the continuous endpoints of viral load ($log_{10}TCID_{50}$/mL) at Day 1, 6, 8, 11, 15, and 40, summary statistics (including mean, **standard deviation,** median, minimum and maximum) will be derived for each treatment group.

Old text:

For the primary analysis, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- Comparison to placebo control from pivotal registration trials

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established to match (in terms of efficacy evaluations, duration of observation etc.) those of the current study. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

  The median time to resolution of all influenza symptoms will be determined for each treatment group, along with its 95% confidence interval. These confidence intervals will be compared individually to the placebo control confidence interval described above as a potential means of establishing treatment effects.

- Assessment of relative efficacy

  The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval. The methodology will be based on the use of the Hodges-Lehmann estimator in the case of censored data.

This confidence interval will provide lower and upper limits for the treatment difference and can be used as the basis for potential inferences. For example, if it does not contain the value zero a difference will have been established. However, given the study sample size this outcome is only likely if a clinically significant difference (e.g. 30%) exists. Although this is not anticipated from the experience in pivotal registration trials in normal healthy patients, it cannot be ruled out as a possibility in immunocompromised transplant recipients.

For the dichotomous secondary endpoints, the proportion experiencing the event or symptom, and its associated 95% confidence interval will be derived for each treatment

group. Where possible, the corresponding confidence intervals from the placebo control population will be presented for comparison to those derived for the active treatment groups in this trial.

The continuous secondary endpoints time to resolution of fever will be analyzed as for the primary endpoint.

For the continuous endpoints of viral load ($log_{10}TCID_{50}$/mL) at Day 1, 6, 8, 11, 15, and 40, summary statistics (including mean, median, minimum and maximum) will be derived for each treatment group.

Section 8.2.7 Analysis of Resistance (new section added)

New text:

For the summary of the development of resistance for each treatment arm, 95% confidence intervals will be provided with the estimated rates. Further details are available in the Resistance Plan for NV20234. These data will be summarized in a report separate from the final study report.

Old text:

None. Text added.

Section 8.2.7.1 Exclusion of Data from Analysis

New text:

Efficacy data collected from any patient who begins treatment with another antiviral for influenza (for example, amantadine, rimantadine, **laninamivir, peramivir,** or zanamavir), after the other antiviral treatment commences, will be excluded from the primary and secondary efficacy analyses. Such patients will be considered failures to oseltamivir treatment in the analyses. Further details will be provided in the **SAP.**

Old text:

Efficacy data collected from any patient who begins treatment with another antiviral for influenza (for example, amantadine, rimantadine, or zanamavir), after the other antiviral treatment commences, will be excluded from the primary and secondary efficacy analyses. Such patients will be considered failures to oseltamivir treatment in the analyses. Further details will be provided in the DRAM.

Section 8.2.8 Other Analyses

New text:

Further exploratory analysis, (including assessments of the rapid diagnostic test, sub group analysis) will be detailed in the **SAP.**

Old text:

The number and percentage of patients with influenza infection (defined as a positive culture from a nasal and/or throat swab or 4 fold or greater rise in antibody titers) will be summarized by treatment group for the Intent-to-treat Population.

Further exploratory analysis, (including assessments of the rapid diagnostic test, sub group analysis) will be detailed in the DRAM.

The last positive viral isolate from each patient will be tested for reduced sensitivity to oseltamivir. Clonal resistance assays will also be used to evaluate the rate of resistance to oseltamivir. These data will be summarized in a report separate from the final study report.

Section 10 References

New text: updated version of IB and references 20-40 added

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Old text: references 4, 9 and 16 deleted

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Section Appendices 6, 7, 8

New text:

Appendix 6 Primary Immunodeficiency Conditions

Category	Conditions
Severe Combined Immunodeficiency (SCID)	Adenosine deaminse (ADA) deficiency
	Artemis deficiency (SCIDA)
	CD45 deficiency
	Cernunnos deficiency
	DNA ligase IV (LIG4) deficiency
	Interleukin receptor γ chain deficiency (X-linked SCID)
	Janus-associated kinase 3 (JAK3) deficiency
	Recombinase activating gene (RAG 1 / 2) deficiency
	Reticular dysgenesis
	TAP- 1 or TAP- 2 deficiency (MHC class I deficiency)
Primary T cell Deficiency	CD8 deficiency
	diGeorge syndrome
	Interleukin 7 receptor α (IL7RA) deficiency
	MHC class II deficiency
	LCK deficiency
	Orai 1 deficiency
	Nude syndrome (wing helix nude deficiency)
	Purine nucleotide phosphorylase (PNP) deficiency
	T cell receptor deficiency (CD 3γ, δ, ε, and ζ deficiencies)
	Zap 70 tyrosine kinase deficiency

Predominantly Antibody Deficiency	**X-Linked CD40 ligand deficiency**
	X- Linked IKK-γ (NEMO) deficiency
	CD40 deficiency
Other Well-Defined immunodeficiency Syndromes	**Interferon γ receptor deficiency**
	X-Linked lymphoproliferative syndrome

Adapted from Table 310-2: p2056 reference 20.

Appendix 7 Hematologic Malignancies and their Effect on Immune System

Malignancy	Effect on immunity
ALL, lymphomas	**suppression of hematopoiesis, neutropenia, lymphocyte dysfunction**
CLL, small lymphocytic lymphoma	**hypogammaglobulinemia, increased susceptibility to infections, autoimmune anemia or thrombocytopenia**
Hairy cell leukemia, myelodysplastic syndromes	**pancytopenia**
peripheral T cell and NK neoplasms	**lymphocyte dysfunction, increase in immature cells**
Hodgkin's disease	**suppression of cell-mediated immunity**
AML	**neutropenia, anemia, thrombocytopenia, predisposed to infections, lymphocyte dysfunction**
CML	**anemia, granulocyte dysfunction in some patients in early phase and in most patients in blast phase**

Adapted from reference 20.

Appendix 8 Immunosuppressive Medications

Class	Category	Drugs
Corticosteroids	**-**	**Glucocorticoids (oral, sc, im, iv)**
Cytotoxic agents[20]	**Alkylating agents**	**cyclophosphamide, busulfan, mechlorethamine, chlorambucil, melphalan, carmustine, lomustine, ifosfamide, procarbazine, dacarbazine, temozolomide, cisplatin, carboplatin, oxaliplatin**
	Anti-metabolites	**methotrexate, 6-mercaptopurine, azathioprine**
	Anti-tumor antibiotics	**bleomycin, actinomycin D, mitomycin C, etoposide, topotecan, irinotecan, doxorubicin, daunorubicin, idarubicin, epirubicin, mitoxantrone**
	Anti-mitotic agents	**vincristine, vinblastine, vinorelbine, paclitaxel, docetaxel, estramustine phosphate, NAB-paclitaxel**
	Molecularly targeted agents	**imatinib, tretinoin, bexarotene, denileukin, diftitox, gefitinib, erlotinib, dasatinib, sorafenib, sunitinib**
Calcineurin inhibitors	**-**	**cyclosporine, tacrolimus**
mTOR inhibitors (proliferation-signal inhibitors)	**-**	**sirolimus, everolimus**

Immunosuppressive antibodies	**-**	**anti lymphocyte and antithymocyte globulins (ALG and ATG)**
Monoclonal antibodies [41, 42]	**Inhibitors of pro inflammatory cytokines**	**Adalimumab**
		Infliximab
		Cetrolizumab
		Etarnecept
		Basiliximab
		Daclizumab
	Adhesion cell modulators	**Natalizumab**
	T-cell inhibitors	**Abatacept**
		Alefacept
		Muromonab
	B-cell inhibitors	**Rituximab**
		^{90}Y-Ibritumomab
		^{131}I-Tositumomab
	Anti-CD33	**Gemtuzumab**
	Anti- CD52	**Alemtuzumab**
Others	**-**	**mycophenolate mofetil, thalidomide**

Compiled from Table 81-2: p521-24 reference 20 and references 41, 42.

Old text:

None. Text added.

10. SUBJECT: REPORTING OF SERIOUS ADVERSE EVENT FROM SCREENING

Reason for change:

To clarify that not only serious adverse events that occur after the first dose of study medication but also events that occur between screening and the first dose are to be reported.

Section 7.2.1 Reporting of Serious Adverse Events [immediately reportable]

New text:

Any clinical AE or abnormal laboratory test value that is *serious* **[as defined in Section 7.1.1.3 above] and which occurs during the course of the study, regardless of the treatment arm, occurring from the enrollment visit (start of study screening procedures),** must be reported to Roche or designee **within *one* working day** of the investigator becoming aware of the event [expedited reporting].

Old text:

Any clinical AE or abnormal laboratory test value that is *serious* and which occurs during the course of the study [as defined in section 7.1.1.3 above], regardless of the treatment arm, must be reported to Roche or designee **within *one* working day** of the investigator becoming aware of the event [expedited reporting].

F. HOFFMANN-LA ROCHE LTD

CLINICAL STUDY PROTOCOL

PROTOCOL NUMBER NV20234C

RO 64-0796

TAMIFLU® (OSELTAMIVIR)

EUDRACT NUMBER 2006-002468-24

Sponsor: **F. HOFFMANN-LA ROCHE LTD**
Grenzacherstrasse 124,
4070 Basel, Switzerland

PROTOCOL APPROVAL

Protocol Number / Version: NV20234 / C

Date: See last date in electronic signature manifestation below.

Protocol approved by: See electronic signature manifestation below

Name	Reason for Signing	Date and Time (UTC)
[redacted]	Company Signatory	28-Mar-2011 15:00:49

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SYNOPSIS OF PROTOCOL NUMBER NV20234C

TITLE	A double-blind, randomized, stratified, multi-center trial evaluating conventional and high dose oseltamivir in the treatment of immunocompromised patients with influenza		
SPONSOR	F. Hoffmann-La Roche LTD	CLINICAL PHASE	IIIb
INDICATION	Treatment of influenza in immunocompromised patients		
OBJECTIVES	Primary: To evaluate prospectively the **safety and tolerability** of oseltamivir for the treatment of influenza in **immunocompromised patients and characterize the effects of oseltamivir in immunocompromised patients on the development of resistant influenza virus** Secondary: To evaluate the effects of conventional and high dose oseltamivir in **immunocompromised patients** on: • **The time to resolution of influenza symptoms** • The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis) • The virologic course of influenza (proportion shedding and viral loads at different time points)		
TRIAL DESIGN	This is a double-blind, randomized, multi-center trial of twice daily, conventional and high dose oseltamivir for the treatment of influenza in immunocompromised patients. Subjects will be stratified by age **(≤ 12, > 12 years), transplant status** (yes, no), time since onset of flu symptoms and treatment start **(up to 96 hours)** (≤ **48** or > **48** hours) and vaccination status **(yes, no)**		
NUMBER OF SUBJECTS	A minimum of **166** (**83** per arm) **to allow an adequate number of influenza A patients per arm; including 50 transplant recipients.**		
NUMBER OF CENTERS	Approximately **100 - 110** centers in the Northern hemisphere		
TARGET POPULATION	**Patients immunocompromised due to a primary or secondary immunodeficiency,** 1 year of age and older. The subjects will be positive for influenza by a rapid diagnostic test, **PCR or virus culture** at baseline.		
LENGTH OF STUDY	10 days of treatment, 30 days of follow up.		

INVESTIGATIONAL MEDICINAL PRODUCT(S) DOSE/ ROUTE/ REGIMEN	Oseltamivir/placebo dry powder (to be reconstituted to a concentration of 12 mg/ml) and 75 mg capsules. The duration of dosing in both adults and children is 10 days. Conventional dose: Children ages 1 - 12 years: Oseltamivir syrup ≤ 15 kg 30 mg twice daily > 15 – 23 kg 45 mg twice daily > 23 – 40 kg 60 mg twice daily > 40 kg 75 mg twice daily Adults and adolescents (age ≥ 13 years): Oseltamivir capsules 75 mg twice daily Subjects randomized to the conventional dose will simultaneously receive matching placebo so as to blind them and the investigator from the high dose arm. **<u>High dose</u>**: Children ages 1 - 12 years: Oseltamivir syrup ≤ 15 kg 60 mg twice daily > 15 – 23 kg 90 mg twice daily > 23 – 40 kg 120 mg twice daily > 40 kg 150 mg twice daily Adults and adolescents (age ≥ 13 years): Oseltamivir capsules 150 mg twice daily Dose adjustments: In both treatment arms, patients **whose CrCl decreases to < 60ml/min/1.73M^2 will discontinue study medication**.
COMPARATOR "DRUG" (or STANDARD OF CARE) DOSE/ ROUTE/ REGIMEN	Placebo (from pivotal registration trials in otherwise healthy adults)
ASSESSMENTS OF:	
- **SAFETY**	**Adverse events, physical exams, vital signs, clinical laboratory evaluations**
- **RESISTANCE**	**Development of resistance**
- **EFFICACY**	**Time to resolution of all clinical influenza symptoms as recorded in the patient diary.**

PROCEDURES (summary):

The key procedures are:

Blood draws for serum chemistry, hematology, and serology.

Nasal and throat swabs for viral culture and RT-PCR.

STATISTICAL ANALYSES:

The sample size has been chosen to provide an adequate number of patients to estimate the development of resistance with reasonable precision. Assuming that 90% of enrolled patients will have laboratory-confirmed influenza, there will be 75 patients in each treatment arm in the population evaluable for the development of resistance. The following table shows the 95% Pearson-Clopper confidence intervals that would result with a sample size of 75 patients in a treatment arm if certain event rates are observed in the study.

During the study the number of influenza A virus infected patients and the rate of development of resistance will be monitored in a blinded fashion, in order to ensure a reasonable precision for the estimation is maintained. Additional patients may be enrolled if necessary.

Observed Rate (%)	95% Confidence Interval
0.0	0% - 4.8%
1.3	0.0% - 7.2%
2.7	0.3% - 9.3%
5.3	1.5% - 13.1%
10.7	4.7%-19.9%

A total of 83 patients will be enrolled per arm and will be evaluable for the assessment of safety. This number of patients would provide estimates of adverse event rates with similar precision.

For the primary objective of evaluating the safety of oseltamivir conventional and high dose treatments, AEs, laboratory tests, and vital signs will be summarized and compared with the known safety profile of the drug. For the summary of the development of resistance for each treatment arm, 95% confidence intervals will be provided with the estimated rates.

For the secondary objective of evaluating the efficacy of oseltamivir as measured by the time to resolution of influenza symptoms, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- **Comparison to placebo control from pivotal registration trials**

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established that is comparable to patients in the current study. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

- **Assessment of relative efficacy**

  The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval.

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GLOSSARY OF ABBREVIATIONS

AIDS	**Acquired immunodeficiency syndrome**
ALT [SGPT]	Alanine aminotransferase
ALL	**Acute lymphocytic leukemia**
AML	**Acute myeloid leukemia**
ANC	Absolute neutrophil count
ANOVA	Analysis of variance
AST [SGOT]	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
b.i.d.	Bis in die (twice daily)
BP	Blood pressure
CARIFS	Canadian acute respiratory infections scale
CDC	**Centers for Disease Control and Prevention**
CI	Confidence interval
C_{max}	Maximum plasma concentration
CML	**Chronic myeloid leukemia**
Cr Cl	Creatinine clearance
CRF	Case report form[s]
ESF	Eligibility screening form
GVHD	Graft versus host disease
hrs	Hours
HA	Hemagglutinin
HIV	**Human immunodeficiency virus**
HSCT	Hematopoietic stem cell transplant
ICH	International Conference on Harmonisation
ITT	Intent to treat
ITTI	Intent to treat influenza infected
IVRS	Interactive voice response system
mg	Milligram

GLOSSARY OF ABBREVIATIONS

mL	Milliliter
PD	Pharmacodynamic
PK	Pharmacokinetic
p.o.	Per os (by mouth)
PR	Pulse rate
QD	Once per day
SAE	Serious adverse event
SAP	**Statistical analysis plan**
SCID	**Severe combined immunodeficiency**
SOT	Solid organ transplant
$TCID_{50}$	50% tissue culture infectious dose
Tmax	Time of maximum plasma concentration
t1/2	Elimination half-life
µg	Microgram

PART I: STUDY DESIGN AND CONDUCT

1. BACKGROUND AND RATIONALE

1.1 Background

Influenza is an acute respiratory infection caused by a virus of the orthomyxovirus family which occurs in three forms, influenza A, B and C. Influenza virus types A and B cause an acute febrile infection of the respiratory tract characterized by the sudden onset of fever, malaise, headaches, myalgias and cough. Influenza causes numerous deaths each year [1]. Although difficult to assess, annual influenza epidemics are thought to result in between three and five million cases of severe illness, and between 250,000 and 500,000 deaths every year around the world [2].

The influenza viruses are segmented, negative sense, single stranded, lipid encapsulated, RNA viruses between 80 and 100 nm in size. Subtypes are defined according to glycoproteins present in the viral lipid coat. The haemagglutinin complex (HA) is the major surface protein of the virus. The neuraminidase (NA) protein is the second major surface protein in the virion and plays a role in enhancing virus penetration of the mucus layer around the target cell and in release of virus from the cell surface. The matrix proteins, M1 and M2 appear to trigger the disintegration of the M1 complex during virus fusion with the cell and may also be involved in the maturation of the HA prior to assembly of new virus particles.

1.1.1 Influenza in the Immunodeficient Population

Influenza infection is usually a self limiting condition. However, in children, the elderly and the immunocompromised, influenza infection can be associated with substantial morbidity and mortality [1]. In patients with compromised immunity, influenza virus may cause severe lower respiratory tract involvement; unusual syndromes, including encephalitis, myocarditis, and hepatitis and death [3].

Conditions that compromise immunity may be classified based on etiology into primary (genetic) and secondary (acquired) immunodeficiency. Of the immunodeficient conditions, the ones that affect cell mediated immunity are likely to have adverse outcomes following viral infections [20].

Primary immunodeficiency

Primary immunodeficiencies are relatively common, may be either congenital or manifest later in life and are classified according to whether the genetic defect affects T or B cells or both [20]. There are four groups of disorders: severe combined immunodeficiency (SCID), primary T cell deficiency (e.g. CD8 deficiency, DiGeorge syndrome), predominantly antibody deficiency (e.g. common variable immunodeficiency, selective IgA deficiency) and other well-defined immunodeficiency syndromes (e.g. Wiskott Aldrich syndrome) [20].

Of the primary immunodeficiencies, antibody deficiencies are the most frequent. However, some of the more common antibody deficiency conditions (isolated IgA deficiency, IgG subclass deficiency and common variable immunodeficiency) have intact cell-mediated immunity and therefore the clinical course of viral infections

(unless complicated by bacterial infections) does not differ significantly from that in the normal host [20]. A list of primary immunodeficiency disorders at risk for viral infections is provided in Appendix 6. The incidence of some of these conditions has been estimated. The incidence of SCID is 1 in 100,000 to 1 in 1,000,000 [20]. The more severe forms of primary immunodeficiency are relatively rare, have their onset early in life, and all too frequently result in death during childhood [20].

Secondary Immunodeficiency

Secondary immunodeficiencies are not caused by intrinsic abnormalities in development of T and B cells [20]. Secondary immunodeficiency may result from diseases (human immunodeficiency virus [HIV], hematologic malignancy) or immunosuppressive and cytotoxic drugs (such as those used for treatment of transplant recipients, collagen vascular disease, malignancies).

Secondary immunodeficiency due to disease

HIV-infected individuals with a CD4+ T cell count of < 200/μL are highly susceptible to opportunistic disease [20]. Studies in HIV/acquired immunodeficiency syndrome (AIDS) subjects have shown an increased risk for heart and lung-related hospitalizations during the influenza season compared to other times of the year, prolonged duration of influenza symptoms, increased risk for influenza-related complications and a higher risk of influenza-related death [21].

Several hematologic malignancies affect the immune system (Appendix 7). Several authors have reported influenza in children and adults with hematologic malignancies [22, 23, 24, 25].

Secondary immunodeficiency due to drugs (e.g. transplant recipients, collagen vascular disease, malignancies)

The enhanced survival of the transplant population following the availability of newer immunosuppressive drugs has made them representative of the immunocompromised population in general; and secondary immunodeficiency due to drugs, in particular.

In transplant recipients influenza is associated with a higher rate of pulmonary complications, extrapulmonary manifestations and an increased risk of graft dysfunction and high attributable mortality. In a cohort study of influenza viral infection in **solid organ transplant (SOT)** subjects, 30 cases of influenza viral infection were identified. Secondary bacterial pneumonia was seen in 17% of subjects and three SOT recipients (2 liver and one kidney) had myositis, myocarditis and bronchiolitis obliterans. Biopsy of the transplanted organ was performed in 21 of 30 cases and revealed variable degrees of acute allograft rejection in 62% of subjects. The study concluded that influenza was associated with significant morbidity in different groups of SOT recipients [5].

Among transplant recipients, subjects with **hematopoetic stem cell transplant (HSCT)** are at the greatest risk for morbidity. In a prospective study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation, 1973 patients were evaluated for respiratory virus infections after stem cell transplant. The

overall mortality was 23% from influenza A infection and the direct influenza A-associated mortality was 15.3% in this study [6]. **A large retrospective study (4797 subjects undergoing HSCT over a 13-year period) identified 62 patients with influenza of whom as many as 29% (18 of 62 patients) developed pneumonia. Ten percent of the subjects with influenza died [7].**

The increased morbidity in transplant patients is associated with increased duration of virus shedding compared to that in healthy subjects. In otherwise healthy adults, elderly subjects and children, the median duration of viral shedding in untreated subjects was 70, 96 and 118 hours respectively [8]. In contrast, in the retrospective HSCT study, influenza virus was shed in nasopharyngeal secretions (evaluated at least weekly) for a median duration of 168 hours (7 days, range 2 to 37 days) [7].

Currently available treatments for influenza infection are the M2 ion channel inhibitors (e.g. amantadine, rimantadine) and the neuraminidase inhibitors (e.g. oseltamivir, zanamivir).

1.1.2 Oseltamivir

Oseltamivir (Tamiflu®, Ro 64-0796) is an ethyl ester prodrug which is rapidly absorbed from the gastrointestinal tract after oral administration and metabolized in the liver by high capacity carboxylesterases to form oseltamivir carboxylate (Ro 64-0802), a potent, stable and selective inhibitor of influenza A and B neuraminidase enzymes. The active form, oseltamivir carboxylate is excreted unchanged by the kidney via glomerular filtration and active tubular secretion by the organic anion transport system. The efficacy and safety of oseltamivir in influenza treatment and prevention has been established in an extensive series of clinical studies in man.

Oseltamivir has been approved for the treatment of influenza in Europe, the United States and most other countries around the world. In adults and adolescents, the recommended dose is 75 mg twice daily for five days. In children 1 year of age and older recommended doses are 30, 45 or 60 mg bid based on body weight. In all age groups the recommended dose is administered bid for 5 days.

The approval of oseltamivir for the treatment of influenza is based on several controlled clinical trials. In the pooled population from these clinical trials encompassing adults aged from 13-97 years, many with significant co-morbidity, 1325 subjects were treated with oseltamivir (75 mg bid) and 1056 subjects received placebo. A total of seven influenza symptoms (both respiratory and constitutional) were captured on the diary card for adults. The time to resolution of all symptoms (on the diary card) decreased by 24 hours; from 124.5 hours in the placebo arm to 100.6 hours with oseltamivir 75 mg bid ($p < 0.0001$) [8]. Further, in adults and adolescents with a proven influenza illness, oseltamivir treatment reduced overall antibiotic use for any reason by 26.7% and the incidence of influenza-related lower respiratory tract complications resulting in antibiotic therapy by 55%. The study concluded that oseltamivir treatment of influenza reduces lower respiratory tract complications, antibiotic use and hospitalizations in healthy and 'at risk' subjects [11].

Likewise, in the influenza-infected pediatric population (1 to 12 years of age), oseltamivir treatment (n = 217) was compared with placebo (n = 235). There was a reduction in the median duration of illness (defined based on resolution of temperature, cough, coryza and return to pre-illness health and activity) of 36 hours; from 137 hours with placebo to 101 hours in the oseltamivir treatment arm (p=<0.0001). The Canadian Acute Respiratory Infections Scale (CARIFS), validated for use in children, was used to collect symptom data on the pediatric diary card. The CARIFS scale comprised a total of 18 symptoms which were evaluated twice daily by the parent or guardian. There was a similar reduction in the time to alleviation of all CARIFS symptoms of 36 hours; from 100 hours in the placebo group to 63 hours in the oseltamivir group (p < 0.0001) [12].

Thus in both adults and children, the time to resolution of all symptoms was significantly reduced in the oseltamivir treatment arm compared with placebo.

Oseltamivir was well-tolerated in clinical trials. Approximately 11,000 subjects have received oseltamivir in the development program. The most common adverse events reported by adults, the elderly, and children were nausea and vomiting. Serious adverse events (SAEs) were reported with a low and equal frequency by subjects taking active drug and placebo. Full details are given in the Investigator Brochure [8].

The safety profile of oseltamivir has been well characterized for the prophylaxis indication in a prospective randomized placebo controlled trial conducted in the adult and pediatric immunocompromised (HSCT and SOT) population. In the oseltamivir group, the indications for transplant included hematologic malignancies (acute and chronic leukemias, multiple myeloma and myelodysplastic syndrome), lymphoid malignancies (Hodgkins or non-Hodgkins lymphomas), primary immunodeficiencies (Wiskott-Aldrich syndrome, X-linked lymphoproliferative disease and severe combined immunodeficiency). Other rare indications included bone marrow aplasia, paroxysmal nocturnal hemoglobinuria, myelofibrosis and multiple sclerosis. In the safety population, there were 239 subjects randomized to the conventional dose of oseltamivir and 238 to placebo. The total number of adverse events reported in the placebo group (361 events) was generally similar to that in the oseltamivir group (323 events). Diarrhea was the most frequently reported adverse event (placebo, 8%; oseltamivir, 6%). There were no deaths in the oseltamivir group. Oseltamivir was found to be safe in immunosuppressed transplant recipients [26].

Limited safety and/or efficacy of oseltamivir for the treatment indication is available from several case reports in children and adolescents. Oseltamivir was shown to be safe and/or effective in HIV infected children (n=10) [27], children (age 3 to 12 years) with acute lymphocytic leukemia (ALL) (n=10) [25], in a nosocomial H1N1 outbreak in a pediatric (children aged 10 months to 13 years) oncology ward (n=8) [24], in children and adolescents (age 2 to 19 years) with malignancies (ALL, neuroblastoma, brain tumor, non-Hodgkin's lymphoma, osteosarcoma, Ewing sarcoma, myelodysplasia, acute myeloid leukemia (AML), Wilms tumor, aplastic anemia, chronic myeloid leukemia (CML) and acute promyelocytic leukemia) (n=51) [22], in children aged 4 to 14 years on immunosuppressive drugs (n=5) [28] and in children (aged 5 months to 5 years) with bone marrow transplant (n=3) [29].

Oseltamivir has also been shown to be safe and/or effective in mixed populations of children and adults (62 patients) with HSCT [7], and in a large epidemiologic study (n=221) with SOT [30].

Finally, oseltamivir has been shown to be safe and/or effective in immunocompromised adults with HSCT [13] and adults with lung transplant [31, 32, 33].

The dose of oseltamivir was the same as the conventional dose in a majority of these reports. In one report as many as 25 adult patients received twice the conventional dose [30] while in another report, three of nine adult patients received twice the conventional dose [33]. Treatment with oseltamivir generally ranged from 5 to 10 days [32, 33, 30] and occasionally until the patient was symptom free [32, 33]. In exceptional cases treatment was given for as long as 20 days [22] or for as long as the patient was positive by RT-PCR [25].

There is some concern about the development of resistance in the immunocompromised population. During the pandemic influenza season, more than 23,000 clinical isolates of novel H1N1 pandemic virus were tested for resistance in the 6 international WHO regions. A total of 225 isolates (from 225 subjects) were resistant (H275Y mutation in the neuraminidase coding sequence) to oseltamivir for an approximate incidence of 1%. Information on immune status was available for 142 of 225 patients. Among the 142 patients, 56 (40%) were immunocompromised [34]. Prolonged viral replication and lack of immune-mediated virus clearance in immunosuppressed patients treated with antivirals can result in a higher incidence of selection of drug-resistant viruses, a phenomenon that has been documented previously [35, 36].

1.2 Rationale for the Study

Because of the increasing body of evidence (Section 1.1.2), oseltamivir is now recommended in national guidelines as an option for the treatment of influenza in the transplant population [40]. As the transplant population is considered representative of the immunocompromised population, the primary objective of this study is to evaluate safety and resistance, while evaluating efficacy as a secondary objective.

2. OBJECTIVES

2.1 Primary Objective

The primary objective of the study is to evaluate prospectively the safety and tolerability of oseltamivir for the treatment of influenza in immunocompromised patients and characterize the effects of oseltamivir in immunocompromised patients on the development of resistant influenza virus.

2.2 Secondary Objectives

The secondary objectives of the study are:

To evaluate the effects of conventional and high dose oseltamivir in **immunocompromised patients** on:

- **The time to resolution of influenza symptoms.**
- The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis).
- The virologic course of influenza (proportion shedding and viral loads at different time points).

3. STUDY DESIGN

3.1 Overview of Study Design and Dosing Regimen

This is a double blind, randomized, stratified, multi-center trial of conventional and high dose oseltamivir for the treatment of influenza in immunocompromised patients. Immunocompromised **patients** who develop an influenza-like illness with a positive rapid diagnostic test, **PCR, or viral culture** for influenza, will be enrolled during the influenza season. Patients will be stratified by transplant **status** [**yes, no**], the time **between** onset of **influenza** symptoms and treatment start **(up to 96 hours)** [≤ **48** hours; > **48** hours], influenza vaccination status for current influenza season [Yes; No] and by age [≤ 12 years; > 12 years]. Eligible patients will be consented and randomized to receive oseltamivir twice daily for 10 days in one of two treatment arms: conventional dose or high dose (double the conventional dose).

3.1.1 Rationale for Study Design

This study incorporates several features that distinguish it from classic placebo controlled trials.

There is no placebo control arm in this study as it was considered unethical for this high risk population. The development of resistance following treatment with oseltamivir (one of the primary objectives of the study) is an objective assessment (determined by laboratory tests) and is unlikely to be impacted by the absence of a placebo arm.

As a placebo control arm was considered unethical in this population, an alternative control arm had to be identified **for efficacy end points**. The response to placebo in pivotal oseltamivir registration trials in the healthy adult population with influenza was chosen as a control in lieu of a placebo arm in the current trial. In order to do this, the end points were designed to be similar to that in the pivotal registration trials.

The following sections provide rationale and justification for specific aspects of the study design **which differ from the currently approved dosing for influenza or from previous pivotal registration trials**. They also provide information on how the results will be used to make recommendations on dose and duration of treatment.

3.1.1.1 Choice of Treatment Arms

The currently approved dose of oseltamivir for the treatment indication is the conventional dose with a duration of five days. In this study a conventional and high dose (twice the conventional dose) are being evaluated. The pivotal registration trials for oseltamivir in healthy adults with influenza had three treatment arms, two active (75 mg and 150 mg) and one placebo. Both treatment arms were found to be significantly better than placebo. No statistical comparisons were made between the two active treatment arms. Evaluation of the results in the two active treatment arms did not reveal any clinically meaningful difference. **However, the proportion of subjects shedding virus on day 4 (3 days after the start of treatment), suggested a possible dose response relationship (36%, 28% and 23% in the placebo, 75 mg and 150 mg dose arms respectively). Because defective immune-mediated virus clearance in immunosuppressed patients treated with antivirals can result in a higher incidence of selection of drug-resistant viruses, it was decided to use both the conventional and high dose arm for this study. A longer duration of treatment was chosen because a large retrospective study has shown that the median duration of viral shedding of 7 days [7] was greater for HSCT recipients than that seen in the healthy children, adult and elderly population with influenza (Section 1.1.1) [8].**

3.1.1.2 Inclusion of Patients Symptomatic up to 96 Hours

In immunocompromised patients, time from onset of symptoms to seeking medical attention (presentation at the clinic) of > 48 hours has been shown in several case reports [37, 31, 32, 33] including two instances of nosocomial outbreaks in children [29, 24]. This notwithstanding, oseltamivir has been shown to be effective in immunocompromised populations that included patients treated with oseltamivir beyond 48 hours of presentation; patients with lung transplant (median time to presentation 3 days) [31], bone marrow transplant (treatment started more than 48 hours after onset of symptoms in all three patients) [29], organ transplant (62 out of 221 patients started treatment after 96 hours) [30] and children with ALL (one child presented after 3 days and two children after 5 days of symptoms) [25].

In a prospective, observational study involving adults hospitalized with influenza, the study authors concluded that 'Weakened host defenses slow viral clearance, whereas antivirals started within the first four days of illness enhance viral clearance' [38].

Additionally, in November 2009, in a communication for clinicians on antiviral treatments for H1N1, the US Centers for Disease Control and Prevention (CDC) stated that while antiviral treatment is most effective when started early; both outpatients with risk factors and hospitalized patients still benefit when treatment with oseltamivir is started more than 48 hours after illness onset [39].

3.1.1.3 Interpretation of Study Results

Safety and tolerability and the development of resistance are the primary objectives of this study. These will be characterized descriptively. For the secondary objective of efficacy, the subset of the population enrolled in the first 48 hours will be compared with placebo patients from pivotal registration trials (where patients were

enrolled in the first two days of illness). Sections 8.2.5, 8.2.6, and 8.2.7 provide details on the statistical considerations and their rationale associated with these comparisons.

Stratification

Stratification in this study has no bearing on any comparisons with results from previous trials. However, in order to evaluate a dose response relationship between the two active treatment arms in this trial, stratification remains essential.

Patients will be stratified by transplant status (yes, no) because transplant patients form a relatively large homogenous group in this study and might influence outcome.

Patients will be stratified by time between onset of symptoms and treatment start (< **48** hours or ≥ **48** hours) because previous experience in otherwise healthy adults and children has shown that patients treated early in their illness respond to treatment better than those who are treated later. Patients will also be stratified by age (≤ 12 years and > 12 years) because otherwise healthy children shed virus longer than otherwise healthy adults. It is expected that a similar difference in the duration of viral shedding would exist between immunocompromised children and adults. As vaccinated subjects may respond faster to therapy, the study is also stratified based on influenza vaccination status for the current influenza season (Yes; No).

3.1.2 Rationale for Dose Selection and Adjustment

Dose Selection

The dose of oseltamivir to be used in this study is the conventional, approved dose for children and adults in the treatment of influenza. There will be a second higher dose for comparison which is two times the conventional dose. This higher dose is used based on theoretical considerations which suggest that the higher dose may be associated with improved efficacy and decreased emergence of resistance.

The anticipated pro-drug and metabolite exposures from this higher dose are not expected to exceed maximum exposures seen previously in the oseltamivir development program. The safety and tolerability of the higher dose regimen has already been demonstrated in treatment studies of immunocompetent adult subjects (n = 447) [14]. In a study to demonstrate cardiac safety, in the highest dose group treated with 450 mg b.i.d. for 5 days [n = 99], no subject had a serious adverse event, nor withdrew prematurely. In Phase I studies in adults, oseltamivir has been administered in multiple doses of up to 500 mg b.i.d. Doses of 200 mg b.i.d. and greater have been associated with increased gastrointestinal adverse effects (nausea and vomiting) [8]. In adult subjects with creatinine clearances of ≤ 30 mL/min, doses of 100 mg b.i.d. for 6 days were well tolerated, despite steady-state oseltamivir carboxylate exposures approximately 10-fold higher than those achieved with standard dosing in renally competent individuals [15]. No other adverse effects were reported more frequently with higher doses and no serious adverse events have been reported within the volunteer studies. Co-administration of oseltamivir with food has been demonstrated to substantially reduce the frequency and severity of gastrointestinal side effects.

Thus, the rationale for the higher dose arm in this study is to explore the possibility of improved efficacy and decreased emergence of resistance without an undue increase in risk.

Drug interactions with immunosuppressive medications have also been evaluated. The pharmacokinetics of oseltamivir and oseltamivir carboxylate after administration of 75 mg oseltamivir (conventional adult dose) in subjects with a well-functioning, stable renal allograft who were being maintained on immunosuppressive therapy were studied. These were similar to those described in the literature for adults with comparable degrees of renal function. Oseltamivir was well tolerated and had no clinically relevant effect on the steady-state pharmacokinetics of cyclosporine A, tacrolimus, or mycophenolate mofetil [8].

Duration of Dosing

The duration of dosing chosen for this population (10 days) is longer than that in the healthy adult and pediatric populations (5 days). This is based on observations that the viral shedding and illness are typically longer in immunocompromised patients than it is in healthy adults [7, 13].

Dose Adjustments

In this study, patients whose CrCl decreases to < 60mL/min/1.73M^2 will be discontinued from treatment.

3.1.3 End of Study

The study comprises 10 days of treatment with follow up visits approximately 5 and 30 days later as shown in the schedule of assessments. A rapid diagnostic test for influenza will be performed at the end of the 10 days of treatment. Subjects still having influenza based on the rapid diagnostic test will be treated per the local standard of care by the principal investigator.

3.2 Number of Subjects/ Assignment to Treatment Groups

A minimum of **166** patients **(approximately 83 per arm) to allow an adequate number of influenza A patients, including 50 transplant recipients** will be enrolled in this study**.** After screening, patients will be randomly assigned to one of the two active treatment groups.

3.3 Centers

This will be a multicenter study with approximately **100-110** centers in the Northern hemisphere.

4. STUDY POPULATION

4.1 Overview

The study population comprises immunocompromised adults (including adolescents) and children who have influenza. Additionally, the subjects must not have other medical conditions that will preclude the assessment of efficacy or safety. Influenza vaccinated and non-vaccinated subjects are eligible to participate in this study. **Principal**

investigators will review oseltamivir resistance patterns of strains circulating in the area and weigh the risk versus the benefit before enrolling patients with a potentially resistant strain.

Under no circumstances are patients who enroll in this study permitted to be re-randomized to this study and enrolled for a second course of treatment.

4.2 Inclusion Criteria

- Age greater than or equal to 1 year
- Rapid diagnostic test, **PCR, or viral culture positive** for influenza in the **96** hours prior to first dose
- Immunocompromised subject **defined as one who meets any of the following**:
    - **Primary immunodeficiency at risk for viral infections (representative examples in Appendix 6) OR**
    - **Secondary immunodeficiency**
        - SOT **with ongoing immunosuppression OR**
        - Allogenic HSCT **with ongoing immunosuppression OR**
        - **HIV with CD4 count < 200/mm^3 OR**
        - **Hematologic malignancies (representative examples in Appendix 7) OR**
        - **Systemic (e.g. enteric, sc, im or iv) immunosuppressive therapy, irrespective of medical indication, started at least 12 weeks prior to, and ongoing at the time of first dose of study drug (representative examples in Appendix 8)**
- Symptoms suggestive of influenza like illness including, but not limited to fever, cough, or coryza
- **In patients with history or clinical presentation at randomization suggestive of renal failure; a CrCl > 60 ml/min/1.73M^2**
- Less than or equal to **96** hours between onset of influenza like illness and first dose of study drug
- Parent/guardian willing and able to comply with study requirements and give consent, (country specific age cut off)
- Patient able to comply with study requirements and willing to give assent, as appropriate (country specific age cut off)
- For adult patients, willing and able to comprehend and give written informed consent
- Patients, in the reproductive age group, must agree to utilize an effective method of contraception throughout the study period and **for females** for one reproductive cycle following cessation of study therapy
- Females of childbearing potential must have a negative urine pregnancy test prior to start of study medication

4.3 Exclusion Criteria

- SOT within 6 months of the time of randomization.
- Have in the investigator's opinion experienced acute rejection in the 4 weeks prior to randomization.

- HSCT patients with no evidence of engraftment (engraftment is defined as the point at which a patient can maintain a sustained absolute neutrophil count (ANC) of $> 500/mm^3$ and sustained platelet count of $\geq 20,000/mm^3$, lasting ≥ 3 consecutive days without transfusions).
- HSCT subjects not discharged from hospital after their initial hospitalization for transplantation.
- Have clinical evidence for hepatic decompensation at the time of randomization (clinical icterus, ascites, hepatic encephalopathy, coagulopathy).
- Have cirrhosis of the liver at the time of randomization.
- Patients currently receiving any form of renal replacement therapy including hemodialysis, peritoneal dialysis or hemofiltration.
- Have evidence of active or uncontrolled opportunistic infections (bacterial, fungal, or viral-including cytomegalovirus [CMV] or polyoma virus [BKV]) at the time of randomization. Patients with HCV or HBV are not excluded.
- Patients with **co-morbid conditions which are** uncontrolled. Uncontrolled is defined as disease requiring change of therapy or hospitalization in the 4 weeks preceding randomization. Change of therapy is defined as dose increase or change of medication prior to onset of present influenza like illness.
- Patients with gastrointestinal disorders which might interfere with their ability to absorb oral medication.
- Allergy to the test medication.
- Patients with hereditary fructose intolerance (for subjects who will be taking the liquid formulation).
- Influenza vaccination **with live attenuated vaccine** in the 2 weeks prior to randomization.
- Antiviral treatment (example: amantadine, rimantadine, **oseltamivir, laninamivir, peramivir,** zanamivir and ribavirin) for influenza in the 2 weeks prior to randomization.
- Patients taking probenecid medication.
- Patients who are pregnant or breast-feeding.
- Participation in a clinical trial or expanded access trial with an investigational drug in the 4 weeks prior to randomization or concomitantly with this study.

4.4 Concomitant Medication and Treatment

Antiviral treatments with activity against influenza (e.g. amantadine, rimantadine, zanamivir, ribavirin, **laninamivir, peramivir,** and additional oseltamivir above that specified for this study) are not allowed during the 10-day treatment phase of the study. Concomitant use of an investigational drug during the study is also excluded. Live attenuated vaccines may not be advisable for this population. It is possible that concurrent use with oseltamivir may render live vaccines ineffective.

Medications required for the routine care of the patient may be continued during the study. Concomitant use of probenecid (irrespective of the indication) is not allowed during the study.

4.5 Criteria for Premature Withdrawal

The investigator must discontinue treatment if the creatinine clearance is **< 60** ml/min in adults or **< 60** ml/min/1.73 M^2 in children. The investigator must also discontinue treatment from all subjects with intercurrent illnesses or adverse events suggestive of hepatic decompensation. The investigator also has the right to discontinue treatment in the event of intercurrent illness, adverse events, treatment failure, protocol violations, administrative reasons or other reasons.

The investigator must discontinue treatment with study drug if the patient is to be treated concomitantly with another antiviral for influenza, for example, amantadine, rimantadine, **laninamivir, peramivir,** or zanamavir. However, all patients, including those who discontinue study drug prematurely and/or are treated with another antiviral, will be required to return for follow up approximately 5 and 30 days after the last dose of study medication (day 15 and day 40 assessments).

Subjects have the right to withdraw from the study at any time for any reason.

An excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of subjects should be avoided. Should a subject decide to withdraw, all efforts will be made to complete and report the observations prior to withdrawal as thoroughly as possible.

The investigator should contact the subject or a responsible relative either by telephone or through a personal visit to establish as completely as possible the reason for the withdrawal. A complete final evaluation at the time of the patient's withdrawal should be made with an explanation of why the subject is withdrawing from the study. If the reason for removal of a subject from the study is an Adverse Event, the principal specific event will be recorded on the CRF.

In the case that the subject decides to prematurely discontinue study treatment ["refuses treatment"], he/she should be asked if he/she can still be contacted for further information. The outcome of that discussion should be documented in both the medical records and in the CRF.

4.6 Replacement Policy [Ensuring Adequate Numbers of Evaluable Subjects]

4.6.1 For Subjects

No subject prematurely discontinued from the study for any reason will be replaced.

4.6.2 For Centers

A center may be replaced for the following administrative reasons:

– Excessively slow recruitment.

– Poor protocol adherence.

5. SCHEDULE OF ASSESSMENTS AND PROCEDURES

Table 1 Schedule of Assessments

	Baseline	Treatment					Follow-up	
Study Day	**1 (pre-dose)**	**1**	**2 or 3[f,g]**	**6[g]**	**8[f, g]**	**11[g, h, i]**	**15[g]**	**40[g, j]**
Informed Consent/Assent	x							
Medical history	x							
Demographics	x							
Height and weight	x					x		
Pregnancy Test [a]	x					x		x
Rapid diagnostic test for influenza virus shedding	x					x		
Safety Labs [b]	x					x		
Physical Examination	x					x		x
Vital Signs (including PR, RR, temperature, Blood pressure)	x		x	x	x	x	x	x
Nasal and throat swabs for viral shedding and viral load [c, d]	x		x	x	x	x	x	x
Review of electronic diary data [e]			x	x	x	x	x	x
Drug Administration		←	—	—	—	→		
Collection of unused study medication and empty containers						x		
Previous Diseases	x							
Previous/Concomitant medications	x	←	—	—	—	—	—	→
Adverse Events/Sec Illnesses and Treatments		←	—	—	—	—	—	→
Rejection, Graft versus host disease (GVHD)		←	—	—	—	—	—	→

[a] Urine pregnancy test for patients of child-bearing potential according to the judgment/discretion of the investigator.

[b] Hematology (CBC with differential) and chemistry (AST, ALT, total bilirubin, urea, Cr). Serum creatinine testing may be done at a local laboratory **when clinically indicated** to calculate creatinine **clearance**.

[c] Baseline swab samples will be assessed for the presence of **resistance mutations.**

[d] Two nasal and one throat swab for viral culture and RT-PCR.

[e] Influenza symptoms, temperature, and date/ time of oseltamivir dose will be recorded by the patient in electronic patient diaries twice daily on days 1 – 10, and once daily thereafter.

[f] A home visit may be made on day 2 or 3 (for patients who are too ill to come into the clinic) and day 8.

[g] Day 2/3 visit window = +1 day. Day 6 visit window = +/- 1 day; Day 8 visit window = +1 day. Day 11 visit window = ± 1 day. Day 15 and day 40 visit occur approximately 5 and 30 days after the last dose respectively. Day 15 window = ± 1 day. Day 40 window ± 2 days.

[h] Only weight to be assessed. Height is not necessary at this visit. Study drug is taken on day 11, only if the first dose was taken after 4 PM on Day 1 [5.3] Subjects who are rapid diagnostic positive can be treated per standard of care. They will be required to come for their follow-up visits on day 15 and day 40.

Table 1 Schedule of Assessments (Cont.)

[i] Subjects who discontinue treatment prematurely will have an end of treatment (day 11) assessment at the time of treatment discontinuation or the following day. They will be required to attend their follow up visits approximately 5 and 30 days after the last dose (day 15 and day 40 assessments).

[j] Subjects who discontinue during follow-up will have an end of follow-up (day 40) assessment. This visit must occur within 30 days of the last dose.

5.1 Screening Examination and Eligibility Screening Form

All subjects must provide written informed consent (assent where applicable) before any study specific assessments or procedures are performed. Screening will take place at the baseline visit prior to first dose. However, if necessary, screening may take place the day before the baseline visit, as long as the patient is randomized and receives his/her first dose of study medication within **96** hours of influenza symptom onset.

Subjects will be assessed for inclusion and exclusion criteria and those who fulfill all the inclusion and none of the exclusion criteria will be randomized into the study.

An Eligibility Screening Form [ESF] documenting the investigator's assessment of each screened subject with regard to the protocol's inclusion and exclusion criteria is to be completed by the investigator.

A screen failure log must be maintained by the investigator.

5.2 Procedures for Enrollment of Eligible Subjects

Once a subject has fulfilled the entry criteria, he/she will be randomized to one of two treatment groups. The subject randomization numbers will be generated by Roche or its designee and incorporated into double-blind labeling.

The investigator or designee will use the CRF pre-printed with the assigned subject number and enter the randomization number provided by IVRS for allocation to the treatment groups in the appropriate place on each subject's CRF.

Randomization will be stratified by transplant **status** (**Yes; No**); time between onset of symptoms and treatment start (≤ **48** hrs or > **48** hrs), influenza vaccination status (Yes; No) and patient's age (≤ 12 years or > 12 years).

5.3 Clinical Assessments and Procedures

At all visits subjects will receive the routine care for their primary illness. Routine patient care (including the adjustment of doses of any of the immunosuppressive drugs) will be dictated by tests performed locally at the trial site. All safety labs will be sent to a central laboratory. Results for these laboratory parameters will be used by the sponsor/designee to assess overall safety.

All assessments and procedures will be performed according to the Schedule of Assessments (Table 1). Additional information regarding these procedures not provided in the Schedule of Assessments is provided below.

Study Day 1

The baseline and study day 1 assessment may be performed at the same visit.

Study medication, electronic diaries, and thermometers will be dispensed. Patients or guardians/parents will be instructed how to complete electronic symptom diaries (Appendix 2) (Appendix 3), temperature recording, and treatment administration details, including time of each oseltamivir dose. The first diary entries will be made at the site before the first dose of study drug.

Baseline nasal and throat swab samples will be assessed for the presence of **oseltamivir-resistance mutations.**

The date of the first dose of study drug is defined as study day 1. Once randomized, the first dose of study drug will be administered in the clinic. Study day 2 will begin at 12 midnight of the same calendar day. If the first dose of study drug is taken after 4 pm on day 1, the next dose of study drug will be taken in the morning of day 2. In this case, the last dose of study drug will be taken on the morning of study day 11.

If the first dose of study drug is taken prior to 4 pm on day 1, the next dose of study drug should be taken in the evening of the same day (i.e. prior to midnight on the same calendar day with a minimum of 7 hours between doses). For these patients the last dose of study drug will be taken in the evening of study day 10. More information on dosing is provided later (Section 6.1).

Study Days 2 - 11

Study day 2 will begin at 12 midnight of study day 1.

It is expected the study staff will maintain close contact with patients, especially during the first few days of the study. The study staff should inquire about any adverse events, check that the patient or parent/guardian is entering data into the electronic diary properly, and assess drug compliance. During the dosing period, diary symptoms should be assessed and temperature should be recorded at the same time that the study drug is taken.

End of Treatment Day 11

The end of treatment visit for all subjects is on day 11 (irrespective of whether they took one or two doses on day 1). Subjects who discontinue study medication prematurely will have all day 11 assessments completed at the time of discontinuation or the following day.

After all day 11 study assessments are completed, a rapid diagnostic test will be performed at this visit. If the rapid diagnostic test is positive, the subject may be treated per standard of care at the discretion of the investigator.

All subjects (including those who discontinue study medication prematurely and those who are positive for influenza on their rapid diagnostic test at the end of treatment visit) will be required to return for follow up approximately 5 and 30 days after the last dose (day 15 and day 40 assessments).

Study Days 12 – 40

Study day 15 is an important visit as it is the first assessment of viral culture after cessation of treatment.

End of Follow-Up Day 40

All subjects must attend an end of follow-up visit on day 40.

If the patient is withdrawn after completion of treatment (after the day 11 assessment), a termination visit should be arranged. This visit should be the end of follow-up visit assessment [Day 40]. This visit must occur within 30 days of the last dose.

Patients or parents/guardians who do not return to the clinic will be contacted by telephone to ascertain their health status, or that of their child's, record information on adverse events, and to ask that they return the electronic diary.

5.3.1 Efficacy Assessments

The primary **efficacy** end point in this study is the time to resolution of all influenza symptoms as recorded in the patient diary.

The clinical efficacy parameters in this study are:

1. Symptoms of influenza-like illness. These are captured in the diary for both adults and children (Appendix 2) (Appendix 3). Influenza symptoms are graded on a nominal scale from zero (absent/no problem) to 3 (severe or major problem). A score of zero is considered asymptomatic. The primary **efficacy** end point, time to alleviation of all symptoms is comprised of all the symptoms captured in the diary.
2. Temperature. This is captured in the diary. Temperature is used for assessment of the primary and several secondary **efficacy** end points.

5.3.2 Safety

Safety parameters in this study include adverse events, vital signs, and clinical laboratory evaluations.

Pre-defined symptoms of influenza captured in the adult and pediatric diaries are not to be reported as adverse events unless they can be further qualified. Thus 'headache due to stress at work' is reported as an adverse event. However, unexplained 'headache' is considered a predefined symptom related to influenza and not an adverse event.

Adverse events such as bronchitis, pneumonia, otitis media and sinusitis are considered secondary illnesses of influenza and should be recorded as adverse events.

Other adverse events to be expected in the transplant population such as rejection and graft versus host disease (in HSCT subjects) will also be collected as adverse events.

5.4 Laboratory Assessments

The laboratory assessments include those for efficacy and safety.

Efficacy

The efficacy laboratory assessments are used for laboratory confirmation of influenza. These are:

Nasal and throat swabs. Two nasal and one throat swab will be collected as described in the Schedule of Assessments. All swabs are sent to a central laboratory for RT-PCR and viral cultures. Influenza virus shedding will be assessed. Baseline samples will be assessed for the presence of oseltamivir-**resistance mutations**.

During the course of treatment, neither the investigator nor the patient will know which participants have ongoing positive viral cultures for influenza. At the end of treatment (day 11), a rapid diagnostic test is permitted for the diagnosis of ongoing influenza.

Safety

The safety laboratory assessments in this study, including the assessment of serum chemistry and hematology will be done at the central laboratory. Serum chemistry assessments comprise AST, ALT, total bilirubin, urea and creatinine. Hematology assessments include CBC and differential count. The total volume of blood loss for laboratory assessments will be approximately 20 mL for the entire duration of the study.

Protection of patient confidentiality (Section 16) will extend to any data generated from the assaying of these samples. Biological samples taken from all patients may be infectious and will be classified as "diagnostic specimens" for dispatch purposes.

The Principal Investigator may draw blood for serum creatinine to be assessed at the local laboratory **when clinically indicated** during the study **to calculate creatinine clearance**.

6. INVESTIGATIONAL MEDICINAL PRODUCT

The Investigational Medical Product in this study is oseltamivir dry powder (to be reconstituted to a concentration of 12 mg/ml) or 75 mg capsules and matching placebo.

The Investigational Medicinal Products will be supplied, packaged individually for each subject and labeled in accordance with Roche Standard and local regulation by Roche Clinical Trial Supply, Basel, Switzerland.

6.1 Dose and Schedule of Study Drug

Oseltamivir will be given twice daily over 10 days for a total of 20 doses. The doses need to be taken at 12 hourly intervals. Under no circumstances is a subject allowed to take two doses within 7 hours of each other.

Patients will be randomized to receive either conventional or high dose of study drug.

Conventional dose:

Children ages 1 - 12 years: Oseltamivir syrup

≤ 15 kg	30 mg twice daily
> 15 – 23 kg	45 mg twice daily

> 23 – 40 kg	60 mg twice daily
> 40 kg	75 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules

75 mg twice daily

Subjects randomized to the conventional dose will simultaneously receive matching placebo so as to blind them and the investigator from the high dose arm.

High dose:

Children ages 1 - 12 years: Oseltamivir syrup

≤ 15 kg	60 mg twice daily
> 15 – 23 kg	90 mg twice daily
> 23 – 40 kg	120 mg twice daily
> 40 kg	150 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules

150 mg twice daily

6.1.1 Dose Modifications

No dose modifications will be allowed on study.

6.2 Preparation and Administration of Study Drug

Oseltamivir will be provided in two forms:

1. Capsules containing 75 mg of active drug and packaging material consisting of pregelatinized starch, povidone, talc and sodium stearyl fumarate. All participants 13 years and older will receive this dosage form. *Oseltamivir capsules should be stored at 25ºC.*
2. A pediatric suspension containing 12 mg oseltamivir per ml of reconstituted solution and the following excipients: sorbitol, titanium dioxide, sodium benzoate, xanthan gum, monosodium citrate, saccharin sodium and Permaseal11900-31 Tutti Frutti (flavor). All participants 12 years and under will receive this dosage form. *Oseltamivir dry powder for suspension [pediatric syrup] should be stored at 25ºC. After reconstitution, the suspension should not be used for longer than 10 days. Store constituted suspension under refrigeration at 2º to 8ºC. Do not freeze.*

Matching placebo will be available as capsules and suspension. Subjects in the conventional dose arm will get the conventional dose and matching placebo so that they are blinded from the high dose arm.

Each subject will be dispensed a medication pack that will provide enough medication to cover 20 doses. For subjects randomized to the conventional dose arm, the medication pack will contain a bottle of oseltamivir dry powder or a blister wallet with oseltamivir

capsules and matching placebo. For subjects randomized to the high dose arm, the medication pack will contain two bottles of oseltamivir dry powder or two blister wallets with oseltamivir capsules. Irrespective of the treatment group the subject is randomized to, for each dose the subject will take the same amount from both bottles or blister wallets provided in the medication pack such that the sum of the amounts from each immediate container constitutes one dose.

One dose is to be administered twice per day at approximately 12-hour intervals with a light snack or glass of milk or fruit juice. The first dose of study medication will be administered in the clinic at the time of randomization.

6.3 Blinding and Unblinding

Randomization will be administered by a central randomization center.

The Randomization List will not be available at the study center, to the study monitors, project statisticians or to the project team at Roche. Emergency codes, or another adequate method of unblinding, will be implemented before study start, if the identity of the test medication is necessary for patient management in the case of a serious adverse event. Emergency codes should not be broken except in the case of emergency situations. Any request from the investigator for information about the treatment administered to study subjects for another purpose must be discussed with Roche/designee.

As per regulatory reporting requirement, Roche/designee will unblind the identity of the study medication for all unexpected [as per IB] serious adverse events that are considered by the investigator to be related to study drug. Details of patients who are unblinded during the study will be included in the Clinical Study Report.

All other individuals directly involved in this study will remain blinded until the final analysis of the primary parameter.

The randomization will be stratified by transplant **status**, duration of symptoms, influenza vaccination status and age and will be provided by the Roche randomization group for the IVRS vendor.

6.4 Assessment of Compliance

Accountability and subject compliance will be assessed by maintaining adequate "drug dispensing" and return records.

Subjects will be asked to return all used and unused drug supply containers at the end of the treatment as a measure of compliance.

A Drug Dispensing Log must be kept current and should contain the following information:

- the identification of the subject [randomization and medication numbers] to whom the study medication was dispensed
- the date[s], quantity of the study medication dispensed *to* the subject
- the date[s] and quantity of the study medication returned *by* the subject

This inventory must be available for inspection by the Monitor. All supplies, including partially used or empty containers and the dispensing logs, must be returned to the monitor at the end of the study.

6.5 Destruction of Study Drug

Local or institutional regulations may require immediate destruction of used investigational product for safety reasons. In these cases, it may be acceptable for investigational site staff to destroy dispensed investigational product before a monitoring inspection provided that source document verification is performed on the remaining inventory and reconciled against the documentation of quantity shipped, dispensed, returned and destroyed. Written authorization must be obtained from the sponsor or designee at study start up before destruction.

Written documentation of destruction must contain the following:

- Identity [batch numbers or subject numbers] of investigational product[s] destroyed
- Quantity of investigational product[s] destroyed
- Date of destruction
- Method of destruction
- Name and signature of responsible person [or company] who destroyed investigational products[s]

7. SAFETY INSTRUCTIONS AND GUIDANCE

7.1 Adverse Events (AEs) and Laboratory Abnormalities

7.1.1 Clinical AEs

Per the International Conference of Harmonization [ICH], an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign [including an abnormal laboratory finding], symptom, or disease temporally associated with the use of a medicinal [investigational] product, whether or not considered related to the medicinal [investigational] product. Pre-existing conditions which worsen during a study are to be reported as AEs. Influenza signs and symptoms reported on the patient diary will be summarized as efficacy end points and need not be captured as adverse events. However, secondary illnesses due to influenza must be reported as adverse events.

7.1.1.1 Intensity

All clinical AEs encountered during the clinical study will be reported on the AE page of the CRF.

Intensity of AEs will be graded on a four -point scale [mild, moderate, severe, life-threatening] and reported in detail on the CRF.

Mild	discomfort noticed but no disruption of normal daily activity.
Moderate	discomfort sufficient to reduce or affect daily activity.
Severe	inability to work or perform normal daily activity
Life Threatening	represents an immediate threat to life

7.1.1.2 Drug - Adverse Event Relationship

Relationship of the AE to the treatment should always be assessed by the investigator. Description of scales can be found in Appendix 4.

7.1.1.3 Serious Adverse Events [Immediately Reportable to Roche]

A Serious Adverse Event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that at any dose fulfils at least one of the following criteria:

- is fatal; [results in death; NOTE: death is an outcome, not an event].
- is Life-Threatening [NOTE: the term "Life-Threatening" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe].
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above.

The full requirements of the ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 will be adhered to (Appendix 5).

7.1.2 Treatment and Follow-up of AEs

AEs, especially those for which the relationship to test “drug” is not “unrelated”, should be followed up until they have returned to baseline status or stabilized. If a clear explanation is established it should be recorded on the CRF.

7.1.3 Laboratory Test Abnormalities

Laboratory test results will appear printed on laboratory reports provided to the site from the central laboratory.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event [SAE] should be reported as such, in addition to being recorded as an AE in the CRF.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the AE page in the CRF:

- Accompanied by clinical symptoms.
- Leading to a change in study medication [e.g. dose modification, interruption or permanent discontinuation].
- Requiring a change in concomitant therapy [e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment].

7.1.4 Follow-up of Abnormal Laboratory Test Values

In the event of medically significant unexplained abnormal laboratory test values, the tests should be repeated and followed up until they have returned to the normal range and/or an adequate explanation of the abnormality is found. If a clear explanation is established it should be recorded on the CRF.

7.2 Handling of Safety Parameters

7.2.1 Reporting of Serious Adverse Events [immediately reportable]

Any clinical AE or abnormal laboratory test value that is *serious* **[as defined in Section 7.1.1.3 above] and which occurs during the course of the study, regardless of the treatment arm, occurring from the enrollment visit (start of study screening procedures),** must be reported to Roche or designee **within *one* working day** of the investigator becoming aware of the event [expedited reporting].

Related Serious Adverse Events ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed.

Unrelated Serious Adverse Events must be collected and reported during the study and up until the follow-up visit.

The definition and reporting requirements of **ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2** will be adhered to (Appendix 5).

7.2.2 Pregnancy

A female subject must be instructed to stop taking the test "drug" and immediately inform the investigator if she becomes pregnant during the study. The investigator should report all pregnancies within 24 hours to the sponsor or designee. The investigator should counsel the subject, and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. **Pregnancies occurring within 28 days of treatment completion should be reported to Roche.**

7.3 Warnings and Precautions

Events such as convulsions and delirium (including symptoms such as altered level of consciousness, confusion, abnormal behavior, delusions, hallucinations, agitation,

anxiety, nightmares) have been reported during oseltamivir use in patients with influenza, predominately in children and adolescents. In rare cases, these events resulted in accidental injury. The contribution of oseltamivir to those events is unknown. Such events have also been reported in patients with influenza who were not taking oseltamivir. Patients, especially children and adolescents, should be closely monitored for signs of abnormal behavior.

Please refer to the attached Investigator's Brochure for additional warnings, precautions, and other reported adverse events.

8. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

Full details of all planned analyses will be specified in a separate **Statistical Analysis Plan (SAP) for the safety and efficacy variables and in the Resistance Plan for the development of resistance.** The methods described below are an outline of the main planned analyses.

8.1 Primary and Secondary Study Endpoints

8.1.1 Primary Endpoints

The primary endpoints for safety will be assessments of adverse events, physical exams, vital signs and clinical laboratory evaluations. The primary endpoint of development of resistance will be determined from the genotypic and phenotypic variables measured post baseline and is described further in the Resistance Plan.

8.1.2 Secondary Endpoints

The following are secondary endpoints **which measure efficacy, the primary of which is time to alleviation of all clinical symptoms.** With the exception **of this variable,** viral load and time to resolution of fever, they are defined as either the occurrence or non-occurrence of the specified events or the presence or absence of the specified symptoms**.**

The time (hours) to alleviation of all clinical influenza symptoms (recorded in the patient diary)

Shedding virus by culture at day 1, 2, 6, 8, 11, 15 and 40

Shedding virus by RT-PCR at day 1, 2, 6, 8, 11, 15, and 40

Viral load by culture ($log_{10}TCID_{50}$/mL) at Day 1, 2, 6, 8, 11, 15, and 40

Viral load by quantitative RT-PCR at day 1, 2, 6, 8, 11, 15 and 40

The time (hours) from first dose of study medication until resolution of fever

Fever

Cough

Coryza

Development of secondary illnesses (otitis media, bronchitis, pneumonia, or sinusitis) at any time during the study

Development of secondary illnesses (otitis media, bronchitis, pneumonia, or sinusitis) at any time during the study that are treated with antibiotics

Initiation of treatment with antibiotics after randomization

Hospitalization, and for those who are hospitalized, the duration of hospitalization

Development of rejection or GVHD

8.1.3 Safety

Safety of the treatment will be evaluated by AEs, **physical exams, clinical** laboratory tests, and vital signs.

In addition to routine safety assessments, the proportion of subjects experiencing a rejection and/or graft versus host disease will be summarized by treatment group.

8.2 Statistical and Analytical Methods

8.2.1 Statistical Model

8.2.1.1 Time to Event Variables

A non-parametric model will be assumed with estimation of medians based on Kaplan-Meier methods. Subjects without alleviation of symptoms will have their time censored at the last available observation that a complete assessment was made.

For the purpose of comparing treatment groups, it will be assumed that their respective distributions for the primary endpoint differ only by a shift in location.

8.2.1.2 Dichotomous Variables and Viral Load

For the endpoints defined dichotomously in terms of events or symptoms **including the development of resistance**, it will be assumed that the number of patients experiencing the event or exhibiting the symptom will have a binomial distribution.

For the continuous endpoints of viral load at Day 1, 2, 6, 8, 11, 15, and 40 no model will be assumed.

8.2.2 Sample Size

The sample size has been chosen to provide an adequate number of patients to estimate the development of resistance with reasonable precision. Assuming that 90% of enrolled patients will have laboratory-confirmed influenza, there will be 75 patients in each treatment arm in the population evaluable for the development of resistance. The following table shows the 95% Pearson-Clopper confidence intervals that would result with a sample size of 75 patients in a treatment arm if certain event rates are observed in the study.

During the study the number of influenza A virus infected patients and the rate of development resistance will be monitored in a blinded fashion, in order to ensure a

reasonable precision for the estimation. An additional number of patients may be enrolled if necessary.

Observed Rate (%)	95% Confidence Interval
0.0	0% - 4.8%
1.3	0.0% - 7.2%
2.7	0.3% - 9.3%
5.3	1.5% - 13.1%
10.7	4.7%-19.9%

A total of 83 patients will be enrolled per arm and will be evaluable for the assessment of safety. This number of patients would provide estimates of adverse event rates with similar precision.

8.2.3 Hypothesis Testing

Formal hypothesis testing will be not performed, instead inferences will be based on comparison of confidence intervals.

8.2.4 Analysis Populations

Three main patient populations will be used for the analysis of data from this study; the Safety Population, the Intent-to treat Population and the Intent-to-Treat Infected Population. Detailed definitions of these populations will be given in the **SAP**.

8.2.4.1 Intent to Treat Population

All patients randomized will be included in the intent to treat population [Patients will be assigned to treatment groups as randomized for analysis purposes].

8.2.4.2 Intent to Treat Infected Population

All patients randomized and with laboratory confirmation of influenza infection, excluding patients infected with oseltamivir-resistant influenza at baseline, will be included in the intent to treat influenza infected population [Patients will be assigned to treatment groups as randomized for analysis purposes].

The ITTI Population will be the primary population for the summary and analysis of the **development of resistance and the** efficacy variables.

8.2.4.3 Subpopulations

A subpopulation of the ITTI population will be defined comprising patients who received their first dose of study medication within 48 hours of influenza symptom onset for the comparison of efficacy endpoints with the age appropriate placebo treated patients (pediatric or adult) from the registration trials.

In order to evaluate the potential for relapse, the following two subpopulations of the ITTI population will be evaluated:

- patients not shedding virus as assessed by culture on Day 11
- patients not shedding virus as assessed by RT-PCR on Day 11

Viral shedding and RT-PCR at days 15 and 40 will be evaluated in these subpopulations.

Based on the proportion of subjects hospitalized, an additional subpopulation may be defined to evaluate the length of hospitalization for hospitalized subjects.

Likewise, based on the proportion of children enrolled additional subpopulations of children and adults will be created to evaluate the course of influenza in children and adults.

8.2.5 Efficacy Analysis

For the analysis **of time to alleviation of all clinical symptoms**, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- Comparison to placebo control from pivotal registration trials

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established **that is comparable to the subpopulation of patients in** the current study **whose first dose of study medication was within 48 hours of symptom onset.** For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

  The median time to resolution of all influenza symptoms will be determined for each treatment group, along with its 95% confidence interval. These confidence intervals will be compared individually to the placebo control confidence interval described above as a potential means of establishing treatment effects.

- Assessment of relative efficacy

  The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval.

This confidence interval will provide lower and upper limits for the treatment difference and can be used as the basis for potential inferences. For example, if it does not contain the value zero a difference will have been established.

For the dichotomous endpoints, the proportion experiencing the event or symptom, and its associated 95% confidence interval will be derived for each treatment group. Where possible, the corresponding confidence intervals from the placebo control population will be presented for comparison to those derived for the active treatment groups in this trial.

The continuous secondary endpoint time to resolution of fever will be analyzed as for the primary **efficacy** endpoint.

For the continuous endpoints of viral load ($log_{10}TCID_{50}$/mL) at Day 1, 6, 8, 11, 15, and 40, summary statistics (including mean, **standard deviation,** median, minimum and maximum) will be derived for each treatment group.

8.2.6 Safety Data Analysis

The safety analysis population will include all subjects who receive at least one dose and had a safety assessment performed post randomization. All safety variables will be summarized and presented in tables based on this safety population.

8.2.7 Analysis of Resistance

For the summary of the development of resistance for each treatment arm, 95% confidence intervals will be provided with the estimated rates. Further details are available in the Resistance Plan for NV20234. These data will be summarized in a report separate from the final study report.

8.2.7.1 Exclusion of Data from Analysis

Efficacy data collected from any patient who begins treatment with another antiviral for influenza (for example, amantadine, rimantadine, **laninamivir, peramivir,** or zanamavir), after the other antiviral treatment commences, will be excluded from the primary and secondary efficacy analyses. Such patients will be considered failures to oseltamivir treatment in the analyses. Further details will be provided in the **SAP**.

8.2.7.2 Interim Analysis

No interim analyses are planned.

8.2.8 Other Analyses

Further exploratory analysis, (including assessments of the rapid diagnostic test, sub group analysis) will be detailed in the **SAP**.

9. DATA QUALITY ASSURANCE

The overall procedures for quality assurance of clinical study data are described in the Roche Standard Operational Procedures.

Accurate and reliable data collection will be assured by verification and cross–check of the CRFs against the investigator's records by the study monitor [source document verification], and the maintenance of a drug–dispensing log by the investigator.

The data collected will be entered into the study database from the working copy of the CRF faxed from the site.

A comprehensive validation check program will verify the data and discrepancy reports will be generated accordingly for resolution by the investigator. As patients complete the study [or prematurely withdraw] and their signed CRFs become available, a second data entry will be performed from the original, signed CRF. A comparison check will be run to identify and resolve any discrepancies between the first and second data entry.

Throughout the study the Study Management Team [SMT] will review data according to the SMT Data Review Plan as described in the Data Quality Plan.

9.1 Assignment of Preferred Terms and Original Terminology

For classification purposes, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using the current version of MedDRA (Medical Dictionary for Regulatory Activities terminology) for adverse events and diseases and the INN (International Non-Proprietary name) drug terms and procedures dictionary for treatments and surgical and medical procedures.

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PART II: ETHICS AND GENERAL STUDY ADMINISTRATION

11. ETHICAL ASPECTS

11.1 Local Regulations/Declaration of Helsinki

The investigator will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study must fully adhere to the principles outlined in "Guideline for Good Clinical Practice" ICH Tripartite Guideline [January 1997] or with local law if it affords greater protection to the subject. For studies conducted in the EU/EEA countries, the investigator will ensure compliance with the EU Clinical Trial Directive [2001/20/EC]. For studies conducted in the USA or under US IND, the investigator will additionally ensure that the basic principles of "Good Clinical Practice" as outlined in the current version of 21 CFR, subchapter D, part 312, "Responsibilities of Sponsors and Investigators", part 50, "Protection of Human Subjects", and part 56, "Institutional Review Boards", are adhered to.

In other countries where "Guideline for Good Clinical Practice" exist Roche and the investigators will strictly ensure adherence to the stated provisions.

11.2 Informed Consent

It is the responsibility of the investigator, or a person designated by the investigator [if acceptable by local regulations], to obtain written informed consent from each subject participating in this study, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. For subjects not qualified or incapable of giving legal consent, written consent must be obtained from the legally acceptable representative. In the case where both the subject and his/her legally acceptable representative are unable to read, an impartial witness should be present during the entire informed consent discussion. After the subject and representative have orally consented to participation in the trial, the witness' signature on the form will attest that the information in the consent form was accurately explained and understood. The investigator or designee must also explain that the subjects are completely free to refuse to enter the study or to withdraw from it at any time, for any reason. The Case Report Forms [CRFs] for this study contain a section for documenting informed subject consent, and this must be completed appropriately. If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All subjects [including those already being treated] should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

If children are old enough to understand the risks and benefits of the study, they should also be informed and should also provide their written consent.

11.3 Independent Ethics Committees/Institutional Review Board

Independent Ethics Committees [non-US]: This protocol and any accompanying material provided to the subject [such as subject information sheets or descriptions of the study used to obtain informed consent] as well as any advertising or compensation given to the patient, will be submitted by the investigator to an Independent Ethics Committee. Approval from the committee must be obtained before starting the study, and should be documented in a letter to the investigator specifying the date on which the committee met and granted the approval.

Any modifications made to the protocol after receipt of the Independent Ethics Committee approval must also be submitted by the investigator to the Committee in accordance with local procedures and regulatory requirements.

When no local review board exists, the investigator is expected to submit the protocol to a regional committee. If no regional committee exists, Roche will assist the investigator in submitting the protocol to the European Ethics Review Committee.

Institutional Review Board [US]: It is the understanding of the sponsor that this protocol [and any modifications] as well as appropriate consent procedures, will be reviewed and approved by an Institutional Review Board. This board must operate in accordance with the current Federal Regulations. A letter or certificate of approval will be sent by the investigator to the sponsor or designee prior to initiation of the study, and also whenever subsequent modifications to the protocol are made.

12. CONDITIONS FOR MODIFYING THE PROTOCOL

Protocol modifications to ongoing studies must be made only after consultation between an appropriate representative of the sponsor and the investigator [investigator representative[s] in the case of a multicenter trial]. Protocol modifications must be prepared by a representative of the sponsor and initially reviewed and approved by the Clinical Science Leader/Clinical Pharmacologist and Biostatistician.

All protocol modifications must be submitted to the appropriate Independent Ethics Committee or Institutional Review Board for information and approval in accordance with local requirements, and to Regulatory Agencies if required. Approval must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial subjects, or when the change[s] involves only logistical or administrative aspects of the trial [e.g. change in monitor[s], change of telephone number[s].

13. CONDITIONS FOR TERMINATING THE STUDY

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange the procedures on an individual study basis after review and consultation. In terminating the study, Roche and the investigator will assure that adequate consideration is given to the protection of the patient's interests.

14. STUDY DOCUMENTATION, CRFS AND RECORD KEEPING

14.1 Investigator's Files / Retention of Documents

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two different separate categories [1] Investigator's Study File, and [2] subject clinical source documents.

The Investigator's Study File will contain the protocol/amendments, Case Report and Query Forms, Independent Ethics Committee/Institutional Review Board and governmental approval with correspondence, sample informed consent, drug records, staff curriculum vitae and authorization forms and other appropriate documents/correspondence etc.

Subject clinical source documents [usually defined by the project in advance to record key efficacy/safety parameters independent of the CRFs] would include patient hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, EEG, X-ray, pathology and special assessment reports, signed informed consent forms, consultant letters, and subject screening and enrollment logs. The Investigator must keep these two categories of documents on file for at least 15 years after completion or discontinuation of the study. After that period of time the documents may be destroyed, subject to local regulations.

Should the Investigator wish to assign the study records to another party or move them to another location, Roche must be notified in advance.

If the Investigator can not guarantee this archiving requirement at the investigational site for any or all of the documents, special arrangements must be made between the Investigator and Roche to store these in a sealed container[s] outside of the site so that they can be returned sealed to the Investigator in case of a regulatory audit. Where source documents are required for the continued care of the patient, appropriate copies should be made for storing outside of the site.

14.2 Source Documents and Background Data

The investigator shall supply the sponsor or designee on request with any required background data from the study documentation or clinic records. This is particularly important when CRFs are illegible or when errors in data transcription are suspected. In case of special problems and/or governmental queries or requests for audit inspections, it is also necessary to have access to the complete study records, provided that patient confidentiality is protected.

14.3 Audits and Inspections

The investigator should understand that source documents for this trial should be made available to appropriately qualified personnel from the Roche Pharma Development Quality Assurance Unit or its designees, or to health authority inspectors after appropriate notification. The verification of the CRF data must be by direct inspection of source documents.

14.4 Case Report Forms

For each patient enrolled, a CRF must be completed and signed by the principal investigator or authorized delegate from the study staff. This also applies to records for those patients who fail to complete the study [even during a pre-randomization screening period if a CRF was initiated]. If a patient withdraws from the study, the reason must be noted on the CRF. If a patient is withdrawn from the study because of a treatment-limiting AE, thorough efforts should be made to clearly document the outcome.

All forms should be typed or filled out using indelible ink, and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or his/her authorized delegate. The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor or designee in the CRFs and in all required reports.

15. MONITORING THE STUDY

It is understood that the responsible Roche monitor [or designee] will contact and visit the investigator regularly and will be allowed, on request, to inspect the various records of the trial [CRFs and other pertinent data] provided that patient confidentiality is maintained in accord with local requirements.

It will be the monitor's responsibility to inspect the CRFs at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitor should have access to laboratory test reports and other patient records needed to verify the entries on the CRF. The investigator [or his/her deputy] agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

16. CONFIDENTIALITY OF TRIAL DOCUMENTS AND SUBJECT RECORDS

The investigator must assure that subjects' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents submitted to the sponsor or designee, subjects should not be identified by their names, but by an identification code. The investigator should keep a subject enrollment log showing codes, names and addresses. The investigator should maintain documents not for submission to Roche, e.g., subjects' written consent forms, in strict confidence.

17. PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to Roche prior to submission. This allows the sponsor or designee to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the investigator.

In accord with standard editorial and ethical practice, Roche will generally support publication of multicenter trials only in their entirety and not as individual center data. In this case, a coordinating investigator will be designated by mutual agreement.

Any formal publication of the study in which input of Roche personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate Roche personnel. Authorship will be determined by mutual agreement.

Appendix 1 Calculation of Creatinine Clearance and Grading of Chronic Extensive GVHD

Calculation of Creatinine Clearance

Conversion Factor for Serum Creatinine:

Conventional Units (mg/dL) = SI Units (μmol/L) ÷ 88.4

Age = number of years (12 years 11 months = 12 years)

Estimated Creatinine Clearance according to Cockcroft-Gault [17]

(for patients ≥ 18 years):

- **Males**

Creatinine

Clearance (mL/min) = [(140 – age) X Body Weight (kg)] ÷ [72 X **Serum Creatinine** (mg/dL)]

- **Females**

Creatinine Clearance = above equation X 0.85

Estimated Creatinine Clearance according to Schwartz equation [18]

(for patients < 18 years):

Creatinine Clearance (mL/min/1.73 M^2) = k X Height (cms) ÷ **Serum Creatinine** (mg/dL)

Value for k:

k	Age (years)
0.45	< 2
0.55	≥ 2 to < 13
0.7	≥ 13 to < 18 (males)
0.55	**≥ 13 to < 18 (females)**

Appendix 1 Calculation of Creatinine Clearance and Grading of Chronic Extensive GVHD (Cont.)

Grading of Chronic GVHD [19]

Type of Disease	Extent of Disease
Limited	Localized skin involvement, liver dysfunction or both
Extensive	Generalized skin involvement
	Localized skin involvement or liver dysfunction plus any one of the following: 1. Chronic aggressive hepatitis, bridging necrosis or cirrhosis 2. Eye involvement (Schirmer's test, < 5 mm) 3. Involvement of mucosalivary glands 4. Mucosal involvement (on lip biopsy) 5. Involvement of other target organs

Appendix 2 Adult Patient Diary Data and Symptom Record

The purpose of the electronic diary is for all adults (13 years and older) to record any symptoms of influenza-like illness for the duration of the study. Temperature and date and time of drug administration will also be recorded on the electronic patient diary.

Scoring of Symptoms.

Please answer All of the questions yourself by checking one box for each row.

The information you provide is very important and will remain strictly confidential.

	absent 0	mild 1	moderate 2	severe 3
1. Nasal Congestion	☐	☐	☐	☐
2. Sore Throat	☐	☐	☐	☐
3. Cough	☐	☐	☐	☐
4. Aches and Pains	☐	☐	☐	☐
5. Fatigue(Tiredness)	☐	☐	☐	☐
6. Headache	☐	☐	☐	☐
7. Chills/sweats (feverish)	☐	☐	☐	☐

Appendix 3 Diary Data for Children

Temperature and date and time of drug administration will also be recorded on the electronic patient diary.

Date of Assessment ☐☐☐☐☐☐ Time of Assessment ☐☐☐☐

dd mm yy h min

Temperature: ☐☐☐.☐ C/F

Symptoms of influenza-like illness

Please mark one box only per question

Item	No Problem 0	Minor Problem 1	Moderate Problem 2	Major Problem 3	Don't Know or not Applicable
1. Poor appetite	☐	☐	☐	☐	☐
2. Not sleeping well	☐	☐	☐	☐	☐
3. Irritable, cranky, fussy	☐	☐	☐	☐	☐
4. Feels unwell	☐	☐	☐	☐	☐
5. Low energy, tired	☐	☐	☐	☐	☐
6. Not playing well	☐	☐	☐	☐	☐
7. Crying more than usual	☐	☐	☐	☐	☐
8. Needing extra care	☐	☐	☐	☐	☐
9. Clinginess	☐	☐	☐	☐	☐
10. Headache	☐	☐	☐	☐	☐
11. Sore throat	☐	☐	☐	☐	☐
12. Muscle aches or pains	☐	☐	☐	☐	☐
13. Fever	☐	☐	☐	☐	☐
14. Cough	☐	☐	☐	☐	☐
15. Nasal congestion, runny nose	☐	☐	☐	☐	☐
16. Vomiting	☐	☐	☐	☐	☐
17. Not interested in what's going on	☐	☐	☐	☐	☐
18. Unable to get to get out of bed	☐	☐	☐	☐	☐

This form was filled out by:

1. ☐ Parent
2. ☐ Other relative
3. ☐ Nanny
4. ☐ Subject
5. ☐ Other *specify*_____

Appendix 4 Adverse Event [AEs] Categories for Determining Relationship to Test Drug

PROBABLE [must have first three]

This category applies to those AEs which are considered, with a high degree of certainty, to be related to the test drug. An AE may be considered probable, if:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It cannot be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
3. It disappears or decreases on cessation or reduction in dose. [There are important exceptions when an AE does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists; e.g., [1] bone marrow depression, [2] tardive dyskinesias].
4. It follows a known pattern of response to the suspected drug.
5. It reappears upon rechallenge.

POSSIBLE [must have first two]

1. This category applies to those AEs in which the connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possible if, or when:
2. It follows a reasonable temporal sequence from administration of the drug.
3. It may have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
4. It follows a known pattern of response to the suspected drug.

REMOTE [must have first two]

1. In general, this category is applicable to an AE which meets the following criteria:
2. It does <u>not</u> follow a reasonable temporal sequence from administration of the drug.
3. It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
4. It does not follow a known pattern of response to the suspected drug.
5. It does not reappear or worsen when the drug is readministered.

UNRELATED

This category is applicable to those AEs which are judged to be clearly and incontrovertibly due only to extraneous causes [disease, environment, etc.] and do not meet the criteria for drug relationship listed under remote, possible, or probable.

Appendix 4 Adverse Event [AEs] Categories for Determining Relationship to Test Drug (Cont.)

	Probable	Possible	Remote	Unrelated
Clearly due to extraneous causes	–	–	–	+
Reasonable temporal association with drug administration	+	+	–	–
May be produced by subject clinical state, etc.	–	+	+	+
Known response pattern to suspected drug	+	+	–	–
Disappears or decreases on cessation or reduction in dose	+	–	–	–
Reappears on rechallenge	+	–	–	–

Appendix 5 ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that at any dose fulfills at least one of the following criteria:

- is fatal; [results in death] [NOTE: death is an outcome, not an event].
- is Life-Threatening [NOTE: the term "Life-Threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe].
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above.

Medical and scientific judgment should be exercised in deciding whether expedited reporting to the sponsor or designee is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definitions above. These situations should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

An unexpected AE is one, the nature or severity of which is not consistent with the applicable product information.

Causality is initially assessed by the investigator. For Serious Adverse Events, possible causes of the event is indicated by selecting one or more options. (Check all that apply)

- Pre-existing/Underlying disease - specify
- Study treatment – specify the drug(s) related to the event
- Other treatment (concomitant or previous) – specify
- Protocol-related procedure
- Other (e.g. accident, new or intercurrent illness) - specify

The term severe is a measure of intensity, thus a severe AE is not necessarily serious. For example, nausea of several hours' duration may be rated as severe, but may not be clinically serious.

Appendix 5 ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 [Cont.]

A serious adverse event occurring during the study or which comes to the attention of the investigator within 15 days after stopping the treatment or during the protocol-defined follow-up period, if this is longer, whether considered treatment-related or not, must be reported. In addition, a serious adverse event that occurs after this time, if considered related to test "drug", should be reported.

Such preliminary reports will be followed by detailed descriptions later which will include copies of hospital case reports, autopsy reports and other documents when requested and applicable.

For serious adverse events, the following must be assessed and recorded on the AEs page of the CRF: intensity, relationship to test substance, action taken, and outcome to date.

The investigator must notify the Ethics Review Committee/Institutional Review Board of a serious adverse event in writing as soon as is practical and in accordance with international and local laws and regulations.

ROCHE LOCAL COUNTRY CONTACT for SAEs: Local Monitor

ROCHE HEADQUARTERS CONTACT for SAEs: Clinical Operations/Clinical Science

Within the US, weekends, holidays and after 5:00 pm, call [redacted] and ask for the physician on call. Outside the US, call the local emergency contact number provided by the Monitor.

Appendix 6 Primary Immunodeficiency Conditions

Category	Conditions
Severe Combined Immunodeficiency (SCID)	Adenosine deaminse (ADA) deficiency
	Artemis deficiency (SCIDA)
	CD45 deficiency
	Cernunnos deficiency
	DNA ligase IV (LIG4) deficiency
	Interleukin receptor γ chain deficiency (X-linked SCID)
	Janus-associated kinase 3 (JAK3) deficiency
	Recombinase activating gene (RAG 1 / 2) deficiency
	Reticular dysgenesis
	TAP- 1 or TAP- 2 deficiency (MHC class I deficiency)
Primary T cell Deficiency	CD8 deficiency
	diGeorge syndrome
	Interleukin 7 receptor α (IL7RA) deficiency
	MHC class II deficiency
	LCK deficiency
	Orai 1 deficiency
	Nude syndrome (wing helix nude deficiency)
	Purine nucleotide phosphorylase (PNP) deficiency
	T cell receptor deficiency (CD 3γ, δ, ε, and ζ deficiencies)
	Zap 70 tyrosine kinase deficiency
Predominantly Antibody Deficiency	X-Linked CD40 ligand deficiency
	X- Linked IKK-γ (NEMO) deficiency
	CD40 deficiency
Other Well-Defined immunodeficiency Syndromes	Interferon γ receptor deficiency
	X-Linked lymphoproliferative syndrome

Adapted from Table 310-2: p2056 reference 20.

Appendix 7 Hematologic Malignancies and their Effect on the Immune System

Malignancy	Effect on immunity
ALL, lymphomas	suppression of hematopoiesis, neutropenia, lymphocyte dysfunction
CLL, small lymphocytic lymphoma	hypogammaglobulinemia, increased susceptibility to infections, autoimmune anemia or thrombocytopenia
Hairy cell leukemia, myelodysplastic syndromes	pancytopenia
peripheral T cell and NK neoplasms	lymphocyte dysfunction, increase in immature cells
Hodgkin's disease	suppression of cell-mediated immunity
AML	neutropenia, anemia, thrombocytopenia, predisposed to infections, lymphocyte dysfunction
CML	anemia, granulocyte dysfunction in some patients in early phase and in most patients in blast phase

Adapted from reference 20.

Appendix 8 Immunosuppressive Medications

Class	Category	Drugs
Corticosteroids	-	Glucocorticoids (oral, sc, im, iv)
Cytotoxic agents[20]	Alkylating agents	cyclophosphamide, busulfan, mechlorethamine, chlorambucil, melphalan, carmustine, lomustine, ifosfamide, procarbazine, dacarbazine, temozolomide, cisplatin, carboplatin, oxaliplatin
	Anti-metabolites	methotrexate, 6-mercaptopurine, azathioprine
	Anti-tumor antibiotics	bleomycin, actinomycin D, mitomycin C, etoposide, topotecan, irinotecan, doxorubicin, daunorubicin, idarubicin, epirubicin, mitoxantrone
	Anti-mitotic agents	vincristine, vinblastine, vinorelbine, paclitaxel, docetaxel, estramustine phosphate, NAB-paclitaxel
	Molecularly targeted agents	imatinib, tretinoin, bexarotene, denileukin, diftitox, gefitinib, erlotinib, dasatinib, sorafenib, sunitinib
Calcineurin inhibitors	-	cyclosporine, tacrolimus
mTOR inhibitors (proliferation-signal inhibitors)	-	sirolimus, everolimus
Immunosuppressive antibodies	-	anti lymphocyte and antithymocyte globulins (ALG and ATG)
Monoclonal antibodies [41, 42]	Inhibitors of pro inflammatory cytokines	Adalimumab
		Infliximab
		Cetrolizumab
		Etarnecept
		Basiliximab
		Daclizumab
	Adhesion cell modulators	Natalizumab
	T-cell inhibitors	Abatacept
		Alefacept
		Muromonab
	B-cell inhibitors	Rituximab
		^{90}Y-Ibritumomab
		^{131}I-Tositumomab
	Anti-CD33	Gemtuzumab
	Anti- CD52	Alemtuzumab
Others	-	mycophenolate mofetil, thalidomide

Compiled from Table 81-2: p521-24 reference 20 and references 41, 42.

PROTOCOL

TITLE: **A DOUBLE BLIND, RANDOMIZED, STRATIFIED, MULTI-CENTER TRIAL EVALUATING CONVENTIONAL AND HIGH DOSE OSELTAMIVIR IN THE TREATMENT OF IMMUNOCOMPROMISED PATIENTS WITH INFLUENZA**

PROTOCOL NUMBER:	NV20234	**VERSION NUMBER:**	D
EUDRACT NUMBER:	2006-002468-24	**IND NUMBER:**	53,093
TEST PRODUCT:	oseltamivir (Tamiflu® RO 64-0796)		
MEDICAL MONITOR:	[redacted], *M.D.*		
SPONSOR:	F. Hoffmann-La Roche Ltd		
DATE FINAL:	Version A: 14 June 2007		
DATES AMENDED:	Version B: 21 July 2008 Version C: 28 March 2011 Version D: See electronic date stamp below		

PROTOCOL AMENDMENT APPROVAL

Approver's Name	Title	Date and Time (UTC)
[redacted]	Clinical Science Leader	28-Sep-2012 14:52:40

CONFIDENTIAL STATEMENT

PROTOCOL AMENDMENT, VERSION D:
RATIONALE

Protocol NV20234 has been amended to include the Southern Hemisphere and allow global enrollment into this clinical trial. In order to further facilitate enrollment, inclusion and exclusion criteria have been amended to include as much of the immunocompromised patient population as possible.

Additional changes to the protocol are as follows:

- Update to the safety reporting section to include SAE reporting timeline changes from within one working day to within 24 hours of the knowledge of its occurrence; this change is in line with new E.U. legislation.
- Allowance for self-swabbing at home when there is a home visit planned, thereby allowing the sample to be shipped in an expedited manner.
- Reintroduction of the pharmacokinetic (PK) study component that was present in Version A of this protocol. However, for this protocol version, a sparse PK sampling schedule will be used in which blood samples are not required to be collected on multiple days. This is possible due to the additional data available on Tamiflu®. This component of the study will only be applicable to patients who provide additional consent, thereby avoiding recruitment issues should a patient not want to take part in the PK sampling aspect of this study.

Additional minor changes have been made to improve clarity and consistency. Substantive new information appears in italics. This amendment represents cumulative changes to the original protocol.

PROTOCOL AMENDMENT, VERSION D: SUMMARY OF CHANGES

GLOBAL CHANGES

References have been updated throughout the document

PROTOCOL SYNOPSIS

The protocol synopsis has been updated to reflect the changes to the protocol, where applicable.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The list of abbreviations and definitions of terms has been updated to reflect the changes to the protocol, where applicable.

SECTION 1.1: BACKGROUND

The neuraminidase (NA) ~~protein is~~ *proteins are* the second major surface ~~protein~~ *proteins* in the virion and ~~plays~~ *play* a role in enhancing virus penetration of the mucus layer around the target cell and in release of virus from the cell surface. ~~The matrix proteins, M1 and M2 appear to trigger the disintegration of the M1 complex during virus fusion with the cell and may also be involved in the maturation of the HA prior to assembly of new virus particles.~~ *The M2 protein triggers the disintegration of the virion during virus entry into the cell and may also be involved in protecting the HA prior to assembly of new virus particles.*

SECTION 1.1.1: Influenza in the Immunodeficient Population

. . .

Secondary immunodeficiency due to disease

HIV-infected individuals with a CD4+ T cell count of < 200/μL *(AIDS defining)* are highly susceptible to opportunistic disease [16]. *However, CD4 counts <500/μL are considered abnormal in HIV-infected individuals, and therefore these individuals are also more susceptible to infection.*

SECTION 1.2: RATIONALE FOR THE STUDY

Because of the increasing body of evidence (Section 1.1.2), oseltamivir is ~~now~~ recommended in national guidelines as an option for the treatment of influenza in the transplant population [37]~~. As the transplant population is considered representative~~*, which comprises a significant portion* of the immunocompromised population~~, the~~. *However, there is limited data on safety and efficacy of oseltamivir use in this population. The* primary objective of this study is to evaluate safety and resistance, while evaluating efficacy as a secondary objective*, in the broader immunocompromised patient population, who are considered at increased risk of viral infection*.

SECTION 2.2: SECONDARY OBJECTIVES

- The time to resolution of influenza symptoms.

- The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis).
- The virologic course of influenza (proportion shedding and viral loads at different time points).
- *To estimate the population pharmacokinetics of oseltamivir and oseltamivir carboxylate (e.g., clearance, volume of distribution) in immunocompromised patients with confirmed influenza infection, through the application of established population pharmacokinetic (PK) models to the sparse plasma concentration data generated.*

SECTION 3.1.1.3: *Rational for Sparse PK Sampling*

Limited PK sampling will be done in this protocol, in patients who have provided additional consent, and is justifiable in this study for the following two reasons:

- *There are already tested and qualified population PK models available [36] that will be used to estimate exposure in the immunocompromised population on the basis of the data collected in this study, meaning extensive PK sampling over a steady-state dosing interval is not required.*
- *The patient population in this study is likely to be very ill with a complex clinical picture and significant additional burden of treatment and monitoring due to their primary condition. In this context, it makes ethical sense to minimize blood draws and assessments to only those absolutely essential to meet the objectives of the study and to ensure patient safety.*

SECTION 3.3: CENTERS

This will be a multicenter study ~~with approximately 100-110 centers~~ *taking place in the* Northern *and Southern* Hemisphere*s at approximately 125 centers*.

SECTION 4.2: INCLUSION CRITERIA

- Rapid diagnostic test, PCR, or viral culture positive for influenza ~~in the 96 hours prior to first dose~~
- Immunocompromised subject defined as one who meets any of the following:
    - Primary immunodeficiency at risk for viral infections (representative examples in Appendix 6) OR
    - Secondary immunodeficiency
        - SOT with ongoing immunosuppression OR
        - Allogenic HSCT with ongoing immunosuppression OR
        - HIV with CD4 count ~~< 200~~ *< 500* /mm^3 *and, in the investigator's opinion, considered immunocompromised* OR
        - Hematologic malignancies (representative examples in Appendix 7) OR
        - Systemic (e.g. enteric, sc, im or iv) immunosuppressive therapy, irrespective of medical indication, started at least 12 weeks prior to, and ongoing at the time of first dose of study drug (representative examples in Appendix 8)

SECTION 4.3: EXCLUSION CRITERIA

- ~~SOT within 6 months of the time of randomization.~~
- ~~Have in the investigator's opinion experienced acute rejection in the 4 weeks prior to randomization.~~
- ~~HSCT patients with no evidence of engraftment (engraftment is defined as the point at which a patient can maintain a sustained absolute neutrophil count (ANC) of > 500/mm3 and sustained platelet count of ≥ 20,000/mm3, lasting ≥ 3 consecutive days without transfusions).~~
- ~~HSCT subjects not discharged from hospital after their initial hospitalization for transplantation~~
- ~~Have clinical evidence for hepatic decompensation at the time of randomization (clinical icterus, ascites, hepatic encephalopathy, coagulopathy).~~
- ~~Have cirrhosis of the liver at the time of randomization.~~
- *Clinical evidence of severe hepatic impairment, defined as Child-Pugh grade C (score >9) or decompensated cirrhosis.*
- Patients currently receiving any form of renal replacement therapy including hemodialysis, peritoneal dialysis or hemofiltration.
- *Have evidence of a serious secondary respiratory or disseminated infection that may confound or overlay the diagnosis and/or symptomatology of influenza.*
- ~~Have evidence of active or uncontrolled opportunistic infections (bacterial, fungal, or viral including cytomegalovirus [CMV] or polyoma virus [BKV]) at the time of randomization. Patients with HCV or HBV are not excluded.~~
- ~~Patients with co morbid conditions which are uncontrolled. Uncontrolled is defined as disease requiring change of therapy or hospitalization in the 4 weeks preceding randomization. Change of therapy is defined as dose increase or change of medication prior to onset of present influenza like illness.~~
- Patients with gastrointestinal disorders which might interfere with their ability to absorb oral medication.
- Allergy to the test medication.
- Patients with hereditary fructose intolerance (for subjects who will be taking the liquid formulation).
- Influenza vaccination with live attenuated vaccine in the 2 weeks prior to randomization.
- Antiviral treatment (example: amantadine, rimantadine, oseltamivir, laninamivir, peramivir, zanamivir and ribavirin) for influenza in the 2 weeks prior to randomization.
- Patients taking probenecid medication.
- Patients who are pregnant or breast-feeding.

- ~~Participation~~ *Participated* in a clinical trial or expanded access trial with an investigational drug *suspected/demonstrated to impact pathways important for the metabolism and excretion of oseltamivir* in the 4 weeks prior to randomization or concomitantly with this study.

TABLE 1: SCHEDULE OF ASSESSMENTS AND PROCEDURES

Table 1 has been revised to reintroduce the PK sampling (blood assessment) that was present in Version A of this protocol, as well as allow for self-swabbing at home when there is a home visit planned..

SECTION 5.3: CLINICAL ASSESSMENTS AND PROCEDURES

At all visits subjects will receive the routine care for their primary illness. Routine patient care (including the adjustment of doses of any of the immunosuppressive drugs) will be dictated by tests performed locally at the trial site. *For subjects unable to attend the clinic, provision will be made for swabbing to be conducted at home, when there is a home visit scheduled. Training will be provided to site staff on how to perform this.* All safety labs will be sent to a central laboratory. Results for these laboratory parameters will be used by the sponsor/designee to assess overall safety.

All assessments and procedures will be performed according to the Schedule of Assessments (Table 1). Additional information regarding these procedures not provided in the Schedule of Assessments is provided below.

Blood samples will be collected from patients who provide additional consent for PK sampling, as outlined in Section 5.5.

SECTION 5.5: *PHARMACOKINETIC ASSESSMENTS /PHARMACODYNAMIC ASSESSMENTS*

Participation in PK assessments is not compulsory for this study. Blood samples for the characterization of oseltamivir and oseltamivir carboxylate pharmacokinetics using a sparse sampling strategy will be collected from all patients who provide additional consent to participate in the PK assessments.

Blood samples will be collected according to the Schedule of Assessments (Table 1) and as described below. If these blood samples are collected at a home visit, site staff should ensure the PK blood sample handling processing is not compromised. The time and date of the dose and blood samples should be captured. Further details on pharmacokinetics/pharmacodynamics can be found in Sections 8.3.1 and 8.3.2.

5.5.1 Pharmacokinetic Assessments

Plasma PK samples for assessment of oseltamivir and oseltamivir carboxylate will be collected at the following timepoints on Day 6, or any day after the 11th dose, using the following sampling approach:

- *Within 30 minutes prior to the dose administration (e.g., 08:30 a.m.)*
- *1.5 hours ±30 minutes post-dose (e.g., 10:30 a.m. ±30 min)*
- *4 hours ±60 minutes post dose (e.g., 1:00 p.m. ±60 min)*
- *8 hours ±1.5 hours post dose (e.g., 5:00 p.m. ±1.5 hr)*

For adults and adolescents, approximately 2 mL of blood will be taken at each timepoint; therefore, the total volume blood loss for PK assessments will be approximately 8 mL. For pediatric subjects, not less than approximately 0.6 mL of blood will be taken at each timepoint; therefore, the total volume blood loss for PK assessments will be approximately 3.6 mL.

The samples from this study are classified as Biological Substance, Category B.

Plasma concentrations of oseltamivir and oseltamivir carboxylate will be measured by a specific and validated method. Details on sampling procedures, sample storage, and shipment are provided in the Sampling Manual.

5.5.2 Pharmacodynamic Assessments

Nasal and throat swabs will be collected from individuals on the days specified in the schedule of assessments. Specimens will be analyzed at a central laboratory. The proportion of patients with viral shedding at each visit will be summarized.

SECTION 6.2: PREPARATION AND ADMINISTRATION OF STUDY DRUG

Oseltamivir will be provided in two forms:

1. *Oseltamivir c*apsules containing 75 mg of active drug and packaging material consisting of pregelatinized starch, povidone, talc and sodium stearyl fumarate. All participants 13 years and older will receive this dosage form. *Oseltamivir capsules should be stored at 25ºC.*
2. A pediatric suspension containing 12 mg oseltamivir per ml of reconstituted solution and the following excipients: sorbitol, titanium dioxide, sodium benzoate, xanthan gum, monosodium citrate, saccharin sodium and Permaseal11900-31 Tutti Frutti (flavor). All participants 12 years and under will receive this dosage form. *Oseltamivir dry powder for oral suspension [pediatric syrup] should be stored ~~at 25ºC. After reconstitution~~below 30 ℃ [86 ℉]. Once reconstituted, the suspension should not be used for longer than 10 days~~. Store constituted suspension~~ if stored under room temperature conditions (below 25 ℃) or for 17 days if stored under refrigeration at 2 ℃ to ~~8ºC. Do~~8 ℃ (36 ℉ to 46 ℉). The suspension is not ~~freeze~~ suitable for freezing.*

For further details, refer to the Tamiflu® Investigator's Brochure.

SECTION 7.1.1.3: Serious Adverse Events [Immediately Reportable to Roche]

Certain events require immediate reporting to allow the Sponsor to take appropriate measures to address potential new risks in a clinical trial. The investigator must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours

after the investigator learns of the event. The following is a list of events that the investigator must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study drug:

- *Serious adverse events*
- *Pregnancies*

SECTION 7.2.1: Reporting of Serious Adverse Events [immediately reportable]

Any clinical AE or abnormal laboratory test value that is *serious* [as defined in Section 7.1.1.3 above] and which occurs during the course of the study, regardless of the treatment arm, occurring from the enrollment visit (start of study screening procedures), must be reported to Roche or designee ~~within *one* working day of~~*immediately (i.e., no more than 24 hours after* the investigator becom*es* aware of the event [expedited reporting]*)*.

SECTION 7.2.2: Pregnancy

A female subject must be instructed to stop taking the test "drug" and immediately inform the investigator if she becomes pregnant during the study. The investigator should report all pregnancies ~~within~~ *immediately (i.e., no more than* 24 hours *after the investigator becomes aware of the pregnancy)* to the Sponsor or designee. The investigator should counsel the subject, and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Pregnancies occurring within 28 days of treatment completion should be reported to Roche.

SECTION 8.1.2: Secondary Endpoints

The following are the model-predicted PK secondary endpoints for both oseltamivir and oseltamivir carboxylate:

- *Steady-state area under the concentration–time curve from 0 to 12 hours (AUC_{0-12})*
- *Maximum plasma concentration (C_{max}) for both oseltamivir and oseltamivir carboxylate*
- *Trough plasma concentration (C_{trough}) for both oseltamivir and oseltamivir carboxylate*

The following model-predicted PK endpoints may be included, if appropriate:

- *Elimination half-life ($t_{1/2}$)*
- *Time to maximum concentration (t_{max})*
- *Elimination constant (k_e)*
- *Apparent clearance (CL/F)*
- *Apparent volume of distribution (Vc/F)*
- *Apparent total clearance of metabolite (CL_m)*
- *Last measurable concentration (C_{last}) and time to last measurable concentration (t_{last})*

SECTION 8.2.4.3: *Pharmacokinetic Evaluable Patient Population (PKEP)*

The PKEP population comprises all patients in the ITT population who have at least one post-dose drug concentration measurement at a scheduled visit timepoint. Patients may be excluded from the PKEP population if they significantly violate the inclusion or exclusion criteria, deviate significantly from the protocol, or have unavailable or incomplete data that may influence the PK analysis.

Decisions on patient exclusion from the PK analysis will be made prior to database closure by the clinical pharmacologist. Excluded patients will be documented along with the reason for exclusion.

SECTION 8.3: *PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSIS*

8.3.1 Pharmacokinetic Analysis

The primary study variables are the model-predicted PK parameters: steady-state $AUC_{0\text{-}12}$, C_{max}, and C_{trough} of oseltamivir and oseltamivir carboxylate.

Secondary model-predicted PK variables may be included for both oseltamivir and oseltamivir carboxylate, if appropriate: $t_{1/2}$, t_{max}, k_e, CL/F, Vc/F, CL_m, C_{last} and t_{last}.

Individual and mean plasma concentrations at each sampling timepoint for oseltamivir and oseltamivir carboxylate will be presented by listings and descriptive summary statistics, including means, geometric means, medians, ranges, standard deviations, and coefficients of variation. Individual and mean concentration-versus-time profiles will be plotted on linear and semi-logarithmic scales.

Plasma concentration data from sparse sampling will be analyzed using an established population PK model to determine key exposure parameters (e.g., C_{max}, C_{trough}, and AUC). For immunocompromised children aged 1 to 18 years, plasma oseltamivir and oseltamivir carboxylate concentrations will be modeled in NONMEM using a structure similar to a comprehensive population PK model, which was previously developed using plasma data of non-immunocompromised children and adults (ages 1 to 80 years) [36]. The basic structure consists of a 2-compartment model with first-order absorption and direct conversion of oseltamivir to oseltamivir carboxylate, while a 1-compartment model is used to account for the renal elimination of oseltamivir carboxylate from the plasma. Body weight, evaluated using a power function and centered around 70 kg, is a statistically significant predictor of the CL/F for oseltamivir, and both CL/F and central volume of distribution (Vc/F) for oseltamivir carboxylate. For oseltamivir carboxylate, CrCl is also a significant predictor of CL/F, while Vc/F decreases linearly with age.

8.3.2 Pharmacodynamic Analysis

If feasible, the relationship between PK exposure of oseltamivir carboxylate and viral shedding response data will be characterized using nonlinear mixed effects modeling (using software

NONMEM). Relevant population pharmacodynamic (PD) parameters will be derived, and the influence of covariates will be investigated. If deemed appropriate and necessary, data may be pooled with data from previous studies investigating oseltamivir and oseltamivir carboxylate in order to improve the model stability.

SAMPLE INFORMED CONSENT FORMS

The sample Informed Consent Forms have been revised to reflect the changes to the protocol.

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PROTOCOL SYNOPSIS

TITLE:	**A DOUBLE BLIND, RANDOMIZED, STRATIFIED, MULTI-CENTER TRIAL EVALUATING CONVENTIONAL AND HIGH DOSE OSELTAMIVIR IN THE TREATMENT OF IMMUNOCOMPROMISED PATIENTS WITH INFLUENZA**
PROTOCOL NUMBER:	NV20234
VERSION NUMBER:	D
EUDRACT NUMBER:	2006-002468-24
IND NUMBER:	53,093
TEST PRODUCT:	oseltamivir (Tamiflu® RO 64-0796)
PHASE:	IIIb
INDICATION:	Treatment of influenza in immunocompromised patients
SPONSOR:	F. Hoffmann-La Roche Ltd

Objectives

Primary

To evaluate prospectively the safety and tolerability of oseltamivir for the treatment of influenza in immunocompromised patients and characterize the effects of oseltamivir in immunocompromised patients on the development of resistant influenza virus

Secondary

To evaluate the effects of conventional and high dose oseltamivir in immunocompromised patients on:

- The time to resolution of influenza symptoms
- The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis)
- The virologic course of influenza (proportion shedding and viral loads at different time points)
- *To estimate the population pharmacokinetics of oseltamivir and oseltamivir carboxylate (e.g., clearance, volume of distribution) in immunocompromised patients with confirmed influenza infection, through the application of established population pharmacokinetic (PK) models to the sparse plasma concentration data generated.*

Study Design

Description of Study

This is a double-blind, randomized, multi-center trial of twice daily, conventional and high dose oseltamivir for the treatment of influenza in immunocompromised patients. Subjects will be stratified by age ($\leq 12, > 12$ years), transplant status (yes, no), time since onset of flu symptoms and treatment start (up to 96 hours) (≤ 48 or > 48 hours) and vaccination status (yes, no)

Number of Patients

A minimum of 166 (83 per arm) to allow an adequate number of influenza A patients per arm; including 50 transplant recipients.

Number of Centers

Approximately *125* centers in the Northern *and Southern* Hemisphere*s*

Target Population

Patients immunocompromised due to a primary or secondary immunodeficiency, 1 year of age and older. The subjects will be positive for influenza by a rapid diagnostic test, PCR or virus culture at baseline.

Length of Study

10 days of treatment, 30 days of follow up.

Procedures (summary)

The key procedures are:

- Blood draws for serum chemistry, hematology, serology*, and PK assessments (for those patients who provide additional consent to participate in the PK assessments)*.
- Nasal and throat swabs for viral culture and RT-PCR.

Assessments of:

Safety

Adverse events, physical exams, vital signs, clinical laboratory evaluations

Resistance

Development of resistance

Efficacy

Time to resolution of all clinical influenza symptoms as recorded in the patient diary.

Investigational Medicinal Products

Test Product:

Oseltamivir/placebo dry powder (to be reconstituted to a concentration of 12 mg/ml) and 75 mg capsules. The duration of dosing in both adults and children is 10 days.

Conventional dose:

Children ages 1 – 12 years: Oseltamivir syrup

≤ 15 kg	30 mg twice daily
> 15 – 23 kg	45 mg twice daily
> 23 – 40 kg	60 mg twice daily
> 40 kg	75 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules 75 mg twice daily

Subjects randomized to the conventional dose will simultaneously receive matching placebo so as to blind them and the investigator from the high dose arm.

High dose:

Children ages 1 - 12 years: Oseltamivir syrup

≤ 15 kg	60 mg twice daily
> 15 – 23 kg	90 mg twice daily
> 23 – 40 kg	120 mg twice daily
> 40 kg	150 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules 150 mg twice daily

Comparator:

Placebo (from pivotal registration trials in otherwise healthy adults)

Statistical Methods

The sample size has been chosen to provide an adequate number of patients to estimate the development of resistance with reasonable precision. Assuming that 90% of enrolled patients will have laboratory-confirmed influenza, there will be 75 patients in each treatment arm in the population evaluable for the development of resistance. The following table shows the 95% Pearson-Clopper confidence intervals that would result with a sample size of 75 patients in a treatment arm if certain event rates are observed in the study.

During the study the number of influenza A virus infected patients and the rate of development of resistance will be monitored in a blinded fashion, in order to ensure a reasonable precision for the estimation is maintained. Additional patients may be enrolled if necessary.

Observed Rate (%)	95% Confidence Interval
0.0	0% - 4.8%
1.3	0.0% - 7.2%
2.7	0.3% - 9.3%
5.3	1.5% - 13.1%
10.7	4.7%-19.9%

A total of 83 patients will be enrolled per arm and will be evaluable for the assessment of safety. This number of patients would provide estimates of adverse event rates with similar precision.

For the primary objective of evaluating the safety of oseltamivir conventional and high dose treatments, AEs, laboratory tests, and vital signs will be summarized and compared with the known safety profile of the drug. For the summary of the development of resistance for each treatment arm, 95% confidence intervals will be provided with the estimated rates.

For the secondary objective of evaluating the efficacy of oseltamivir as measured by the time to resolution of influenza symptoms, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- Comparison to placebo control from pivotal registration trials

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established that is comparable to patients in the current study. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

- Assessment of relative efficacy

  The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval.

GLOSSARY OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ALT [SGPT]	Alanine aminotransferase
ALL	Acute lymphocytic leukemia
AML	Acute myeloid leukemia
ANOVA	Analysis of variance
AST [SGOT]	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
$AUC_{0\text{-}12}$	*Steady-state area under the concentration–time curve from 0 to 12 hours*
b.i.d.	Bis in die (twice daily)
BP	Blood pressure
CARIFS	Canadian acute respiratory infections scale
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CL/F	*Apparent clearance*
C_{last}	*Last measurable concentration*
CL_m	*Apparent total clearance of metabolite*
C_{max}	Maximum plasma concentration
CML	Chronic myeloid leukemia
Cr Cl	Creatinine clearance
CRF	Case report form[s]
C_{trough}	*Trough plasma concentration*
ESF	Eligibility screening form
GVHD	Graft versus host disease
hrs	Hours
HA	Hemagglutinin
HIV	Human immunodeficiency virus
HSCT	Hematopoietic stem cell transplant
ICH	International Conference on Harmonisation
ITT	Intent to treat
ITTI	Intent to treat influenza infected
IVRS	Interactive voice response system
k_e	*Elimination constant*
mg	Milligram
mL	Milliliter
PD	Pharmacodynamic
PK	Pharmacokinetic

GLOSSARY OF ABBREVIATIONS

PKEP	*Pharmacokinetic Evaluable Patient Population*
p.o.	Per os (by mouth)
QD	Once per day
SAE	Serious adverse event
SAP	Statistical analysis plan
SCID	Severe combined immunodeficiency
SOT	Solid organ transplant
$TCID_{50}$	50% tissue culture infectious dose
t_{last}	*Time to last measurable concentration*
t_{max}	Time to maximum plasma concentration
$t_{1/2}$	Elimination half-life
µg	Microgram
Vc/F	*Apparent volume of distribution*

PART I: STUDY DESIGN AND CONDUCT

1. BACKGROUND AND RATIONALE

1.1 BACKGROUND

Influenza is an acute respiratory infection caused by a virus of the orthomyxovirus family which occurs in three forms, influenza A, B and C. Influenza virus types A and B cause an acute febrile infection of the respiratory tract characterized by the sudden onset of fever, malaise, headaches, myalgias and cough. Influenza causes numerous deaths each year [1]. Although difficult to assess, annual influenza epidemics are thought to result in between three and five million cases of severe illness, and between 250,000 and 500,000 deaths every year around the world [2].

The influenza viruses are segmented, negative sense, single stranded, lipid encapsulated, RNA viruses between 80 and 100 nm in size. Subtypes are defined according to glycoproteins present in the viral lipid coat. The haemagglutinin complex (HA) is the major surface protein of the virus. The neuraminidase (NA) protein*s are* the second major surface protein*s* in the virion and play a role in enhancing virus penetration of the mucus layer around the target cell and in release of virus from the cell surface. *The M2 protein triggers the disintegration of the virion during virus entry into the cell and may also be involved in protecting the HA prior to assembly of new virus particles.*

1.1.1 Influenza in the Immunodeficient Population

Influenza infection is usually a self limiting condition. However, in children, the elderly and the immunocompromised, influenza infection can be associated with substantial morbidity and mortality [1]. In patients with compromised immunity, influenza virus may cause severe lower respiratory tract involvement; unusual syndromes, including encephalitis, myocarditis, and hepatitis and death [3].

Conditions that compromise immunity may be classified based on etiology into primary (genetic) and secondary (acquired) immunodeficiency. Of the immunodeficient conditions, the ones that affect cell mediated immunity are likely to have adverse outcomes following viral infections [16].

Primary immunodeficiency

Primary immunodeficiencies are relatively common, may be either congenital or manifest later in life and are classified according to whether the genetic defect affects T or B cells or both [16]. There are four groups of disorders: severe combined immunodeficiency (SCID), primary T cell deficiency (e.g. CD8 deficiency, DiGeorge syndrome), predominantly *B-cell-related* antibody deficiency (e.g. common variable immunodeficiency, selective IgA deficiency) and other well-defined immunodeficiency syndromes (e.g. Wiskott Aldrich syndrome) [16].

Of the primary immunodeficiencies, antibody deficiencies are the most frequent. However, some of the more common antibody deficiency conditions (isolated IgA deficiency, IgG subclass deficiency and common variable immunodeficiency) have intact cell-mediated immunity and therefore the clinical course of viral infections (unless complicated by bacterial infections) does not differ significantly from that in the normal host [16]. A list of primary immunodeficiency disorders at risk for viral infections is provided in Appendix 6. The incidence of some of these conditions has been estimated. The incidence of SCID is 1 in 100,000 to 1 in 1,000,000 [16]. The more severe forms of primary immunodeficiency are relatively rare, have their onset early in life, and all too frequently result in death during childhood [16].

Secondary Immunodeficiency

Secondary immunodeficiencies are not caused by intrinsic abnormalities in development of T and B cells [16]. Secondary immunodeficiency may result from diseases (human immunodeficiency virus [HIV], hematologic malignancy) or immunosuppressive and cytotoxic drugs (such as those used for treatment of transplant recipients, collagen vascular disease, malignancies).

Secondary immunodeficiency due to disease

HIV-infected individuals with a CD4+ T cell count of < 200/μL *(AIDS defining)* are highly susceptible to opportunistic disease [16]. *However, CD4 counts <500/μL are considered abnormal in HIV-infected individuals, and therefore these individuals are also more susceptible to infection.* Studies in HIV/AIDS subjects have shown an increased risk for heart and lung-related hospitalizations during the influenza season compared to other times of the year, prolonged duration of influenza symptoms, increased risk for influenza-related complications and a higher risk of influenza-related death [17].

Several hematologic malignancies affect the immune system (Appendix 7). Several authors have reported influenza in children and adults with hematologic malignancies [18, 19, 20, 21].

Secondary immunodeficiency due to drugs (e.g. transplant recipients, collagen vascular disease, malignancies)

The enhanced survival of the transplant population following the availability of newer immunosuppressive drugs has made them representative of the immunocompromised population in general; and secondary immunodeficiency due to drugs, in particular.

In transplant recipients influenza is associated with a higher rate of pulmonary complications, extrapulmonary manifestations and an increased risk of graft dysfunction and high attributable mortality. In a cohort study of influenza viral infection in solid organ transplant (SOT) subjects, 30 cases of influenza viral infection were identified. Secondary bacterial pneumonia was seen in 17% of subjects and three SOT recipients

(2 liver and one kidney) had myositis, myocarditis and bronchiolitis obliterans. Biopsy of the transplanted organ was performed in 21 of 30 cases and revealed variable degrees of acute allograft rejection in 62% of subjects. The study concluded that influenza was associated with significant morbidity in different groups of SOT recipients [4].

Among transplant recipients, subjects with *hematopoietic* stem cell transplant (HSCT) are at the greatest risk for morbidity. In a prospective study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation, 1973 patients were evaluated for respiratory virus infections after stem cell transplant*ation*. The overall mortality was 23% from influenza A infection and the direct influenza A-associated mortality was 15.3% in this study [5]. A large retrospective study (4797 subjects undergoing HSCT over a 13-year period) identified 62 patients with influenza of whom as many as 29% (18 of 62 patients) developed pneumonia. Ten percent of the subjects with influenza died [6].

The increased morbidity in transplant patients is associated with increased duration of virus shedding compared to that in healthy subjects. In otherwise healthy adults, elderly subjects and children, the median duration of viral shedding in untreated subjects was 70, 96 and 118 hours respectively [7]. In contrast, in the retrospective HSCT study, influenza virus was shed in nasopharyngeal secretions (evaluated at least weekly) for a median duration of 168 hours (7 days, range 2 to 37 days) [6].

Currently available treatments for influenza infection are the M2 ion channel inhibitors (e.g. amantadine, rimantadine) and the neuraminidase inhibitors (e.g. oseltamivir, zanamivir).

1.1.2 Oseltamivir

Oseltamivir (Tamiflu®, Ro 64-0796) is an ethyl ester prodrug which is rapidly absorbed from the gastrointestinal tract after oral administration and metabolized in the liver by high capacity carboxylesterases to form oseltamivir carboxylate (Ro 64-0802), a potent, stable and selective inhibitor of influenza A and B neuraminidase enzymes. The active form, oseltamivir carboxylate is excreted unchanged by the kidney via glomerular filtration and active tubular secretion by the organic anion transport system. The efficacy and safety of oseltamivir in influenza treatment and prevention has been established in an extensive series of clinical studies in man.

Oseltamivir has been approved for the treatment of influenza in Europe, the United States and most other countries around the world. In adults and adolescents, the recommended dose is 75 mg twice daily for five days. In children 1 year of age and older recommended doses are 30, 45 or 60 mg bid based on body weight. In all age groups the recommended dose is administered bid for 5 days.

The approval of oseltamivir for the treatment of influenza is based on several controlled clinical trials. In the pooled population from these clinical trials encompassing adults

aged from 13-97 years, many with significant co-morbidity, 1325 subjects were treated with oseltamivir (75 mg bid) and 1056 subjects received placebo. A total of seven influenza symptoms (both respiratory and constitutional) were captured on the diary card for adults. The time to resolution of all symptoms (on the diary card) decreased by 24 hours; from 124.5 hours in the placebo arm to 100.6 hours with oseltamivir 75 mg bid ($p < 0.0001$) [7]. Further, in adults and adolescents with a proven influenza illness, oseltamivir treatment reduced overall antibiotic use for any reason by 26.7% and the incidence of influenza-related lower respiratory tract complications resulting in antibiotic therapy by 55%. The study concluded that oseltamivir treatment of influenza reduces lower respiratory tract complications, antibiotic use and hospitalizations in healthy and 'at risk' subjects [8].

Likewise, in the influenza-infected pediatric population (1 to 12 years of age), oseltamivir treatment ($n = 217$) was compared with placebo ($n = 235$). There was a reduction in the median duration of illness (defined based on resolution of temperature, cough, coryza and return to pre-illness health and activity) of 36 hours; from 137 hours with placebo to 101 hours in the oseltamivir treatment arm ($p \leq 0.0001$). The Canadian Acute Respiratory Infections Scale (CARIFS), validated for use in children, was used to collect symptom data on the pediatric diary card. The CARIFS scale comprised a total of 18 symptoms which were evaluated twice daily by the parent or guardian. There was a similar reduction in the time to alleviation of all CARIFS symptoms of 36 hours; from 100 hours in the placebo group to 63 hours in the oseltamivir group ($p < 0.0001$) [9].

Thus in both adults and children, the time to resolution of all symptoms was significantly reduced in the oseltamivir treatment arm compared with placebo.

Oseltamivir was well-tolerated in clinical trials. Approximately 11,000 subjects have received oseltamivir in the development program. The most common adverse events reported by adults, the elderly, and children were nausea and vomiting. Serious adverse events (SAEs) were reported with a low and equal frequency by subjects taking active drug and placebo. Full details are given in the Investigator Brochure [7].

The safety profile of oseltamivir has been well characterized for the prophylaxis indication in a prospective randomized placebo controlled trial conducted in the adult and pediatric immunocompromised (HSCT and SOT) population. In the oseltamivir group, the indications for transplant included hematologic malignancies (acute and chronic leukemias, multiple myeloma and myelodysplastic syndrome), lymphoid malignancies (Hodgkins or non-Hodgkins lymphomas), primary immunodeficiencies (Wiskott-Aldrich syndrome, X-linked lymphoproliferative disease and severe combined immunodeficiency). Other rare indications included bone marrow aplasia, paroxysmal nocturnal hemoglobinuria, myelofibrosis and multiple sclerosis. In the safety population, there were 239 subjects randomized to the conventional dose of oseltamivir and 238 to placebo. The total number of adverse events reported in the placebo group (361 events) was generally similar to that in the oseltamivir group (323 events). Diarrhea was the

most frequently reported adverse event (placebo, 8%; oseltamivir, 6%). There were no deaths in the oseltamivir group. Oseltamivir was found to be safe in immunosuppressed transplant recipients [22].

Limited safety and/or efficacy of oseltamivir for the treatment indication is available from several case reports in children and adolescents. Oseltamivir was shown to be safe and/or effective in HIV infected children (n = 10) [23], children (age 3 to 12 years) with acute lymphocytic leukemia (ALL) (n = 10) [21], in a nosocomial H1N1 outbreak in a pediatric (children aged 10 months to 13 years) oncology ward (n = 8) [20], in children and adolescents (age 2 to 19 years) with malignancies (ALL, neuroblastoma, brain tumor, non-Hodgkin's lymphoma, osteosarcoma, Ewing sarcoma, myelodysplasia, acute myeloid leukemia (AML), Wilms tumor, aplastic anemia, chronic myeloid leukemia (CML) and acute promyelocytic leukemia) (n = 51) [18], in children aged 4 to 14 years on immunosuppressive drugs (n = 5) [24] and in children (aged 5 months to 5 years) with bone marrow transplant (n = 3) [25].

Oseltamivir has also been shown to be safe and/or effective in mixed populations of children and adults (62 patients) with HSCT [6], and in a large epidemiologic study (n = 221) with SOT [26].

Finally, oseltamivir has been shown to be safe and/or effective in immunocompromised adults with HSCT [10] and adults with lung transplant [27, 28, 29].

The dose of oseltamivir was the same as the conventional dose in a majority of these reports. In one report as many as 25 adult patients received twice the conventional dose [26] while in another report, three of nine adult patients received twice the conventional dose [29]. Treatment with oseltamivir generally ranged from 5 to 10 days [28, 29, 26] and occasionally until the patient was symptom free [28, 29]. In exceptional cases treatment was given for as long as 20 days [18] or for as long as the patient was positive by RT-PCR [21].

There is some concern about the development of resistance in the immunocompromised population. During the pandemic influenza season, more than 23,000 clinical isolates of novel H1N1 pandemic virus were tested for resistance in the 6 international WHO regions. A total of 225 isolates (from 225 subjects) were resistant (H275Y mutation in the neuraminidase coding sequence) to oseltamivir for an approximate incidence of 1%. Information on immune status was available for 142 of 225 patients. Among the 142 patients, 56 (40%) were immunocompromised [30]. Prolonged viral replication and lack of immune-mediated virus clearance in immunosuppressed patients treated with antivirals can result in a higher incidence of selection of drug-resistant viruses, a phenomenon that has been documented previously [31, 32].

1.2 RATIONALE FOR THE STUDY

Because of the increasing body of evidence (Section 1.1.2), oseltamivir is recommended in national guidelines as an option for the treatment of influenza in the transplant population [37]*, which comprises a significant portion of the immunocompromised population. However, there is limited data on safety and efficacy of oseltamivir use in this population. T*he primary objective of this study is to evaluate safety and resistance, while evaluating efficacy as a secondary objective*, in the broader immunocompromised patient population, who are considered at increased risk of viral infection*.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective of the study is to evaluate prospectively the safety and tolerability of oseltamivir for the treatment of influenza in immunocompromised patients and characterize the effects of oseltamivir in immunocompromised patients on the development of resistant influenza virus.

2.2 SECONDARY OBJECTIVES

The secondary objectives of the study are:

To evaluate the effects of conventional and high dose oseltamivir in immunocompromised patients on:

- The time to resolution of influenza symptoms.
- The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis).
- The virologic course of influenza (proportion shedding and viral loads at different time points).
- *To estimate the population pharmacokinetics of oseltamivir and oseltamivir carboxylate (e.g., clearance, volume of distribution) in immunocompromised patients with confirmed influenza infection, through the application of established population pharmacokinetic (PK) models to the sparse plasma concentration data generated.*

3. STUDY DESIGN

3.1 OVERVIEW OF STUDY DESIGN AND DOSING REGIMEN

This is a double blind, randomized, stratified, multi-center trial of conventional and high dose oseltamivir for the treatment of influenza in immunocompromised patients. Immunocompromised patients who develop an influenza-like illness with a positive rapid diagnostic test, PCR, or viral culture for influenza, will be enrolled during the influenza season. Patients will be stratified by transplant status [yes, no], the time between onset of influenza symptoms and treatment start (up to 96 hours) [$\leq$48 hours; $>$48 hours], influenza vaccination status for current influenza season [Yes; No] and by age [$\leq$12 years; $>$12 years]. Eligible patients will be consented and randomized to receive

oseltamivir twice daily for 10 days in one of two treatment arms: conventional dose or high dose (double the conventional dose).

3.1.1 Rationale for Study Design

This study incorporates several features that distinguish it from classic placebo controlled trials.

There is no placebo control arm in this study as it was considered unethical for this high risk population. The development of resistance following treatment with oseltamivir (one of the primary objectives of the study) is an objective assessment (determined by laboratory tests) and is unlikely to be impacted by the absence of a placebo arm.

As a placebo control arm was considered unethical in this population, an alternative control arm had to be identified for efficacy end points. The response to placebo in pivotal oseltamivir registration trials in the healthy adult population with influenza was chosen as a control in lieu of a placebo arm in the current trial. In order to do this, the end points were designed to be similar to that in the pivotal registration trials.

The following sections provide rationale and justification for specific aspects of the study design which differ from the currently approved dosing for influenza or from previous pivotal registration trials. They also provide information on how the results will be used to make recommendations on dose and duration of treatment.

3.1.1.1 Choice of Treatment Arms

The currently approved dose of oseltamivir for the treatment indication is the conventional dose with a duration of five days. In this study a conventional and high dose (twice the conventional dose) are being evaluated. The pivotal registration trials for oseltamivir in healthy adults with influenza had three treatment arms, two active (75 mg and 150 mg) and one placebo. Both treatment arms were found to be significantly better than placebo. No statistical comparisons were made between the two active treatment arms. Evaluation of the results in the two active treatment arms did not reveal any clinically meaningful difference. However, the proportion of subjects shedding virus on day 4 (3 days after the start of treatment), suggested a possible dose response relationship (36%, 28% and 23% in the placebo, 75 mg and 150 mg dose arms respectively). Because defective immune-mediated virus clearance in immunosuppressed patients treated with antivirals can result in a higher incidence of selection of drug-resistant viruses, it was decided to use both the conventional and high dose arm for this study. A longer duration of treatment was chosen because a large retrospective study has shown that the median duration of viral shedding of 7 days [6] was greater for HSCT recipients than that seen in the healthy children, adult and elderly population with influenza (Section 1.1.1) [7].

3.1.1.2 Inclusion of Patients Symptomatic up to 96 Hours

In immunocompromised patients, time from onset of symptoms to seeking medical attention (presentation at the clinic) of >48 hours has been shown in several case reports [33, 27, 28, 29] including two instances of nosocomial outbreaks in children [25, 20]. This notwithstanding, oseltamivir has been shown to be effective in immunocompromised populations that included patients treated with oseltamivir beyond 48 hours of presentation; patients with lung transplant (median time to presentation 3 days) [27], bone marrow transplant (treatment started more than 48 hours after onset of symptoms in all three patients) [25], organ transplant (62 out of 221 patients started treatment after 96 hours) [26] and children with ALL (one child presented after 3 days and two children after 5 days of symptoms) [21].

In a prospective, observational study involving adults hospitalized with influenza, the study authors concluded that 'Weakened host defenses slow viral clearance, whereas antivirals started within the first four days of illness enhance viral clearance' [34].

Additionally, in November 2009, in a communication for clinicians on antiviral treatments for H1N1, the US Centers for Disease Control and Prevention (CDC) stated that while antiviral treatment is most effective when started early; both outpatients with risk factors and hospitalized patients still benefit when treatment with oseltamivir is started more than 48 hours after illness onset [35].

3.1.1.3 *Rational for Sparse PK Sampling*

Limited PK sampling will be done in this protocol, in patients who have provided additional consent, and is justifiable in this study for the following two reasons:

- *There are already tested and qualified population PK models available [36] that will be used to estimate exposure in the immunocompromised population on the basis of the data collected in this study, meaning extensive PK sampling over a steady-state dosing interval is not required.*
- *The patient population in this study is likely to be very ill with a complex clinical picture and significant additional burden of treatment and monitoring due to their primary condition. In this context, it makes ethical sense to minimize blood draws and assessments to only those absolutely essential to meet the objectives of the study and to ensure patient safety.*

3.1.1.4 Interpretation of Study Results

Safety and tolerability and the development of resistance are the primary objectives of this study. These will be characterized descriptively. For the secondary objective of efficacy, the subset of the population enrolled in the first 48 hours will be compared with placebo patients from pivotal registration trials (where patients were enrolled in the first two days of illness). Sections 8.2.5, 8.2.6, and 8.2.7 provide details on the statistical considerations and their rationale associated with these comparisons.

Stratification

Stratification in this study has no bearing on any comparisons with results from previous trials. However, in order to evaluate a dose response relationship between the two active treatment arms in this trial, stratification remains essential.

Patients will be stratified by transplant status (yes, no) because transplant patients form a relatively large homogenous group in this study and might influence outcome.

Patients will be stratified by time between onset of symptoms and treatment start (<48 hours or ≥ 48 hours) because previous experience in otherwise healthy adults and children has shown that patients treated early in their illness respond to treatment better than those who are treated later. Patients will also be stratified by age (≤ 12 years and >12 years) because otherwise healthy children shed virus longer than otherwise healthy adults. It is expected that a similar difference in the duration of viral shedding would exist between immunocompromised children and adults. As vaccinated subjects may respond faster to therapy, the study is also stratified based on influenza vaccination status for the current influenza season (Yes; No).

3.1.2 Rationale for Dose Selection and Adjustment

Dose Selection

The dose of oseltamivir to be used in this study is the conventional, approved dose for children and adults in the treatment of influenza. There will be a second higher dose for comparison which is two times the conventional dose. This higher dose is used based on theoretical considerations which suggest that the higher dose may be associated with improved efficacy and decreased emergence of resistance.

The anticipated pro-drug and metabolite exposures from this higher dose are not expected to exceed maximum exposures seen previously in the oseltamivir development program. The safety and tolerability of the higher dose regimen has already been demonstrated in treatment studies of immunocompetent adult subjects ($n=447$) [11]. In a study to demonstrate cardiac safety, in the highest dose group treated with 450 mg b.i.d. for 5 days [$n=99$], no subject had a serious adverse event, nor withdrew prematurely. In Phase I studies in adults, oseltamivir has been administered in multiple doses of up to 500 mg b.i.d. Doses of 200 mg b.i.d. and greater have been associated with increased gastrointestinal adverse effects (nausea and vomiting) [7]. In adult subjects with creatinine clearances of ≤ 30 mL/min, doses of 100 mg b.i.d. for 6 days were well tolerated, despite steady-state oseltamivir carboxylate exposures approximately 10-fold higher than those achieved with standard dosing in renally competent individuals [12]. No other adverse effects were reported more frequently with higher doses and no serious adverse events have been reported within the volunteer studies. Co-administration of oseltamivir with food has been demonstrated to substantially reduce the frequency and severity of gastrointestinal side effects.

Thus, the rationale for the higher dose arm in this study is to explore the possibility of improved efficacy and decreased emergence of resistance without an undue increase in risk.

Drug interactions with immunosuppressive medications have also been evaluated. The pharmacokinetics of oseltamivir and oseltamivir carboxylate after administration of 75 mg oseltamivir (conventional adult dose) in subjects with a well-functioning, stable renal allograft who were being maintained on immunosuppressive therapy were studied. These were similar to those described in the literature for adults with comparable degrees of renal function. Oseltamivir was well tolerated and had no clinically relevant effect on the steady-state pharmacokinetics of cyclosporine A, tacrolimus, or mycophenolate mofetil [7].

Duration of Dosing

The duration of dosing chosen for this population (10 days) is longer than that in the healthy adult and pediatric populations (5 days). This is based on observations that the viral shedding and illness are typically longer in immunocompromised patients than it is in healthy adults [6, 10].

Dose Adjustments

In this study, patients whose CrCl decreases to $<$60mL/min/1.73M^2 will be discontinued from treatment.

3.1.3 End of Study

The study comprises 10 days of treatment with follow up visits approximately 5 and 30 days later as shown in the schedule of assessments. A rapid diagnostic test for influenza will be performed at the end of the 10 days of treatment. Subjects still having influenza based on the rapid diagnostic test will be treated per the local standard of care by the principal investigator.

3.2 NUMBER OF SUBJECTS/ ASSIGNMENT TO TREATMENT GROUPS

A minimum of 166 patients (approximately 83 per arm) to allow an adequate number of influenza A patients, including 50 transplant recipients will be enrolled in this study. After screening, patients will be randomly assigned to one of the two active treatment groups.

3.3 CENTERS

This will be a multicenter study *taking place in the* Northern *and Southern* Hemisphere*s at approximately 125 centers*.

4. STUDY POPULATION

4.1 OVERVIEW

The study population comprises immunocompromised adults (including adolescents) and children who have influenza. Additionally, the subjects must not have other medical conditions that will preclude the assessment of efficacy or safety. Influenza vaccinated and non-vaccinated subjects are eligible to participate in this study. Principal investigators will review oseltamivir resistance patterns of strains circulating in the area and weigh the risk versus the benefit before enrolling patients with a potentially resistant strain.

Under no circumstances are patients who enroll in this study permitted to be re-randomized to this study and enrolled for a second course of treatment.

4.2 INCLUSION CRITERIA

- Age greater than or equal to 1 year
- Rapid diagnostic test, PCR, or viral culture positive for influenza
- Immunocompromised subject defined as one who meets any of the following:
    - Primary immunodeficiency at risk for viral infections (representative examples in Appendix 6) OR
    - Secondary immunodeficiency
        - SOT with ongoing immunosuppression OR
        - Allogenic HSCT with ongoing immunosuppression OR
        - HIV with CD4 count $<500/mm^3$ *and, in the investigator's opinion, considered immunocompromised* OR
        - Hematologic malignancies (representative examples in Appendix 7) OR
        - Systemic (e.g. enteric, sc, im or iv) immunosuppressive therapy, irrespective of medical indication, started at least 12 weeks prior to, and ongoing at the time of first dose of study drug (representative examples in Appendix 8)
- Symptoms suggestive of influenza like illness including, but not limited to fever, cough, or coryza
- In patients with history or clinical presentation at randomization suggestive of renal failure; a CrCl >60 ml/min/$1.73M^2$
- Less than or equal to 96 hours between onset of influenza like illness and first dose of study drug
- Parent/guardian willing and able to comply with study requirements and give consent, (country specific age cut off)

- Patient able to comply with study requirements and willing to give assent, as appropriate (country specific age cut off)
- For adult patients, willing and able to comprehend and give written informed consent
- Patients, in the reproductive age group, must agree to utilize an effective method of contraception throughout the study period and for females for one reproductive cycle following cessation of study therapy
- Females of childbearing potential must have a negative urine pregnancy test prior to start of study medication

4.3 EXCLUSION CRITERIA

- *Clinical evidence of severe hepatic impairment, defined as Child-Pugh grade C (score >9) or decompensated cirrhosis.*
- Patients currently receiving any form of renal replacement therapy including hemodialysis, peritoneal dialysis or hemofiltration.
- *Have evidence of a serious secondary respiratory or disseminated infection that may confound or overlay the diagnosis and/or symptomatology of influenza.*
- Patients with gastrointestinal disorders which might interfere with their ability to absorb oral medication.
- Allergy to the test medication.
- Patients with hereditary fructose intolerance (for subjects who will be taking the liquid formulation).
- Influenza vaccination with live attenuated vaccine in the 2 weeks prior to randomization.
- Antiviral treatment (example: amantadine, rimantadine, oseltamivir, laninamivir, peramivir, zanamivir and ribavirin) for influenza in the 2 weeks prior to randomization.
- Patients taking probenecid medication.
- Patients who are pregnant or breast-feeding.
- *Participated* in a clinical trial or expanded access trial with an investigational drug *suspected/demonstrated to impact pathways important for the metabolism and excretion of oseltamivir* in the 4 weeks prior to randomization or concomitantly with this study.

4.4 CONCOMITANT MEDICATION AND TREATMENT

Antiviral treatments with activity against influenza (e.g. amantadine, rimantadine, zanamivir, ribavirin, laninamivir, peramivir, and additional oseltamivir above that specified for this study) are not allowed during the 10-day treatment phase of the study. Concomitant use of an investigational drug during the study is also excluded. Live attenuated vaccines may not be advisable for this population. It is possible that concurrent use with oseltamivir may render live vaccines ineffective.

Medications required for the routine care of the patient may be continued during the study. Concomitant use of probenecid (irrespective of the indication) is not allowed during the study.

4.5 CRITERIA FOR PREMATURE WITHDRAWAL

The investigator must discontinue treatment if the creatinine clearance is $<$60 ml/min in adults or $<$60 ml/min/1.73 M^2 in children. The investigator must also discontinue treatment from all subjects with intercurrent illnesses or adverse events suggestive of hepatic decompensation. The investigator also has the right to discontinue treatment in the event of intercurrent illness, adverse events, treatment failure, protocol violations, administrative reasons or other reasons.

The investigator must discontinue treatment with study drug if the patient is to be treated concomitantly with another antiviral for influenza, for example, amantadine, rimantadine, laninamivir, peramivir, or zanamavir. However, all patients, including those who discontinue study drug prematurely and/or are treated with another antiviral, will be required to return for follow up approximately 5 and 30 days after the last dose of study medication (day 15 and day 40 assessments).

Subjects have the right to withdraw from the study at any time for any reason.

An excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of subjects should be avoided. Should a subject decide to withdraw, all efforts will be made to complete and report the observations prior to withdrawal as thoroughly as possible.

The investigator should contact the subject or a responsible relative either by telephone or through a personal visit to establish as completely as possible the reason for the withdrawal. A complete final evaluation at the time of the patient's withdrawal should be made with an explanation of why the subject is withdrawing from the study. If the reason for removal of a subject from the study is an Adverse Event, the principal specific event will be recorded on the CRF.

In the case that the subject decides to prematurely discontinue study treatment ["refuses treatment"], he/she should be asked if he/she can still be contacted for further information. The outcome of that discussion should be documented in both the medical records and in the CRF.

4.6 REPLACEMENT POLICY [ENSURING ADEQUATE NUMBERS OF EVALUABLE SUBJECTS]

4.6.1 <u>For Subjects</u>

No subject prematurely discontinued from the study for any reason will be replaced.

4.6.2 For Centers

A center may be replaced for the following administrative reasons:

- Excessively slow recruitment.
- Poor protocol adherence.

5. SCHEDULE OF ASSESSMENTS AND PROCEDURES

Table 1 Schedule of Assessments

	Baseline	Treatment					Follow-up	
Study Day	**1 (pre-dose)**	**1**	**2 or 3[f,g]**	**6[f,g]**	**8[f,g]**	**11[g,h,i]**	**15[g]**	**40[g,j]**
Informed Consent/Assent [l]	x							
Medical history	x							
Demographics	x							
Height and weight	x					x		
Pregnancy Test [a]	x					x		x
Rapid diagnostic test for influenza virus shedding	x					x		
Safety Labs [b]	x					x		
Physical Examination	x					x		x
Vital Signs (including *pulse*, RR, temperature, Blood pressure)	x		x	x	x	x	x	x
Nasal and throat swabs for viral shedding and viral load [c,d,k]	x		x	x	x	x	x	x
PK sampling (blood) [l]				x[m]				
Review of electronic diary data [e]			x	x	x	x	x	x
Drug Administration		←	—	—	—	→		
Collection of unused study medication and empty containers						x		
Previous Diseases	x							
Previous/Concomitant medications	x	←	—	—	—	—	—	→
Adverse Events/Sec Illnesses and Treatments		←	—	—	—	—	—	→
Rejection, Graft versus host disease (GVHD)		←	—	—	—	—	—	→

a. Urine pregnancy test for patients of child-bearing potential according to the judgment/discretion of the investigator.

b. Hematology (CBC with differential) and chemistry (AST, ALT, total bilirubin, urea, Cr). Serum creatinine testing may be done at a local laboratory when clinically indicated to calculate creatinine clearance.

c. Baseline swab samples will be assessed for the presence of resistance mutations.

d. Two nasal and one throat swab for viral culture and RT-PCR.

e. Influenza symptoms, temperature, and date/ time of oseltamivir dose will be recorded by the patient in electronic patient diaries twice daily on days 1 – 10, and once daily thereafter.

Table 1 Schedule of Assessments (cont.)

[f] A home visit may be made on day 2 or 3 (for patients who are too ill to come into the clinic) and*/or, day 6, and/or* day 8*; however, it is recommended that the PK blood draw be performed at the clinic*.

[g.] Day 2/3 visit window = +1 day. Day 6 visit window = +/- 1 day; Day 8 visit window = +1 day. Day 11 visit window = ± 1 day. Day 15 and day 40 visit occur approximately 5 and 30 days after the last dose respectively. Day 15 window = ± 1 day. Day 40 window ± 2 days.

[h.] Only weight to be assessed. Height is not necessary at this visit. Study drug is taken on day 11, only if the first dose was taken after 4 PM on Day 1 [5.3] Subjects who are rapid diagnostic positive can be treated per standard of care. They will be required to come for their follow-up visits on day 15 and day 40.

[i.] Subjects who discontinue treatment prematurely will have an end of treatment (day 11) assessment at the time of treatment discontinuation or the following day. They will be required to attend their follow up visits approximately 5 and 30 days after the last dose (day 15 and day 40 assessments).

[j.] Subjects who discontinue during follow-up will have an end of follow-up (day 40) assessment. This visit must occur within 30 days of the last dose.

[k.] *For subjects who are unable to attend the clinic, swabs may be taken at home on those days where there is a home visit by site personnel.*

[l.] *Plasma PK samples for assessment of oseltamivir and oseltamivir carboxylate will be collected from patients who give additional consent to participate in PK sampling using a sparse PK sampling approach (Section 5.5).*

[m.] *Serial PK samples taken at steady-state no earlier than day 6 (i.e., not before the 11th dose) consisting of as many of the four timepoints as possible: within 30 minutes prior to the dose administration, 1.5 hours ±30 minutes post dose, 4 hours ±60 minutes post dose, and 8 hours ±1.5 hours post dose (for patients who have given additional consent).*

5.1 SCREENING EXAMINATION AND ELIGIBILITY SCREENING FORM

All subjects must provide written informed consent (assent where applicable) before any study specific assessments or procedures are performed. Screening will take place at the baseline visit prior to first dose. However, if necessary, screening may take place the day before the baseline visit, as long as the patient is randomized and receives his/her first dose of study medication within 96 hours of influenza symptom onset.

Subjects will be assessed for inclusion and exclusion criteria and those who fulfill all the inclusion and none of the exclusion criteria will be randomized into the study.

An Eligibility Screening Form [ESF] documenting the investigator's assessment of each screened subject with regard to the protocol's inclusion and exclusion criteria is to be completed by the investigator.

A screen failure log must be maintained by the investigator.

5.2 PROCEDURES FOR ENROLLMENT OF ELIGIBLE SUBJECTS

Once a subject has fulfilled the entry criteria, he/she will be randomized to one of two treatment groups. The subject randomization numbers will be generated by Roche or its designee and incorporated into double-blind labeling.

The investigator or designee will use the CRF pre-printed with the assigned subject number and enter the randomization number provided by IVRS for allocation to the treatment groups in the appropriate place on each subject's CRF.

Randomization will be stratified by transplant status (Yes; No); time between onset of symptoms and treatment start ($\leq$48 hrs or $>$48 hrs), influenza vaccination status (Yes; No) and patient's age ($\leq$12 years or $>$12 years).

5.3 CLINICAL ASSESSMENTS AND PROCEDURES

At all visits subjects will receive the routine care for their primary illness. Routine patient care (including the adjustment of doses of any of the immunosuppressive drugs) will be dictated by tests performed locally at the trial site. *For subjects unable to attend the clinic, provision will be made for swabbing to be conducted at home, when there is a home visit scheduled. Training will be provided to site staff on how to perform this.* All safety labs will be sent to a central laboratory. Results for these laboratory parameters will be used by the sponsor/designee to assess overall safety.

All assessments and procedures will be performed according to the Schedule of Assessments (Table 1). Additional information regarding these procedures not provided in the Schedule of Assessments is provided below.

Blood samples will be collected from patients who provide additional consent for PK sampling, as outlined in Section 5.5.

Study Day 1

The baseline and study day 1 assessment may be performed at the same visit.

Study medication, electronic diaries, and thermometers will be dispensed. Patients or guardians/parents will be instructed how to complete electronic symptom diaries (Appendix 2) (Appendix 3), temperature recording, and treatment administration details, including time of each oseltamivir dose. The first diary entries will be made at the site before the first dose of study drug.

Baseline nasal and throat swab samples will be assessed for the presence of oseltamivir-resistance mutations.

The date of the first dose of study drug is defined as study day 1. Once randomized, the first dose of study drug will be administered in the clinic. Study day 2 will begin at 12 midnight of the same calendar day. If the first dose of study drug is taken after 4 pm on

day 1, the next dose of study drug will be taken in the morning of day 2. In this case, the last dose of study drug will be taken on the morning of study day 11.

If the first dose of study drug is taken prior to 4 pm on day 1, the next dose of study drug should be taken in the evening of the same day (i.e. prior to midnight on the same calendar day with a minimum of 7 hours between doses). For these patients the last dose of study drug will be taken in the evening of study day 10. More information on dosing is provided later (Section 6.1).

Study Days 2 - 11

Study day 2 will begin at 12 midnight of study day 1.

It is expected the study staff will maintain close contact with patients, especially during the first few days of the study. The study staff should inquire about any adverse events, check that the patient or parent/guardian is entering data into the electronic diary properly, and assess drug compliance. During the dosing period, diary symptoms should be assessed and temperature should be recorded at the same time that the study drug is taken.

End of Treatment Day 11

The end of treatment visit for all subjects is on day 11 (irrespective of whether they took one or two doses on day 1). Subjects who discontinue study medication prematurely will have all day 11 assessments completed at the time of discontinuation or the following day.

After all day 11 study assessments are completed, a rapid diagnostic test will be performed at this visit. If the rapid diagnostic test is positive, the subject may be treated per standard of care at the discretion of the investigator.

All subjects (including those who discontinue study medication prematurely and those who are positive for influenza on their rapid diagnostic test at the end of treatment visit) will be required to return for follow up approximately 5 and 30 days after the last dose (day 15 and day 40 assessments).

Study Days 12 – 40

Study day 15 is an important visit as it is the first assessment of viral culture after cessation of treatment.

End of Follow-Up Day 40

All subjects must attend an end of follow-up visit on day 40.

If the patient is withdrawn after completion of treatment (after the day 11 assessment), a termination visit should be arranged. This visit should be the end of follow-up visit assessment [Day 40]. This visit must occur within 30 days of the last dose.

Patients or parents/guardians who do not return to the clinic will be contacted by telephone to ascertain their health status, or that of their child's, record information on adverse events, and to ask that they return the electronic diary.

5.3.1 Efficacy Assessments

The primary efficacy end point in this study is the time to resolution of all influenza symptoms as recorded in the patient diary.

The clinical efficacy parameters in this study are:

1. Symptoms of influenza-like illness. These are captured in the diary for both adults and children (Appendix 2) (Appendix 3). Influenza symptoms are graded on a nominal scale from zero (absent/no problem) to 3 (severe or major problem). A score of zero is considered asymptomatic. The primary efficacy end point, time to alleviation of all symptoms is comprised of all the symptoms captured in the diary.
2. Temperature. This is captured in the diary. Temperature is used for assessment of the primary and several secondary efficacy end points.

5.3.2 Safety

Safety parameters in this study include adverse events, vital signs, and clinical laboratory evaluations.

Pre-defined symptoms of influenza captured in the adult and pediatric diaries are not to be reported as adverse events unless they can be further qualified. Thus 'headache due to stress at work' is reported as an adverse event. However, unexplained 'headache' is considered a predefined symptom related to influenza and not an adverse event.

Adverse events such as bronchitis, pneumonia, otitis media and sinusitis are considered secondary illnesses of influenza and should be recorded as adverse events.

Other adverse events to be expected in the transplant population such as rejection and graft versus host disease (in HSCT subjects) will also be collected as adverse events.

5.4 LABORATORY ASSESSMENTS

The laboratory assessments include those for efficacy and safety.

Efficacy

The efficacy laboratory assessments are used for laboratory confirmation of influenza. These are:

Nasal and throat swabs. Two nasal and one throat swab will be collected as described in the Schedule of Assessments. All swabs are sent to a central laboratory for RT-PCR and viral cultures. Influenza virus shedding will be assessed. Baseline samples will be assessed for the presence of oseltamivir-resistance mutations.

During the course of treatment, neither the investigator nor the patient will know which participants have ongoing positive viral cultures for influenza. At the end of treatment (day 11), a rapid diagnostic test is permitted for the diagnosis of ongoing influenza.

Safety

The safety laboratory assessments in this study, including the assessment of serum chemistry and hematology will be done at the central laboratory. Serum chemistry assessments comprise AST, ALT, total bilirubin, urea and creatinine. Hematology assessments include CBC and differential count. The total volume of blood loss for laboratory assessments will be approximately 20 mL for the entire duration of the study.

Protection of patient confidentiality (Section 16) will extend to any data generated from the assaying of these samples. Biological samples taken from all patients may be infectious and will be classified as "diagnostic specimens" for dispatch purposes.

The Principal Investigator may draw blood for serum creatinine to be assessed at the local laboratory when clinically indicated during the study to calculate creatinine clearance.

5.5 *PHARMACOKINETIC ASSESSMENTS /PHARMACODYNAMIC ASSESSMENTS*

Participation in PK assessments is not compulsory for this study. Blood samples for the characterization of oseltamivir and oseltamivir carboxylate pharmacokinetics using a sparse sampling strategy will be collected from all patients who provide additional consent to participate in the PK assessments.

Blood samples will be collected according to the Schedule of Assessments (Table 1) and as described below. If these blood samples are collected at a home visit, site staff should ensure the PK blood sample handling processing is not compromised. The time and date of the dose and blood samples should be captured. Further details on pharmacokinetics/pharmacodynamics can be found in Sections 8.3.1and 8.3.2.

5.5.1 *Pharmacokinetic Assessments*

Plasma PK samples for assessment of oseltamivir and oseltamivir carboxylate will be collected at the following timepoints on Day 6, or any day after the 11th dose, using the following sampling approach:

- *Within 30 minutes prior to the dose administration (e.g., 08:30 a.m.)*
- *1.5 hours ±30 minutes post-dose (e.g., 10:30 a.m. ±30 min)*
- *4 hours ±60 minutes post dose (e.g., 1:00 p.m. ±60 min)*
- *8 hours ±1.5 hours post dose (e.g., 5:00 p.m. ±1.5 hr)*

For adults and adolescents, approximately 2 mL of blood will be taken at each timepoint; therefore, the total volume blood loss for PK assessments will be approximately 8 mL. For pediatric subjects, not less than approximately 0.6 mL of blood will be taken at each timepoint; therefore, the total volume blood loss for PK assessments will be approximately 3.6 mL.

The samples from this study are classified as Biological Substance, Category B.

Plasma concentrations of oseltamivir and oseltamivir carboxylate will be measured by a specific and validated method. Details on sampling procedures, sample storage, and shipment are provided in the Sampling Manual.

5.5.2 *Pharmacodynamic Assessments*

Nasal and throat swabs will be collected from individuals on the days specified in the schedule of assessments. Specimens will be analyzed at a central laboratory. The proportion of patients with viral shedding at each visit will be summarized.

6. INVESTIGATIONAL MEDICINAL PRODUCT

The Investigational Medical Product in this study is oseltamivir dry powder (to be reconstituted to a concentration of 12 mg/ml) or 75 mg capsules and matching placebo.

The Investigational Medicinal Products will be supplied, packaged individually for each subject and labeled in accordance with Roche Standard and local regulation by Roche Clinical Trial Supply, Basel, Switzerland.

6.1 DOSE AND SCHEDULE OF STUDY DRUG

Oseltamivir will be given twice daily over 10 days for a total of 20 doses. The doses need to be taken at 12 hourly intervals. Under no circumstances is a subject allowed to take two doses within 7 hours of each other.

Patients will be randomized to receive either conventional or high dose of study drug.

Conventional dose:

Children ages 1 – 12 years: Oseltamivir syrup

≤ 15 kg	30 mg twice daily
> 15 – 23 kg	45 mg twice daily
> 23 – 40 kg	60 mg twice daily
> 40 kg	75 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules

75 mg twice daily

Subjects randomized to the conventional dose will simultaneously receive matching placebo so as to blind them and the investigator from the high dose arm.

High dose:

Children ages 1 – 12 years: Oseltamivir syrup

≤ 15 kg 60 mg twice daily

> 15 – 23 kg 90 mg twice daily

> 23 – 40 kg 120 mg twice daily

> 40 kg 150 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules

150 mg twice daily

6.1.1 Dose Modifications

No dose modifications will be allowed on study.

6.2 PREPARATION AND ADMINISTRATION OF STUDY DRUG

Oseltamivir will be provided in two forms:

1. *Oseltamivir c*apsules containing 75 mg of active drug and packaging material consisting of pregelatinized starch, povidone, talc and sodium stearyl fumarate. All participants 13 years and older will receive this dosage form. *Oseltamivir capsules should be stored at 25ºC.*
2. A pediatric suspension containing 12 mg oseltamivir per ml of reconstituted solution and the following excipients: sorbitol, titanium dioxide, sodium benzoate, xanthan gum, monosodium citrate, saccharin sodium and Permaseal11900-31 Tutti Frutti (flavor). All participants 12 years and under will receive this dosage form. *Oseltamivir dry powder for oral suspension [pediatric syrup] should be stored below 30 ℃ [86 ℉]. Once reconstituted, the suspension should not be used for longer than 10 days if stored under room temperature conditions (below 25 ℃) or for 17 days if stored under refrigeration at 2 ℃ to 8 ℃ (36 ℉ to 46 ℉). The suspension is not suitable for freezing.*

For further details, refer to the Tamiflu® Investigator's Brochure.

Matching placebo will be available as capsules and suspension. Subjects in the conventional dose arm will get the conventional dose and matching placebo so that they are blinded from the high dose arm.

Each subject will be dispensed a medication pack that will provide enough medication to cover 20 doses. For subjects randomized to the conventional dose arm, the medication pack will contain a bottle of oseltamivir dry powder or a blister wallet with oseltamivir capsules and matching placebo. For subjects randomized to the high dose arm, the medication pack will contain two bottles of oseltamivir dry powder or two blister wallets with oseltamivir capsules. Irrespective of the treatment group the subject is randomized to, for each dose the subject will take the same amount from both bottles or blister wallets provided in the medication pack such that the sum of the amounts from each immediate container constitutes one dose.

One dose is to be administered twice per day at approximately 12-hour intervals with a light snack or glass of milk or fruit juice. The first dose of study medication will be administered in the clinic at the time of randomization.

6.3 BLINDING AND UNBLINDING

Randomization will be administered by a central randomization center.

The Randomization List will not be available at the study center, to the study monitors, project statisticians or to the project team at Roche. Emergency codes, or another adequate method of unblinding, will be implemented before study start, if the identity of the test medication is necessary for patient management in the case of a serious adverse event. Emergency codes should not be broken except in the case of emergency situations. Any request from the investigator for information about the treatment administered to study subjects for another purpose must be discussed with Roche/designee.

As per regulatory reporting requirement, Roche/designee will unblind the identity of the study medication for all unexpected [as per IB] serious adverse events that are considered by the investigator to be related to study drug. Details of patients who are unblinded during the study will be included in the Clinical Study Report.

All other individuals directly involved in this study will remain blinded until the final analysis of the primary parameter.

The randomization will be stratified by transplant status, duration of symptoms, influenza vaccination status and age and will be provided by the Roche randomization group for the IVRS vendor.

6.4 ASSESSMENT OF COMPLIANCE

Accountability and subject compliance will be assessed by maintaining adequate “drug dispensing” and return records.

Subjects will be asked to return all used and unused drug supply containers at the end of the treatment as a measure of compliance.

A Drug Dispensing Log must be kept current and should contain the following information:

- the identification of the subject [randomization and medication numbers] to whom the study medication was dispensed
- the date[s], quantity of the study medication dispensed *to* the subject
- the date[s] and quantity of the study medication returned *by* the subject

This inventory must be available for inspection by the Monitor. All supplies, including partially used or empty containers and the dispensing logs, must be returned to the monitor at the end of the study.

6.5 DESTRUCTION OF STUDY DRUG

Local or institutional regulations may require immediate destruction of used investigational product for safety reasons. In these cases, it may be acceptable for investigational site staff to destroy dispensed investigational product before a monitoring inspection provided that source document verification is performed on the remaining inventory and reconciled against the documentation of quantity shipped, dispensed, returned and destroyed. Written authorization must be obtained from the sponsor or designee at study start up before destruction.

Written documentation of destruction must contain the following:

- Identity [batch numbers or subject numbers] of investigational product[s] destroyed
- Quantity of investigational product[s] destroyed
- Date of destruction
- Method of destruction
- Name and signature of responsible person [or company] who destroyed investigational products[s]

7. SAFETY INSTRUCTIONS AND GUIDANCE

7.1 ADVERSE EVENTS (AES) AND LABORATORY ABNORMALITIES

7.1.1 Clinical AEs

Per the International Conference of Harmonization [ICH], an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign [including an abnormal laboratory finding], symptom, or disease temporally associated with the use of a medicinal [investigational] product, whether or not considered related to the medicinal [investigational] product. Pre-existing conditions which worsen during a study are to be reported as AEs. Influenza signs and symptoms reported on the patient diary will be

summarized as efficacy end points and need not be captured as adverse events. However, secondary illnesses due to influenza must be reported as adverse events.

7.1.1.1 Intensity

All clinical AEs encountered during the clinical study will be reported on the AE page of the CRF.

Intensity of AEs will be graded on a four -point scale [mild, moderate, severe, life-threatening] and reported in detail on the CRF.

Mild	discomfort noticed but no disruption of normal daily activity.
Moderate	discomfort sufficient to reduce or affect daily activity.
Severe	inability to work or perform normal daily activity
Life Threatening	represents an immediate threat to life

7.1.1.2 Drug - Adverse Event Relationship

Relationship of the AE to the treatment should always be assessed by the investigator. Description of scales can be found in Appendix 4.

7.1.1.3 Serious Adverse Events [Immediately Reportable to Roche]

Certain events require immediate reporting to allow the Sponsor to take appropriate measures to address potential new risks in a clinical trial. The investigator must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours after the investigator learns of the event. The following is a list of events that the investigator must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study drug:

- *Serious adverse events*
- *Pregnancies*

A Serious Adverse Event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that at any dose fulfils at least one of the following criteria:

– is fatal; [results in death; NOTE: death is an outcome, not an event].

– is Life-Threatening [NOTE: the term "Life-Threatening" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe].

– required in-patient hospitalization or prolongation of existing hospitalization;

– results in persistent or significant disability/incapacity;

– is a congenital anomaly/birth defect;

– is medically significant or requires intervention to prevent one or other of the outcomes listed above.

The full requirements of the ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 will be adhered to (Appendix 5).

7.1.2 Treatment and Follow-up of AEs

AEs, especially those for which the relationship to test "drug" is not "unrelated", should be followed up until they have returned to baseline status or stabilized. If a clear explanation is established it should be recorded on the CRF.

7.1.3 Laboratory Test Abnormalities

Laboratory test results will appear printed on laboratory reports provided to the site from the central laboratory.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event [SAE] should be reported as such, in addition to being recorded as an AE in the CRF.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the AE page in the CRF:

- Accompanied by clinical symptoms.
- Leading to a change in study medication [e.g. dose modification, interruption or permanent discontinuation].
- Requiring a change in concomitant therapy [e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment].

7.1.4 Follow-up of Abnormal Laboratory Test Values

In the event of medically significant unexplained abnormal laboratory test values, the tests should be repeated and followed up until they have returned to the normal range and/or an adequate explanation of the abnormality is found. If a clear explanation is established it should be recorded on the CRF.

7.2 HANDLING OF SAFETY PARAMETERS

7.2.1 Reporting of Serious Adverse Events [immediately reportable]

Any clinical AE or abnormal laboratory test value that is *serious* [as defined in Section 7.1.1.3 above] and which occurs during the course of the study, regardless of the treatment arm, occurring from the enrollment visit (start of study screening procedures), must be reported to Roche or designee *immediately (i.e., no more than 24 hours after* the investigator become*s* aware of the event [expedited reporting]*)*.

Related Serious Adverse Events ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed.

Unrelated Serious Adverse Events must be collected and reported during the study and up until the follow-up visit.

The definition and reporting requirements of ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 will be adhered to (Appendix 5).

7.2.2 Pregnancy

A female subject must be instructed to stop taking the test "drug" and immediately inform the investigator if she becomes pregnant during the study. The investigator should report all pregnancies *immediately (i.e., no more than 24 hours after the investigator becomes aware of the pregnancy)* to the Sponsor or designee. The investigator should counsel the subject, and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Pregnancies occurring within 28 days of treatment completion should be reported to Roche.

7.3 WARNINGS AND PRECAUTIONS

Events such as convulsions and delirium (including symptoms such as altered level of consciousness, confusion, abnormal behavior, delusions, hallucinations, agitation, anxiety, nightmares) have been reported during oseltamivir use in patients with influenza, predominately in children and adolescents. In rare cases, these events resulted in accidental injury. The contribution of oseltamivir to those events is unknown. Such events have also been reported in patients with influenza who were not taking oseltamivir. Patients, especially children and adolescents, should be closely monitored for signs of abnormal behavior.

Please refer to the attached Investigator's Brochure for additional warnings, precautions, and other reported adverse events.

8. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

Full details of all planned analyses will be specified in a separate Statistical Analysis Plan (SAP) for the safety and efficacy variables and in the Resistance Plan for the development of resistance. The methods described below are an outline of the main planned analyses.

8.1 PRIMARY AND SECONDARY STUDY ENDPOINTS

8.1.1 Primary Endpoints

The primary endpoints for safety will be assessments of adverse events, physical exams, vital signs and clinical laboratory evaluations. The primary endpoint of development of

resistance will be determined from the genotypic and phenotypic variables measured post baseline and is described further in the Resistance Plan.

8.1.2 Secondary Endpoints

The following are secondary endpoints which measure efficacy, the primary of which is time to alleviation of all clinical symptoms. With the exception of this variable, viral load and time to resolution of fever, they are defined as either the occurrence or non-occurrence of the specified events or the presence or absence of the specified symptoms.

- The time (hours) to alleviation of all clinical influenza symptoms (recorded in the patient diary)
- Shedding virus by culture at day 1, 2, 6, 8, 11, 15 and 40
- Shedding virus by RT-PCR at day 1, 2, 6, 8, 11, 15, and 40
- Viral load by culture ($log_{10}TCID_{50}$/mL) at Day 1, 2, 6, 8, 11, 15, and 40
- Viral load by quantitative RT-PCR at day 1, 2, 6, 8, 11, 15 and 40
- The time (hours) from first dose of study medication until resolution of fever
- Fever
- Cough
- Coryza
- Development of secondary illnesses (otitis media, bronchitis, pneumonia, or sinusitis) at any time during the study
- Development of secondary illnesses (otitis media, bronchitis, pneumonia, or sinusitis) at any time during the study that are treated with antibiotics
- Initiation of treatment with antibiotics after randomization
- Hospitalization, and for those who are hospitalized, the duration of hospitalization
- Development of rejection or GVHD

The following are the model-predicted PK secondary endpoints for both oseltamivir and oseltamivir carboxylate:

- *Steady-state area under the concentration–time curve from 0 to 12 hours (AUC_{0-12})*
- *Maximum plasma concentration (C_{max}) for both oseltamivir and oseltamivir carboxylate*
- *Trough plasma concentration (C_{trough}) for both oseltamivir and oseltamivir carboxylate*

The following model-predicted PK endpoints may be included, if appropriate:

- *Elimination half-life ($t_{1/2}$)*
- *Time to maximum concentration (t_{max})*
- *Elimination constant (k_e)*
- *Apparent clearance (CL/F)*

- *Apparent volume of distribution (Vc/F)*
- *Apparent total clearance of metabolite (CL_m)*
- *Last measurable concentration (C_{last}) and time to last measurable concentration (t_{last})*

8.1.3 Safety

Safety of the treatment will be evaluated by AEs, physical exams, clinical laboratory tests, and vital signs.

In addition to routine safety assessments, the proportion of subjects experiencing a rejection and/or graft versus host disease will be summarized by treatment group.

8.2 STATISTICAL AND ANALYTICAL METHODS

8.2.1 Statistical Model

8.2.1.1 Time to Event Variables

A non-parametric model will be assumed with estimation of medians based on Kaplan-Meier methods. Subjects without alleviation of symptoms will have their time censored at the last available observation that a complete assessment was made.

For the purpose of comparing treatment groups, it will be assumed that their respective distributions for the primary endpoint differ only by a shift in location.

8.2.1.2 Dichotomous Variables and Viral Load

For the endpoints defined dichotomously in terms of events or symptoms including the development of resistance, it will be assumed that the number of patients experiencing the event or exhibiting the symptom will have a binomial distribution.

For the continuous endpoints of viral load at Day 1, 2, 6, 8, 11, 15, and 40 no model will be assumed.

8.2.2 Sample Size

The sample size has been chosen to provide an adequate number of patients to estimate the development of resistance with reasonable precision. Assuming that 90% of enrolled patients will have laboratory-confirmed influenza, there will be 75 patients in each treatment arm in the population evaluable for the development of resistance. The following table shows the 95% Pearson-Clopper confidence intervals that would result with a sample size of 75 patients in a treatment arm if certain event rates are observed in the study.

During the study the number of influenza A virus infected patients and the rate of development resistance will be monitored in a blinded fashion, in order to ensure a reasonable precision for the estimation. An additional number of patients may be enrolled if necessary.

Observed Rate (%)	95% Confidence Interval
0.0	0% - 4.8%
1.3	0.0% - 7.2%
2.7	0.3% - 9.3%
5.3	1.5% - 13.1%
10.7	4.7%-19.9%

A total of 83 patients will be enrolled per arm and will be evaluable for the assessment of safety. This number of patients would provide estimates of adverse event rates with similar precision.

8.2.3 Hypothesis Testing

Formal hypothesis testing will be not performed, instead inferences will be based on comparison of confidence intervals.

8.2.4 Analysis Populations

Three main patient populations will be used for the analysis of data from this study; the Safety Population, the Intent-to treat Population and the Intent-to-Treat Infected Population. Detailed definitions of these populations will be given in the SAP.

8.2.4.1 Intent to Treat Population

All patients randomized will be included in the intent to treat population [Patients will be assigned to treatment groups as randomized for analysis purposes].

8.2.4.2 Intent to Treat Infected Population

All patients randomized and with laboratory confirmation of influenza infection, excluding patients infected with oseltamivir-resistant influenza at baseline, will be included in the intent to treat influenza infected population [Patients will be assigned to treatment groups as randomized for analysis purposes].

The ITTI Population will be the primary population for the summary and analysis of the development of resistance and the efficacy variables.

8.2.4.3 *Pharmacokinetic Evaluable Patient Population (PKEP)*

The PKEP population comprises all patients in the ITT population who have at least one post-dose drug concentration measurement at a scheduled visit timepoint. Patients may be excluded from the PKEP population if they significantly violate the inclusion or exclusion criteria, deviate significantly from the protocol, or have unavailable or incomplete data that may influence the PK analysis.

Decisions on patient exclusion from the PK analysis will be made prior to database closure by the clinical pharmacologist. Excluded patients will be documented along with the reason for exclusion.

8.2.4.4 Subpopulations

A subpopulation of the ITTI population will be defined comprising patients who received their first dose of study medication within 48 hours of influenza symptom onset for the comparison of efficacy endpoints with the age appropriate placebo treated patients (pediatric or adult) from the registration trials.

In order to evaluate the potential for relapse, the following two subpopulations of the ITTI population will be evaluated:

- patients not shedding virus as assessed by culture on Day 11
- patients not shedding virus as assessed by RT-PCR on Day 11

Viral shedding and RT-PCR at days 15 and 40 will be evaluated in these subpopulations.

Based on the proportion of subjects hospitalized, an additional subpopulation may be defined to evaluate the length of hospitalization for hospitalized subjects.

Likewise, based on the proportion of children enrolled additional subpopulations of children and adults will be created to evaluate the course of influenza in children and adults.

8.2.5 Efficacy Analysis

For the analysis of time to alleviation of all clinical symptoms, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- Comparison to placebo control from pivotal registration trials

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established that is comparable to the subpopulation of patients in the current study whose first dose of study medication was within 48 hours of symptom onset. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

  The median time to resolution of all influenza symptoms will be determined for each treatment group, along with its 95% confidence interval. These confidence intervals will be compared individually to the placebo control confidence interval described above as a potential means of establishing treatment effects.

- Assessment of relative efficacy

  The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval.

This confidence interval will provide lower and upper limits for the treatment difference and can be used as the basis for potential inferences. For example, if it does not contain the value zero a difference will have been established.

For the dichotomous endpoints, the proportion experiencing the event or symptom, and its associated 95% confidence interval will be derived for each treatment group. Where possible, the corresponding confidence intervals from the placebo control population will be presented for comparison to those derived for the active treatment groups in this trial.

The continuous secondary endpoint time to resolution of fever will be analyzed as for the primary efficacy endpoint.

For the continuous endpoints of viral load ($log_{10}TCID_{50}$/mL) at Day 1, 6, 8, 11, 15, and 40, summary statistics (including mean, standard deviation, median, minimum and maximum) will be derived for each treatment group.

8.2.6 Safety Data Analysis

The safety analysis population will include all subjects who receive at least one dose and had a safety assessment performed post randomization. All safety variables will be summarized and presented in tables based on this safety population.

8.2.7 Analysis of Resistance

For the summary of the development of resistance for each treatment arm, 95% confidence intervals will be provided with the estimated rates. Further details are available in the Resistance Plan for NV20234. These data will be summarized in a report separate from the final study report.

8.2.7.1 Exclusion of Data from Analysis

Efficacy data collected from any patient who begins treatment with another antiviral for influenza (for example, amantadine, rimantadine, laninamivir, peramivir, or zanamavir), after the other antiviral treatment commences, will be excluded from the primary and secondary efficacy analyses. Such patients will be considered failures to oseltamivir treatment in the analyses. Further details will be provided in the SAP.

8.2.7.2 Interim Analysis

No interim analyses are planned.

8.2.8 Other Analyses

Further exploratory analysis (including assessments of the rapid diagnostic test, sub group analysis) will be detailed in the SAP.

8.3 *PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSIS*

8.3.1 *Pharmacokinetic Analysis*

The primary study variables are the model-predicted PK parameters: steady-state $AUC_{0\text{-}12}$, C_{max}, and C_{trough} of oseltamivir and oseltamivir carboxylate.

Secondary model-predicted PK variables may be included for both oseltamivir and oseltamivir carboxylate, if appropriate: $t_{1/2}$, t_{max}, k_e, CL/F, Vc/F, CL_m, C_{last} and t_{last}.

Individual and mean plasma concentrations at each sampling timepoint for oseltamivir and oseltamivir carboxylate will be presented by listings and descriptive summary statistics, including means, geometric means, medians, ranges, standard deviations, and coefficients of variation. Individual and mean concentration-versus-time profiles will be plotted on linear and semi-logarithmic scales.

Plasma concentration data from sparse sampling will be analyzed using an established population PK model to determine key exposure parameters (e.g., C_{max}, C_{trough}, and AUC). For immunocompromised children aged 1 to 18 years, plasma oseltamivir and oseltamivir carboxylate concentrations will be modeled in NONMEM using a structure similar to a comprehensive population PK model, which was previously developed using plasma data of non-immunocompromised children and adults (ages 1 to 80 years) [36]. The basic structure consists of a 2-compartment model with first-order absorption and direct conversion of oseltamivir to oseltamivir carboxylate, while a 1-compartment model is used to account for the renal elimination of oseltamivir carboxylate from the plasma. Body weight, evaluated using a power function and centered around 70 kg, is a statistically significant predictor of the CL/F for oseltamivir, and both CL/F and central volume of distribution (Vc/F) for oseltamivir carboxylate. For oseltamivir carboxylate, CrCl is also a significant predictor of CL/F, while Vc/F decreases linearly with age.

8.3.2 *Pharmacodynamic Analysis*

If feasible, the relationship between PK exposure of oseltamivir carboxylate and viral shedding response data will be characterized using nonlinear mixed effects modeling (using software NONMEM). Relevant population pharmacodynamic (PD) parameters will be derived, and the influence of covariates will be investigated. If deemed appropriate and necessary, data may be pooled with data from previous studies investigating oseltamivir and oseltamivir carboxylate in order to improve the model stability.

9. DATA QUALITY ASSURANCE

The overall procedures for quality assurance of clinical study data are described in the Roche Standard Operational Procedures.

Accurate and reliable data collection will be assured by verification and cross–check of the CRFs against the investigator's records by the study monitor [source document verification], and the maintenance of a drug–dispensing log by the investigator.

The data collected will be entered into the study database from the working copy of the CRF faxed from the site.

A comprehensive validation check program will verify the data and discrepancy reports will be generated accordingly for resolution by the investigator. As patients complete the study [or prematurely withdraw] and their signed CRFs become available, a second data entry will be performed from the original, signed CRF. A comparison check will be run to identify and resolve any discrepancies between the first and second data entry.

Throughout the study the Study Management Team [SMT] will review data according to the SMT Data Review Plan as described in the Data Quality Plan.

9.1 ASSIGNMENT OF PREFERRED TERMS AND ORIGINAL TERMINOLOGY

For classification purposes, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using the current version of MedDRA (Medical Dictionary for Regulatory Activities terminology) for adverse events and diseases and the INN (International Non-Proprietary name) drug terms and procedures dictionary for treatments and surgical and medical procedures.

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PART II: ETHICS AND GENERAL STUDY ADMINISTRATION

11. ETHICAL ASPECTS

11.1 LOCAL REGULATIONS/DECLARATION OF HELSINKI

The investigator will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study must fully adhere to the principles outlined in "Guideline for Good Clinical Practice" ICH Tripartite Guideline [January 1997] or with local law if it affords greater protection to the subject. For studies conducted in the EU/EEA countries, the investigator will ensure compliance with the EU Clinical Trial Directive [2001/20/EC]. For studies conducted in the USA or under US IND, the investigator will additionally ensure that the basic principles of "Good Clinical Practice" as outlined in the current version of 21 CFR, subchapter D, part 312, "Responsibilities of Sponsors and Investigators", part 50, "Protection of Human Subjects", and part 56, "Institutional Review Boards", are adhered to.

In other countries where "Guideline for Good Clinical Practice" exist Roche and the investigators will strictly ensure adherence to the stated provisions.

11.2 INFORMED CONSENT

It is the responsibility of the investigator, or a person designated by the investigator [if acceptable by local regulations], to obtain written informed consent from each subject participating in this study, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. For subjects not qualified or incapable of giving legal consent, written consent must be obtained from the legally acceptable representative. In the case where both the subject and his/her legally acceptable representative are unable to read, an impartial witness should be present during the entire informed consent discussion. After the subject and representative have orally consented to participation in the trial, the witness' signature on the form will attest that the information in the consent form was accurately explained and understood. The investigator or designee must also explain that the subjects are completely free to refuse to enter the study or to withdraw from it at any time, for any reason. The Case Report Forms [CRFs] for this study contain a section for documenting informed subject consent, and this must be completed appropriately. If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All subjects [including those already being treated] should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

If children are old enough to understand the risks and benefits of the study, they should also be informed and should also provide their written consent.

11.3 INDEPENDENT ETHICS COMMITTEES/INSTITUTIONAL REVIEW BOARD

Independent Ethics Committees [non-US]: This protocol and any accompanying material provided to the subject [such as subject information sheets or descriptions of the study used to obtain informed consent] as well as any advertising or compensation given to the patient, will be submitted by the investigator to an Independent Ethics Committee. Approval from the committee must be obtained before starting the study, and should be documented in a letter to the investigator specifying the date on which the committee met and granted the approval.

Any modifications made to the protocol after receipt of the Independent Ethics Committee approval must also be submitted by the investigator to the Committee in accordance with local procedures and regulatory requirements.

When no local review board exists, the investigator is expected to submit the protocol to a regional committee. If no regional committee exists, Roche will assist the investigator in submitting the protocol to the European Ethics Review Committee.

Institutional Review Board [US]: It is the understanding of the sponsor that this protocol [and any modifications] as well as appropriate consent procedures, will be reviewed and approved by an Institutional Review Board. This board must operate in accordance with the current Federal Regulations. A letter or certificate of approval will be sent by the investigator to the Sponsor or designee prior to initiation of the study, and also whenever subsequent modifications to the protocol are made.

12. CONDITIONS FOR MODIFYING THE PROTOCOL

Protocol modifications to ongoing studies must be made only after consultation between an appropriate representative of the sponsor and the investigator [investigator representative[s] in the case of a multicenter trial]. Protocol modifications must be prepared by a representative of the sponsor and initially reviewed and approved by the Clinical Science Leader/Clinical Pharmacologist and Biostatistician.

All protocol modifications must be submitted to the appropriate Independent Ethics Committee or Institutional Review Board for information and approval in accordance with local requirements, and to Regulatory Agencies if required. Approval must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial subjects, or when the change[s] involves only logistical or administrative aspects of the trial [e.g. change in monitor[s], change of telephone number[s].

13. CONDITIONS FOR TERMINATING THE STUDY

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange the procedures on an individual study

basis after review and consultation. In terminating the study, Roche and the investigator will assure that adequate consideration is given to the protection of the patient's interests.

14. STUDY DOCUMENTATION, CRFS AND RECORD KEEPING

14.1 INVESTIGATOR'S FILES / RETENTION OF DOCUMENTS

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two different separate categories [1] Investigator's Study File, and [2] subject clinical source documents.

The Investigator's Study File will contain the protocol/amendments, Case Report and Query Forms, Independent Ethics Committee/Institutional Review Board and governmental approval with correspondence, sample informed consent, drug records, staff curriculum vitae and authorization forms and other appropriate documents/correspondence etc.

Subject clinical source documents [usually defined by the project in advance to record key efficacy/safety parameters independent of the CRFs] would include patient hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, EEG, X-ray, pathology and special assessment reports, signed informed consent forms, consultant letters, and subject screening and enrollment logs. The Investigator must keep these two categories of documents on file for at least 15 years after completion or discontinuation of the study. After that period of time the documents may be destroyed, subject to local regulations.

Should the Investigator wish to assign the study records to another party or move them to another location, Roche must be notified in advance.

If the Investigator cannot guarantee this archiving requirement at the investigational site for any or all of the documents, special arrangements must be made between the Investigator and Roche to store these in a sealed container[s] outside of the site so that they can be returned sealed to the Investigator in case of a regulatory audit. Where source documents are required for the continued care of the patient, appropriate copies should be made for storing outside of the site.

14.2 SOURCE DOCUMENTS AND BACKGROUND DATA

The investigator shall supply the sponsor or designee on request with any required background data from the study documentation or clinic records. This is particularly important when CRFs are illegible or when errors in data transcription are suspected. In case of special problems and/or governmental queries or requests for audit inspections, it is also necessary to have access to the complete study records, provided that patient confidentiality is protected.

14.3 AUDITS AND INSPECTIONS

The investigator should understand that source documents for this trial should be made available to appropriately qualified personnel from the Roche Pharma Development Quality Assurance Unit or its designees, or to health authority inspectors after appropriate notification. The verification of the CRF data must be by direct inspection of source documents.

14.4 CASE REPORT FORMS

For each patient enrolled, a CRF must be completed and signed by the principal investigator or authorized delegate from the study staff. This also applies to records for those patients who fail to complete the study [even during a pre-randomization screening period if a CRF was initiated]. If a patient withdraws from the study, the reason must be noted on the CRF. If a patient is withdrawn from the study because of a treatment-limiting AE, thorough efforts should be made to clearly document the outcome.

All forms should be typed or filled out using indelible ink, and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or his/her authorized delegate. The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor or designee in the CRFs and in all required reports.

15. MONITORING THE STUDY

It is understood that the responsible Roche monitor [or designee] will contact and visit the investigator regularly and will be allowed, on request, to inspect the various records of the trial [CRFs and other pertinent data] provided that patient confidentiality is maintained in accord with local requirements.

It will be the monitor's responsibility to inspect the CRFs at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitor should have access to laboratory test reports and other patient records needed to verify the entries on the CRF. The investigator [or his/her deputy] agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

16. CONFIDENTIALITY OF TRIAL DOCUMENTS AND SUBJECT RECORDS

The investigator must assure that subjects' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents submitted to the sponsor or designee, subjects should not be identified by their names, but by an identification code. The investigator should keep a subject enrollment log showing codes, names and addresses. The investigator should maintain documents not for submission to Roche, e.g., subjects' written consent forms, in strict confidence.

17. PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to Roche prior to submission. This allows the sponsor or designee to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the investigator.

In accord with standard editorial and ethical practice, Roche will generally support publication of multicenter trials only in their entirety and not as individual center data. In this case, a coordinating investigator will be designated by mutual agreement.

Any formal publication of the study in which input of Roche personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate Roche personnel. Authorship will be determined by mutual agreement.

Appendix 1 Calculation of Creatinine Clearance and Grading of Chronic Extensive GVHD

Calculation of Creatinine Clearance

Conversion Factor for Serum Creatinine:
Conventional Units (mg/dL) = SI Units (μmol/L) ÷ 88.4
Age = number of years (12 years 11 months = 12 years)

Estimated Creatinine Clearance according to Cockcroft-Gault [13]

(for patients ≥ 18 years):

- **Males**

Creatinine Clearance (mL/min) = **[(140 − age) × Body Weight (kg)] ÷ [72 × Serum Creatinine (mg/dL)]**

- **Females**

Creatinine Clearance = above equation × 0.85

Estimated Creatinine Clearance according to Schwartz equation [14]
(for patients < 18 years):

Creatinine Clearance (mL/min/1.73 M^2) = $k \times$ Height (cms) ÷ **Serum Creatinine** (mg/dL)

Value for k:

k	Age (years)
0.45	< 2
0.55	≥ 2 to < 13
0.7	≥ 13 to < 18 (males)
0.55	**≥ 13 to < 18 (females)**

Appendix 1 Calculation of Creatinine Clearance and Grading of Chronic Extensive GVHD (cont.)

Grading of Chronic GVHD [15]

Type of Disease	Extent of Disease
Limited	Localized skin involvement, liver dysfunction or both
Extensive	Generalized skin involvement
	Localized skin involvement or liver dysfunction plus any one of the following: 1. Chronic aggressive hepatitis, bridging necrosis or cirrhosis 2. Eye involvement (Schirmer's test, $<$5 mm) 3. Involvement of mucosalivary glands 4. Mucosal involvement (on lip biopsy) 5. Involvement of other target organs

Appendix 2 Adult Patient Diary Data and Symptom Record

The purpose of the electronic diary is for all adults (13 years and older) to record any symptoms of influenza-like illness for the duration of the study. Temperature and date and time of drug administration will also be recorded on the electronic patient diary.

Scoring of Symptoms.

Please answer All of the questions yourself by checking one box for each row.

The information you provide is very important and will remain strictly confidential.

	absent	mild	moderate	severe
	0	1	2	3
1. Nasal Congestion	☐	☐	☐	☐
2. Sore Throat	☐	☐	☐	☐
3. Cough	☐	☐	☐	☐
4. Aches and Pains	☐	☐	☐	☐
5. Fatigue(Tiredness)	☐	☐	☐	☐
6. Headache	☐	☐	☐	☐
7. Chills/sweats (feverish)	☐	☐	☐	☐

Appendix 3 Diary Data for Children

Temperature and date and time of drug administration will also be recorded on the electronic patient diary.

Date of Assessment ☐☐☐☐☐☐ (dd mm yy) Time of Assessment ☐☐☐☐ (h min)

Temperature: ☐☐☐.☐ °C/F

Symptoms of influenza-like illness

Please mark one box only per question

Item	No Problem 0	Minor Problem 1	Moderate Problem 2	Major Problem 3	Don't Know or not Applicable
1. Poor appetite	☐	☐	☐	☐	☐
2. Not sleeping well	☐	☐	☐	☐	☐
3. Irritable, cranky, fussy	☐	☐	☐	☐	☐
4. Feels unwell	☐	☐	☐	☐	☐
5. Low energy, tired	☐	☐	☐	☐	☐
6. Not playing well	☐	☐	☐	☐	☐
7. Crying more than usual	☐	☐	☐	☐	☐
8. Needing extra care	☐	☐	☐	☐	☐
9. Clinginess	☐	☐	☐	☐	☐
10. Headache	☐	☐	☐	☐	☐
11. Sore throat	☐	☐	☐	☐	☐
12. Muscle aches or pains	☐	☐	☐	☐	☐
13. Fever	☐	☐	☐	☐	☐
14. Cough	☐	☐	☐	☐	☐
15. Nasal congestion, runny nose	☐	☐	☐	☐	☐
16. Vomiting	☐	☐	☐	☐	☐
17. Not interested in what's going on	☐	☐	☐	☐	☐
18. Unable to get to get out of bed	☐	☐	☐	☐	☐

This form was filled out by:

1. ☐ Parent
2. ☐ Other relative
3. ☐ Nanny
4. ☐ Subject
5. ☐ Other

*specify*_____

Appendix 4 Adverse Event [AEs] Categories for Determining Relationship to Test Drug

PROBABLE [must have first three]

This category applies to those AEs which are considered, with a high degree of certainty, to be related to the test drug. An AE may be considered probable, if:

3. It follows a reasonable temporal sequence from administration of the drug.
4. It cannot be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
5. It disappears or decreases on cessation or reduction in dose. [There are important exceptions when an AE does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists; e.g., [1] bone marrow depression, [2] tardive dyskinesias].
6. It follows a known pattern of response to the suspected drug.
7. It reappears upon rechallenge.

POSSIBLE [must have first two]

1. This category applies to those AEs in which the connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possible if, or when:
2. It follows a reasonable temporal sequence from administration of the drug.
3. It may have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
4. It follows a known pattern of response to the suspected drug.

REMOTE [must have first two]

1. In general, this category is applicable to an AE which meets the following criteria:
2. It does <u>not</u> follow a reasonable temporal sequence from administration of the drug.
3. It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
4. It does not follow a known pattern of response to the suspected drug.
5. It does not reappear or worsen when the drug is readministered.

UNRELATED

This category is applicable to those AEs which are judged to be clearly and incontrovertibly due only to extraneous causes [disease, environment, etc.] and do not meet the criteria for drug relationship listed under remote, possible, or probable.

Appendix 4 Adverse Event [AEs] Categories for Determining Relationship to Test Drug (cont.)

	Probable	Possible	Remote	Unrelated
Clearly due to extraneous causes	–	–	–	+
Reasonable temporal association with drug administration	+	+	–	–
May be produced by subject clinical state, etc.	–	+	+	+
Known response pattern to suspected drug	+	+	–	–
Disappears or decreases on cessation or reduction in dose	+	–	–	–
Reappears on rechallenge	+	–	–	–

Appendix 5 ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that at any dose fulfills at least one of the following criteria:

- is fatal; [results in death] [NOTE: death is an outcome, not an event].
- is Life-Threatening [NOTE: the term "Life-Threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe].
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above.

Medical and scientific judgment should be exercised in deciding whether expedited reporting to the sponsor or designee is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definitions above. These situations should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

An unexpected AE is one, the nature or severity of which is not consistent with the applicable product information.

Causality is initially assessed by the investigator. For Serious Adverse Events, possible causes of the event is indicated by selecting one or more options. (Check all that apply)

- Pre-existing/Underlying disease - specify
- Study treatment – specify the drug(s) related to the event
- Other treatment (concomitant or previous) – specify
- Protocol-related procedure
- Other (e.g. accident, new or intercurrent illness) - specify

The term severe is a measure of intensity, thus a severe AE is not necessarily serious. For example, nausea of several hours' duration may be rated as severe, but may not be clinically serious.

Appendix 5 ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 (cont.)

A serious adverse event occurring during the study or which comes to the attention of the investigator within 15 days after stopping the treatment or during the protocol-defined follow-up period, if this is longer, whether considered treatment-related or not, must be reported. In addition, a serious adverse event that occurs after this time, if considered related to test "drug", should be reported.

Such preliminary reports will be followed by detailed descriptions later which will include copies of hospital case reports, autopsy reports and other documents when requested and applicable.

For serious adverse events, the following must be assessed and recorded on the AEs page of the CRF: intensity, relationship to test substance, action taken, and outcome to date.

The investigator must notify the Ethics Review Committee/Institutional Review Board of a serious adverse event in writing as soon as is practical and in accordance with international and local laws and regulations.

ROCHE LOCAL COUNTRY CONTACT for SAEs: Local Monitor

ROCHE HEADQUARTERS CONTACT for SAEs: Clinical Operations/Clinical Science

Within the US, weekends, holidays and after 5:00 pm, call [redacted] and ask for the physician on call. Outside the US, call the local emergency contact number provided by the Monitor.

Appendix 6 Primary Immunodeficiency Conditions

Category	Conditions
Severe Combined Immunodeficiency (SCID)	Adenosine deaminse (ADA) deficiency
	Artemis deficiency (SCIDA)
	CD45 deficiency
	Cernunnos deficiency
	DNA ligase IV (LIG4) deficiency
	Interleukin receptor γ chain deficiency (X-linked SCID)
	Janus-associated kinase 3 (JAK3) deficiency
	Recombinase activating gene (RAG 1 / 2) deficiency
	Reticular dysgenesis
	TAP- 1 or TAP- 2 deficiency (MHC class I deficiency)
Primary T cell Deficiency	CD8 deficiency
	diGeorge syndrome
	Interleukin 7 receptor α (IL7RA) deficiency
	MHC class II deficiency
	LCK deficiency
	Orai 1 deficiency
	Nude syndrome (wing helix nude deficiency)
	Purine nucleotide phosphorylase (PNP) deficiency
	T cell receptor deficiency (CD 3γ, δ, ε, and ζ deficiencies)
	Zap 70 tyrosine kinase deficiency
Predominantly Antibody Deficiency	X-Linked CD40 ligand deficiency
	X- Linked IKK-γ (NEMO) deficiency
	CD40 deficiency
Other Well-Defined immunodeficiency Syndromes	Interferon γ receptor deficiency
	X-Linked lymphoproliferative syndrome

Adapted from Table 310-2: p2056 reference 16.

Appendix 7 Hematologic Malignancies and their Effect on the Immune System

Malignancy	Effect on immunity
ALL, lymphomas	suppression of hematopoiesis, neutropenia, lymphocyte dysfunction
CLL, small lymphocytic lymphoma	hypogammaglobulinemia, increased susceptibility to infections, autoimmune anemia or thrombocytopenia
Hairy cell leukemia, myelodysplastic syndromes	pancytopenia
peripheral T cell and NK neoplasms	lymphocyte dysfunction, increase in immature cells
Hodgkin's disease	suppression of cell-mediated immunity
AML	neutropenia, anemia, thrombocytopenia, predisposed to infections, lymphocyte dysfunction
CML	anemia, granulocyte dysfunction in some patients in early phase and in most patients in blast phase

Adapted from reference 16.

Appendix 8 Immunosuppressive Medications

Class	Category	Drugs
Corticosteroids	-	Glucocorticoids (oral, sc, im, iv)
Cytotoxic agents[16]	Alkylating agents	cyclophosphamide, busulfan, mechlorethamine, chlorambucil, melphalan, carmustine, lomustine, ifosfamide, procarbazine, dacarbazine, temozolomide, cisplatin, carboplatin, oxaliplatin
	Anti-metabolites	methotrexate, 6-mercaptopurine, azathioprine
	Anti-tumor antibiotics	bleomycin, actinomycin D, mitomycin C, etoposide, topotecan, irinotecan, doxorubicin, daunorubicin, idarubicin, epirubicin, mitoxantrone
	Anti-mitotic agents	vincristine, vinblastine, vinorelbine, paclitaxel, docetaxel, estramustine phosphate, NAB-paclitaxel
	Molecularly targeted agents	imatinib, tretinoin, bexarotene, denileukin, diftitox, gefitinib, erlotinib, dasatinib, sorafenib, sunitinib
Calcineurin inhibitors	-	cyclosporine, tacrolimus
mTOR inhibitors (proliferation-signal inhibitors)	-	sirolimus, everolimus
Immunosuppressive antibodies	-	anti lymphocyte and antithymocyte globulins (ALG and ATG)
Monoclonal antibodies [38, 39]	Inhibitors of pro inflammatory cytokines	Adalimumab
		Infliximab
		Cetrolizumab
		Etarnecept
		Basiliximab
		Daclizumab
	Adhesion cell modulators	Natalizumab
	T-cell inhibitors	Abatacept
		Alefacept
		Muromonab
	B-cell inhibitors	Rituximab
		^{90}Y-Ibritumomab
		^{131}I-Tositumomab
	Anti-CD33	Gemtuzumab
	Anti- CD52	Alemtuzumab
Others	-	mycophenolate mofetil, thalidomide

Compiled from Table 81-2: p521-24 reference 16 and references 38, 39.

PROTOCOL

TITLE:	**A DOUBLE BLIND, RANDOMIZED, STRATIFIED, MULTI-CENTER TRIAL EVALUATING CONVENTIONAL AND DOUBLE DOSE OSELTAMIVIR IN THE TREATMENT OF IMMUNOCOMPROMISED PATIENTS WITH INFLUENZA**
PROTOCOL NUMBER:	NV20234
VERSION NUMBER:	E
EUDRACT NUMBER:	2006-002468-24
IND NUMBER:	53,093
TEST PRODUCT:	Oseltamivir (Tamiflu® RO 64-0796)
MEDICAL MONITOR:	[redacted], M.D.
SPONSOR:	F. Hoffmann-La Roche Ltd
DATE FINAL:	Version A: 14 June 2007
DATES AMENDED:	Version B: 31 July 2008 Version C: 28 March 2011 Version D: 28 September 2012 Version E: see electronic stamp below

PROTOCOL AMENDMENT APPROVAL

Approver's Name	Title	Date and Time (UTC)
[redacted]	Clinical Science Leader	30-Oct-2013 16:46:29

PROTOCOL AMENDMENT, VERSION E
RATIONALE

Protocol NV20234 has been amended to provide clarity and additional guidance on the inclusion criteria regarding CD4 counts for patients with HIV and on the discontinuation of patients with a creatinine clearance (CrCl) value of <60 mL/min.

Additional changes to the protocol are as follows:

- The statistical methods section was updated to include an overview of planned pharmacokinetic analysis
- CD4 $+$T$-$cell percentage values that will be used for patients ≤ 5 years with HIV were added
- The exclusion criterion 'have evidence of a serious secondary respiratory or disseminated infection that may confound or overlay the diagnosis and/or symptomatology of influenza' was removed. This was removed to prevent ambiguity around which patients should not be enrolled into this clinical protocol, since patients on antibiotics or with other secondary infections are not allowed to be enrolled. Removal of this criterion is not expected to impact the benefit-risk profile of NV20234 as high-risk patients suspected of having influenza would receive oseltamivir as part of their clinical care (as per WHO guidance) and these data are available through various publications.
- Reference to patient electronic diary cards and to all uses of paper diaries in emergency situations were removed
- The total PK blood volume for pediatric patients was corrected from 3.6 mL to 2.4 mL
- Appendices 2 and 3 have been updated to include an example of the current patient diary text
- Updated emergency medical call-in information in Appendix 5
- Added Child-Pugh Classification

Additional minor changes have been made to improve clarity and consistency. Substantive new information appears in italics. This amendment represents cumulative changes to the original protocol.

PROTOCOL AMENDMENT, VERSION E
SUMMARY OF CHANGES

GLOBAL CHANGES

Throughout the protocol high dose was changed to double dose to more accurately reflect the dose amount. Pharmacokinetic assessment reference to oseltamivir was removed as only oseltamivir carboxylate is evaluated in plasma samples. The term medication was changed to drug to better identify which medication and all references to electronic diary were changed to patient diary. References were added as appropriate from newly added text.

PROTOCOL SYNOPSIS

The protocol synopsis has been updated to reflect the changes to the protocol, where applicable.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The list of abbreviations and definitions of terms has been updated to reflect the changes to the protocol, where applicable.

SECTION 1.1.1: Influenza in the Immunodeficient Population

Secondary immunodeficiency due to disease

HIV-infected individuals with a CD4+ T-cell count of $<200/mm^3$ ~~µL~~ (AIDS defining) are highly susceptible to opportunistic disease [16]. However, CD4 counts $<500/mm^3$ ~~µL~~ are considered abnormal in HIV-infected individuals, and therefore these individuals are also more susceptible to infection. *CD4+T cell counts are higher in infants and young children than in adults and decline over the first few years of life [40, 41]. because of this age-dependent variation in absolute CD4+ T cell count, calculation of CD4 percentage is used as a measure for young children because it has shown less variability. HIV-infected children (12 months to 5 years old) with <15% CD4+ T cells are classed as severely immunosuppressed and highly susceptible to opportunistic disease, although antiretroviral therapy is recommended in children with <25% CD4+ T cells as they are still classified as immunosuppressed [42].* Studies in patients with HIV/AIDS have shown an increased risk for heart and lung-related hospitalizations during the influenza season compared with other times of the year, prolonged duration of influenza symptoms, increased risk for influenza-related complications and a higher risk of influenza-related death [17].

SECTION 2.2: SECONDARY OBJECTIVES

To evaluate the effects of conventional and ~~high~~ *double* dose oseltamivir in immunocompromised patients on:

- *The population pharmacokinetics of* ~~oseltamivir and~~ *oseltamivir carboxylate (e.g., clearance, volume of distribution) in immunocompromised patients with confirmed influenza infection, through the application of established population pharmacokinetic (PK) models to the sparse plasma concentration data generated*

- *The virologic course of influenza (proportion shedding and viral loads at different time points)*
- ~~The virologic course of influenza (proportion shedding and viral loads at different time points).~~
- ~~To estimate the population pharmacokinetics of oseltamivir and oseltamivir carboxylate (e.g., clearance, volume of dstribution) in immunocompromised patients with confirmed influenza infection, through the application of established population pharmacokinetic (PK) models to the sparse plasma concentration data generated.~~
- *To explore the relationship of metrics of exposure (e.g. AUC, C_{min}) to relevant pharmacodynamic (PD) endpoints*

SECTION 3.1.1.1: Choice of Treatment Arms

The currently approved dose of oseltamivir for the treatment indication is the conventional dose with a duration of 5 days. In this study a conventional and ~~high~~ *double* dose ~~(twice the conventional dose)~~ are being evaluated. The pivotal registration trials for oseltamivir in healthy adults with influenza had three treatment arms, two active (75 mg and 150 mg) and one placebo. Both treatment arms were found to be significantly better than placebo. No statistical comparisons were made between the two active treatment arms. Evaluation of the results in the two active treatment arms did not reveal any clinically meaningful difference. However, the proportion of patients shedding virus on Day 4 (3 days after the start of treatment), suggested a possible dose-response relationship (36%, 28% and 23% in the placebo, 75 mg and 150 mg dose arms respectively). Because defective immune-mediated virus clearance in immunosuppressed patients treated with antivirals can result in a higher incidence of selection of drug-resistant viruses, it was decided to use both the conventional and ~~high~~ *double* dose arm for this study.

SECTION 3.1.1.2: Inclusion of Patients Symptomatic up to 96 Hours

In immunocompromised patients, time from onset of symptoms to seeking medical attention (presentation at the clinic) of $>$48 hours has been shown in several case reports [33, 27, 28,29] including two instances of nosocomial outbreaks in children [25,20]. This notwithstanding, oseltamivir has been shown to *offer benefit*~~be effective~~ in immunocompromised populations that included patients treated with oseltamivir beyond 48 hours of presentation; patients with lung transplant (median time to presentation 3 days) [27], bone marrow transplant (treatment started more than 48 hours after onset of symptoms in all 3 patients) [25], organ transplant (62 of 221 patients started treatment after 96 hours) [26] and children with ~~ALL~~*acute lymphocytic leukemia* (one child presented after 3 days and two children presented after 5 days of symptoms) [21].

SECTION 3.1.1.4: Interpretation of Study Results

Safety and tolerability and the development of resistance are the primary objectives of this study. These will be characterized descriptively. For the secondary objective of efficacy, the subset of the population enrolled in the first 48 hours will be compared with

placebo patients from pivotal registration trials (where patients were enrolled in the first 2 days of illness). Sections 8.2.5, 8.2.6, and 8.2.7 provide details on the statistical considerations ~~and their rationale associated with these comparisons~~.

SECTION 3.1.2: Rationale for Dose Selection and Adjustment

Dose Selection

Thus, the rationale for the *double* ~~higher~~ dose arm in this study is to explore the possibility of improved efficacy and decreased emergence of resistance without an undue increase in risk. *For patients with a creatinine clearance level of 45 mL/min or above, oseltamivir carboxylate exposures for both the 75-mg and 150-mg doses are not expected to exceed exposures previously tested. Patients with a creatinine clearance as low as 45 ml/min, who receive the 150-mg BID dose, will have exposures of oseltamivir carboxylate that are within (albeit in the upper end) the established safety margin. This corresponds to an AUC produced by the 450 mg BID dose in adults with normal renal function (AUC $_{0\text{-}12\ hrs}$ of approximately 15,000 ng •hr/mL).*

Dose Adjustments

As this is a double blind ~~In this~~ study, patients whose *creatinine clearance* (CrCl) decreases to *<* ~~60mL~~*45mL*/min *(adults) using the Cockcroft-Gault method or* <*45mL/min*/1.73m^2 *(<18 years old) using the Schwartz equation* will be discontinued from *study* treatment. *A lower limit of 45 mL/min for creatinine clearance will be used to allow for patients with a mild to moderate renal impairment.*

SECTION 3.1.3: End of Study

The study comprises 10 days of treatment with follow-up visits approximately 5 and 30 days later as shown in the schedule of assessments. *Study medication will be administered twice daily over 10 days for a total of 20 doses.* A rapid diagnostic test for influenza will be performed at the end of the 10 days of treatment. Patients still having influenza based on the rapid diagnostic test will be treated per the local standard of care by the principal investigator, *and any medication provided during the follow-up period should be captured in the CRF*.

SECTION 4.2: INCLUSION CRITERIA

- Immunocompromised patient defined as one who meets any of the following:
    - Primary immunodeficiency at risk for viral infections (representative examples in Appendix 6) OR
    - Secondary immunodeficiency
        - SOT with ongoing immunosuppression OR
        - Allogenic HSCT with ongoing immunosuppression OR

- HIV with *a most recent* CD4 count $<$ 500/mm^3 *(or <25% in children ≤ 5 years old) within the last 6 months* and, in the investigator's opinion, considered immunocompromised OR

- In patients with history or clinical presentation at randomization suggestive of renal failure; a CrCl $>$ 60 mL/min *(>18 years old) or* >*60mL/min/*1.73m^2*(<18 years old) within the last 3 months*

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SECTION 4.3: EXCLUSION CRITERIA

- ~~Have evidence of a serious secondary respiratory or disseminated infection that may confound or overlay the diagnosis and/or symptomatology of influenza.~~
- Participat~~ed~~*ion* in a clinical trial or expanded access trial with an investigational drug suspected/demonstrated to impact pathways important for the metabolism and excretion of oseltamivir in the 4 weeks prior to randomization or concomitantly with this study

SECTION 4.4: CONCOMITANT MEDICATION AND TREATMENT

Antiviral treatments with activity against influenza (e.g. amantadine, rimantadine, zanamivir, ribavirin, laninamivir, peramivir, and additional oseltamivir above that specified for this study) are not allowed during the 10-day treatment phase of the study. Concomitant use of an investigational drug during the study is also excluded~~.~~ *if suspected/demonstrated to impact pathways important for the metabolism and excretion of oseltamivir.*

SECTION 4.5: CRITERIA FOR PREMATURE WITHDRAWAL

The investigator must discontinue treatment if the creatinine clearance is $<$ ~~60~~ *45* mL/min in adults *using the Cockcroft-Gault method* or $<$ ~~60~~ *45* mL/min/1.73 m^2 *using the Schwartz equation* in children. *A lower limit of 45 mL/min for creatinine clearance will be used to allow for patients with a mild to moderate renal impairment.*

SECTION 5.1: SCREENING EXAMINATION AND ELIGIBILITY SCREENING FORM

Patients will be assessed for inclusion and exclusion criteria and those who fulfill all the inclusion and none of the exclusion criteria will be randomized into the study. *Influenza Rapid Diagnostic test, PCR, or viral culture for the purposes of inclusion criteria fulfillment will be done by the site or the local laboratory. Historical CrCl values within 3 months and CD4+ T-cell counts (where applicable) within 6 months before randomization are acceptable for inclusion into the study.*

SECTION 5.3: CLINICAL ASSESSMENTS AND PROCEDURES

Study Day 1

The baseline and study Day 1 assessment may be performed at the same visit. *Laboratory assessments (safety labs and nasal and throat swabs for virology) should be performed after randomization but prior to the patient receiving first dose of study drug.*

Study ~~medication, electronic~~ *drug, patient* diaries, and thermometers will be dispensed. Patients or guardians/parents will be instructed on how to complete ~~electronic symptom~~ *the patient* diaries (Appendix 2 andAppendix 3), temperature recording, and treatment

End of Treatment Day 11

The end of treatment visit for all patients is on Day 11 (irrespective of whether they took one or two doses on Day 1). *Study medication will be administered twice daily over 10 days for a total of 20 doses.* Patients who discontinue study ~~medication~~*drug* prematurely will have all Day 11 assessments completed at the time of discontinuation or the following day.

After all Day 11 study assessments are completed, a rapid diagnostic test will be performed at this visit. If the rapid diagnostic test is positive, the patient may be treated per standard of care at the discretion of the investigator. *If treatment is provided, this should be captured in the CRF.*

Study Days 12 – 40

Study Day 15 is an important visit as it is the first assessment of viral culture after cessation of treatment. *It is therefore important that this visit is completed.*

SECTION 5.3.1: ~~Efficacy Assessments~~

~~The primary efficacy end point in this study is the time to resolution of all influenza symptoms as recorded in the patient diary.~~

~~The clinical efficacy parameters in this study are:~~

1. ~~Symptoms of influenza-like illness. These are captured in the *patient* diary for both adults and children (Appendix 2) (Appendix 3). Influenza symptoms are graded on a nominal scale from zero (absent/no problem) to 3 (severe or major problem). A score of zero is considered asymptomatic. The primary efficacy end point, time to alleviation of all symptoms is comprised of all the symptoms captured in the *patient* diary.~~
2. ~~Temperature. This is captured in the *patient* diary. Temperature is used for assessment of the primary and several secondary efficacy end points.~~

SECTION 5.3.1: Safety

Safety is one of the primary end-points of this study. Safety parameters ~~in this study~~ include adverse events, vital signs, and clinical laboratory evaluations.

Nasal and throat swab samples will be assessed from each study visit for the presence of oseltamivir-resistant mutations.

SECTION 5.3.2: Efficacy Assessments

The clinical efficacy parameters in this study are:

1. *Symptoms of influenza-like illness. These are captured in the patient diary for both adults and children (Appendix 2and Appendix 3). Influenza symptoms are graded on a nominal scale from zero (absent/no problem) to 3 (severe or major problem). A score of zero is considered asymptomatic. The efficacy end point, time to alleviation of all symptoms is comprised of all the symptoms captured in the patient diary.*
2. *Temperature. This is captured in the patient diary. Temperature is used for assessment of several efficacy endpoints.*

SECTION 5.4: LABORATORY ASSESSMENTS

The laboratory assessments include those for ~~efficacy and~~ safety, *resistance, and efficacy.*

Safety

The safety laboratory assessments in this study, including the assessment of serum chemistry and hematology, will be carried out at a central laboratory. Serum chemistry assessments comprise AST, ALT, total bilirubin, urea and creatinine. Hematology assessments include CBC and differential count. The total volume of blood loss for laboratory assessments will be approximately 20 mL for the entire duration of the study.

Protection of patient confidentiality (Section16) will extend to any data generated from the assay of these samples. Biological samples collected from all patients may be infectious and will be classified as "diagnostic specimens" for dispatch purposes.

When clinically indicated, the investigator may draw blood for serum creatinine to be assessed at the local laboratory during the study to calculate creatinine clearance.

Efficacy

The efficacy laboratory assessments are used for laboratory confirmation of influenza. These are:

Nasal and throat swabs. Two nasal and one throat swab will be collected as described in the Schedule of Assessments. All swabs are sent to a central laboratory for RT-PCR and viral cultures. Influenza virus shedding will be assessed. ~~Baseline samples will be assessed for the presence of oseltamivir resistance mutations.~~

~~During the course of treatment, neither the investigator nor the patient will know which participants have ongoing positive viral cultures for influenza.~~ At the end of treatment (~~day~~*Day* 11), a rapid diagnostic test is permitted for the diagnosis of ongoing influenza.

~~Safety~~

~~The safety laboratory assessments in this study, including the assessment of serum chemistry and hematology will be done at the central laboratory. Serum chemistry assessments comprise AST, ALT, total bilirubin, urea and creatinine. Hematology assessments include CBC and differential count. The total volume of blood loss for laboratory assessments will be approximately 20 mL for the entire duration of the study.~~

~~Protection of patient confidentiality (Section16) will extend to any data generated from the assaying of these samples. Biological samples taken from all patients may be infectious and will be classified as "diagnostic specimens" for dispatch purposes.~~

~~The Principal Investigator may draw blood for serum creatinine to be assessed at the local laboratory when clinically indicated during the study to calculate creatinine clearance.~~

SECTION 5.5.1: Pharmacokinetic Assessments

Plasma PK samples for assessment of ~~oseltamivir and~~ oseltamivir carboxylate will be collected at the following timepoints on Day 6, or any day after the 11th dose, using the following sampling approach:

- Within 30 minutes prior to the dose administration (e.g., 8:30 a.m~~.~~). *dose 09:00 am)*

For adults and adolescents, approximately 2 mL of blood will be collected at each timepoint; therefore, the total volume blood loss for PK assessments will be approximately 8 mL. For pediatric patients, not less than approximately 0.6 mL of blood will be collected at each timepoint; therefore, the total volume blood loss for PK assessments will be approximately ~~3.6~~*2.4* mL

SECTION 5.5.2: Pharmacodynamic Assessments

Nasal and throat swabs will be collected from individuals on the days specified in the schedule of assessments. Specimens will be analyzed at a central laboratory. *As immunocompromised patients are expected to shed virus for a longer duration, viral samples will be collected at all visits.* The proportion of patients with viral shedding at each visit will be summarized

SECTION 6.2: PREPARATION AND ADMINISTRATION OF STUDY DRUG

1. Oseltamivir capsules containing 75 mg of active drug and packaging material consisting of pregelatinized starch, povidone, talc and sodium stearyl fumarate. All participants 13 years and older will receive this dosage form. *Oseltamivir capsules should be stored at*~~t~~*between 2-25ºC.*

2. A pediatric suspension containing 12 mg oseltamivir per mL of reconstituted solution and the following excipients: sorbitol, titanium dioxide, sodium benzoate, xanthan gum, monosodium citrate, saccharin sodium and Permaseal11900-31 Tutti Frutti (flavor). All participants 12 years and under will receive this dosage form. *Oseltamivir dry powder for* oral *suspension [pediatric syrup] should be stored below 30°C [86°F]*). Once reconstituted, *the suspension should not be used for longer*

than 10 days if stored ~~under~~*at* room temperature conditions (below 25°C~~) or for 17 days if~~*). If stored under* ~~refrigeration~~*refrigerated conditions at 2–8 °C (36–46 °F) the suspension should not be used for longer than* 17 days.

SECTION 6.3: BLINDING AND UNBLINDING

~~Emergency codes should not be broken except in the case of emergency situations.~~
Any request from the investigator for information about the treatment administered to study patients for another purpose must be discussed with Roche/designee.

SECTION 7.2.1: Reporting of Serious Adverse Events [immediately reportable]

The definition and reporting requirements of ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 will be adhered to (Appendix 5). *The serious adverse events should be reported in the most up -to-date version of the SAE form.*

SECTION 7.2.2: Pregnancy

A female patient must be instructed to stop taking the test "drug" and immediately inform the investigator if she becomes pregnant during the study. The investigator should report all pregnancies immediately (i.e., no more than 24 hours after the investigator becomes aware of the pregnancy) to the Sponsor or designee*, using the most up -to-date version of the pregnancy form.*

SECTION 8: STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

For continuous endpoints summary statistics including mean, standard deviation, median, minimum and maximum will be derived for each treatment group. For categorical data the number and percentage of events and/or patients for each treatment group will be presented.

SECTION 8.1.1: Primary Endpoints

The primary endpoints for safety will be *descriptive* assessments of:

- Adverse events
- Physical exam*ination*~~s~~,
- Vital signs ~~and~~
- Clinical laboratory evaluations
- *Tissue rejection and/or graft versus host disease in transplant patients*
-

SECTION 8.1.2: Secondary Endpoints

The following endpoints will be analyzed descriptively at relevant timepoints ~~The following are secondary endpoints which measure efficacy, the primary of which is time to alleviation of all clinical symptoms. With the exception of this variable, viral load and time to resolution of fever, they are defined as either the occurrence or non-occurrence of the specified events or the presence or absence of the specified symptoms.~~

~~The time (hours) to alleviation of all clinical influenza symptoms (recorded in the patient diary)~~

- Shedding virus by culture *and RT-PCR* ~~at day~~Day 1, 2, 6, 8, 11, 15 and 40
- ~~Shedding virus by RT-PCR at day~~Day 1, 2, 6, 8, 11, 15, and 40
- Viral load by culture ($log_{10}TCID_{50}$/mL) *and quantitative RT-PCR*
- *Individual symptom scores* ~~at Day 1, 2, 6, 8, 11, 15, and 40~~
- ~~Viral load by quantitative RT-PCR at day~~Day 1, 2, 6, 8, 11, 15 and 40
- The time (hours) from first dose of study ~~medication~~*drug* until resolution of fever
- ~~Fever~~
- ~~Cough~~
- ~~Coryza~~

SECTION 8.1.3: ~~Safety~~

~~Safety of the treatment will be evaluated by AEs, physical exams, clinical laboratory tests, and vital signs.~~

~~In addition to routine safety assessments, the proportion of subjects experiencing a rejection and/or graft versus host disease will be summarized by treatment group.~~

SECTION 8.2.1.1: Time to Event Variables

~~For the purpose of comparing treatment groups, it will be assumed that their respective distributions for the primary endpoint differ only by a shift in location.~~

SECTION 8.2.3: Hypothesis Testing

Formal hypothesis testing will be not performed, *any comparisons between groups* ~~instead inferences~~ will be based on comparison of confidence intervals.

SECTION 8.2.4.4: Subpopulations

~~In order to evaluate the potential for relapse, the following two subpopulations of the ITTI population will be evaluated:~~

- ~~patients not shedding virus as assessed by culture on Day 11~~
- ~~patients not shedding virus as assessed by RT-PCR on Day 11~~

~~Viral shedding and RT-PCR at days~~*Days* ~~15 and 40 will be evaluated in these subpopulations.~~

~~Based on the proportion of subjects hospitalized, an additional subpopulation may be defined to evaluate the length of hospitalization for hospitalized subjects.~~

Likewise, based on the proportion of children enrolled additional subpopulations of children and adults will be created to evaluate the course of influenza in children and adults.

Efficacy Analysis

For the analysis of time to alleviation of all clinical symptoms, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

Comparison to placebo control from pivotal registration trials

From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established that is comparable to the subpopulation of patients in the current study whose first dose of study medication$drug$ was within 48 hours of symptom onset. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

The median time to resolution of all influenza symptoms will be determined for each treatment group, along with its 95% confidence interval. These confidence intervals will be compared individually to the placebo control confidence interval described above as a potential means of establishing treatment effects.

Assessment of relative efficacy

The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval.

This confidence interval will provide lower and upper limits for the treatment difference and can be used as the basis for potential inferences. For example, if it does not contain the value zero a difference will have been established.

For the dichotomous endpoints, the proportion experiencing the event or symptom, and its associated 95% confidence interval will be derived for each treatment group. Where possible, the corresponding confidence intervals from the placebo control population will be presented for comparison to those derived for the active treatment groups in this trial.

The continuous secondary endpoint time to resolution of fever will be analyzed as for the primary efficacy endpoint.

For the continuous endpoints of viral load ($log_{10}TCID_{50}$/mL) at Day 1, 6, 8, 11, 15, and 40, summary statistics (including mean, standard deviation, median, minimum and maximum) will be derived for each treatment group.

SECTION 8.2.5: Safety Data Analysis

The safety analysis population will include all patients who receive at least one dose *of study* ~~medication~~*drug* and had a safety assessment performed post randomization. All safety variables will be summarized and presented in tables based on this safety population:

- *Adverse events*
- *Physical examination*
- *Vital signs*
- *Clinical laboratory evaluations*
- *Rejection and/or graft versus host disease*

SECTION 8.2.7: *Efficacy Analysis*

For the analysis of time to alleviation of all clinical symptoms, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- *Comparison to placebo control from pivotal registration trials*

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established that is comparable to the subpopulation of patients in the current study whose first dose of study drug was within 48 hours of symptom onset. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

  The median time to resolution of all influenza symptoms will be determined for each treatment group, along with its 95% confidence interval.

- *The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval.*

For the dichotomous endpoints, the proportion of patients experiencing the event or symptom, and its associated 95% confidence interval will be derived for each treatment group. Where possible, the corresponding confidence intervals from the placebo control population will be presented for comparison to those derived for the active treatment groups in this trial.

The continuous secondary endpoint time to resolution of fever will be analyzed as for the time to alleviation of all symptoms.

SECTION 8.2.8: Other Analyses

Further exploratory analyses ~~(including assessments of the rapid diagnostic test, sub group analysis)~~ will be detailed in the SAP

SECTION 8.3.1: Pharmacokinetic Analysis

The primary study variables are the model-~~predicted~~ *derived* PK parameters: steady-state AUC_{0-12}, C_{max}, and C_{trough} of ~~oseltamivir and~~ oseltamivir carboxylate.

~~Secondary model predicted PK variables may be included for both oseltamivir and oseltamivir carboxylate, if appropriate: $t_{1/2}$, t_{max}, k_e, CL/F, Vc/F, CL_m, C_{last} and t_{last}.~~

Plasma concentration data from sparse sampling will be analyzed using an established population PK model to determine key exposure parameters (e.g., C_{max}, C_{trough}, and AUC). For immunocompromised children aged 1 to 18 years, plasma ~~oseltamivir and~~ oseltamivir carboxylate concentrations will be modeled in NONMEM using a structure similar to a comprehensive population PK model, which was previously developed using plasma data from non-immunocompromised children and adults (ages 1 to 80 years) [36]. The basic structure consists of a 2-compartment model with first-order absorption and direct conversion of oseltamivir to oseltamivir carboxylate, while a 1-compartment model is used to account for the renal elimination of oseltamivir carboxylate from the plasma. Body weight, evaluated using a power function and centered around 70 kg, is a statistically significant predictor of ~~the CL/F for oseltamivir, and~~ both CL/F and central volume of distribution (Vc/F) for oseltamivir carboxylate. For oseltamivir carboxylate, CrCl is also a significant predictor of CL/F, while Vc/F decreases linearly with age.

SECTION 8.3.2: *Pharmacokinetic*/Pharmacodynamic Analysis

If feasible, *exposure-response relationships [43]will be evaluated between independent variables of exposure (e.g., AUC, C_{min}) and dependent variables including continuous (area under the viral titer curve, and peak viral titer) and time-to-event (cessation of viral shedding) virologic endpoints. Exploratory PK/PD analyses will be performed using appropriate methodologies. In order to account for potential non-linearity and non-monotonicity, each continuous independent variable (e.g., AUC) will be evaluated in its original form, as a categorical variable based on quartiles, as two- and three-group categorical variables.*~~the relationship between PK exposure of oseltamivir carboxylate and viral shedding response data will be characterized using nonlinear mixed effects modeling (using software NONMEM). Relevant population pharmacodynamic (PD) parameters will be derived, and the influence of covariates will be investigated. If deemed appropriate and necessary, data may be pooled with data from previous studies investigating oseltamivir and oseltamivir carboxylate in order to improve the model stability~~

TABLE 1: Schedule of Assessments

Table 1 has been revised to remove reference to the electronic patient diary and testing by RIDT PCR/ Culture for confirmation of influenza virus was added.

APPENDIX 2: Patient Diary Data and Symptom Record *for Adults*

Appendix 2 has been revised to reflect the changes to the protocol.

APPENDIX 3: *Patient* Diary Data *and Symptom Record* for Children

Appendix 3 has been revised to reflect the changes to the protocol.

APPENDIX 5: ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2

Appendix 5 has been updated to remove the call number and information regarding the Emergency Medical Call Center Help Desk were added.

APPENDIX 9: *Child-Pugh Classification of Severity of Liver Disease*

Appendix 9 has been added.

SAMPLE INFORMED CONSENT FORMS

The sample Informed Consent Forms have been revised to reflect the changes to the protocol.

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PROTOCOL AMENDMENT ACCEPTANCE FORM

TITLE:	**A DOUBLE BLIND, RANDOMIZED, STRATIFIED, MULTI-CENTER TRIAL EVALUATING CONVENTIONAL AND DOUBLE DOSE OSELTAMIVIR IN THE TREATMENT OF IMMUNOCOMPROMISED PATIENTS WITH INFLUENZA**
PROTOCOL NUMBER:	NV20234
VERSION NUMBER:	E
EUDRACT NUMBER:	2006-002468-24
IND NUMBER:	53,093
TEST PRODUCT:	Oseltamivir (Tamiflu® RO 64-0796)
MEDICAL MONITOR:	Vedran Pavlovic, M.D.
SPONSOR:	F. Hoffmann-La Roche Ltd

I agree to conduct the study in accordance with the current protocol.

_____

Principal Investigator's Name (print)

_____ _____

Principal Investigator's Signature Date

Please return the signed original of this form as instructed by your local study monitor. . Please retain a copy for your study files.

PROTOCOL SYNOPSIS

TITLE:	**A DOUBLE BLIND, RANDOMIZED, STRATIFIED, MULTI-CENTER TRIAL EVALUATING CONVENTIONAL AND DOUBLE DOSE OSELTAMIVIR IN THE TREATMENT OF IMMUNOCOMPROMISED PATIENTS WITH INFLUENZA**
PROTOCOL NUMBER:	NV20234
VERSION NUMBER:	E
EUDRACT NUMBER:	2006-002468-24
IND NUMBER:	53,093
TEST PRODUCT:	oseltamivir (Tamiflu® RO 64-0796)
PHASE:	IIIb
INDICATION:	Treatment of influenza in immunocompromised patients
SPONSOR:	F. Hoffmann-La Roche Ltd

Objectives

Primary

To evaluate prospectively the safety and tolerability of oseltamivir for the treatment of influenza in immunocompromised patients and characterize the effects of oseltamivir in immunocompromised patients on the development of resistant influenza virus

Secondary

To evaluate the effects of conventional and *double* dose oseltamivir in immunocompromised patients on:

- *The population pharmacokinetics of oseltamivir carboxylate (e.g., clearance, volume of distribution) in immunocompromised patients with confirmed influenza infection, through the application of established population pharmacokinetic (PK) models to the sparse plasma concentration data generated.*
- *The virologic course of influenza (proportion shedding and viral loads at different time points*)
- The time to resolution of influenza symptoms
- The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis)
- *To explore the relationship of metrics of exposure (e.g. AUC, C_{min}) to relevant pharmacodynamic endpoints*

Study Design

Description of Study

This is a double-blind, randomized, multi-center trial of twice daily, conventional and *double* dose oseltamivir for the treatment of influenza in immunocompromised patients. Patientss will be stratified by age ($\leq 12, > 12$ years), transplant status (yes, no), time since onset of flu symptoms and treatment start (up to 96 hours) (≤ 48 or > 48 hours) and vaccination status (yes, no)

Number of Patients

A minimum of 166 (83 per arm) to allow an adequate number of influenza A patients per arm; including 50 transplant recipients.

Number of Centers

Approximately 125 centers in the Northern and Southern Hemispheres

Target Population

Patients immunocompromised due to a primary or secondary immunodeficiency, 1 year of age and older. The patientss will be positive for influenza by a rapid diagnostic test, PCR or virus culture at baseline.

Length of Study

The study comprises 10 days of treatment with follow up visits approximately 5 and 30 days later as shown in the schedule of assessments. *Study medication will be administered twice daily over 10 days for a total of 20 doses.* A rapid diagnostic test for influenza will be performed at the end of the 10 days of treatment. Patients still having influenza based on the rapid diagnostic test will be treated per the local standard of care by the principal investigator, *and any medication provided during the follow-up period should be captured in the CRF.*

Procedures (summary)

The key procedures are:

- Blood draws for serum chemistry, hematology, serology, and PK assessments (for those patients who provide additional consent to participate in the PK assessments).
- Nasal and throat swabs for viral culture and RT-PCR.

Assessments of:

Safety

The safety laboratory assessments in this study, including the assessment of serum chemistry and hematology will be carried out at a central laboratory. Serum chemistry assessments comprise AST, ALT, total bilirubin, urea and creatinine. Hematology assessments include CBC and differential count. The total volume of blood loss for laboratory assessments will be approximately 20 mL for the entire duration of the study.

Protection of patient confidentiality (Section16) will extend to any data generated from the assaying of these samples. Biological samples taken from all patients may be infectious and will be classified as "diagnostic specimens" for dispatch purposes.

When clinically indicated, the investigator may draw blood for serum creatinine to be assessed at the local laboratory during the study to calculate creatinine clearance

Resistance

Development of resistance

Efficacy

The efficacy laboratory assessments are used for laboratory confirmation of influenza. These are:

Nasal and throat swabs. Two nasal and one throat swab will be collected as described in the Schedule of Assessments. All swabs are sent to a central laboratory for RT-PCR and viral cultures. Influenza virus shedding will be assessed.

At the end of treatment (Day 11), a rapid diagnostic test is permitted for the diagnosis of ongoing influenza.

Investigational Medicinal Products

Test Product:

Oseltamivir/placebo dry powder (to be reconstituted to a concentration of 12 mg/ml) and 75 mg capsules. The duration of dosing in both adults and children is 10 days.

Conventional dose:

Children ages 1 – 12 years: Oseltamivir syrup

≤ 15 kg	30 mg twice daily
> 15 – 23 kg	45 mg twice daily
> 23 – 40 kg	60 mg twice daily
> 40 kg	75 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules 75 mg twice daily

Patients randomized to the conventional dose will simultaneously receive matching placebo so as to blind them and the investigator from the *double* dose arm.

Double dose:

Children ages 1 - 12 years: Oseltamivir syrup

≤ 15 kg	60 mg twice daily
> 15 – 23 kg	90 mg twice daily
> 23 – 40 kg	120 mg twice daily
> 40 kg	150 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules 150 mg twice daily

Comparator:

Placebo (from pivotal registration trials in otherwise healthy adults)

Statistical Methods

The sample size has been chosen to provide an adequate number of patients to estimate the development of resistance with reasonable precision. Assuming that 90% of enrolled patients will have laboratory-confirmed influenza, there will be 75 patients in each treatment arm in the population evaluable for the development of resistance. The following table shows the 95% Pearson-Clopper confidence intervals that would result with a sample size of 75 patients in a treatment arm if certain event rates are observed in the study.

During the study the number of influenza A virus infected patients and the rate of development of resistance will be monitored in a blinded fashion, in order to ensure a reasonable precision for the estimation is maintained. Additional patients may be enrolled if necessary.

Observed Rate (%)	95% Confidence Interval
0.0	0% - 4.8%
1.3	0.0% - 7.2%
2.7	0.3% - 9.3%
5.3	1.5% - 13.1%
10.7	4.7%-19.9%

A total of 83 patients will be enrolled per arm and will be evaluable for the assessment of safety. This number of patients would provide estimates of adverse event rates with similar precision.

For the primary objective of evaluating the safety of oseltamivir conventional and *double* dose treatments, AEs, *physical examinations, tissue rejection and/or graft versus host disease in transplant patients,* laboratory tests, and vital signs will be summarized and compared with the known safety profile of the drug. For the summary of the development of resistance for each treatment arm, 95% confidence intervals will be provided with the estimated rates.

For the secondary objective of evaluating the efficacy of oseltamivir as measured by the time to resolution of influenza symptoms, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- Comparison to placebo control from pivotal registration trials

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established that is comparable to patients in the current study *whose first dose of study drug was within 48 hours of symptom onset*. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

- Assessment of relative efficacy

  The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval.

  The following are the model-derived PK secondary endpoints for oseltamivir carboxylate: steady-state AUC, C_{max}, and C_{trough}.

GLOSSARY OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ALT [SGPT]	Alanine aminotransferase
ALL	Acute lymphocytic leukemia
AML	Acute myeloid leukemia
ANOVA	Analysis of variance
AST [SGOT]	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
AUC_{0-12}	Steady-state area under the concentration–time curve from 0 to 12 hours
BID.	Bis in die (twice daily)
BP	Blood pressure
CARIFS	Canadian Acute Respiratory Infections Scale
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CL/F	Apparent clearance
C_{last}	Last measurable concentration
CL_m	Apparent total clearance of metabolite
C_{max}	Maximum plasma concentration
CML	Chronic myeloid leukemia
CrCl	Creatinine clearance
CRF	Case report form[s]
C_{trough}	Trough plasma concentration
ESF	Eligibility screening form
GVHD	Graft versus host disease
HA	Hemagglutinin
HIV	Human immunodeficiency virus
HSCT	Hematopoietic stem cell transplant
ICH	International Conference on Harmonisation
ITT	Intent to treat
ITTI	Intent to treat influenza infected
IVRS	Interactive voice response system
k_e	Elimination constant
PD	Pharmacodynamic
PK	Pharmacokinetic
PKEP	Pharmacokinetic Evaluable Patient Population
p.o.	Per os (by mouth)
QD	Once per day

GLOSSARY OF ABBREVIATIONS

RIDT	*Rapid Influenza Diagnostic Test*
SAE	Serious adverse event
SAP	Statistical analysis plan
SCID	Severe combined immunodeficiency
SOT	Solid organ transplant
$TCID_{50}$	50% tissue culture infectious dose
t_{last}	Time to last measurable concentration
t_{max}	Time to maximum plasma concentration
$t_{1/2}$	Elimination half-life
Vc/F	Apparent volume of distribution

PART I: STUDY DESIGN AND CONDUCT

1. BACKGROUND AND RATIONALE

1.1 BACKGROUND

Influenza is an acute respiratory infection caused by a virus of the orthomyxovirus family which occurs in three forms, influenza A, B and C. Influenza virus types A and B cause an acute febrile infection of the respiratory tract characterized by the sudden onset of fever, malaise, headaches, myalgias and cough. Influenza causes numerous deaths each year [1]. Although difficult to assess, annual influenza epidemics are thought to result in between three and five million cases of severe illness, and between 250,000 and 500,000 deaths every year around the world [2].

The influenza viruses are segmented, negative sense, single stranded, lipid encapsulated, RNA viruses between 80 and 100 nm in size. Subtypes are defined according to glycoproteins present in the viral lipid coat. The haemagglutinin complex (HA) is the major surface protein of the virus. The neuraminidase (NA) proteins are the second major surface proteins in the virion and play a role in enhancing virus penetration of the mucus layer around the target cell and in release of virus from the cell surface. The M2 protein triggers the disintegration of the virion during virus entry into the cell and may also be involved in protecting the HA prior to assembly of new virus particles.

1.1.1 Influenza in the Immunodeficient Population

Influenza infection is usually a self–limiting condition. However, in children, the elderly and the immunocompromised, influenza infection can be associated with substantial morbidity and mortality [1]. In patients with compromised immunity, influenza virus may cause severe lower respiratory tract involvement; unusual syndromes, including encephalitis, myocarditis, and hepatitis and death [3].

Conditions that compromise immunity may be classified based on etiology into primary (genetic) and secondary (acquired) immunodeficiency. Of the immunodeficient conditions, the ones that affect cell mediated immunity are likely to have adverse outcomes following viral infections [16].

Primary immunodeficiency

Primary immunodeficiencies are relatively common, may be either congenital or manifest later in life and are classified according to whether the genetic defect affects T or B cells or both [16]. There are four groups of disorders: severe combined immunodeficiency (SCID), primary T cell deficiency (e.g. CD8 deficiency, DiGeorge syndrome), predominantly B-cell-related antibody deficiency (e.g. common variable immunodeficiency, selective IgA deficiency) and other well-defined immunodeficiency syndromes (e.g., Wiskott Aldrich syndrome) [16].

Of the primary immunodeficiencies, antibody deficiencies are the most frequent. However, some of the more common antibody deficiency conditions (isolated IgA deficiency, IgG subclass deficiency and common variable immunodeficiency) have intact cell-mediated immunity and therefore the clinical course of viral infections (unless complicated by bacterial infections) does not differ significantly from that in the normal host [16]. A list of primary immunodeficiency disorders at risk for viral infections is provided in Appendix 6. The incidence of some of these conditions has been estimated. The incidence of SCID is 1 in 100,000 to 1 in 1,000,000 [16]. The more severe forms of primary immunodeficiency are relatively rare, have their onset early in life, and all too frequently result in death during childhood [16].

Secondary Immunodeficiency

Secondary immunodeficiencies are not caused by intrinsic abnormalities in development of T and B cells [16]. Secondary immunodeficiency may result from diseases (human immunodeficiency virus [HIV], hematologic malignancy) or immunosuppressive and cytotoxic drugs (such as those used for treatment of transplant recipients, collagen vascular disease, malignancies).

Secondary immunodeficiency due to disease

HIV-infected individuals with a CD4+ T-cell count of $<200/mm^3$ (AIDS defining) are highly susceptible to opportunistic disease [16]. However, CD4 counts $<500/mm^3$ are considered abnormal in HIV-infected individuals, and therefore these individuals are also more susceptible to infection. *CD4+T cell counts are higher in infants and young children than in adults and decline over the first few years of life [40 ,41]. Because of this age-dependent variation in absolute CD4+ T cell count, calculation of CD4 percentage is used as a measure for young children because it has shown less variability. HIV-infected children (12 months to 5 years old) with <15% CD4+ T cells are classed as severely immunosuppressed and highly susceptible to opportunistic disease, although antiretroviral therapy is recommended in children with <25% CD4+ T cells as they are still classified as immunosuppressed [42].* Studies in patients with HIV/AIDS have shown an increased risk for heart and lung-related hospitalizations during the influenza season compared with other times of the year, prolonged duration of influenza symptoms, increased risk for influenza-related complications and a higher risk of influenza-related death [17].

Several hematologic malignancies affect the immune system (Appendix 7). Several authors have reported influenza in children and adults with hematologic malignancies [18, 19, 20, 21].

Secondary immunodeficiency due to drugs (e.g. transplant recipients, collagen vascular disease, malignancies)

The enhanced survival of the transplant population following the availability of newer immunosuppressive drugs has made them representative of the immunocompromised population in general; and secondary immunodeficiency due to drugs, in particular.

In transplant recipients influenza is associated with a higher rate of pulmonary complications, extrapulmonary manifestations and an increased risk of graft dysfunction and high attributable mortality. In a cohort study of influenza viral infection in solid organ transplant (SOT) patients, 30 cases of influenza viral infection were identified. Secondary bacterial pneumonia was seen in 17% of patients and three SOT recipients (2 liver and one kidney) had myositis, myocarditis and bronchiolitis obliterans. Biopsy of the transplanted organ was performed in 21 of 30 cases and revealed variable degrees of acute allograft rejection in 62% of patients. The study concluded that influenza was associated with significant morbidity in different groups of SOT recipients [4].

Among transplant recipients, patients with hematopoietic stem cell transplant (HSCT) are at the greatest risk for morbidity. In a prospective study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation, 1973 patients were evaluated for respiratory virus infections after stem cell transplantation. The overall mortality was 23% from influenza A infection and the direct influenza A-associated mortality was 15.3% in this study [5]. A large retrospective study (4797 patients undergoing HSCT over a 13-year period) identified 62 patients with influenza of whom as many as 29% (18 of 62 patients) developed pneumonia. Ten percent of the patients with influenza died [6].

The increased morbidity in transplant patients is associated with increased duration of virus shedding compared to that in healthy subjects. In otherwise healthy adults, elderly patients and children, the median duration of viral shedding in untreated patients was 70, 96 and 118 hours respectively [7]. In contrast, in the retrospective HSCT study, influenza virus was shed in nasopharyngeal secretions (evaluated at least weekly) for a median duration of 168 hours (7 days, range 2 to 37 days) [6].

Currently available treatments for influenza infection are the M2 ion channel inhibitors (e.g. amantadine, rimantadine) and the neuraminidase inhibitors (e.g. oseltamivir, zanamivir).

1.1.2 Oseltamivir

Oseltamivir (Tamiflu®, Ro 64-0796) is an ethyl ester prodrug which is rapidly absorbed from the gastrointestinal tract after oral administration and metabolized in the liver by high capacity carboxylesterases to form oseltamivir carboxylate (Ro 64-0802), a potent, stable and selective inhibitor of influenza A and B neuraminidase enzymes. The active form, oseltamivir carboxylate is excreted unchanged by the kidney via glomerular

filtration and active tubular secretion by the organic anion transport system. The efficacy and safety of oseltamivir in influenza treatment and prevention has been established in an extensive series of clinical studies in man.

Oseltamivir has been approved for the treatment of influenza in Europe, the United States and most other countries around the world. In adults and adolescents, the recommended dose is 75 mg twice daily for five days. In children 1 year of age and older recommended doses are 30, 45 or 60 mg bid based on body weight. In all age groups the recommended dose is administered bid for 5 days.

The approval of oseltamivir for the treatment of influenza is based on several controlled clinical trials. In the pooled population from these clinical trials encompassing adults aged from 13-97 years, many with significant co-morbidity, 1325 patients were treated with oseltamivir (75 mg bid) and 1056 patients received placebo. A total of seven influenza symptoms (both respiratory and constitutional) were captured on the diary card for adults. The time to resolution of all symptoms (on the diary card) decreased by 24 hours; from 124.5 hours in the placebo arm to 100.6 hours with oseltamivir 75 mg bid ($p < 0.0001$) [7]. Further, in adults and adolescents with a proven influenza illness, oseltamivir treatment reduced overall antibiotic use for any reason by 26.7% and the incidence of influenza-related lower respiratory tract complications resulting in antibiotic therapy by 55%. The study concluded that oseltamivir treatment of influenza reduces lower respiratory tract complications, antibiotic use and hospitalizations in healthy and 'at risk' patients [8].

Likewise, in the influenza-infected pediatric population (1 to 12 years of age), oseltamivir treatment ($n = 217$) was compared with placebo ($n = 235$). There was a reduction in the median duration of illness (defined based on resolution of temperature, cough, coryza and return to pre-illness health and activity) of 36 hours; from 137 hours with placebo to 101 hours in the oseltamivir treatment arm ($p \leq 0.0001$). The Canadian Acute Respiratory Infections Scale (CARIFS), validated for use in children, was used to collect symptom data on the pediatric diary card. The CARIFS scale comprised a total of 18 symptoms which were evaluated twice daily by the parent or guardian. There was a similar reduction in the time to alleviation of all CARIFS symptoms of 36 hours; from 100 hours in the placebo group to 63 hours in the oseltamivir group ($p < 0.0001$) [9].

Thus in both adults and children, the time to resolution of all symptoms was significantly reduced in the oseltamivir treatment arm compared with placebo.

Oseltamivir was well-tolerated in clinical trials. Approximately 11,000 patients have received oseltamivir in the development program. The most common adverse events reported by adults, the elderly, and children were nausea and vomiting. Serious adverse events (SAEs) were reported with a low and equal frequency by patients taking active drug and placebo. Full details are given in the Investigator Brochure [7].

The safety profile of oseltamivir has been well characterized for the prophylaxis indication in a prospective randomized placebo controlled trial conducted in the adult and pediatric immunocompromised (HSCT and SOT) population. In the oseltamivir group, the indications for transplant included hematologic malignancies (acute and chronic leukemias, multiple myeloma and myelodysplastic syndrome), lymphoid malignancies (Hodgkins or non-Hodgkins lymphomas), primary immunodeficiencies (Wiskott-Aldrich syndrome, X-linked lymphoproliferative disease and severe combined immunodeficiency). Other rare indications included bone marrow aplasia, paroxysmal nocturnal hemoglobinuria, myelofibrosis and multiple sclerosis. In the safety population, there were 239 patients randomized to the conventional dose of oseltamivir and 238 to placebo. The total number of adverse events reported in the placebo group (361 events) was generally similar to that in the oseltamivir group (323 events). Diarrhea was the most frequently reported adverse event (placebo, 8%; oseltamivir, 6%). There were no deaths in the oseltamivir group. Oseltamivir was found to be safe in immunosuppressed transplant recipients [22].

Limited safety and/or efficacy of oseltamivir for the treatment indication is available from several case reports in children and adolescents. Oseltamivir was shown to be safe and/or effective in HIV infected children (n = 10) [23], children (age 3 to 12 years) with acute lymphocytic leukemia (ALL) (n = 10) [21], in a nosocomial H1N1 outbreak in a pediatric (children aged 10 months to 13 years) oncology ward (n = 8) [20], in children and adolescents (age 2 to 19 years) with malignancies (ALL, neuroblastoma, brain tumor, non-Hodgkin's lymphoma, osteosarcoma, Ewing sarcoma, myelodysplasia, acute myeloid leukemia (AML), Wilms tumor, aplastic anemia, chronic myeloid leukemia (CML) and acute promyelocytic leukemia) (n = 51) [18], in children aged 4 to 14 years on immunosuppressive drugs (n = 5) [24] and in children (aged 5 months to 5 years) with bone marrow transplant (n = 3) [25].

Oseltamivir has also been shown to be safe and/or effective in mixed populations of children and adults (62 patients) with HSCT [6], and in a large epidemiologic study (n = 221) with SOT [26].

Finally, oseltamivir has been shown to be safe and/or effective in immunocompromised adults with HSCT [10] and adults with lung transplant [27, 28, 29].

The dose of oseltamivir was the same as the conventional dose in a majority of these reports. In one report as many as 25 adult patients received twice the conventional dose [26] while in another report, three of nine adult patients received twice the conventional dose [29]. Treatment with oseltamivir generally ranged from 5 to 10 days [28, 29, 26] and occasionally until the patient was symptom free [28, 29]. In exceptional cases treatment was given for as long as 20 days [18] or for as long as the patient was positive by RT-PCR [21].

There is some concern about the development of resistance in the immunocompromised population. During the pandemic influenza season, more than 23,000 clinical isolates of novel H1N1 pandemic virus were tested for resistance in the 6 international WHO regions. A total of 225 isolates (from 225 patients) were resistant (H275Y mutation in the neuraminidase coding sequence) to oseltamivir for an approximate incidence of 1%. Information on immune status was available for 142 of 225 patients. Among the 142 patients, 56 (40%) were immunocompromised [30]. Prolonged viral replication and lack of immune-mediated virus clearance in immunosuppressed patients treated with antivirals can result in a higher incidence of selection of drug-resistant viruses, a phenomenon that has been documented previously [31, 32].

1.2 RATIONALE FOR THE STUDY

Because of the increasing body of evidence (Section 1.1.2), oseltamivir is recommended in national guidelines as an option for the treatment of influenza in the transplant population [37], which comprises a significant portion of the immunocompromised population. However, there is limited data on safety and efficacy of oseltamivir use in this population. The primary objective of this study is to evaluate safety and resistance, while evaluating efficacy as a secondary objective, in the broader immunocompromised patient population, who are considered at increased risk of viral infection.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective of the study is to evaluate prospectively the safety and tolerability of oseltamivir for the treatment of influenza in immunocompromised patients and characterize the effects of oseltamivir in immunocompromised patients on the development of resistant influenza virus.

2.2 SECONDARY OBJECTIVES

The secondary objectives of the study are:

To evaluate the effects of conventional and *double* dose oseltamivir in immunocompromised patients on:

- *The population pharmacokinetics of oseltamivir carboxylate (e.g., clearance, volume of distribution) in immunocompromised patients with confirmed influenza infection, through the application of established population pharmacokinetic (PK) models to the sparse plasma concentration data generated*
- *The virologic course of influenza (proportion shedding and viral loads at different time points)*
- The time to resolution of influenza symptoms
- The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis)

- *To explore the relationship of metrics of exposure (e.g., AUC, C_{min}) to relevant pharmacodynamic (PD) endpoints*

3. STUDY DESIGN

3.1 OVERVIEW OF STUDY DESIGN AND DOSING REGIMEN

This is a double blind, randomized, stratified, multi-center trial of conventional and *double* dose oseltamivir for the treatment of influenza in immunocompromised patients. Immunocompromised patients, who develop an influenza-like illness with a positive rapid diagnostic test, PCR, or viral culture for influenza, will be enrolled during the influenza season. Patients will be stratified by transplant status [yes, no], the time between onset of influenza symptoms and treatment start (up to 96 hours) [$\leq$48 hours; $>$48 hours], influenza vaccination status for current influenza season [Yes; No] and by age [$\leq$12 years; $>$12 years]. Eligible patients will be consented and randomized to receive oseltamivir twice daily for 10 days in one of two treatment arms: conventional dose or *double* dose (double the conventional dose).

3.1.1 Rationale for Study Design

This study incorporates several features that distinguish it from classic placebo controlled trials.

There is no placebo control arm in this study as it was considered unethical for this high risk population. The development of resistance following treatment with oseltamivir (one of the primary objectives of the study) is an objective assessment (determined by laboratory tests) and is unlikely to be impacted by the absence of a placebo arm.

As a placebo control arm was considered unethical in this population, an alternative control arm had to be identified for efficacy end points. The response to placebo in pivotal oseltamivir registration trials in the healthy adult population with influenza was chosen as a control in lieu of a placebo arm in the current trial. In order to do this, the end points were designed to be similar to that in the pivotal registration trials.

The following sections provide rationale and justification for specific aspects of the study design which differ from the currently approved dosing for influenza or from previous pivotal registration trials. They also provide information on how the results will be used to make recommendations on dose and duration of treatment.

3.1.1.1 Choice of Treatment Arms

The currently approved dose of oseltamivir for the treatment indication is the conventional dose with a duration of 5 days. In this study a conventional and *double* dose are being evaluated. The pivotal registration trials for oseltamivir in healthy adults with influenza had three treatment arms; two active (75 mg and 150 mg) and one placebo. Both treatment arms were found to be significantly better than placebo. No statistical comparisons were made between the two active treatment arms. Evaluation

of the results in the two active treatment arms did not reveal any clinically meaningful difference. However, the proportion of patients shedding virus on Day 4 (3 days after the start of treatment), suggested a possible dose-response relationship (36%, 28% and 23% in the placebo, 75 mg and 150 mg dose arms respectively). Because defective immune-mediated virus clearance in immunosuppressed patients treated with antivirals can result in a higher incidence of selection of drug-resistant viruses, it was decided to use both the conventional and *double* dose arm for this study. A longer duration of treatment was chosen because a large retrospective study has shown that the median duration of viral shedding of 7 days [6] was greater for HSCT recipients than that seen in the healthy children, adult and elderly population with influenza (Section 1.1.1) [7].

3.1.1.2 Inclusion of Patients Symptomatic up to 96 Hours

In immunocompromised patients, time from onset of symptoms to seeking medical attention (presentation at the clinic) of $>$48 hours has been shown in several case reports [33, 27, 28, 29] including two instances of nosocomial outbreaks in children [25, 20]. This notwithstanding, oseltamivir has been shown to *offer benefit* in immunocompromised populations that included patients treated with oseltamivir beyond 48 hours of presentation; patients with lung transplant (median time to presentation 3 days) [27], bone marrow transplant (treatment started more than 48 hours after onset of symptoms in all 3 patients) [25], organ transplant (62 of 221 patients started treatment after 96 hours) [26] and children with *acute lymphocytic leukemia* (one child presented after 3 days and two children presented after 5 days of symptoms) [21].

In a prospective, observational study involving adults hospitalized with influenza, the study authors concluded that 'Weakened host defenses slow viral clearance, whereas antivirals started within the first four days of illness enhance viral clearance' [34].

Additionally, in November 2009, in a communication for clinicians on antiviral treatments for H1N1, the US Centers for Disease Control and Prevention (CDC) stated that while antiviral treatment is most effective when started early; both outpatients with risk factors and hospitalized patients still benefit when treatment with oseltamivir is started more than 48 hours after illness onset [35].

3.1.1.3 Rational for Sparse PK Sampling

Limited PK sampling will be done in this protocol, in patients who have provided additional consent, and is justifiable in this study for the following two reasons:

- There are already tested and qualified population PK models available [36] that will be used to estimate exposure in the immunocompromised population on the basis of the data collected in this study, meaning extensive PK sampling over a steady-state dosing interval is not required.
- The patient population in this study is likely to be very ill with a complex clinical picture and significant additional burden of treatment and monitoring due to their primary condition. In this context, it makes ethical sense to minimize blood draws

and assessments to only those absolutely essential to meet the objectives of the study and to ensure patient safety.

3.1.1.4 Interpretation of Study Results

Safety and tolerability and the development of resistance are the primary objectives of this study. These will be characterized descriptively. For the secondary objective of efficacy, the subset of the population enrolled in the first 48 hours will be compared with placebo patients from pivotal registration trials (where patients were enrolled in the first 2 days of illness). Section 8.2.5 , Section 8.2.6 , and Section 8.2.7 provide details on the statistical considerations.

Stratification

Stratification in this study has no bearing on any comparisons with results from previous trials. However, in order to evaluate a dose response relationship between the two active treatment arms in this trial, stratification remains essential.

Patients will be stratified by transplant status (yes, no) because transplant patients form a relatively large homogenous group in this study and might influence outcome.

Patients will be stratified by time between onset of symptoms and treatment start (<48 hours or ≥ 48 hours) because previous experience in otherwise healthy adults and children has shown that patients treated early in their illness respond to treatment better than those who are treated later. Patients will also be stratified by age (≤ 12 years and >12 years) because otherwise healthy children shed virus longer than otherwise healthy adults. It is expected that a similar difference in the duration of viral shedding would exist between immunocompromised children and adults. As vaccinated patients may respond faster to therapy, the study is also stratified based on influenza vaccination status for the current influenza season (Yes; No).

3.1.2 Rationale for Dose Selection and Adjustment

Dose Selection

The dose of oseltamivir to be used in this study is the conventional, approved dose for children and adults in the treatment of influenza. There will be a second higher dose for comparison which is two times the conventional dose. This higher dose is used based on theoretical considerations which suggest that the higher dose may be associated with improved efficacy and decreased emergence of resistance.

The anticipated pro-drug and metabolite exposures from this higher dose are not expected to exceed maximum exposures seen previously in the oseltamivir development program. The safety and tolerability of the higher dose regimen has already been demonstrated in treatment studies of immunocompetent adult subjects ($n = 447$) [11]. In a study to demonstrate cardiac safety, in the highest dose group treated with 450 mg BID. for 5 days [$n = 99$], no subject had a serious adverse event, nor withdrew

prematurely. In Phase I studies in adults, oseltamivir has been administered in multiple doses of up to 500 mg BID. Doses of 200 mg BID. and greater have been associated with increased gastrointestinal adverse effects (nausea and vomiting) [7]. In adult subjects with creatinine clearances of ≤ 30 mL/min, doses of 100 mg BID for 6 days were well tolerated, despite steady-state oseltamivir carboxylate exposures approximately 10-fold higher than those achieved with standard dosing in renally competent individuals [12]. No other adverse effects were reported more frequently with higher doses and no serious adverse events have been reported within the volunteer studies. Co-administration of oseltamivir with food has been demonstrated to substantially reduce the frequency and severity of gastrointestinal side effects.

Thus, the rationale for the *double* dose arm in this study is to explore the possibility of improved efficacy and decreased emergence of resistance without an undue increase in risk. *For patients with a creatinine clearance level of 45 mL/min or above, oseltamivir carboxylate exposures for both the 75-mg and 150-mg doses are not expected to exceed exposures previously tested. Patients with a creatinine clearance as low as 45 mL/min, who receive the 150-mg BID dose, will have exposures of oseltamivir carboxylate that are within (albeit in the upper end) the established safety margin. This corresponds to an AUC produced by the 450-mg BID dose in adults with normal renal function (AUC $_{0\text{-}12\ hrs}$ of approximately 15,000 ng •hr/mL).*

Drug interactions with immunosuppressive medications have also been evaluated. The pharmacokinetics of oseltamivir and oseltamivir carboxylate after administration of 75 mg oseltamivir (conventional adult dose) in patients with a well-functioning, stable renal allograft who were being maintained on immunosuppressive therapy were studied. These were similar to those described in the literature for adults with comparable degrees of renal function. Oseltamivir was well tolerated and had no clinically relevant effect on the steady-state pharmacokinetics of cyclosporine A, tacrolimus, or mycophenolate mofetil [7].

Duration of Dosing

The duration of dosing chosen for this population (10 days) is longer than that in the healthy adult and pediatric populations (5 days). This is based on observations that the viral shedding and illness are typically longer in immunocompromised patients than it is in healthy adults [6, 10].

Dose Adjustments

As this is a double blind study, patients whose *creatinine clearance* (CrCl) decreases to *<45mL*/min *(adults) using the Cockcroft-Gault method or* <*45 mL/min*/1.73m^2 *(<18 years old) using the Schwartz equation* will be discontinued from *study* treatment. *A lower limit of 45 mL/min for CrCl will be used to allow for patients with a mild to moderate renal impairment.*

3.1.3 End of Study

The study comprises 10 days of treatment with follow-up visits approximately 5 and 30 days later as shown in the schedule of assessments. *Study medication will be administered BID over 10 days for a total of 20 doses.* A rapid diagnostic test for influenza will be performed at the end of the 10 days of treatment. Patients still having influenza based on the rapid diagnostic test will be treated per the local standard of care by the principal investigator, *and any medication provided during the follow-up period should be captured in the CRF.*

3.2 NUMBER OF PATIENTS/ ASSIGNMENT TO TREATMENT GROUPS

A minimum of 166 patients (approximately 83 per arm) to allow an adequate number of influenza A patients, including 50 transplant recipients will be enrolled in this study**.** After screening, patients will be randomly assigned to one of the two active treatment groups.

3.3 CENTERS

This will be a multicenter study taking place in the Northern and Southern Hemispheres at approximately 125 centers.

4. STUDY POPULATION

4.1 OVERVIEW

The study population comprises immunocompromised adults (including adolescents) and children who have influenza. Additionally, the patients must not have other medical conditions that will preclude the assessment of efficacy or safety. Influenza vaccinated and non-vaccinated patients are eligible to participate in this study. Principal investigators will review oseltamivir resistance patterns of strains circulating in the area and weigh the risk versus the benefit before enrolling patients with a potentially resistant strain.

Under no circumstances are patients who enroll in this study permitted to be re-randomized to this study and enrolled for a second course of treatment.

4.2 INCLUSION CRITERIA

- Age greater than or equal to 1 year
- Rapid diagnostic test, PCR, or viral culture positive for influenza
- Immunocompromised patient defined as one who meets any of the following:
    - Primary immunodeficiency at risk for viral infections (representative examples in Appendix 6) OR
    - Secondary immunodeficiency
        - SOT with ongoing immunosuppression OR
        - Allogenic HSCT with ongoing immunosuppression OR

- HIV with *a most recent* CD4 count < 500/mm^3 *(or <25% in children ≤ 5 years old) within the last 6 months* and, in the investigator's opinion, considered immunocompromised OR
- Hematologic malignancies (representative examples in Appendix 7) OR
- Systemic (e.g. enteric, sc, im or iv) immunosuppressive therapy, irrespective of medical indication, started at least 12 weeks prior to, and ongoing at the time of first dose of study drug (representative examples in Appendix 8)

- Symptoms suggestive of influenza like illness including, but not limited to fever, cough, or coryza
- In patients with history or clinical presentation at randomization suggestive of renal failure; a CrCl > 60 mL/min *(>18 years old) or >60mL/min/*1.73m^2*(<18 years old) within the last 3 months*
- Less than or equal to 96 hours between onset of influenza like illness and first dose of study drug
- Parent/guardian willing and able to comply with study requirements and give consent, (country specific age cut off)
- Patient able to comply with study requirements and willing to give assent, as appropriate (country specific age cut off)
- For adult patients, willing and able to comprehend and give written informed consent
- Patients, in the reproductive age group, must agree to utilize an effective method of contraception throughout the study period and for females for one reproductive cycle following cessation of study therapy
- Females of childbearing potential must have a negative urine pregnancy test prior to start of study drug

4.3 EXCLUSION CRITERIA

- Clinical evidence of severe hepatic impairment, defined as Child-Pugh grade C (score > 9) or decompensated cirrhosis *(see Appendix 9).*
- Patients currently receiving any form of renal replacement therapy including hemodialysis, peritoneal dialysis or hemofiltration.
- Patients with gastrointestinal disorders which might interfere with their ability to absorb oral medication.
- Allergy to the test medication.
- Patients with hereditary fructose intolerance (for patients who will be taking the liquid formulation).
- Influenza vaccination with live attenuated vaccine in the 2 weeks prior to randomization.

- Antiviral treatment (example: amantadine, rimantadine, oseltamivir, laninamivir, peramivir, zanamivir and ribavirin) for influenza in the 2 weeks prior to randomization.
- Patients taking probenecid medication.
- Patients who are pregnant or breast-feeding.
- Participation in a clinical trial or expanded access trial with an investigational drug suspected/demonstrated to impact pathways important for the metabolism and excretion of oseltamivir in the 4 weeks prior to randomization or concomitantly with this study.

4.4 CONCOMITANT MEDICATION AND TREATMENT

Antiviral treatments with activity against influenza (e.g., amantadine, rimantadine, zanamivir, ribavirin, laninamivir, peramivir, and additional oseltamivir above that specified for this study) are not allowed during the 10-day treatment phase of the study. Concomitant use of an investigational drug during the study is also excluded *if suspected/demonstrated to impact pathways important for the metabolism and excretion of oseltamivir.* Live attenuated vaccines may not be advisable for this population. It is possible that concurrent use with oseltamivir may render live vaccines ineffective.

Medications required for the routine care of the patient may be continued during the study. Concomitant use of probenecid (irrespective of the indication) is not allowed during the study.

4.5 CRITERIA FOR PREMATURE WITHDRAWAL

The investigator must discontinue treatment if the creatinine clearance is *<45* mL/min in adults *using the Cockcroft-Gault method* or *< 45* mL/min/1.73 m^2 *using the Schwartz equation* in children. *A lower limit of 45 mL/min for creatinine clearance will be used to allow for patients with a mild to moderate renal impairment.* The investigator must also discontinue treatment from all patients with intercurrent illnesses or adverse events suggestive of hepatic decompensation. The investigator also has the right to discontinue treatment in the event of intercurrent illness, adverse events, treatment failure, protocol violations, administrative reasons or other reasons.

The investigator must discontinue treatment with study drug if the patient is to be treated concomitantly with another antiviral for influenza, for example, amantadine, rimantadine, laninamivir, peramivir, or zanamavir. However, all patients, including those who discontinue study drug prematurely and/or are treated with another antiviral, will be required to return for follow up approximately 5 and 30 days after the last dose of study drug *(*Day 15 and Day 40 assessments).

Patients have the right to withdraw from the study at any time for any reason.

An excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of subjects should be avoided. Should a patient decide to withdraw, all efforts will be made to complete and report the observations prior to withdrawal as thoroughly as possible.

The investigator should contact the patient or a responsible relative either by telephone or through a personal visit to establish as completely as possible the reason for the withdrawal. A complete final evaluation at the time of the patient's withdrawal should be made with an explanation of why the patient is withdrawing from the study. If the reason for removal of a patient from the study is an Adverse Event, the principal specific event will be recorded on the CRF.

In the case that the patient decides to prematurely discontinue study treatment ["refuses treatment"], he/she should be asked if he/she can still be contacted for further information. The outcome of that discussion should be documented in both the medical records and in the CRF.

4.6 REPLACEMENT POLICY [ENSURING ADEQUATE NUMBERS OF EVALUABLE PATIENTS]

4.6.1 For Patients

No patient prematurely discontinued from the study for any reason will be replaced.

4.6.2 For Centers

A center may be replaced for the following administrative reasons:

- Excessively slow recruitment.
- Poor protocol adherence.

5. SCHEDULE OF ASSESSMENTS AND PROCEDURES

Table 1 Schedule of Assessments

	Baseline	Treatment					Follow-up	
Study Day	**1 (pre-dose)**	**1**	**2 or 3[f,g]**	**6[f, g]**	**8[f, g]**	**11[g, h, i]**	**15[g]**	**40[g, j]**
Informed Consent/Assent [l]	x							
Medical history	x							
Demographics	x							
Height and weight	x					x		
Pregnancy Test [a]	x					x		x
Rapid diagnostic test for influenza virus shedding						x		
RIDTPCR/Culture for confirmation of influenza virus	*x*							
Safety Labs [b]	x					x		
Physical Examination	x					x		x
Vital Signs (including pulse, RR, temperature, Blood pressure)	x		x	x	x	x	x	x
Nasal and throat swabs for viral shedding and viral load [c, d k]	x		x	x	x	x	x	x
PK sampling (blood) [l]					x[m]			
Review of *patient diary* data [e]			x	x	x	x	x	x
Drug Administration		←	—	—	—	→		
Collection of unused study *drug* and empty containers						x		
Previous Diseases	x							
Previous/Concomitant medications	x	←	—	—	—	—	—	→
Adverse Events/Sec Illnesses and Treatments		←	—	—	—	—	—	→
Rejection, Graft versus host disease (GVHD)		←	—	—	—	—	—	→

a. Urine pregnancy test for patients of child-bearing potential according to the judgment/discretion of the investigator.

b. Hematology (CBC with differential) and chemistry (AST, ALT, total bilirubin, urea, Cr). Serum creatinine testing may be done at a local laboratory when clinically indicated to calculate creatinine clearance.

c. Baseline swab samples will be assessed for the presence of resistance mutations.

d. Two nasal and one throat swab for viral culture and RT-PCR.

e. Influenza symptoms, temperature, and date/ time of oseltamivir dose will be recorded by the patient in *the* patient diaries twice daily on Days 1 – 10, and once daily thereafter.

Table 1 Schedule of Assessments (cont.)

f. A home visit may be made on Day 2 or 3 (for patients who are too ill to come into the clinic) and/or, Day 6, and/or Day 8; however, it is recommended that the PK blood draw be performed at the clinic.

g. Day 2/3 visit window = +1 day. Day 6 visit window = ± 1 day; Day 8 visit window = +1 day. Day 11 visit window = ± 1 day. Day 15 and Day 40 visit occur approximately 5 and 30 days after the last dose respectively. Day 15 window = ± 1 day. Day 40 window ± 2 days.

h. Only weight to be assessed. Height is not necessary at this visit. Study drug is taken on Day 11, only if the first dose was taken after 4 PM on Day 1 [5.3] Patients who are rapid diagnostic positive can be treated per standard of care. They will be required to come for their follow-up visits on Day 15 and Day 40.

i. Patients who discontinue treatment prematurely will have an end of treatment (Day 11) assessment at the time of treatment discontinuation or the following day. They will be required to attend their follow up visits approximately 5 and 30 days after the last dose (Day 15 and Day 40 assessments).

j. Patients who discontinue during follow-up will have an end of follow-up (Day 40) assessment. This visit must occur within 30 days of the last dose.

k. For patients who are unable to attend the clinic, swabs may be taken at home on those days where there is a home visit by site personnel.

l. Plasma PK samples for assessment of oseltamivir carboxylate will be collected from patients who give additional consent to participate in PK sampling using a sparse PK sampling approach (Section 5.4).

m. Serial PK samples taken at steady-state no earlier than Day 6 (i.e., not before the 11th dose) consisting of as many of the four timepoints as possible: within 30 minutes prior to the dose administration, 1.5 hours ± 30 minutes post dose, 4 hours ± 60 minutes post dose, and 8 hours ± 1.5 hours post dose (for patients who have given additional consent).

5.1 SCREENING EXAMINATION AND ELIGIBILITY SCREENING FORM

All patients must provide written informed consent (assent where applicable) before any study specific assessments or procedures are performed. Screening will take place at the baseline visit prior to first dose. However, if necessary, screening may take place the day before the baseline visit, as long as the patient is randomized and receives his/her first dose of study drug within 96 hours of influenza symptom onset.

Patients will be assessed for inclusion and exclusion criteria and those who fulfill all the inclusion and none of the exclusion criteria will be randomized into the study. *Influenza Rapid Diagnostic test, PCR, or viral culture for the purposes of inclusion criteria fulfillment will be done by the site or the local laboratory. Historical CrCl values within 3 months and CD4+ T-cell counts (where applicable) within 6 months before randomization are acceptable for inclusion into the study.*

An Eligibility Screening Form [ESF] documenting the investigator's assessment of each screened patient with regard to the protocol's inclusion and exclusion criteria is to be completed by the investigator.

A screen failure log must be maintained by the investigator.

5.2 PROCEDURES FOR ENROLLMENT OF ELIGIBLE PATIENTS

Once a patient has fulfilled the entry criteria, he/she will be randomized to one of two treatment groups. The patient randomization numbers will be generated by Roche or its designee and incorporated into double-blind labeling.

The investigator or designee will use the CRF pre-printed with the assigned patient number and enter the randomization number provided by IVRS for allocation to the treatment groups in the appropriate place on each patient's CRF.

Randomization will be stratified by transplant status (Yes; No); time between onset of symptoms and treatment start ($\leq$48 hrs or $>$48 hrs), influenza vaccination status (Yes; No) and patient's age ($\leq$12 years or $>$12 years).

5.3 CLINICAL ASSESSMENTS AND PROCEDURES

At all visits patients will receive the routine care for their primary illness. Routine patient care (including the adjustment of doses of any of the immunosuppressive drugs) will be dictated by tests performed locally at the trial site. For patients unable to attend the clinic, provision will be made for swabbing to be conducted at home, when there is a home visit scheduled. Training will be provided to site staff on how to perform this. All safety labs will be sent to a central laboratory. Results for these laboratory parameters will be used by the sponsor/designee to assess overall safety.

All assessments and procedures will be performed according to the Schedule of Assessments (Table 1). Additional information regarding these procedures not provided in the Schedule of Assessments is provided below.

Blood samples will be collected from patients who provide additional consent for PK sampling, as outlined in Section 5.5 .

Study Day 1

The baseline and study Day 1 assessment may be performed at the same visit.
Laboratory assessments (safety labs and nasal and throat swabs for virology) should be performed after randomization but prior to the patient receiving first dose of study drug.

Study drug, patient diaries, and thermometers will be dispensed. Patients or guardians/parents will be instructed on how to complete the patient diaries (Appendix 2) and(Appendix 3), temperature recording, and treatment administration details, including

time of each oseltamivir dose. The first diary entries will be made at the site before the first dose of study drug.

Baseline nasal and throat swab samples will be assessed for the presence of oseltamivir-resistance mutations.

The date of the first dose of study drug is defined as study Day 1. Once randomized, the first dose of study drug will be administered in the clinic. Study Day 2 will begin at 12 midnight of the same calendar day. If the first dose of study drug is taken after 4 pm on Day 1, the next dose of study drug will be taken in the morning of Day 2. In this case, the last dose of study drug will be taken on the morning of study Day 11.

If the first dose of study drug is taken prior to 4 pm on Day 1, the next dose of study drug should be taken in the evening of the same day (i.e. prior to midnight on the same calendar day with a minimum of 7 hours between doses). For these patients the last dose of study drug will be taken in the evening of study Day 10. More information on dosing is provided later (Section 6.1).

Study Days 2 - 11

Study Day 2 will begin at 12 midnight of study Day 1.

It is expected the study staff will maintain close contact with patients, especially during the first few days of the study. The study staff should inquire about any adverse events, check that the patient or parent/guardian is entering data into the patient diary properly, and assess drug compliance. During the dosing period, diary symptoms should be assessed and temperature should be recorded at the same time that the study drug is taken.

End of Treatment Day 11

The end-of-treatment visit for all patients is on Day 11 (irrespective of whether they took one or two doses on *Day* 1). *Study medication will be administered twice daily over 10 days for a total of 20 doses.* Patients who discontinue study drug prematurely will have all Day 11 assessments completed at the time of discontinuation or the following day.

After all Day 11 study assessments are completed, a rapid diagnostic test will be performed at this visit. If the rapid diagnostic test is positive, the patient may be treated per standard of care at the discretion of the investigator. *If treatment is provided, this should be captured in the CRF.*

All patients (including those who discontinue study drug prematurely and those who are positive for influenza on their rapid diagnostic test at the end of treatment visit) will be required to return for follow up approximately 5 and 30 days after the last dose (Day 15 and Day 40 assessments).

Study Days 12 – 40

Study Day 15 is an important visit as it is the first assessment of viral culture after cessation of treatment. *It is therefore important that this visit is completed.*

End of Follow-Up Day 40

All patients must attend an end of follow-up visit on Day 40.

If the patient is withdrawn after completion of treatment (after the Day 11 assessment), a termination visit should be arranged. This visit should be the end of follow-up visit assessment [Day 40]. This visit must occur within 30 days of the last dose.

Patients or parents/guardians who do not return to the clinic will be contacted by telephone to ascertain their health status, or that of their child's, record information on adverse events, and to ask that they return the patient diary.

5.3.1 Safety

Safety is one of the primary end-points of this study. Safety parameters include adverse events, vital signs, and clinical laboratory evaluations.

Pre-defined symptoms of influenza captured in the adult and pediatric diaries are not to be reported as adverse events unless they can be further qualified. Thus 'headache due to stress at work' is reported as an adverse event. However, unexplained 'headache' is considered a predefined symptom related to influenza and not an adverse event.

Adverse events such as bronchitis, pneumonia, otitis media and sinusitis are considered secondary illnesses of influenza and should be recorded as adverse events.

Other adverse events to be expected in the transplant population such as rejection and graft versus host disease (in patients with HSCT) will also be collected as adverse events. *Nasal and throat swab samples will be assessed from each study visit for the presence of oseltamivir-resistant mutations.*

5.3.2 *Efficacy Assessments*

The clinical efficacy parameters in this study are:

1. *Symptoms of influenza-like illness. These are captured in the patient diary for both adults and children (Appendix 2) and (Appendix 3). Influenza symptoms are graded on a nominal scale from zero (absent/no problem) to 3 (severe or major problem). A score of zero is considered asymptomatic. The efficacy end point, time to alleviation of all symptoms is comprised of all the symptoms captured in the patient diary.*
2. *Temperature. This is captured in the patient diary. Temperature is used for assessment of several efficacy endpoints.*

5.4 LABORATORY ASSESSMENTS

The laboratory assessments include those for safety, *resistance, and efficacy.*

Safety

The safety laboratory assessments in this study, including the assessment of serum chemistry and hematology, will be carried out at a central laboratory. Serum chemistry assessments comprise AST, ALT, total bilirubin, urea and creatinine. Hematology assessments include CBC and differential count. The total volume of blood loss for laboratory assessments will be approximately 20 mL for the entire duration of the study.

Protection of patient confidentiality (Section 16) will extend to any data generated from the assay of these samples. Biological samples collected from all patients may be infectious and will be classified as "diagnostic specimens" for dispatch purposes.

When clinically indicated, the investigator may draw blood for serum creatinine to be assessed at the local laboratory during the study to calculate creatinine clearance.

Efficacy

The efficacy laboratory assessments are used for laboratory confirmation of influenza. These are:

Nasal and throat swabs. Two nasal and one throat swab will be collected as described in the Schedule of Assessments. All swabs are sent to a central laboratory for RT-PCR and viral cultures. Influenza virus shedding will be assessed.

At the end of treatment (Day 11), a rapid diagnostic test is permitted for the diagnosis of ongoing influenza.

5.5 PHARMACOKINETIC ASSESSMENTS /PHARMACODYNAMIC ASSESSMENTS

Participation in PK assessments is not compulsory for this study. Blood samples for the characterization of oseltamivir carboxylate pharmacokinetics using a sparse sampling strategy will be collected from all patients who provide additional consent to participate in the PK assessments.

Blood samples will be collected according to the Schedule of Assessments (Table 1) and as described below. If these blood samples are collected at a home visit, site staff should ensure the PK blood sample handling processing is not compromised. The time and date of the dose and blood samples should be captured. Further details on pharmacokinetics/pharmacodynamics can be found in Sections 8.3.1and 8.3.2.

5.5.1 Pharmacokinetic Assessments

Plasma PK samples for assessment of oseltamivir carboxylate will be collected at the following timepoints on Day 6, or any day after the 11th dose, using the following sampling approach:

- Within 30 minutes prior to the dose administration (e.g., 8:30 a.m. *dose 9:00 a.m.)*
- 1.5 hours ± 30 minutes post-dose (e.g., 10:30 a.m. ± 30 min)
- 4 hours ± 60 minutes post dose (e.g., 1:00 p.m. ± 60 min)
- 8 hours ± 1.5 hours post dose (e.g., 5:00 p.m. ± 1.5 hr)

For adults and adolescents, approximately 2 mL of blood will be collected at each timepoint; therefore, the total volume blood loss for PK assessments will be approximately 8 mL. For pediatric patients, not less than approximately 0.6 mL of blood will be tcollected at each timepoint; therefore, the total volume blood loss for PK assessments will be approximately *2.4* mL.

The samples from this study are classified as Biological Substance, Category B.

Plasma concentrations of oseltamivir carboxylate will be measured by a specific and validated method. Details on sampling procedures, sample storage, and shipment are provided in the Sampling Manual.

5.5.2 Pharmacodynamic Assessments

Nasal and throat swabs will be collected from individuals on the days specified in the schedule of assessments. Specimens will be analyzed at a central laboratory. *As immunocompromised patients are expected to shed virus for a longer duration, viral samples will be collected at all visits.* The proportion of patients with viral shedding at each visit will be summarized.

6. INVESTIGATIONAL MEDICINAL PRODUCT

The Investigational Medical Product in this study is oseltamivir dry powder (to be reconstituted to a concentration of 12 mg/mL) or 75 mg capsules and matching placebo.

The Investigational Medicinal Products will be supplied, packaged individually for each subject and labeled in accordance with Roche Standard and local regulation by Roche Clinical Trial Supply, Basel, Switzerland.

6.1 DOSE AND SCHEDULE OF STUDY DRUG

Oseltamivir will be given twice daily over 10 days for a total of 20 doses. The doses need to be taken at 12 hourly intervals. Under no circumstances is a patient allowed to take two doses within 7 hours of each other.

Patients will be randomized to receive either conventional or *double* dose of study drug.

Conventional dose:

Children ages 1 – 12 years: Oseltamivir syrup

≤ 15 kg	30 mg twice daily
> 15 – 23 kg	45 mg twice daily
> 23 – 40 kg	60 mg twice daily
> 40 kg	75 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules

75 mg twice daily

Patients randomized to the conventional dose will simultaneously receive matching placebo so as to blind them and the investigator from the *double* dose arm.

***Double* dose:**

Children ages 1 – 12 years: Oseltamivir syrup

≤ 15 kg	60 mg twice daily
> 15 – 23 kg	90 mg twice daily
> 23 – 40 kg	120 mg twice daily
> 40 kg	150 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules

150 mg twice daily

6.1.1 Dose Modifications

No dose modifications will be allowed on study.

6.2 PREPARATION AND ADMINISTRATION OF STUDY DRUG

Oseltamivir will be provided in two forms:

1. Oseltamivir capsules containing 75 mg of active drug and packaging material consisting of pregelatinized starch, povidone, talc and sodium stearyl fumarate. All participants 13 years and older will receive this dosage form. *Oseltamivir capsules should be stored between 2-25ºC.*

2. A pediatric suspension containing 12 mg oseltamivir per ml of reconstituted solution and the following excipients: sorbitol, titanium dioxide, sodium benzoate, xanthan gum, monosodium citrate, saccharin sodium and Permaseal11900-31 Tutti Frutti (flavor). All participants 12 years and under will receive this dosage form. *Oseltamivir dry powder for* oral *suspension [pediatric syrup] should be stored below 30°C [86°F]*). Once reconstituted, *the suspension should not be used for longer than 10 days* if stored *at* room temperature conditions (below 25°C*). If stored under refrigerated conditions at 2–8 °C (36–46 °F) the suspension should not be used for longer than* 17 days. The suspension is not suitable for freezing.

For further details, refer to the Tamiflu® Investigator's Brochure.

Matching placebo will be available as capsules and suspension. Patients in the conventional dose arm will get the conventional dose and matching placebo so that they are blinded from the *double* dose arm.

Each patient will be dispensed a medication pack that will provide enough medication to cover 20 doses. For patients randomized to the conventional dose arm, the medication pack will contain a bottle of oseltamivir dry powder or a blister wallet with oseltamivir capsules and matching placebo. For patients randomized to the *double* dose arm, the medication pack will contain two bottles of oseltamivir dry powder or two blister wallets with oseltamivir capsules. Irrespective of the treatment group the patient is randomized to, for each dose the patient will take the same amount from both bottles or blister wallets provided in the medication pack such that the sum of the amounts from each immediate container constitutes one dose.

One dose is to be administered twice per day at approximately 12-hour intervals with a light snack or glass of milk or fruit juice. The first dose of study *drug* will be administered in the clinic at the time of randomization.

6.3 BLINDING AND UNBLINDING

Randomization will be administered by a central randomization center.

The Randomization List will not be available at the study center, to the study monitors, project statisticians or to the project team at Roche. Emergency codes, or another adequate method of unblinding, will be implemented before study start, if the identity of the test medication is necessary for patient management in the case of a serious adverse event. Any request from the investigator for information about the treatment administered to study patients for another purpose must be discussed with Roche/designee.

As per regulatory reporting requirement, Roche/designee will unblind the identity of the study drug for all unexpected [as per IB] serious adverse events that are considered by the investigator to be related to study drug. Details of patients who are unblinded during the study will be included in the Clinical Study Report.

All other individuals directly involved in this study will remain blinded until the final analysis of the primary parameter.

The randomization will be stratified by transplant status, duration of symptoms, influenza vaccination status and age and will be provided by the Roche randomization group for the IVRS vendor.

6.4 ASSESSMENT OF COMPLIANCE

Accountability and patient compliance will be assessed by maintaining adequate “drug dispensing” and return records.

Patients will be asked to return all used and unused drug supply containers at the end of the treatment as a measure of compliance.

A Drug Dispensing Log must be kept current and should contain the following information:

- the identification of the patient [randomization and medication numbers] to whom the study drug was dispensed
- the date[s], quantity of the study drug dispensed to the patient
- the date[s] and quantity of the study drug returned by the patient

This inventory must be available for inspection by the Monitor. All supplies, including partially used or empty containers and the dispensing logs, must be returned to the monitor at the end of the study.

6.5 DESTRUCTION OF STUDY DRUG

Local or institutional regulations may require immediate destruction of used investigational product for safety reasons. In these cases, it may be acceptable for investigational site staff to destroy dispensed investigational product before a monitoring inspection provided that source document verification is performed on the remaining inventory and reconciled against the documentation of quantity shipped, dispensed, returned and destroyed. Written authorization must be obtained from the sponsor or designee at study start up before destruction.

Written documentation of destruction must contain the following:

- Identity [batch numbers or patient numbers] of investigational product[s] destroyed
- Quantity of investigational product[s] destroyed
- Date of destruction
- Method of destruction
- Name and signature of responsible person [or company] who destroyed investigational products[s]

7. SAFETY INSTRUCTIONS AND GUIDANCE

7.1 ADVERSE EVENTS AND LABORATORY ABNORMALITIES

7.1.1 Clinical AEs

Per the International Conference of Harmonization [ICH], an Adverse Event (AE) is any untoward medical occurrence in a patient or clinical investigation patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign [including an abnormal laboratory finding], symptom, or disease temporally associated with the use of a medicinal [investigational] product, whether or not considered related to the medicinal [investigational] product. Pre-existing conditions which worsen during a study are to be reported as AEs. Influenza signs and symptoms reported on the patient diary will be summarized as efficacy end points and need not be captured as adverse events. However, secondary illnesses due to influenza must be reported as adverse events.

7.1.1.1 Intensity

All clinical AEs encountered during the clinical study will be reported on the AE page of the CRF.

Intensity of AEs will be graded on a four -point scale [mild, moderate, severe, life-threatening] and reported in detail on the CRF.

Mild	discomfort noticed but no disruption of normal daily activity.
Moderate	discomfort sufficient to reduce or affect daily activity.
Severe	inability to work or perform normal daily activity
Life Threatening	represents an immediate threat to life

7.1.1.2 Drug - Adverse Event Relationship

Relationship of the AE to the treatment should always be assessed by the investigator. Description of scales can be found in Appendix 4.

7.1.1.3 Serious Adverse Events [Immediately Reportable to Roche]

Certain events require immediate reporting to allow the Sponsor to take appropriate measures to address potential new risks in a clinical trial. The investigator must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours after the investigator learns of the event. The following is a list of events that the investigator must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study drug:

- Serious adverse events
- Pregnancies

A Serious Adverse Event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that at any dose fulfils at least one of the following criteria:

- is fatal; [results in death; NOTE: death is an outcome, not an event].
- is Life-Threatening [NOTE: the term "Life-Threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe].
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above.

The full requirements of the ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 will be adhered to (Appendix 5).

7.1.2 Treatment and Follow-up of AEs

AEs, especially those for which the relationship to test “drug” is not “unrelated”, should be followed up until they have returned to baseline status or stabilized. If a clear explanation is established it should be recorded on the CRF.

7.1.3 Laboratory Test Abnormalities

Laboratory test results will appear printed on laboratory reports provided to the site from the central laboratory.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event [SAE] should be reported as such, in addition to being recorded as an AE in the CRF.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the AE page in the CRF:

- Accompanied by clinical symptoms.
- Leading to a change in study drug [e.g. dose modification, interruption or permanent discontinuation].
- Requiring a change in concomitant therapy [e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment].

7.1.4 Follow-up of Abnormal Laboratory Test Values

In the event of medically significant unexplained abnormal laboratory test values, the tests should be repeated and followed up until they have returned to the normal range and/or an adequate explanation of the abnormality is found. If a clear explanation is established it should be recorded on the CRF.

7.2 HANDLING OF SAFETY PARAMETERS

7.2.1 Reporting of Serious Adverse Events [immediately reportable]

Any clinical AE or abnormal laboratory test value that is serious [as defined in Section 7.1.1.3 above] and which occurs during the course of the study, regardless of the treatment arm, occurring from the enrollment visit (start of study screening procedures), must be reported to Roche or designee immediately (i.e., no more than 24 hours after the investigator becomes aware of the event [expedited reporting]).

Related Serious Adverse Events ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed.

Unrelated Serious Adverse Events must be collected and reported during the study and up until the follow-up visit.

The definition and reporting requirements of ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 will be adhered to (Appendix 5). *The serious adverse events should be reported in the most up -to-date version of the SAE form.*

7.2.2 Pregnancy

A female patient must be instructed to stop taking the test "drug" and immediately inform the investigator if she becomes pregnant during the study. The investigator should report all pregnancies immediately (i.e., no more than 24 hours after the investigator becomes aware of the pregnancy) to the Sponsor or designee*, using the most up-to-date version of the pregnancy form*. The investigator should counsel the patient, and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Pregnancies occurring within 28 days of treatment completion should be reported to Roche.

7.3 WARNINGS AND PRECAUTIONS

Events such as convulsions and delirium (including symptoms such as altered level of consciousness, confusion, abnormal behavior, delusions, hallucinations, agitation, anxiety, nightmares) have been reported during oseltamivir use in patients with influenza, predominately in children and adolescents. In rare cases, these events resulted in accidental injury. The contribution of oseltamivir to those events is unknown. Such events have also been reported in patients with influenza who were not taking

oseltamivir. Patients, especially children and adolescents, should be closely monitored for signs of abnormal behavior.

Please refer to the attached Investigator's Brochure for additional warnings, precautions, and other reported adverse events.

8. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

Full details of all planned analyses will be specified in a separate Statistical Analysis Plan (SAP) for the safety and efficacy variables and in the Resistance Plan for the development of resistance. The methods described below are an outline of the main planned analyses. *For continuous endpoints summary statistics including mean, standard deviation, median, minimum, and maximum will be derived for each treatment group. For categorical data, the number and percentage of events and/or patients for each treatment group will be presented.*

8.1 PRIMARY AND SECONDARY STUDY ENDPOINTS

8.1.1 Primary Endpoints

The primary endpoints for safety will be *descriptive* assessments of:

- Adverse events
- Physical exam*ination*~~s~~
- Vital signs
- Clinical laboratory evaluations
- *Tissue rejection and/or graft versus host disease in transplant patients*

The primary endpoint of development of resistance will be determined from the genotypic and phenotypic variables measured post baseline and is described further in the Resistance Plan.

8.1.2 Secondary Endpoints

The following endpoints will be analyzed descriptively at relevant timepoints:

- Shedding virus by culture *and RT-PCR*
- Viral load by culture ($log_{10}TCID_{50}$/mL) *and quantitative RT-PCR*
- *Individual symptom scores*
- The time (hours) from first dose of study drug until resolution of fever
- Development of secondary illnesses (otitis media, bronchitis, pneumonia, or sinusitis) at any time during the study
- Development of secondary illnesses (otitis media, bronchitis, pneumonia, or sinusitis) at any time during the study that are treated with antibiotics
- Initiation of treatment with antibiotics after randomization

- Hospitalization, and for those who are hospitalized, the duration of hospitalization
- Development of rejection or GVHD

The following are the model-predicted PK secondary endpoints for oseltamivir carboxylate:

- Steady-state area under the concentration–time curve from 0 to 12 hours (AUC_{0-12})
- Maximum plasma concentration (C_{max}) for oseltamivir carboxylate
- Trough plasma concentration (C_{trough}) for oseltamivir carboxylate

The following model-predicted PK endpoints may be included, if appropriate:

- Elimination half-life ($t_{1/2}$)
- Time to maximum concentration (t_{max})
- Elimination constant (k_e)
- Apparent clearance (CL/F)
- Apparent volume of distribution (Vc/F)
- Apparent total clearance of metabolite (CL_m)
- Last measurable concentration (C_{last}) and time to last measurable concentration (t_{last})

8.2 STATISTICAL AND ANALYTICAL METHODS

8.2.1 Statistical Model

8.2.1.1 Time to Event Variables

A non-parametric model will be assumed with estimation of medians based on Kaplan-Meier methods. Patients without alleviation of symptoms will have their time censored at the last available observation that a complete assessment was made.

8.2.1.2 Dichotomous Variables and Viral Load

For the endpoints defined dichotomously in terms of events or symptoms including the development of resistance, it will be assumed that the number of patients experiencing the event or exhibiting the symptom will have a binomial distribution.

For the continuous endpoints of viral load at Day 1, 2, 6, 8, 11, 15, and 40 no model will be assumed.

8.2.2 Sample Size

The sample size has been chosen to provide an adequate number of patients to estimate the development of resistance with reasonable precision. Assuming that 90% of enrolled patients will have laboratory-confirmed influenza, there will be 75 patients in each treatment arm in the population evaluable for the development of resistance. The following table shows the 95% Pearson-Clopper confidence intervals that would result with a sample size of 75 patients in a treatment arm if certain event rates are observed in the study.

During the study the number of influenza A virus infected patients and the rate of development resistance will be monitored in a blinded fashion, in order to ensure a reasonable precision for the estimation. An additional number of patients may be enrolled if necessary.

Observed Rate (%)	95% Confidence Interval
0.0	0% - 4.8%
1.3	0.0% - 7.2%
2.7	0.3% - 9.3%
5.3	1.5% - 13.1%
10.7	4.7%-19.9%

A total of 83 patients will be enrolled per arm and will be evaluable for the assessment of safety. This number of patients would provide estimates of adverse event rates with similar precision.

8.2.3 Hypothesis Testing

Formal hypothesis testing will be not performed; *any comparisons between groups* will be based on comparison of confidence intervals.

8.2.4 Analysis Populations

Three main patient populations will be used for the analysis of data from this study; the Safety Population, the Intent-to treat Population and the Intent-to-Treat Infected Population. Detailed definitions of these populations will be given in the SAP.

8.2.4.1 Intent to Treat Population

All patients randomized will be included in the intent to treat population [Patients will be assigned to treatment groups as randomized for analysis purposes].

8.2.4.2 Intent to Treat Infected Population

All patients randomized and with laboratory confirmation of influenza infection, excluding patients infected with oseltamivir-resistant influenza at baseline, will be included in the intent to treat influenza infected population [Patients will be assigned to treatment groups as randomized for analysis purposes].

The ITTI Population will be the primary population for the summary and analysis of the development of resistance and the efficacy variables.

8.2.4.3 Pharmacokinetic Evaluable Patient Population

The Pharmacokinetic evaluable patient population (PKEP) population comprises all patients in the ITT population who have at least one post-dose drug concentration measurement at a scheduled visit timepoint. Patients may be excluded from the PKEP population if they significantly violate the inclusion or exclusion criteria, deviate significantly from the protocol, or have unavailable or incomplete data that may influence the PK analysis.

Decisions on patient exclusion from the PK analysis will be made prior to database closure by the clinical pharmacologist. Excluded patients will be documented along with the reason for exclusion.

8.2.4.4 Subpopulations

A subpopulation of the ITTI population will be defined comprising patients who received their first dose of study drug within 48 hours of influenza symptom onset for the comparison of efficacy endpoints with the age appropriate placebo treated patients (pediatric or adult) from the registration trials.

8.2.5 Safety Data Analysis

The safety analysis population will include all patients who receive at least one dose of study drug and had a safety assessment performed post randomization. All safety variables will be summarized and presented in tables based on this safety population:

- *Adverse events*
- *Physical examination*
- *Vital signs*
- *Clinical laboratory evaluations*
- *Rejection and/or graft versus host disease*

8.2.6 Analysis of Resistance

For the summary of the development of resistance for each treatment arm, 95% confidence intervals will be provided with the estimated rates. Further details are available in the Resistance Plan for NV20234. These data will be summarized in a report separate from the final study report.

8.2.7 *Efficacy Analysis*

For the analysis of time to alleviation of all clinical symptoms, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- *Comparison to placebo control from pivotal registration trials*

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo-treated patients will be established that is comparable to the subpopulation of patients in the current study whose first dose of study drug was within 48 hours of symptom onset. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

  The median time to resolution of all influenza symptoms will be determined for each treatment group, along with its 95% confidence interval.

- *The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval.*

For the dichotomous endpoints, the proportion of patients experiencing the event or symptom, and its associated 95% confidence interval will be derived for each treatment group. Where possible, the corresponding confidence intervals from the placebo control population will be presented for comparison to those derived for the active treatment groups in this trial.

The continuous secondary endpoint time to resolution of fever will be analyzed as for the time to alleviation of all symptoms.

8.2.7.1 Exclusion of Data from Analysis

Efficacy data collected from any patient who begins treatment with another antiviral for influenza (for example, amantadine, rimantadine, laninamivir, peramivir, or zanamavir), after the other antiviral treatment commences, will be excluded from the primary and secondary efficacy analyses. Such patients will be considered failures to oseltamivir treatment in the analyses. Further details will be provided in the SAP.

8.2.7.2 Interim Analysis

No interim analyses are planned.

8.2.8 Other Analyses

Further exploratory analyses will be detailed in the SAP.

8.3 PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSIS

8.3.1 Pharmacokinetic Analysis

The primary study variables are the model-*derived* PK parameters: steady-state AUC, C_{max}, and C_{trough} of oseltamivir carboxylate.

Individual and mean plasma concentrations at each sampling timepoint for oseltamivir carboxylate will be presented by listings and descriptive summary statistics, including means, geometric means, medians, ranges, standard deviations, and coefficients of variation. Individual and mean concentration-versus-time profiles will be plotted on linear and semi-logarithmic scales.

Plasma concentration data from sparse sampling will be analyzed using an established population PK model to determine key exposure parameters (e.g., C_{max}, C_{trough}, and AUC). For immunocompromised children aged 1 to 18 years, plasma oseltamivir carboxylate concentration will be modeled in NONMEM using a structure similar to a comprehensive population PK model, which was previously developed using plasma data from non-immunocompromised children and adults (ages 1 to 80 years) [36]. The basic structure consists of a 2-compartment model with first-order absorption and direct conversion of oseltamivir to oseltamivir carboxylate, while a 1-compartment model is used to account for the renal elimination of oseltamivir carboxylate from the plasma. Body weight, evaluated using a power function and centered around 70 kg, is a statistically significant predictor of both CL/F and central volume of distribution (Vc/F) for oseltamivir carboxylate. For oseltamivir carboxylate, CrCl is also a significant predictor of CL/F, while Vc/F decreases linearly with age.

8.3.2 *Pharmacokinetic*/Pharmacodynamic Analysis

If feasible, *exposure-response relationships [43]will be evaluated between independent variables of exposure (e.g., AUC, C_{min}) and dependent variables including continuous (area under the viral titer curve, and peak viral titer) and time-to-event (cessation of viral shedding) virologic endpoints. Exploratory PK/PD analyses will be performed using appropriate methodologies. In order to account for potential non-linearity and non-monotonicity, each continuous independent variable (e.g., AUC) will be evaluated in its original form, as a categorical variable based on quartiles, as two- and three-group categorical variables.*

9. DATA QUALITY ASSURANCE

The overall procedures for quality assurance of clinical study data are described in the Roche Standard Operational Procedures.

Accurate and reliable data collection will be assured by verification and cross–check of the CRFs against the investigator's records by the study monitor [source document verification], and the maintenance of a drug–dispensing log by the investigator.

The data collected will be entered into the study database from the working copy of the CRF faxed from the site.

A comprehensive validation check program will verify the data and discrepancy reports will be generated accordingly for resolution by the investigator. As patients complete the study [or prematurely withdraw] and their signed CRFs become available, a second data entry will be performed from the original, signed CRF. A comparison check will be run to identify and resolve any discrepancies between the first and second data entry.

Throughout the study the Study Management Team [SMT] will review data according to the SMT Data Review Plan as described in the Data Quality Plan.

9.1 ASSIGNMENT OF PREFERRED TERMS AND ORIGINAL TERMINOLOGY

For classification purposes, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using the current version of MedDRA (Medical Dictionary for Regulatory Activities terminology) for adverse events and diseases and the INN (International Non-Proprietary name) drug terms and procedures dictionary for treatments and surgical and medical procedures.

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PART II: ETHICS AND GENERAL STUDY ADMINISTRATION

11. ETHICAL ASPECTS

11.1 LOCAL REGULATIONS/DECLARATION OF HELSINKI

The investigator will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study must fully adhere to the principles outlined in "Guideline for Good Clinical Practice" ICH Tripartite Guideline [January 1997] or with local law if it affords greater protection to the patient. For studies conducted in the European Union/European Economic Areacountries, the investigator will ensure compliance with the EU Clinical Trial Directive [2001/20/EC]. For studies conducted in the United States or under US IND, the investigator will additionally ensure that the basic principles of "Good Clinical Practice" as outlined in the current version of 21 CFR, subchapter D, part 312, "Responsibilities of Sponsors and Investigators", part 50, "Protection of Human Subjects", and part 56, "Institutional Review Boards", are adhered to.

In other countries where "Guideline for Good Clinical Practice" exist Roche and the investigators will strictly ensure adherence to the stated provisions.

11.2 INFORMED CONSENT

It is the responsibility of the investigator, or a person designated by the investigator [if acceptable by local regulations], to obtain written informed consent from each patient participating in this study, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. For patients not qualified or incapable of giving legal consent, written consent must be obtained from the legally acceptable representative. In the case where both the patient and his/her legally acceptable representative are unable to read, an impartial witness should be present during the entire informed consent discussion. After the patient and representative have orally consented to participation in the trial, the witness' signature on the form will attest that the information in the consent form was accurately explained and understood. The investigator or designee must also explain that the patients are completely free to refuse to enter the study or to withdraw from it at any time, for any reason. The Case Report Forms [CRFs] for this study contain a section for documenting informed patient consent, and this must be completed appropriately. If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All patients [including those already being treated] should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

If children are old enough to understand the risks and benefits of the study, they should also be informed and should also provide their written consent.

11.3 INDEPENDENT ETHICS COMMITTEES/INSTITUTIONAL REVIEW BOARD

Independent Ethics Committees [non-US]: This protocol and any accompanying material provided to the patient [such as patient information sheets or descriptions of the study used to obtain informed consent] as well as any advertising or compensation given to the patient, will be submitted by the investigator to an Independent Ethics Committee. Approval from the committee must be obtained before starting the study, and should be documented in a letter to the investigator specifying the date on which the committee met and granted the approval.

Any modifications made to the protocol after receipt of the Independent Ethics Committee approval must also be submitted by the investigator to the Committee in accordance with local procedures and regulatory requirements.

When no local review board exists, the investigator is expected to submit the protocol to a regional committee. If no regional committee exists, Roche will assist the investigator in submitting the protocol to the European Ethics Review Committee.

Institutional Review Board [US]: It is the understanding of the sponsor that this protocol [and any modifications] as well as appropriate consent procedures, will be reviewed and approved by an Institutional Review Board. This board must operate in accordance with the current Federal Regulations. A letter or certificate of approval will be sent by the investigator to the Sponsor or designee prior to initiation of the study, and also whenever subsequent modifications to the protocol are made.

12. CONDITIONS FOR MODIFYING THE PROTOCOL

Protocol modifications to ongoing studies must be made only after consultation between an appropriate representative of the sponsor and the investigator [investigator representative[s] in the case of a multicenter trial]. Protocol modifications must be prepared by a representative of the sponsor and initially reviewed and approved by the Clinical Science Leader/Clinical Pharmacologist and Biostatistician.

All protocol modifications must be submitted to the appropriate Independent Ethics Committee or Institutional Review Board for information and approval in accordance with local requirements, and to Regulatory Agencies if required. Approval must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial patients, or when the change[s] involves only logistical or administrative aspects of the trial [e.g. change in monitor[s], change of telephone number[s].

13. CONDITIONS FOR TERMINATING THE STUDY

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange the procedures on an individual study

basis after review and consultation. In terminating the study, Roche and the investigator will assure that adequate consideration is given to the protection of the patient's interests.

14. STUDY DOCUMENTATION, CRFS AND RECORD KEEPING

14.1 INVESTIGATOR'S FILES / RETENTION OF DOCUMENTS

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two different separate categories [1] Investigator's Study File, and [2] patient clinical source documents.

The Investigator's Study File will contain the protocol/amendments, Case Report and Query Forms, Independent Ethics Committee/Institutional Review Board and governmental approval with correspondence, sample informed consent, drug records, staff curriculum vitae and authorization forms and other appropriate documents/correspondence etc.

Patient clinical source documents [usually defined by the project in advance to record key efficacy/safety parameters independent of the CRFs] would include patient hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, EEG, X-ray, pathology and special assessment reports, signed informed consent forms, consultant letters, and patient screening and enrollment logs. The Investigator must keep these two categories of documents on file for at least 15 years after completion or discontinuation of the study. After that period of time the documents may be destroyed, subject to local regulations.

Should the Investigator wish to assign the study records to another party or move them to another location, Roche must be notified in advance.

If the Investigator cannot guarantee this archiving requirement at the investigational site for any or all of the documents, special arrangements must be made between the Investigator and Roche to store these in a sealed container[s] outside of the site so that they can be returned sealed to the Investigator in case of a regulatory audit. Where source documents are required for the continued care of the patient, appropriate copies should be made for storing outside of the site.

14.2 SOURCE DOCUMENTS AND BACKGROUND DATA

The investigator shall supply the sponsor or designee on request with any required background data from the study documentation or clinic records. This is particularly important when CRFs are illegible or when errors in data transcription are suspected. In case of special problems and/or governmental queries or requests for audit inspections, it is also necessary to have access to the complete study records, provided that patient confidentiality is protected.

14.3 AUDITS AND INSPECTIONS

The investigator should understand that source documents for this trial should be made available to appropriately qualified personnel from the Roche Pharma Development Quality Assurance Unit or its designees, or to health authority inspectors after appropriate notification. The verification of the CRF data must be by direct inspection of source documents.

14.4 CASE REPORT FORMS

For each patient enrolled, a CRF must be completed and signed by the principal investigator or authorized delegate from the study staff. This also applies to records for those patients who fail to complete the study [even during a pre-randomization screening period if a CRF was initiated]. If a patient withdraws from the study, the reason must be noted on the CRF. If a patient is withdrawn from the study because of a treatment-limiting AE, thorough efforts should be made to clearly document the outcome.

All forms should be typed or filled out using indelible ink, and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or his/her authorized delegate. The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor or designee in the CRFs and in all required reports.

15. MONITORING THE STUDY

It is understood that the responsible Roche monitor [or designee] will contact and visit the investigator regularly and will be allowed, on request, to inspect the various records of the trial [CRFs and other pertinent data] provided that patient confidentiality is maintained in accord with local requirements.

It will be the monitor's responsibility to inspect the CRFs at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitor should have access to laboratory test reports and other patient records needed to verify the entries on the CRF. The investigator [or his/her deputy] agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

16. CONFIDENTIALITY OF TRIAL DOCUMENTS AND PATIENT RECORDS

The investigator must assure that patients' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents submitted to the sponsor or designee, patients should not be identified by their names, but by an identification code. The investigator should keep a patient enrollment log showing codes, names and addresses. The investigator should maintain documents not for submission to Roche, e.g., patients' written consent forms, in strict confidence.

17. PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to Roche prior to submission. This allows the sponsor or designee to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the investigator.

In accord with standard editorial and ethical practice, Roche will generally support publication of multicenter trials only in their entirety and not as individual center data. In this case, a coordinating investigator will be designated by mutual agreement.

Any formal publication of the study in which input of Roche personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate Roche personnel. Authorship will be determined by mutual agreement.

Appendix 1 Calculation of Creatinine Clearance and Grading of Chronic Extensive GVHD

Calculation of Creatinine Clearance

Conversion Factor for Serum Creatinine:
Conventional Units (mg/dL) = SI Units (μmol/L) ÷ 88.4
Age = number of years (12 years 11 months = 12 years)

Estimated Creatinine Clearance according to Cockcroft-Gault [13]

(for patients ≥ 18 years):

- **Males**

Creatinine Clearance (mL/min) = **[(140 − age) × Body Weight (kg)] ÷ [72 × Serum Creatinine (mg/dL)]**

- **Females**

Creatinine Clearance = above equation × 0.85

Estimated Creatinine Clearance according to Schwartz equation [14]
(for patients < 18 years):

Creatinine Clearance
(mL/min/1.73 M^2) = k × Height (cms) ÷ **Serum Creatinine** (mg/dL)

Value for k:

k	Age (years)
0.45	< 2
0.55	≥ 2 to < 13
0.7	≥ 13 to < 18 (males)
0.55	**≥ 13 to < 18 (females)**

Appendix 1 Calculation of Creatinine Clearance and Grading of Chronic Extensive GVHD (cont.)

Grading of Chronic GVHD [15]

Type of Disease	Extent of Disease
Limited	Localized skin involvement, liver dysfunction or both
Extensive	Generalized skin involvement
	Localized skin involvement or liver dysfunction plus any one of the following: 1. Chronic aggressive hepatitis, bridging necrosis or cirrhosis 2. Eye involvement (Schirmer's test, $<$5 mm) 3. Involvement of mucosalivary glands 4. Mucosal involvement (on lip biopsy) 5. Involvement of other target organs

Appendix 2 Patient Diary Data and Symptom Record *for Adults*

The purpose of the *patient* diary is for all adults (13 years and older) to record any symptoms of influenza-like illness for the duration of the study. Temperature and date and time of drug administration *should* also be recorded on the patient diary.

Date of assessments ⌊ ⌋⌊ ⌋⌊ ⌋ (dd mm yy) Time of assessments (24 hr clock) ⌊ ⌋⌊ ⌋ (h min)

Vital signs

Temperature ⌊ ⌋■⌊ ⌋ °C

Symptoms of influenza-like illness

Please record the worst you have felt since the last assessment.
Please mark one box only per symptom.
The information you provide is very important and will remain strictly confidential.

	absent 0	mild 1	moderate 2	severe 3
1. Nasal congestion	☐	☐	☐	☐
2. Sore throat	☐	☐	☐	☐
3. Cough	☐	☐	☐	☐
4. Aches and pains	☐	☐	☐	☐
5. Fatigue (tiredness)	☐	☐	☐	☐
6. Headache	☐	☐	☐	☐
7. Chills/sweats (feverish)	☐	☐	☐	☐

Please inform study staff at your next visit of any medications that you have taken today or any medical problems that you have experienced.

Appendix 3 *Patient* Diary Data *and Symptom Record* for Children

Temperature and date and time of drug administration will also be recorded on the patient diary.

Study medication administration (if applicable)

Date of dose	Time of dose (24 hr clock)	Bottle A amount mg	Bottle B amount mg
dd mm yy	h : min		

Date of assessments dd mm yy

Time of assessments (24 hr clock) h : min

Vital signs

Temperature ▪ °C

Symptoms of influenza-like illness

Please record the worst you have felt since the last assessment.
Please mark one box only per symptom.
The information you provide is very important and will remain strictly confidential.

	No Problem 0	Minor Problem 1	Moderate Problem 2	Major Problem 3	Don't Know or not Applicable 4
1. Poor appetite	☐	☐	☐	☐	☐
2. Not sleeping well	☐	☐	☐	☐	☐
3. Irritable, cranky, fussy	☐	☐	☐	☐	☐
4. Feels unwell	☐	☐	☐	☐	☐
5. Low energy, tired	☐	☐	☐	☐	☐
6. Not playing well	☐	☐	☐	☐	☐
7. Crying more than usual	☐	☐	☐	☐	☐
8. Needing extra care	☐	☐	☐	☐	☐
9. Clinginess	☐	☐	☐	☐	☐
10. Headache	☐	☐	☐	☐	☐
11. Sore throat	☐	☐	☐	☐	☐
12. Muscle aches or pains	☐	☐	☐	☐	☐
13. Fever	☐	☐	☐	☐	☐
14. Cough	☐	☐	☐	☐	☐
15. Nasal congestion, runny nose	☐	☐	☐	☐	☐
16. Vomiting	☐	☐	☐	☐	☐
17. Not interested in what's going on	☐	☐	☐	☐	☐
18. Unable to get out of bed	☐	☐	☐	☐	☐

This form was filled out by:

1 ☐ Parent
2 ☐ Other relative
3 ☐ Nanny
4 ☐ Subject
5 ☐ Other *specify*_____

Please inform study staff at your/your child's next visit of any medications that you/your child have taken today or any medical problems that you/your child have experienced.

Appendix 4 Adverse Event [AE] Categories for Determining Relationship to Test Drug

PROBABLE [must have first three]

This category applies to those AEs which are considered, with a high degree of certainty, to be related to the test drug. An AE may be considered probable, if:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It cannot be reasonably explained by the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
3. It disappears or decreases on cessation or reduction in dose. [There are important exceptions when an AE does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists; e.g., [1] bone marrow depression, [2] tardive dyskinesias].
4. It follows a known pattern of response to the suspected drug.
5. It reappears upon rechallenge.

POSSIBLE [must have first two]

1. This category applies to those AEs in which the connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possible if, or when:
2. It follows a reasonable temporal sequence from administration of the drug.
3. It may have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
4. It follows a known pattern of response to the suspected drug.

REMOTE [must have first two]

1. In general, this category is applicable to an AE which meets the following criteria:
2. It does <u>not</u> follow a reasonable temporal sequence from administration of the drug.
3. It may readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
4. It does not follow a known pattern of response to the suspected drug.
5. It does not reappear or worsen when the drug is readministered.

UNRELATED

This category is applicable to those AEs which are judged to be clearly and incontrovertibly due only to extraneous causes [disease, environment, etc.] and do not meet the criteria for drug relationship listed under remote, possible, or probable.

Appendix 4 Adverse Event [AE] Categories for Determining Relationship to Test Drug (cont.)

	Probable	Possible	Remote	Unrelated
Clearly due to extraneous causes	–	–	–	+
Reasonable temporal association with drug administration	+	+	–	–
May be produced by patient clinical state, etc.	–	+	+	+
Known response pattern to suspected drug	+	+	–	–
Disappears or decreases on cessation or reduction in dose	+	–	–	–
Reappears on rechallenge	+	–	–	–

Appendix 5 ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that at any dose fulfills at least one of the following criteria:

- is fatal; [results in death] [NOTE: death is an outcome, not an event].
- is Life-Threatening [NOTE: the term "Life-Threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe].
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above.

Medical and scientific judgment should be exercised in deciding whether expedited reporting to the sponsor or designee is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definitions above. These situations should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

An unexpected AE is one, the nature or severity of which is not consistent with the applicable product information.

Causality is initially assessed by the investigator. For Serious Adverse Events, possible causes of the event is indicated by selecting one or more options. (Check all that apply)

- Pre-existing/Underlying disease - specify
- Study treatment – specify the drug(s) related to the event
- Other treatment (concomitant or previous) – specify
- Protocol-related procedure
- Other (e.g. accident, new or intercurrent illness) - specify

The term severe is a measure of intensity, thus a severe AE is not necessarily serious. For example, nausea of several hours' duration may be rated as severe, but may not be clinically serious.

Appendix 5 ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 (cont.)

A serious adverse event occurring during the study or which comes to the attention of the investigator within 15 days after stopping the treatment or during the protocol-defined follow-up period, if this is longer, whether considered treatment-related or not, must be reported. In addition, a serious adverse event that occurs after this time, if considered related to test "drug", should be reported.

Such preliminary reports will be followed by detailed descriptions later which will include copies of hospital case reports, autopsy reports and other documents when requested and applicable.

For serious adverse events, the following must be assessed and recorded on the AEs page of the CRF: intensity, relationship to test substance, action taken, and outcome to date.

The investigator must notify the Ethics Review Committee/Institutional Review Board of a serious adverse event in writing as soon as is practical and in accordance with international and local laws and regulations.

ROCHE LOCAL COUNTRY CONTACT for SAEs: Local Monitor

ROCHE HEADQUARTERS CONTACT for SAEs: Clinical Operations/Clinical Science

To ensure the safety of study patients, an Emergency Medical Call Center Help Desk will access the Roche Medical Emergency List, escalate emergency medical calls, provide medical translation service (if necessary), connect the investigator with a Roche Medical Monitor, and track all calls. The Emergency Medical Call Center Help Desk will be available 24 hours per day, 7 days per week. Toll-free numbers for the Help Desk and Medical Monitor contact information will be distributed to all investigators.

Appendix 6 Primary Immunodeficiency Conditions

Category	Conditions
Severe Combined Immunodeficiency (SCID)	Adenosine deaminse (ADA) deficiency
	Artemis deficiency (SCIDA)
	CD45 deficiency
	Cernunnos deficiency
	DNA ligase IV (LIG4) deficiency
	Interleukin receptor γ chain deficiency (X-linked SCID)
	Janus-associated kinase 3 (JAK3) deficiency
	Recombinase activating gene (RAG 1 / 2) deficiency
	Reticular dysgenesis
	TAP- 1 or TAP- 2 deficiency (MHC class I deficiency)
Primary T cell Deficiency	CD8 deficiency
	diGeorge syndrome
	Interleukin 7 receptor α (IL7RA) deficiency
	MHC class II deficiency
	LCK deficiency
	Orai 1 deficiency
	Nude syndrome (wing helix nude deficiency)
	Purine nucleotide phosphorylase (PNP) deficiency
	T cell receptor deficiency (CD 3γ, δ, ε, and ζ deficiencies)
	Zap 70 tyrosine kinase deficiency
Predominantly Antibody Deficiency	X-Linked CD40 ligand deficiency
	X- Linked IKK-γ (NEMO) deficiency
	CD40 deficiency
Other Well-Defined immunodeficiency Syndromes	Interferon γ receptor deficiency
	X-Linked lymphoproliferative syndrome

Adapted from Table 310-2: p2056 reference 16.

Appendix 7 Hematologic Malignancies and their Effect on the Immune System

Malignancy	Effect on immunity
ALL, lymphomas	suppression of hematopoiesis, neutropenia, lymphocyte dysfunction
CLL, small lymphocytic lymphoma	hypogammaglobulinemia, increased susceptibility to infections, autoimmune anemia or thrombocytopenia
Hairy cell leukemia, myelodysplastic syndromes	pancytopenia
peripheral T cell and NK neoplasms	lymphocyte dysfunction, increase in immature cells
Hodgkin's disease	suppression of cell-mediated immunity
AML	neutropenia, anemia, thrombocytopenia, predisposed to infections, lymphocyte dysfunction
CML	anemia, granulocyte dysfunction in some patients in early phase and in most patients in blast phase

Adapted from reference 16.

Appendix 8 Immunosuppressive Medications

Class	Category	Drugs
Corticosteroids	-	Glucocorticoids (oral, sc, im, iv)
Cytotoxic agents[16]	Alkylating agents	cyclophosphamide, busulfan, mechlorethamine, chlorambucil, melphalan, carmustine, lomustine, ifosfamide, procarbazine, dacarbazine, temozolomide, cisplatin, carboplatin, oxaliplatin
	Anti-metabolites	methotrexate, 6-mercaptopurine, azathioprine
	Anti-tumor antibiotics	bleomycin, actinomycin D, mitomycin C, etoposide, topotecan, irinotecan, doxorubicin, daunorubicin, idarubicin, epirubicin, mitoxantrone
	Anti-mitotic agents	vincristine, vinblastine, vinorelbine, paclitaxel, docetaxel, estramustine phosphate, NAB-paclitaxel
	Molecularly targeted agents	imatinib, tretinoin, bexarotene, denileukin, diftitox, gefitinib, erlotinib, dasatinib, sorafenib, sunitinib
Calcineurin inhibitors	-	cyclosporine, tacrolimus
mTOR inhibitors (proliferation-signal inhibitors)	-	sirolimus, everolimus
Immunosuppressive antibodies	-	anti lymphocyte and antithymocyte globulins (ALG and ATG)
Monoclonal antibodies [38, 39]	Inhibitors of pro inflammatory cytokines	Adalimumab
		Infliximab
		Cetrolizumab
		Etarnecept
		Basiliximab
		Daclizumab
	Adhesion cell modulators	Natalizumab
	T-cell inhibitors	Abatacept
		Alefacept
		Muromonab
	B-cell inhibitors	Rituximab
		^{90}Y-Ibritumomab
		^{131}I-Tositumomab
	Anti-CD33	Gemtuzumab
	Anti- CD52	Alemtuzumab
Others	-	mycophenolate mofetil, thalidomide

Compiled from Table 81-2: p521-24 reference 16 and references 38, 39.

Appendix 9 *Child-Pugh Classification of Severity of Liver Disease*

Child–Pugh Scoring on Select Parameters

	Points Scored for Increasing Abnormality		
Clinical and biochemical measurements	***1***	***2***	***3***
Encephalopathy (grade) [a]	*None*	*1 or 2*	*3 or 4*
Ascites [b]	*Absent*	*Slight*	*Moderate*
Bilirubin (mg/100 mL)	*< 2*	*2–3*	*> 3*
Albumin (g/100 mL)	*> 3.5*	*2.8–3.5*	*< 2.8*
Prothrombin time (International Normalized Ratio) [c]	*< 1.7*	*1.7–2.3*	*> 2.3*

Note: With increasing abnormality of each of the five parameters measured, 1, 2, or 3 points are scored. Grade A: 5 or 6 points. Grade B: 7–9 points. Grade C: 10–15 points.

[a] *According to grading of Trey, Burns, and Saunders (1966).*

[b] *As determined by physical examination alone.*

[c] *Prothrombin time results should be reported and used for calculations only as International Normalized Ratios because of variations in the methods used and reference ranges for controls (expressed in seconds).*

PROTOCOL

TITLE:	**A DOUBLE BLIND, RANDOMIZED, STRATIFIED, MULTI-CENTER TRIAL EVALUATING CONVENTIONAL AND DOUBLE DOSE OSELTAMIVIR IN THE TREATMENT OF IMMUNOCOMPROMISED PATIENTS WITH INFLUENZA**
PROTOCOL NUMBER:	NV20234
VERSION NUMBER:	F
EUDRACT NUMBER:	2006-002468-24
IND NUMBER:	53,093
TEST PRODUCT:	Oseltamivir (Tamiflu® RO 64-0796)
MEDICAL MONITOR:	[redacted], M.D.
SPONSOR:	F. Hoffmann-La Roche Ltd
DATE FINAL:	Version A: 14 June 2007
DATES AMENDED:	Version B: 31 July 2008 Version C: 28 March 2011 Version D: 28 September 2012 Version E: 30 October 2013 Version F: See electronic stamp below.

Approver's Name	Title	Date and Time (UTC)
[redacted]	Clinical Science Leader	18-Jun-2014 10:08:43

PROTOCOL AMENDMENT APPROVAL

PROTOCOL AMENDMENT, VERSION F
RATIONALE

Protocol NV20234 has been amended to reintroduce the oseltamivir parent compound to the pharmacokinetic analysis, as this was inadvertently removed in Protocol Version E.

Additional minor changes have been made to improve clarity and consistency. Substantive new information appears in italics. This amendment represents cumulative changes to the original protocol.

PROTOCOL AMENDMENT, VERSION F
SUMMARY OF CHANGES

GLOBAL CHANGES

Throughout the document, oseltamivir has been added back to all the pharmacokinetic analysis sections, as applicable, as both oseltamivir (parent) and oseltamivir carboxylate (metabolite) plasma concentrations will be determined from the blood samples.

PROTOCOL SYNOPSIS

The protocol synopsis has been updated to reflect the changes to the protocol, where applicable.

SECTION 2.2: SECONDARY OBJECTIVES:

To evaluate the effects of conventional and double dose oseltamivir in immunocompromised patients on:

- The population pharmacokinetics of *oseltamivir and* oseltamivir carboxylate ~~(e.g., clearance, volume of distribution)~~ in immunocompromised patients with confirmed influenza infection, through the application of established population pharmacokinetic (PK) models to the sparse plasma concentration data generated

SECTION 3.2: NUMBER OF PATIENTS/ASSIGNMENT TO TREATMENT GROUPS

A minimum of 166 patients (approximately 83 per arm) to allow an adequate number of influenza A patients, including 50 transplant recipients *and at least 15 pediatric patients*, will be enrolled in this study. After screening, patients will be randomly assigned to one of the two active treatment groups.

SECTION 4.1: OVERVIEW

The study population comprises immunocompromised adults (including adolescents) and children *(pediatric)* who have influenza *(the pediatric and adolescent patients will be defined as 1 year to less than 18 years of age at randomization)*….

SECTION 5.5.1: <u>Pharmacokinetic Assessments</u>

Plasma PK samples for assessment of *oseltamivir and* oseltamivir carboxylate will be collected at the following timepoints on Day 6, or any day after the 11th dose, using the following sampling approach:

- Within 30 minutes prior to the dose administration (e.g., 8:30 a.m. *if dosing is scheduled for* ~~dose~~ 9:00 a.m.)
- 1.5 hours ± 30 minutes post-dose (e.g., 10:30 a.m. ± 30 min *if dosing is scheduled for 9:00 a.m.*)
- 4 hours ± 60 minutes post dose (e.g., 1:00 p.m. ± 60 min *if dosing is scheduled for 9:00 a.m.*)
- 8 hours ± 1.5 hours post dose (e.g., 5:00 p.m. ± 1.5 hr *if dosing is scheduled for 9:00 a.m.*)

SECTION 6: INVESTIGATIONAL MEDICINAL PRODUCT

The Investigational Medical Product in this study is oseltamivir dry powder (to be reconstituted to a concentration of 12 mg/mL *or 6 mg/mL [the 6 mg/mL will only be used if applicable and not before 2015]*) or 75 mg capsules and matching placebo.

SECTION 6.2: PREPARATION AND ADMINISTRATION OF STUDY DRUG

Oseltamivir will be provided in two forms:

1. Oseltamivir capsules containing 75 mg of active drug and packaging material consisting of pregelatinized starch, povidone, talc, and sodium stearyl fumarate. All participants 13 years and older will receive this dosage form. *Oseltamivir capsules should be stored below* ~~between 2–25°C~~ *[77°F]. Do not store refrigerated.*
2. A pediatric suspension containing 12 mg oseltamivir per mL *or 6 mg oseltamivir per mL (the 6 mg/mL will only be used if applicable and not before 2015),* of reconstituted solution and the following excipients: sorbitol, titanium dioxide, sodium benzoate, xanthan gum, monosodium citrate, saccharin sodium, and Permaseal11900-31 Tutti Frutti (flavor). All participants 12 years and under will receive this dosage form. *Oseltamivir dry powder for* oral *suspension [pediatric syrup] should be stored below 30°C [86°F]*). Once reconstituted, *the suspension should not be used for longer than 10 days* if stored at room temperature conditions (below 25°C/*77°F*). If stored under refrigerated conditions at 2–8°C (36–46°F) the suspension should not be used for longer than 17 days. The suspension is not suitable for freezing.

SECTION 8.1.2: Secondary Endpoints

The following endpoints will be analyzed descriptively at relevant timepoints~~:~~.

The pharmacodynamics assessments based on nose and throat swabs, collected on the days specified in the schedule of assessments:

The following are the model-predicted PK secondary endpoints for *oseltamivir and* oseltamivir carboxylate:

- Steady-state area under the concentration–time curve from 0 to 12 hours (AUC_{0-12})
- Maximum plasma concentration (C_{max}) ~~for oseltamivir carboxylate~~
- Trough plasma concentration (C_{trough}) ~~for oseltamivir carboxylate~~

SECTION 8.3.1: Pharmacokinetic Analysis

… Body weight, evaluated using a power function and centered around 70 kg, is a statistically significant predictor of ~~both~~ *the* CL/F *for oseltamivir and both CL/F* and apparent volume of distribution (Vc/F) for oseltamivir carboxylate. For oseltamivir carboxylate, CrCl is also a significant predictor of CL/F, while Vc/F decreases linearly with age.

SECTION 16: CONFIDENTIALITY OF TRIAL DOCUMENTS AND PATIENT RECORDS

The investigator must assure that patients' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents *(both paper and electronic)* submitted to the sponsor or designee, patients should not be identified by their names but by an identification code.…

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PROTOCOL SYNOPSIS

TITLE:	**A DOUBLE BLIND, RANDOMIZED, STRATIFIED, MULTI-CENTER TRIAL EVALUATING CONVENTIONAL AND DOUBLE DOSE OSELTAMIVIR IN THE TREATMENT OF IMMUNOCOMPROMISED PATIENTS WITH INFLUENZA**
PROTOCOL NUMBER:	NV20234
VERSION NUMBER:	F
EUDRACT NUMBER:	2006-002468-24
IND NUMBER:	53,093
TEST PRODUCT:	oseltamivir (Tamiflu® RO 64-0796)
PHASE:	IIIb
INDICATION:	Treatment of influenza in immunocompromised patients
SPONSOR:	F. Hoffmann-La Roche Ltd

Objectives

Primary

To evaluate prospectively the safety and tolerability of oseltamivir for the treatment of influenza in immunocompromised patients and characterize the effects of oseltamivir in immunocompromised patients on the development of resistant influenza virus

Secondary

To evaluate the effects of conventional and double dose oseltamivir in immunocompromised patients on:

- The population pharmacokinetics of *both oseltamivir and* oseltamivir carboxylate in immunocompromised patients with confirmed influenza infection, through the application of established population pharmacokinetic (PK) models to the sparse plasma concentration data generated.
- The virologic course of influenza (proportion shedding and viral loads at different timepoints)
- The time to resolution of influenza symptoms
- The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis)
- To explore the relationship of metrics of exposure (e.g., AUC, C_{min}) to relevant pharmacodynamic endpoints

Study Design

Description of Study

This is a double-blind, randomized, multi-center trial of twice daily, conventional and double dose oseltamivir for the treatment of influenza in immunocompromised patients. Patients will be stratified by age ($\leq 12, > 12$ years), transplant status (yes, no), time since onset of flu symptoms and treatment start (up to 96 hours) (≤ 48 or > 48 hours) and vaccination status (yes, no)

Number of Patients

A minimum of 166 (83 per arm) to allow an adequate number of influenza A patients per arm; including 50 transplant recipients *and at least 15 pediatric patients*.

Number of Centers

Approximately 125 centers in the Northern and Southern Hemispheres

Target Population

Patients immunocompromised due to a primary or secondary immunodeficiency, 1 year of age and older *(the pediatric and adolescent patients will be defined as 1 year to less than 18 years of age)*. The patients will be positive for influenza by a rapid diagnostic test, PCR or virus culture at baseline.

Length of Study

The study comprises 10 days of treatment with follow up visits approximately 5 and 30 days later as shown in the schedule of assessments. Study medication will be administered twice daily over 10 days for a total of 20 doses. A rapid diagnostic test for influenza will be performed at the end of the 10 days of treatment. Patients still having influenza based on the rapid diagnostic test will be treated per the local standard of care by the principal investigator, and any medication provided during the follow-up period should be captured in the CRF.

Procedures (summary)

The key procedures are:

- Blood draws for serum chemistry, hematology, serology, and PK assessments (for those patients who provide additional consent to participate in the PK assessments).
- Nasal and throat swabs for viral culture and RT-PCR.

Assessments of:

Safety

The safety laboratory assessments in this study, including the assessment of serum chemistry and hematology will be carried out at a central laboratory. Serum chemistry assessments comprise AST, ALT, total bilirubin, urea and creatinine. Hematology assessments include CBC and differential count. The total volume of blood loss for laboratory assessments will be approximately 20 mL for the entire duration of the study.

Protection of patient confidentiality (Section16) will extend to any data generated from the assaying of these samples. Biological samples taken from all patients may be infectious and will be classified as “diagnostic specimens” for dispatch purposes.

When clinically indicated, the investigator may draw blood for serum creatinine to be assessed at the local laboratory during the study to calculate creatinine clearance

Resistance

Development of resistance

Efficacy

The efficacy laboratory assessments are used for laboratory confirmation of influenza. These are:

Nasal and throat swabs. Two nasal and one throat swab will be collected as described in the Schedule of Assessments. All swabs are sent to a central laboratory for RT-PCR and viral cultures. Influenza virus shedding will be assessed.

At the end of treatment (Day 11), a rapid diagnostic test is permitted for the diagnosis of ongoing influenza.

Investigational Medicinal Products

Test Product:

Oseltamivir/placebo dry powder (to be reconstituted to a concentration of 12 mg/mL *or 6 mg/mL [the 6 mg/mL will only be used if applicable and not before 2015]*) and 75 mg capsules. The duration of dosing in both adults and children is 10 days.

Conventional dose:

Children ages 1 –12 years: Oseltamivir syrup

≤ 15 kg	30 mg twice daily
> 15–23 kg	45 mg twice daily
> 23–40 kg	60 mg twice daily
> 40 kg	75 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules 75 mg twice daily

Patients randomized to the conventional dose will simultaneously receive matching placebo so as to blind them and the investigator from the double dose arm.

Double dose:

Children ages 1 - 12 years: Oseltamivir syrup

≤ 15 kg	60 mg twice daily
> 15–23 kg	90 mg twice daily
> 23–40 kg	120 mg twice daily
> 40 kg	150 mg twice daily

Adults and adolescents (age ≥ 13 years): Oseltamivir capsules 150 mg twice daily

Comparator:

Placebo (from pivotal registration trials in otherwise healthy adults)

Statistical Methods

The sample size has been chosen to provide an adequate number of patients to estimate the development of resistance with reasonable precision. Assuming that 90% of enrolled patients will have laboratory-confirmed influenza, there will be 75 patients in each treatment arm in the population evaluable for the development of resistance. The following table shows the 95% Pearson-Clopper confidence intervals that would result with a sample size of 75 patients in a treatment arm if certain event rates are observed in the study.

During the study the number of influenza A virus infected patients and the rate of development of resistance will be monitored in a blinded fashion, in order to ensure a reasonable precision for the estimation is maintained. Additional patients may be enrolled if necessary.

Observed Rate (%)	95% Confidence Interval
0.0	0.0%–4.8%
1.3	0.0%–7.2%
2.7	0.3%–9.3%
5.3	1.5%–13.1%
10.7	4.7%–19.9%

A total of 83 patients will be enrolled per arm and will be evaluable for the assessment of safety. This number of patients would provide estimates of adverse event rates with similar precision.

For the primary objective of evaluating the safety of oseltamivir conventional and double dose treatments, AEs, physical examinations, tissue rejection and/or graft versus host disease in transplant patients, laboratory tests, and vital signs will be summarized and compared with the known safety profile of the drug. For the summary of the development of resistance for each treatment arm, 95% confidence intervals will be provided with the estimated rates.

For the secondary objective of evaluating the efficacy of oseltamivir as measured by the time to resolution of influenza symptoms, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- Comparison to placebo control from pivotal registration trials

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo treated patients will be established that is comparable to patients in the current study whose first dose of study drug was within 48 hours of symptom onset. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

- Assessment of relative efficacy

  The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval.

  The following are the model-derived PK secondary endpoints for *oseltamivir and* oseltamivir carboxylate: steady-state AUC, C_{max}, and C_{trough}.

GLOSSARY OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ALT [SGPT]	Alanine aminotransferase
ALL	Acute lymphocytic leukemia
AML	Acute myeloid leukemia
ANOVA	Analysis of variance
AST [SGOT]	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
AUC_{0-12}	Steady-state area under the concentration–time curve from 0 to 12 hours
BID	Bis in die (twice daily)
BP	Blood pressure
CARIFS	Canadian Acute Respiratory Infections Scale
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CL/F	Apparent clearance
C_{last}	Last measurable concentration
CL_m	Apparent total clearance of metabolite
C_{max}	Maximum plasma concentration
CML	Chronic myeloid leukemia
CrCl	Creatinine clearance
CRF	Case report form[s]
C_{trough}	Trough plasma concentration
ESF	Eligibility screening form
GVHD	Graft versus host disease
HA	Hemagglutinin
HIV	Human immunodeficiency virus
HSCT	Hematopoietic stem cell transplant
ICH	International Conference on Harmonisation
ITT	Intent to treat
ITTI	Intent to treat influenza infected
IVRS	Interactive voice response system
k_e	Elimination constant
PD	Pharmacodynamic
PK	Pharmacokinetic
PKEP	Pharmacokinetic Evaluable Patient Population
p.o.	Per os (by mouth)
QD	Once per day

GLOSSARY OF ABBREVIATIONS

RIDT	Rapid Influenza Diagnostic Test
SAE	Serious adverse event
SAP	Statistical analysis plan
SCID	Severe combined immunodeficiency
SOT	Solid organ transplant
$TCID_{50}$	50% tissue culture infectious dose
t_{last}	Time to last measurable concentration
t_{max}	Time to maximum plasma concentration
$t_{1/2}$	Elimination half-life
Vc/F	Apparent volume of distribution

PART I: STUDY DESIGN AND CONDUCT

1. BACKGROUND AND RATIONALE

1.1 BACKGROUND

Influenza is an acute respiratory infection caused by a virus of the orthomyxovirus family which occurs in three forms, influenza A, B, and C. Influenza virus types A and B cause an acute febrile infection of the respiratory tract characterized by the sudden onset of fever, malaise, headaches, myalgias and cough. Influenza causes numerous deaths each year [1]. Although difficult to assess, annual influenza epidemics are thought to result in between three and five million cases of severe illness and between 250,000 and 500,000 deaths every year around the world [2].

The influenza viruses are segmented, negative sense, single stranded, lipid encapsulated, RNA viruses between 80 and 100 nm in size. Subtypes are defined according to glycoproteins present in the viral lipid coat. The hemagglutinin complex (HA) is the major surface protein of the virus. The neuraminidase (NA) proteins are the second major surface proteins in the virion and play a role in enhancing virus penetration of the mucus layer around the target cell and in release of virus from the cell surface. The M2 protein triggers the disintegration of the virion during virus entry into the cell and may also be involved in protecting the HA prior to assembly of new virus particles.

1.1.1 Influenza in the Immunodeficient Population

Influenza infection is usually a self–limiting condition. However, in children, the elderly, and the immunocompromised, influenza infection can be associated with substantial morbidity and mortality [1]. In patients with compromised immunity, influenza virus may cause severe lower respiratory tract involvement; unusual syndromes, including encephalitis, myocarditis, and hepatitis; and death [3].

Conditions that compromise immunity may be classified based on etiology into primary (genetic) and secondary (acquired) immunodeficiency. Of the immunodeficient conditions, the ones that affect cell mediated immunity are likely to have adverse outcomes following viral infections [16].

Primary immunodeficiency

Primary immunodeficiencies are relatively common, may be either congenital or manifest later in life, and are classified according to whether the genetic defect affects T or B cells or both [16]. There are four groups of disorders: severe combined immunodeficiency (SCID), primary T cell deficiency (e.g., CD8 deficiency, DiGeorge syndrome), predominantly B-cell-related antibody deficiency (e.g., common variable immunodeficiency, selective IgA deficiency), and other well-defined immunodeficiency syndromes (e.g., Wiskott Aldrich syndrome) [16].

Of the primary immunodeficiencies, antibody deficiencies are the most frequent. However, some of the more common antibody deficiency conditions (isolated IgA deficiency, IgG subclass deficiency, and common variable immunodeficiency) have intact cell-mediated immunity and therefore the clinical course of viral infections (unless complicated by bacterial infections) does not differ significantly from that in the normal host [16]. A list of primary immunodeficiency disorders at risk for viral infections is provided in Appendix 6. The incidence of some of these conditions has been estimated. The incidence of SCID is 1 in 100,000 to 1 in 1,000,000 [16]. The more severe forms of primary immunodeficiency are relatively rare, have their onset early in life, and all too frequently result in death during childhood [16].

Secondary Immunodeficiency

Secondary immunodeficiencies are not caused by intrinsic abnormalities in development of T and B cells [16]. Secondary immunodeficiency may result from diseases (human immunodeficiency virus [HIV], hematologic malignancy) or immunosuppressive and cytotoxic drugs (such as those used for treatment of transplant recipients, collagen vascular disease, malignancies).

Secondary immunodeficiency due to disease

HIV-infected individuals with a CD4+ T-cell count of $< 200/mm^3$ (AIDS defining) are highly susceptible to opportunistic disease [16]. However, CD4 counts $< 500/mm^3$ are considered abnormal in HIV-infected individuals, and therefore these individuals are also more susceptible to infection. CD4+ T-cell counts are higher in infants and young children than in adults and decline over the first few years of life [40, 41]. Because of this age-dependent variation in absolute CD4+ T-cell count, calculation of CD4 percentage is used as a measure for young children because it has shown less variability. HIV-infected children (12 months to 5 years old) with $<$ 15% CD4+ T cells are classed as severely immunosuppressed and highly susceptible to opportunistic disease, although antiretroviral therapy is recommended in children with $<$ 25% CD4+ T cells as they are still classified as immunosuppressed [42]. Studies in patients with HIV/AIDS have shown an increased risk for heart and lung-related hospitalizations during the influenza season compared with other times of the year, prolonged duration of influenza symptoms, increased risk for influenza-related complications, and a higher risk of influenza-related death [17].

Several hematologic malignancies affect the immune system (Appendix 7). Several authors have reported influenza in children and adults with hematologic malignancies [18, 19, 20, 21].

Secondary immunodeficiency due to drugs (e.g., transplant recipients, collagen vascular disease, malignancies)

The enhanced survival of the transplant population following the availability of newer immunosuppressive drugs has made them representative of the immunocompromised population in general and secondary immunodeficiency due to drugs, in particular.

In transplant recipients, influenza is associated with a higher rate of pulmonary complications, extrapulmonary manifestations, and an increased risk of graft dysfunction and high attributable mortality. In a cohort study of influenza viral infection in solid organ transplant (SOT) patients, 30 cases of influenza viral infection were identified. Secondary bacterial pneumonia was seen in 17% of patients and three SOT recipients (2 liver and 1 kidney) had myositis, myocarditis, and bronchiolitis obliterans. Biopsy of the transplanted organ was performed in 21 of 30 cases and revealed variable degrees of acute allograft rejection in 62% of patients. The study concluded that influenza was associated with significant morbidity in different groups of SOT recipients [4].

Among transplant recipients, patients with hematopoietic stem cell transplant (HSCT) are at the greatest risk for morbidity. In a prospective study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation, 1973 patients were evaluated for respiratory virus infections after stem cell transplantation. The overall mortality was 23% from influenza A infection and the direct influenza A-associated mortality was 15.3% in this study [5]. A large retrospective study (4797 patients undergoing HSCT over a 13-year period) identified 62 patients with influenza of whom as many as 29% (18 of 62 patients) developed pneumonia. Ten percent of the patients with influenza died [6].

The increased morbidity in transplant patients is associated with increased duration of virus shedding compared to that in healthy subjects. In otherwise healthy adults, elderly patients and children, the median duration of viral shedding in untreated patients was 70, 96, and 118 hours, respectively [7]. In contrast, in the retrospective HSCT study, influenza virus was shed in nasopharyngeal secretions (evaluated at least weekly) for a median duration of 168 hours (7 days, range 2 to 37 days) [6].

Currently available treatments for influenza infection are the M2 ion channel inhibitors (e.g., amantadine, rimantadine) and the neuraminidase inhibitors (e.g., oseltamivir, zanamivir).

1.1.2 Oseltamivir

Oseltamivir (Tamiflu®, Ro 64-0796) is an ethyl ester prodrug that is rapidly absorbed from the gastrointestinal tract after oral administration and metabolized in the liver by high capacity carboxylesterases to form oseltamivir carboxylate (Ro 64-0802), a potent, stable, and selective inhibitor of influenza A and B neuraminidase enzymes. The active form, oseltamivir carboxylate is excreted unchanged by the kidney via glomerular

filtration and active tubular secretion by the organic anion transport system. The efficacy and safety of oseltamivir in influenza treatment and prevention has been established in an extensive series of clinical studies in man.

Oseltamivir has been approved for the treatment of influenza in Europe, the United States and most other countries around the world. In adults and adolescents, the recommended dose is 75 mg twice daily for 5 days. In children 1 year of age and older, recommended doses are 30, 45, or 60 mg BID based on body weight. In all age groups, the recommended dose is administered BID for 5 days.

The approval of oseltamivir for the treatment of influenza is based on several controlled clinical trials. In the pooled population from these clinical trials encompassing adults aged from 13–97 years, many with significant co-morbidity, 1325 patients were treated with oseltamivir (75 mg BID) and 1056 patients received placebo. A total of seven influenza symptoms (both respiratory and constitutional) were captured on the diary card for adults. The time to resolution of all symptoms (on the diary card) decreased by 24 hours; from 124.5 hours in the placebo arm to 100.6 hours with oseltamivir 75 mg BID ($p < 0.0001$) [7]. Further, in adults and adolescents with a proven influenza illness, oseltamivir treatment reduced overall antibiotic use for any reason by 26.7% and the incidence of influenza-related lower respiratory tract complications resulting in antibiotic therapy by 55%. The study concluded that oseltamivir treatment of influenza reduces lower respiratory tract complications, antibiotic use, and hospitalizations in healthy and 'at risk' patients [8].

Likewise, in the influenza-infected pediatric population (1–12 years of age), oseltamivir treatment ($n = 217$) was compared with placebo ($n = 235$). There was a reduction in the median duration of illness (defined based on resolution of temperature, cough, coryza and return to pre-illness health and activity) of 36 hours; from 137 hours with placebo to 101 hours in the oseltamivir treatment arm ($p \leq 0.0001$). The Canadian Acute Respiratory Infections Scale (CARIFS), validated for use in children, was used to collect symptom data on the pediatric diary card. The CARIFS scale comprised a total of 18 symptoms that were evaluated twice daily by the parent or guardian. There was a similar reduction in the time to alleviation of all CARIFS symptoms of 36 hours; from 100 hours in the placebo group to 63 hours in the oseltamivir group ($p < 0.0001$) [9].

Thus in both adults and children, the time to resolution of all symptoms was significantly reduced in the oseltamivir treatment arm compared with placebo.

Oseltamivir was well-tolerated in clinical trials. Approximately 11,000 patients have received oseltamivir in the development program. The most common adverse events reported by adults, the elderly, and children were nausea and vomiting. Serious adverse events (SAEs) were reported with a low and equal frequency by patients taking active drug and placebo. Full details are given in the Investigator Brochure [7].

The safety profile of oseltamivir has been well characterized for the prophylaxis indication in a prospective randomized placebo controlled trial conducted in the adult and pediatric immunocompromised (HSCT and SOT) population. In the oseltamivir group, the indications for transplant included hematologic malignancies (acute and chronic leukemias, multiple myeloma, and myelodysplastic syndrome), lymphoid malignancies (Hodgkin's or non-Hodgkin's lymphomas), primary immunodeficiencies (Wiskott-Aldrich syndrome, X-linked lymphoproliferative disease, and severe combined immunodeficiency). Other rare indications included bone marrow aplasia, paroxysmal nocturnal hemoglobinuria, myelofibrosis, and multiple sclerosis. In the safety population, there were 239 patients randomized to the conventional dose of oseltamivir and 238 to placebo. The total number of adverse events reported in the placebo group (361 events) was generally similar to that in the oseltamivir group (323 events). Diarrhea was the most frequently reported adverse event (placebo, 8%; oseltamivir, 6%). There were no deaths in the oseltamivir group. Oseltamivir was found to be safe in immunosuppressed transplant recipients [22].

Limited safety and/or efficacy of oseltamivir for the treatment indication is available from several case reports in children and adolescents. Oseltamivir was shown to be safe and/or effective in HIV infected children (n = 10) [23], children (ages 3 to 12 years) with acute lymphocytic leukemia (ALL) (n = 10) [21], in a nosocomial H1N1 outbreak in a pediatric (children aged 10 months to 13 years) oncology ward (n = 8) [20], in children and adolescents (ages 2 to 19 years) with malignancies (ALL, neuroblastoma, brain tumor, non-Hodgkin's lymphoma, osteosarcoma, Ewing sarcoma, myelodysplasia, acute myeloid leukemia (AML), Wilms tumor, aplastic anemia, chronic myeloid leukemia (CML), and acute promyelocytic leukemia) (n = 51) [18], in children aged 4 to 14 years on immunosuppressive drugs (n = 5) [24], and in children (aged 5 months to 5 years) with bone marrow transplant (n = 3) [25].

Oseltamivir has also been shown to be safe and/or effective in mixed populations of children and adults (62 patients) with HSCT [6] and in a large epidemiologic study (n = 221) with SOT [26].

Finally, oseltamivir has been shown to be safe and/or effective in immunocompromised adults with HSCT [10] and adults with lung transplant [27, 28, 29].

The dose of oseltamivir was the same as the conventional dose in a majority of these reports. In one report, as many as 25 adult patients received twice the conventional dose [26] while in another report three of nine adult patients received twice the conventional dose [29]. Treatment with oseltamivir generally ranged from 5 to 10 days [28, 29, 26] and occasionally until the patient was symptom free [28, 29]. In exceptional cases treatment was given for as long as 20 days [18] or for as long as the patient was positive by RT-PCR [21].

There is some concern about the development of resistance in the immunocompromised population. During the pandemic influenza season, more than 23,000 clinical isolates of novel H1N1 pandemic virus were tested for resistance in the 6 international WHO regions. A total of 225 isolates (from 225 patients) were resistant (H275Y mutation in the neuraminidase coding sequence) to oseltamivir for an approximate incidence of 1%. Information on immune status was available for 142 of 225 patients. Among the 142 patients, 56 (40%) were immunocompromised [30]. Prolonged viral replication and lack of immune-mediated virus clearance in immunosuppressed patients treated with antivirals can result in a higher incidence of selection of drug-resistant viruses, a phenomenon that has been documented previously [31, 32].

1.2 RATIONALE FOR THE STUDY

Because of the increasing body of evidence (Section 1.1.2), oseltamivir is recommended in national guidelines as an option for the treatment of influenza in the transplant population [37], which comprises a significant portion of the immunocompromised population. However, there is limited data on safety and efficacy of oseltamivir use in this population. The primary objective of this study is to evaluate safety and resistance, while evaluating efficacy as a secondary objective, in the broader immunocompromised patient population, who are considered at increased risk of viral infection.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective of the study is to evaluate prospectively the safety and tolerability of oseltamivir for the treatment of influenza in immunocompromised patients and characterize the effects of oseltamivir in immunocompromised patients on the development of resistant influenza virus.

2.2 SECONDARY OBJECTIVES

The secondary objectives of the study are:

To evaluate the effects of conventional and double dose oseltamivir in immunocompromised patients on:

- The population pharmacokinetics of $oseltamivir\ and$ oseltamivir carboxylate in immunocompromised patients with confirmed influenza infection, through the application of established population pharmacokinetic (PK) models to the sparse plasma concentration data generated
- The virologic course of influenza (proportion shedding and viral loads at different timepoints)
- The time to resolution of influenza symptoms
- The clinical course of influenza (fever, symptoms, secondary illnesses as evidenced by otitis media, bronchitis, pneumonia, or sinusitis)

- To explore the relationship of metrics of exposure (e.g., AUC, C_{min}) to relevant pharmacodynamic (PD) endpoints

3. STUDY DESIGN

3.1 OVERVIEW OF STUDY DESIGN AND DOSING REGIMEN

This is a double blind, randomized, stratified, multi-center trial of conventional and double dose oseltamivir for the treatment of influenza in immunocompromised patients. Immunocompromised patients, who develop an influenza-like illness with a positive rapid diagnostic test, PCR, or viral culture for influenza, will be enrolled during the influenza season. Patients will be stratified by transplant status [yes, no], the time between onset of influenza symptoms and treatment start (up to 96 hours) [$\leq$48 hours; $>$48 hours], influenza vaccination status for current influenza season [yes; no] and by age [$\leq$12 years; $>$12 years]. Eligible patients will be consented and randomized to receive oseltamivir twice daily for 10 days in one of two treatment arms: conventional dose or double dose (double the conventional dose).

3.1.1 Rationale for Study Design

This study incorporates several features that distinguish it from classic placebo controlled trials.

There is no placebo control arm in this study as it was considered unethical for this high risk population. The development of resistance following treatment with oseltamivir (one of the primary objectives of the study) is an objective assessment (determined by laboratory tests) and is unlikely to be impacted by the absence of a placebo arm.

As a placebo control arm was considered unethical in this population, an alternative control arm had to be identified for efficacy endpoints. The response to placebo in pivotal oseltamivir registration trials in the healthy adult population with influenza was chosen as a control in lieu of a placebo arm in the current trial. In order to do this, the endpoints were designed to be similar to that in the pivotal registration trials.

The following sections provide rationale and justification for specific aspects of the study design that differ from the currently approved dosing for influenza or from previous pivotal registration trials. They also provide information on how the results will be used to make recommendations on dose and duration of treatment.

3.1.1.1 Choice of Treatment Arms

The currently approved dose of oseltamivir for the treatment indication is the conventional dose with a duration of 5 days. In this study a conventional and double dose are being evaluated. The pivotal registration trials for oseltamivir in healthy adults with influenza had three treatment arms; two active (75 mg and 150 mg) and one placebo. Both treatment arms were found to be significantly better than placebo. No statistical comparisons were made between the two active treatment arms. Evaluation

of the results in the two active treatment arms did not reveal any clinically meaningful difference. However, the proportion of patients shedding virus on Day 4 (3 days after the start of treatment), suggested a possible dose-response relationship (36%, 28%, and 23% in the placebo, 75 mg, and 150 mg dose arms, respectively). Because defective immune-mediated virus clearance in immunosuppressed patients treated with antivirals can result in a higher incidence of selection of drug-resistant viruses, it was decided to use both the conventional and double dose arm for this study. A longer duration of treatment was chosen because a large retrospective study has shown that the median duration of viral shedding of 7 days [6] was greater for HSCT recipients than that seen in the healthy children, adult, and elderly population with influenza (Section 1.1.1) [7].

3.1.1.2 Inclusion of Patients Symptomatic up to 96 Hours

In immunocompromised patients, time from onset of symptoms to seeking medical attention (presentation at the clinic) of $>$48 hours has been shown in several case reports [33, 27, 28, 29] including two instances of nosocomial outbreaks in children [25, 20]. This notwithstanding, oseltamivir has been shown to offer benefit in immunocompromised populations that included patients treated with oseltamivir beyond 48 hours of presentation; patients with lung transplant (median time to presentation 3 days) [27], bone marrow transplant (treatment started more than 48 hours after onset of symptoms in all 3 patients) [25], organ transplant (62 of 221 patients started treatment after 96 hours) [26], and children with acute lymphocytic leukemia (one child presented after 3 days and two children presented after 5 days of symptoms) [21].

In a prospective, observational study involving adults hospitalized with influenza, the study authors concluded that 'Weakened host defenses slow viral clearance, whereas antivirals started within the first four days of illness enhance viral clearance' [34].

Additionally, in November 2009, in a communication for clinicians on antiviral treatments for H1N1, the U.S. Centers for Disease Control and Prevention (CDC) stated that while antiviral treatment is most effective when started early; both outpatients with risk factors and hospitalized patients still benefit when treatment with oseltamivir is started more than 48 hours after illness onset [35].

3.1.1.3 Rational for Sparse PK Sampling

Limited PK sampling will be done in this protocol, in patients who have provided additional consent, and is justifiable in this study for the following two reasons:

- There are already tested and qualified population PK models available [36] that will be used to estimate exposure in the immunocompromised population on the basis of the data collected in this study, meaning extensive PK sampling over a steady-state dosing interval is not required.
- The patient population in this study is likely to be very ill with a complex clinical picture and significant additional burden of treatment and monitoring due to their primary condition. In this context, it makes ethical sense to minimize blood draws

and assessments to only those absolutely essential to meet the objectives of the study and to ensure patient safety.

3.1.1.4 Interpretation of Study Results

Safety and tolerability and the development of resistance are the primary objectives of this study. These will be characterized descriptively. For the secondary objective of efficacy, the subset of the population enrolled in the first 48 hours will be compared with placebo patients from pivotal registration trials (where patients were enrolled in the first 2 days of illness). Section 8.2.5 , Section 8.2.6 , and Section 8.2.7 provide details on the statistical considerations.

Stratification

Stratification in this study has no bearing on any comparisons with results from previous trials. However, in order to evaluate a dose response relationship between the two active treatment arms in this trial, stratification remains essential.

Patients will be stratified by transplant status (yes, no) because transplant patients form a relatively large homogenous group in this study and might influence outcome.

Patients will be stratified by time between onset of symptoms and treatment start (<48 hours or ≥ 48 hours) because previous experience in otherwise healthy adults and children has shown that patients treated early in their illness respond to treatment better than those who are treated later. Patients will also be stratified by age (≤ 12 years and >12 years) because otherwise healthy children shed virus longer than otherwise healthy adults. It is expected that a similar difference in the duration of viral shedding would exist between immunocompromised children and adults. As vaccinated patients may respond faster to therapy, the study is also stratified based on influenza vaccination status for the current influenza season (yes; no).

3.1.2 Rationale for Dose Selection and Adjustment

Dose Selection

The dose of oseltamivir to be used in this study is the conventional, approved dose for children and adults in the treatment of influenza. There will be a second higher dose for comparison, which is two times the conventional dose. This higher dose is used based on theoretical considerations, which suggest that the higher dose may be associated with improved efficacy and decreased emergence of resistance.

The anticipated pro-drug and metabolite exposures from this higher dose are not expected to exceed maximum exposures seen previously in the oseltamivir development program. The safety and tolerability of the higher dose regimen has already been demonstrated in treatment studies of immunocompetent adult subjects ($n=447$) [11]. In a study to demonstrate cardiac safety, in the highest dose group treated with 450 mg BID for 5 days [$n=99$], no subject had a serious adverse event, nor withdrew

prematurely. In Phase I studies in adults, oseltamivir has been administered in multiple doses of up to 500 mg BID. Doses of 200 mg BID and greater have been associated with increased gastrointestinal adverse effects (nausea and vomiting) [7]. In adult subjects with creatinine clearances of ≤ 30 mL/min, doses of 100 mg BID for 6 days were well tolerated, despite steady-state oseltamivir carboxylate exposures approximately 10-fold higher than those achieved with standard dosing in renally competent individuals [12]. No other adverse effects were reported more frequently with higher doses and no serious adverse events have been reported within the volunteer studies. Co-administration of oseltamivir with food has been demonstrated to substantially reduce the frequency and severity of gastrointestinal side effects.

Thus, the rationale for the double dose arm in this study is to explore the possibility of improved efficacy and decreased emergence of resistance without an undue increase in risk. For patients with a creatinine clearance level of 45 mL/min or above, oseltamivir carboxylate exposures for both the 75-mg and 150-mg doses are not expected to exceed exposures previously tested. Patients with a creatinine clearance as low as 45 mL/min, who receive the 150-mg BID dose, will have exposures of oseltamivir carboxylate that are within (albeit in the upper end) the established safety margin. This corresponds to an AUC produced by the 450-mg BID dose in adults with normal renal function (AUC $_{0\text{-}12\ hrs}$ of approximately 15,000 ng • hr/mL).

Drug interactions with immunosuppressive medications have also been evaluated. The pharmacokinetics of oseltamivir and oseltamivir carboxylate after administration of 75 mg oseltamivir (conventional adult dose) in patients with a well-functioning, stable renal allograft who were being maintained on immunosuppressive therapy were studied. These were similar to those described in the literature for adults with comparable degrees of renal function. Oseltamivir was well tolerated and had no clinically relevant effect on the steady-state pharmacokinetics of cyclosporine A, tacrolimus, or mycophenolate mofetil [7].

Duration of Dosing

The duration of dosing chosen for this population (10 days) is longer than that in the healthy adult and pediatric populations (5 days). This is based on observations that the viral shedding and illness are typically longer in immunocompromised patients than it is in healthy adults [6, 10].

Dose Adjustments

As this is a double blind study, patients whose creatinine clearance (CrCl) decreases to <45mL/min (adults) using the Cockcroft-Gault method or <45 mL/min/1.73m^2 (<18 years old) using the Schwartz equation will be discontinued from study treatment. A lower limit of 45 mL/min for CrCl will be used to allow for patients with a mild to moderate renal impairment.

3.1.3 End of Study

The study comprises 10 days of treatment with follow-up visits approximately 5 and 30 days later as shown in the schedule of assessments. Study medication will be administered BID over 10 days for a total of 20 doses. A rapid diagnostic test for influenza will be performed at the end of the 10 days of treatment. Patients still having influenza based on the rapid diagnostic test will be treated per the local standard of care by the principal investigator, and any medication provided during the follow-up period should be captured in the CRF.

3.2 NUMBER OF PATIENTS/ASSIGNMENT TO TREATMENT GROUPS

A minimum of 166 patients (approximately 83 per arm) to allow an adequate number of influenza A patients, including 50 transplant recipients *and at least 15 pediatric patients*, will be enrolled in this study. After screening, patients will be randomly assigned to one of the two active treatment groups.

3.3 CENTERS

This will be a multicenter study taking place in the Northern and Southern Hemispheres at approximately 125 centers.

4. STUDY POPULATION

4.1 OVERVIEW

The study population comprises immunocompromised adults (including adolescents) and children *(pediatric)* who have influenza *(the pediatric and adolescent patients will be defined as 1 year to less than 18 years of age at randomization)*. Additionally, the patients must not have other medical conditions that will preclude the assessment of efficacy or safety. Influenza vaccinated and non-vaccinated patients are eligible to participate in this study. Principal investigators will review oseltamivir resistance patterns of strains circulating in the area and weigh the risk versus the benefit before enrolling patients with a potentially resistant strain.

Under no circumstances are patients who enroll in this study permitted to be re-randomized to this study and enrolled for a second course of treatment.

4.2 INCLUSION CRITERIA

- Age greater than or equal to 1 year
- Rapid diagnostic test, PCR, or viral culture positive for influenza
- Immunocompromised patient defined as one who meets any of the following:
    - Primary immunodeficiency at risk for viral infections (representative examples in Appendix 6) OR

- Secondary immunodeficiency
  - SOT with ongoing immunosuppression OR
  - Allogenic HSCT with ongoing immunosuppression OR
  - HIV with a most recent CD4 count $<$ 500/mm^3 (or $<$25% in children $\leq$ 5 years old) within the last 6 months and, in the investigator's opinion, considered immunocompromised OR
  - Hematologic malignancies (representative examples in Appendix 7) OR
  - Systemic (e.g., enteric, sc, im or iv) immunosuppressive therapy, irrespective of medical indication, started at least 12 weeks prior to, and ongoing at the time of first dose of study drug (representative examples in Appendix 8)

- Symptoms suggestive of influenza like illness including, but not limited to fever, cough, or coryza
- In patients with history or clinical presentation at randomization suggestive of renal failure; a CrCl $>$ 60 mL/min ($>$ 18 years old) or $>$ 60 mL/min/1.73m^2 ($<$ 18 years old) within the last 3 months
- Less than or equal to 96 hours between onset of influenza-like illness and first dose of study drug
- Parent/guardian willing and able to comply with study requirements and give consent (country specific age cut off)
- Patient able to comply with study requirements and willing to give assent, as appropriate (country specific age cut off)
- For adult patients willing and able to comprehend and give written informed consent
- Patients, in the reproductive age group, must agree to utilize an effective method of contraception throughout the study period and for females for one reproductive cycle following cessation of study therapy
- Females of childbearing potential must have a negative urine pregnancy test prior to start of study drug

4.3 EXCLUSION CRITERIA

- Clinical evidence of severe hepatic impairment, defined as Child-Pugh grade C (score $>$ 9) or decompensated cirrhosis (see Appendix 9)
- Patients currently receiving any form of renal replacement therapy including hemodialysis, peritoneal dialysis or hemofiltration
- Patients with gastrointestinal disorders that might interfere with their ability to absorb oral medication
- Allergy to the test medication
- Patients with hereditary fructose intolerance (for patients who will be taking the liquid formulation)

- Influenza vaccination with live attenuated vaccine in the 2 weeks prior to randomization
- Antiviral treatment (example: amantadine, rimantadine, oseltamivir, laninamivir, peramivir, zanamivir, and ribavirin) for influenza in the 2 weeks prior to randomization
- Patients taking probenecid medication
- Patients who are pregnant or breast-feeding
- Participation in a clinical trial or expanded access trial with an investigational drug suspected/demonstrated to impact pathways important for the metabolism and excretion of oseltamivir in the 4 weeks prior to randomization or concomitantly with this study

4.4 CONCOMITANT MEDICATION AND TREATMENT

Antiviral treatments with activity against influenza (e.g., amantadine, rimantadine, zanamivir, ribavirin, laninamivir, peramivir, and additional oseltamivir above that specified for this study) are not allowed during the 10-day treatment phase of the study. Concomitant use of an investigational drug during the study is also excluded if suspected/demonstrated to impact pathways important for the metabolism and excretion of oseltamivir. Live attenuated vaccines may not be advisable for this population. It is possible that concurrent use with oseltamivir may render live vaccines ineffective.

Medications required for the routine care of the patient may be continued during the study. Concomitant use of probenecid (irrespective of the indication) is not allowed during the study.

4.5 CRITERIA FOR PREMATURE WITHDRAWAL

The investigator must discontinue treatment if the creatinine clearance is $<$45 mL/min in adults using the Cockcroft-Gault method or $<$ 45 mL/min/1.73 m^2 using the Schwartz equation in children. A lower limit of 45 mL/min for creatinine clearance will be used to allow for patients with a mild to moderate renal impairment. The investigator must also discontinue treatment from all patients with intercurrent illnesses or adverse events suggestive of hepatic decompensation. The investigator also has the right to discontinue treatment in the event of intercurrent illness, adverse events, treatment failure, protocol violations, administrative reasons, or other reasons.

The investigator must discontinue treatment with study drug if the patient is to be treated concomitantly with another antiviral for influenza, for example, amantadine, rimantadine, laninamivir, peramivir, or zanamavir. However, all patients, including those who discontinue study drug prematurely and/or are treated with another antiviral, will be required to return for follow-up approximately 5 and 30 days after the last dose of study drug (Day 15 and Day 40 assessments).

Patients have the right to withdraw from the study at any time for any reason.

An excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of subjects should be avoided. Should a patient decide to withdraw, all efforts will be made to complete and report the observations prior to withdrawal as thoroughly as possible.

The investigator should contact the patient or a responsible relative either by telephone or through a personal visit to establish as completely as possible the reason for the withdrawal. A complete final evaluation at the time of the patient's withdrawal should be made with an explanation of why the patient is withdrawing from the study. If the reason for removal of a patient from the study is an Adverse Event, the principal specific event will be recorded on the CRF.

In the case that the patient decides to prematurely discontinue study treatment ["refuses treatment"], he/she should be asked if he/she can still be contacted for further information. The outcome of that discussion should be documented in both the medical records and in the CRF.

4.6 REPLACEMENT POLICY [ENSURING ADEQUATE NUMBERS OF EVALUABLE PATIENTS]

4.6.1 For Patients

No patient prematurely discontinued from the study for any reason will be replaced.

4.6.2 For Centers

A center may be replaced for the following administrative reasons:

- Excessively slow recruitment
- Poor protocol adherence

5. SCHEDULE OF ASSESSMENTS AND PROCEDURES

Table 1 Schedule of Assessments

	Baseline	Treatment					Follow-up	
Study Day	**1 (pre-dose)**	**1**	**2 or 3[f,g]**	**6[f, g]**	**8[f, g]**	**11[g, h, i]**	**15[g]**	**40[g, j]**
Informed Consent/Assent [l]	x							
Medical history	x							
Demographics	x							
Height and weight	x					x		
Pregnancy Test [a]	x					x		x
Rapid diagnostic test for influenza virus shedding						x		
RIDTPCR/Culture for confirmation of influenza virus	x							
Safety Labs [b]	x					x		
Physical Examination	x					x		x
Vital Signs (including pulse, RR, temperature, Blood pressure)	x		x	x	x	x	x	x
Nasal and throat swabs for viral shedding and viral load [c, d k]	x		x	x	x	x	x	x
PK sampling (blood) [l]					x[m]			
Review of patient diary data [e]			x	x	x	x	x	x
Drug Administration		←	—	—	—	→		
Collection of unused study drug and empty containers						x		
Previous Diseases	x							
Previous/Concomitant medications	x	←	—	—	—	—	—	→
Adverse Events/Sec Illnesses and Treatments		←	—	—	—	—	—	→
Rejection, Graft versus host disease (GVHD)		←	—	—	—	—	—	→

a. Urine pregnancy test for patients of child-bearing potential according to the judgment/discretion of the investigator.

b. Hematology (CBC with differential) and chemistry (AST, ALT, total bilirubin, urea, Cr). Serum creatinine testing may be done at a local laboratory when clinically indicated to calculate creatinine clearance.

c. Baseline swab samples will be assessed for the presence of resistance mutations.

d. Two nasal and one throat swab for viral culture and RT-PCR.

e. Influenza symptoms, temperature, and date/time of oseltamivir dose will be recorded by the patient in the patient diaries twice daily on Days 1–10, and once daily thereafter.

Table 1 Schedule of Assessments (cont.)

f. A home visit may be made on Day 2 or 3 (for patients who are too ill to come into the clinic) and/or, Day 6, and/or Day 8; however, it is recommended that the PK blood draw be performed at the clinic.

g. Day 2/3 visit window = +1 day. Day 6 visit window = ± 1 day; Day 8 visit window = +1 day. Day 11 visit window = ± 1 day. Day 15 and Day 40 visit occur approximately 5 and 30 days after the last dose respectively. Day 15 window = ± 1 day. Day 40 window ± 2 days.

h. Only weight to be assessed. Height is not necessary at this visit. Study drug is taken on Day 11, only if the first dose was taken after 4 p.m. on Day 1 [5.3] Patients who are rapid diagnostic positive can be treated per standard of care. They will be required to come for their follow-up visits on Day 15 and Day 40.

i. Patients who discontinue treatment prematurely will have an end of treatment (Day 11) assessment at the time of treatment discontinuation or the following day. They will be required to attend their follow up visits approximately 5 and 30 days after the last dose (Day 15 and Day 40 assessments).

j. Patients who discontinue during follow-up will have an end of follow-up (Day 40) assessment. This visit must occur within 30 days of the last dose.

k. For patients who are unable to attend the clinic, swabs may be taken at home on those days where there is a home visit by site personnel.

l. Plasma PK samples for assessment of *oseltamivir and* oseltamivir carboxylate will be collected from patients who give additional consent to participate in PK sampling using a sparse PK sampling approach (Section 5.5).

m. Serial PK samples taken at steady-state no earlier than Day 6 (i.e., not before the 11th dose) consisting of as many of the four timepoints as possible: within 30 minutes prior to the dose administration, 1.5 hours ± 30 minutes post dose, 4 hours ± 60 minutes post dose, and 8 hours ± 1.5 hours post dose (for patients who have given additional consent).

5.1 SCREENING EXAMINATION AND ELIGIBILITY SCREENING FORM

All patients must provide written informed consent (assent where applicable) before any study specific assessments or procedures are performed. Screening will take place at the baseline visit prior to first dose. However, if necessary, screening may take place the day before the baseline visit, as long as the patient is randomized and receives his/her first dose of study drug within 96 hours of influenza symptom onset.

Patients will be assessed for inclusion and exclusion criteria and those who fulfill all the inclusion and none of the exclusion criteria will be randomized into the study. Influenza Rapid Diagnostic test, PCR, or viral culture for the purposes of inclusion criteria fulfillment will be done by the site or the local laboratory. Historical CrCl values within 3 months and CD4+ T-cell counts (where applicable) within 6 months before randomization are acceptable for inclusion into the study.

An Eligibility Screening Form [ESF] documenting the investigator's assessment of each screened patient with regard to the protocol's inclusion and exclusion criteria is to be completed by the investigator.

A screen failure log must be maintained by the investigator.

5.2 PROCEDURES FOR ENROLLMENT OF ELIGIBLE PATIENTS

Once a patient has fulfilled the entry criteria, he/she will be randomized to one of two treatment groups. The patient randomization numbers will be generated by Roche or its designee and incorporated into double-blind labeling.

The investigator or designee will use the CRF pre-printed with the assigned patient number and enter the randomization number provided by IVRS for allocation to the treatment groups in the appropriate place on each patient's CRF.

Randomization will be stratified by transplant status (Yes; No); time between onset of symptoms and treatment start ($\leq$48 hrs or $>$48 hrs), influenza vaccination status (Yes; No) and patient's age ($\leq$12 years or $>$12 years).

5.3 CLINICAL ASSESSMENTS AND PROCEDURES

At all visits patients will receive the routine care for their primary illness. Routine patient care (including the adjustment of doses of any of the immunosuppressive drugs) will be dictated by tests performed locally at the trial site. For patients unable to attend the clinic, provision will be made for swabbing to be conducted at home when there is a home visit scheduled. Training will be provided to site staff on how to perform this. All safety labs will be sent to a central laboratory. Results for these laboratory parameters will be used by the sponsor/designee to assess overall safety.

All assessments and procedures will be performed according to the Schedule of Assessments (Table 1). Additional information regarding these procedures not provided in the Schedule of Assessments is provided below.

Blood samples will be collected from patients who provide additional consent for PK sampling, as outlined in Section 5.5.

Study Day 1

The baseline and study Day 1 assessment may be performed at the same visit. Laboratory assessments (safety labs and nasal and throat swabs for virology) should be performed after randomization but prior to the patient receiving first dose of study drug.

Study drug, patient diaries, and thermometers will be dispensed. Patients or guardians/parents will be instructed on how to complete the patient diaries (Appendix 2 and Appendix 3), temperature recording, and treatment administration details, including

time of each oseltamivir dose. The first diary entries will be made at the site before the first dose of study drug.

Baseline nasal and throat swab samples will be assessed for the presence of oseltamivir-resistance mutations.

The date of the first dose of study drug is defined as study Day 1. Once randomized, the first dose of study drug will be administered in the clinic. Study Day 2 will begin at 12 midnight of the same calendar day. If the first dose of study drug is taken after 4 p.m. on Day 1, the next dose of study drug will be taken in the morning of Day 2. In this case, the last dose of study drug will be taken on the morning of study Day 11.

If the first dose of study drug is taken prior to 4 p.m. on Day 1, the next dose of study drug should be taken in the evening of the same day (i.e., prior to midnight on the same calendar day with a minimum of 7 hours between doses). For these patients, the last dose of study drug will be taken in the evening of study Day 10. More information on dosing is provided later (Section 6.1).

Study Days 2–11

Study Day 2 will begin at 12 midnight of study Day 1.

It is expected the study staff will maintain close contact with patients, especially during the first few days of the study. The study staff should inquire about any adverse events, check that the patient or parent/guardian is entering data into the patient diary properly, and assess drug compliance. During the dosing period, diary symptoms should be assessed and temperature should be recorded at the same time that the study drug is taken.

End of Treatment Day 11

The end-of-treatment visit for all patients is on Day 11 (irrespective of whether they took one or two doses on Day 1). Study medication will be administered twice daily over 10 days for a total of 20 doses. Patients who discontinue study drug prematurely will have all Day 11 assessments completed at the time of discontinuation or the following day.

After all Day 11 study assessments are completed, a rapid diagnostic test will be performed at this visit. If the rapid diagnostic test is positive, the patient may be treated per standard of care at the discretion of the investigator. If treatment is provided, this should be captured in the CRF.

All patients (including those who discontinue study drug prematurely and those who are positive for influenza on their rapid diagnostic test at the end of treatment visit) will be required to return for follow-up approximately 5 and 30 days after the last dose (Day 15 and Day 40 assessments).

Study Days 12–40

Study Day 15 is an important visit as it is the first assessment of viral culture after cessation of treatment. It is therefore important that this visit is completed.

End of Follow-Up Day 40

All patients must attend an end of follow-up visit on Day 40.

If the patient is withdrawn after completion of treatment (after the Day 11 assessment), a termination visit should be arranged. This visit should be the end of follow-up visit assessment [Day 40]. This visit must occur within 30 days of the last dose.

Patients or parents/guardians who do not return to the clinic will be contacted by telephone to ascertain their health status or that of their child's, record information on adverse events, and to ask that they return the patient diary.

5.3.1 Safety

Safety is one of the primary endpoints of this study. Safety parameters include adverse events, vital signs, and clinical laboratory evaluations.

Pre-defined symptoms of influenza captured in the adult and pediatric diaries are not to be reported as adverse events unless they can be further qualified. Thus 'headache due to stress at work' is reported as an adverse event. However, unexplained 'headache' is considered a predefined symptom related to influenza and not an adverse event.

Adverse events such as bronchitis, pneumonia, otitis media, and sinusitis are considered secondary illnesses of influenza and should be recorded as adverse events.

Other adverse events to be expected in the transplant population such as rejection and graft versus host disease (in patients with HSCT) will also be collected as adverse events. Nasal and throat swab samples will be assessed from each study visit for the presence of oseltamivir-resistant mutations.

5.3.2 Efficacy Assessments

The clinical efficacy parameters in this study are:

1. Symptoms of influenza-like illness. These are captured in the patient diary for both adults and children (Appendix 2) and (Appendix 3). Influenza symptoms are graded on a nominal scale from zero (absent/no problem) to 3 (severe or major problem). A score of zero is considered asymptomatic. The efficacy endpoint, time to alleviation of all symptoms is comprised of all the symptoms captured in the patient diary.
2. Temperature. This is captured in the patient diary. Temperature is used for assessment of several efficacy endpoints.

5.4 LABORATORY ASSESSMENTS

The laboratory assessments include those for safety, resistance, and efficacy.

Safety

The safety laboratory assessments in this study, including the assessment of serum chemistry and hematology, will be carried out at a central laboratory. Serum chemistry assessments comprise AST, ALT, total bilirubin, urea, and creatinine. Hematology assessments include CBC and differential count. The total volume of blood loss for laboratory assessments will be approximately 20 mL for the entire duration of the study.

Protection of patient confidentiality (Section 16) will extend to any data generated from the assay of these samples. Biological samples collected from all patients may be infectious and will be classified as “diagnostic specimens” for dispatch purposes.

When clinically indicated, the investigator may draw blood for serum creatinine to be assessed at the local laboratory during the study to calculate creatinine clearance.

Efficacy

The efficacy laboratory assessments are used for laboratory confirmation of influenza. These are:

Nasal and throat swabs. Two nasal and one throat swab will be collected as described in the Schedule of Assessments. All swabs are sent to a central laboratory for RT-PCR and viral cultures. Influenza virus shedding will be assessed.

At the end of treatment (Day 11), a rapid diagnostic test is permitted for the diagnosis of ongoing influenza.

5.5 PHARMACOKINETIC ASSESSMENTS/PHARMACODYNAMIC ASSESSMENTS

Participation in PK assessments is not compulsory for this study. Blood samples for the characterization of *oseltamivir and* oseltamivir carboxylate pharmacokinetics using a sparse sampling strategy will be collected from all patients who provide additional consent to participate in the PK assessments.

Blood samples will be collected according to the Schedule of Assessments (Table 1) and as described below. If these blood samples are collected at a home visit, site staff should ensure the PK blood sample handling processing is not compromised. The time and date of the dose and blood samples should be captured. Further details on pharmacokinetics/pharmacodynamics can be found in Sections 8.3.1 and 8.3.2.

5.5.1 Pharmacokinetic Assessments

Plasma PK samples for assessment of *oseltamivir and* oseltamivir carboxylate will be collected at the following timepoints on Day 6, or any day after the 11th dose, using the following sampling approach:

- Within 30 minutes prior to the dose administration (e.g., 8:30 a.m. *if dosing is scheduled for* 9:00 a.m.)
- 1.5 hours ± 30 minutes post-dose (e.g., 10:30 a.m. ± 30 min *if dosing is scheduled for 9:00 a.m.*)
- 4 hours ± 60 minutes post dose (e.g., 1:00 p.m. ± 60 min *if dosing is scheduled for 9:00 a.m.*)
- 8 hours ± 1.5 hours post dose (e.g., 5:00 p.m. ± 1.5 hr *if dosing is scheduled for 9:00 a.m.*)

For adults and adolescents, approximately 2 mL of blood will be collected at each timepoint; therefore, the total volume blood loss for PK assessments will be approximately 8 mL. For pediatric patients, not less than approximately 0.6 mL of blood will be collected at each timepoint; therefore, the total volume blood loss for PK assessments will be approximately 2.4 mL.

The samples from this study are classified as Biological Substance, Category B.

Plasma concentrations of *oseltamivir and* oseltamivir carboxylate will be measured by a specific and validated method. Details on sampling procedures, sample storage, and shipment are provided in the Sampling Manual.

5.5.2 Pharmacodynamic Assessments

Nasal and throat swabs will be collected from individuals on the days specified in the schedule of assessments. Specimens will be analyzed at a central laboratory. As immunocompromised patients are expected to shed virus for a longer duration, viral samples will be collected at all visits. The proportion of patients with viral shedding at each visit will be summarized.

6. INVESTIGATIONAL MEDICINAL PRODUCT

The Investigational Medical Product in this study is oseltamivir dry powder (to be reconstituted to a concentration of 12 mg/mL *or 6 mg/mL [the 6 mg/mL will only be used if applicable and not before 2015]*) or 75 mg capsules and matching placebo.

The Investigational Medicinal Products will be supplied, packaged individually for each subject and labeled in accordance with Roche Standard and local regulation by Roche Clinical Trial Supply, Basel, Switzerland.

6.1 DOSE AND SCHEDULE OF STUDY DRUG

Oseltamivir will be given twice daily over 10 days for a total of 20 doses. The doses need to be taken at 12 hourly intervals. Under no circumstances is a patient allowed to take two doses within 7 hours of each other.

Patients will be randomized to receive either conventional or double dose of study drug.

Conventional dose:

Children ages 1–12 years: Oseltamivir syrup

≤15 kg	30 mg twice daily
>15–23 kg	45 mg twice daily
>23–40 kg	60 mg twice daily
>40 kg	75 mg twice daily

Adults and adolescents (age ≥13 years): Oseltamivir capsules

75 mg twice daily

Patients randomized to the conventional dose will simultaneously receive matching placebo so as to blind them and the investigator from the double dose arm.

Double dose:

Children ages 1–12 years: Oseltamivir syrup

≤15 kg	60 mg twice daily
>15–23 kg	90 mg twice daily
>23–40 kg	120 mg twice daily
>40 kg	150 mg twice daily

Adults and adolescents (age ≥13 years): Oseltamivir capsules

150 mg twice daily

6.1.1 Dose Modifications

No dose modifications will be allowed on study.

6.2 PREPARATION AND ADMINISTRATION OF STUDY DRUG

Oseltamivir will be provided in two forms:

1. Oseltamivir capsules containing 75 mg of active drug and packaging material consisting of pregelatinized starch, povidone, talc, and sodium stearyl fumarate. All participants 13 years and older will receive this dosage form. *Oseltamivir capsules should be stored below 25°C [77°F]. Do not store refrigerated.*
2. A pediatric suspension containing 12 mg oseltamivir per mL *or 6 mg oseltamivir per mL (the 6 mg/mL will only be used if applicable and not before 2015)*, of reconstituted solution and the following excipients: sorbitol, titanium dioxide, sodium benzoate, xanthan gum, monosodium citrate, saccharin sodium, and Permaseal11900-31 Tutti Frutti (flavor). All participants 12 years and under will receive this dosage form. *Oseltamivir dry powder for* oral *suspension [pediatric syrup] should be stored below 30°C [86°F]*). Once reconstituted, *the suspension should not be used for longer than 10 days* if stored at room temperature conditions (below 25°C/*77°F*). If stored under refrigerated conditions at 2–8°C (36–46°F) the suspension should not be used for longer than 17 days. The suspension is not suitable for freezing.

For further details, refer to the Tamiflu® Investigator's Brochure.

Matching placebo will be available as capsules and suspension. Patients in the conventional dose arm will get the conventional dose and matching placebo so that they are blinded from the double dose arm.

Each patient will be dispensed a medication pack that will provide enough medication to cover 20 doses. For patients randomized to the conventional dose arm, the medication pack will contain a bottle of oseltamivir dry powder or a blister wallet with oseltamivir capsules and matching placebo. For patients randomized to the double dose arm, the medication pack will contain two bottles of oseltamivir dry powder or two blister wallets with oseltamivir capsules. Irrespective of the treatment group the patient is randomized to, for each dose the patient will take the same amount from both bottles or blister wallets provided in the medication pack such that the sum of the amounts from each immediate container constitutes one dose.

One dose is to be administered twice per day at approximately 12-hour intervals with a light snack or glass of milk or fruit juice. The first dose of study drug will be administered in the clinic at the time of randomization.

6.3 BLINDING AND UNBLINDING

Randomization will be administered by a central randomization center.

The Randomization List will not be available at the study center, to the study monitors, project statisticians, or to the project team at Roche. Emergency codes, or another adequate method of unblinding, will be implemented before study start, if the identity of

the test medication is necessary for patient management in the case of a serious adverse event. Any request from the investigator for information about the treatment administered to study patients for another purpose must be discussed with Roche/designee.

As per regulatory reporting requirement, Roche/designee will unblind the identity of the study drug for all unexpected [as per IB] serious adverse events that are considered by the investigator to be related to study drug. Details of patients who are unblinded during the study will be included in the Clinical Study Report.

All other individuals directly involved in this study will remain blinded until the final analysis of the primary parameter.

The randomization will be stratified by transplant status, duration of symptoms, influenza vaccination status and age and will be provided by the Roche randomization group for the IVRS vendor.

6.4 ASSESSMENT OF COMPLIANCE

Accountability and patient compliance will be assessed by maintaining adequate “drug dispensing” and return records.

Patients will be asked to return all used and unused drug supply containers at the end of the treatment as a measure of compliance.

A Drug Dispensing Log must be kept current and should contain the following information:

- the identification of the patient [randomization and medication numbers] to whom the study drug was dispensed
- the date[s], quantity of the study drug dispensed to the patient
- the date[s] and quantity of the study drug returned by the patient

This inventory must be available for inspection by the Monitor. All supplies, including partially used or empty containers and the dispensing logs, must be returned to the monitor at the end of the study.

6.5 DESTRUCTION OF STUDY DRUG

Local or institutional regulations may require immediate destruction of used investigational product for safety reasons. In these cases, it may be acceptable for investigational site staff to destroy dispensed investigational product before a monitoring inspection provided that source document verification is performed on the remaining inventory and reconciled against the documentation of quantity shipped, dispensed, returned, and destroyed. Written authorization must be obtained from the sponsor or designee at study start up before destruction.

Written documentation of destruction must contain the following:

- Identity [batch numbers or patient numbers] of investigational product[s] destroyed
- Quantity of investigational product[s] destroyed
- Date of destruction
- Method of destruction
- Name and signature of responsible person [or company] who destroyed investigational products[s]

7. SAFETY INSTRUCTIONS AND GUIDANCE

7.1 ADVERSE EVENTS AND LABORATORY ABNORMALITIES

7.1.1 Clinical AEs

Per the International Conference of Harmonization [ICH], an Adverse Event (AE) is any untoward medical occurrence in a patient or clinical investigation patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign [including an abnormal laboratory finding], symptom, or disease temporally associated with the use of a medicinal [investigational] product, whether or not considered related to the medicinal [investigational] product. Pre-existing conditions that worsen during a study are to be reported as AEs. Influenza signs and symptoms reported on the patient diary will be summarized as efficacy endpoints and need not be captured as adverse events. However, secondary illnesses due to influenza must be reported as adverse events.

7.1.1.1 Intensity

All clinical AEs encountered during the clinical study will be reported on the AE page of the CRF.

Intensity of AEs will be graded on a four-point scale [mild, moderate, severe, life-threatening] and reported in detail on the CRF.

Mild	discomfort noticed but no disruption of normal daily activity.
Moderate	discomfort sufficient to reduce or affect daily activity.
Severe	inability to work or perform normal daily activity.
Life Threatening	represents an immediate threat to life.

7.1.1.2 Drug - Adverse Event Relationship

Relationship of the AE to the treatment should always be assessed by the investigator. Description of scales can be found in Appendix 4.

7.1.1.3 Serious Adverse Events [Immediately Reportable to Roche]

Certain events require immediate reporting to allow the Sponsor to take appropriate measures to address potential new risks in a clinical trial. The investigator must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours after the investigator learns of the event. The following is a list of events that the investigator must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study drug:

- Serious adverse events
- Pregnancies

A Serious Adverse Event is any experience that suggests a significant hazard, contraindication, side effect, or precaution. It is any AE that at any dose fulfills at least one of the following criteria:

– is fatal; [results in death; NOTE: death is an outcome, not an event].

– is Life-Threatening [NOTE: the term "Life-Threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe].

– required in-patient hospitalization or prolongation of existing hospitalization;

– results in persistent or significant disability/incapacity;

– is a congenital anomaly/birth defect;

– is medically significant or requires intervention to prevent one or other of the outcomes listed above.

The full requirements of the ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 will be adhered to (Appendix 5).

7.1.2 Treatment and Follow-up of AEs

AEs, especially those for which the relationship to test “drug” is not “unrelated,” should be followed up until they have returned to baseline status or stabilized. If a clear explanation is established it should be recorded on the CRF.

7.1.3 Laboratory Test Abnormalities

Laboratory test results will appear printed on laboratory reports provided to the site from the central laboratory.

Any laboratory result abnormality fulfilling the criteria for a serious adverse event [SAE] should be reported as such, in addition to being recorded as an AE in the CRF.

Any treatment-emergent abnormal laboratory result which is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the AE page in the CRF:

- Accompanied by clinical symptoms.
- Leading to a change in study drug [e.g., dose modification, interruption, or permanent discontinuation].
- Requiring a change in concomitant therapy [e.g., addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy, or treatment].

7.1.4 Follow-up of Abnormal Laboratory Test Values

In the event of medically significant unexplained abnormal laboratory test values, the tests should be repeated and followed up until they have returned to the normal range and/or an adequate explanation of the abnormality is found. If a clear explanation is established it should be recorded on the CRF.

7.2 HANDLING OF SAFETY PARAMETERS

7.2.1 Reporting of Serious Adverse Events [Immediately Reportable]

Any clinical AE or abnormal laboratory test value that is serious [as defined in Section 7.1.1.3 above] and which occurs during the course of the study, regardless of the treatment arm, occurring from the enrollment visit (start of study screening procedures), must be reported to Roche or designee immediately (i.e., no more than 24 hours after the investigator becomes aware of the event [expedited reporting]).

Related Serious Adverse Events ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed.

Unrelated Serious Adverse Events must be collected and reported during the study and up until the follow-up visit.

The definition and reporting requirements of ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 will be adhered to (Appendix 5). The serious adverse events should be reported in the most up-to-date version of the SAE form.

7.2.2 Pregnancy

A female patient must be instructed to stop taking the test “drug” and immediately inform the investigator if she becomes pregnant during the study. The investigator should report all pregnancies immediately (i.e., no more than 24 hours after the investigator becomes aware of the pregnancy) to the Sponsor or designee, using the most up-to-date version of the pregnancy form. The investigator should counsel the patient, and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy.

Pregnancies occurring within 28 days of treatment completion should be reported to Roche.

7.3 WARNINGS AND PRECAUTIONS

Events such as convulsions and delirium (including symptoms such as altered level of consciousness, confusion, abnormal behavior, delusions, hallucinations, agitation, anxiety, nightmares) have been reported during oseltamivir use in patients with influenza, predominately in children and adolescents. In rare cases, these events resulted in accidental injury. The contribution of oseltamivir to those events is unknown. Such events have also been reported in patients with influenza who were not taking oseltamivir. Patients, especially children and adolescents, should be closely monitored for signs of abnormal behavior.

Please refer to the attached Investigator's Brochure for additional warnings, precautions, and other reported adverse events.

8. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

Full details of all planned analyses will be specified in a separate Statistical Analysis Plan (SAP) for the safety and efficacy variables and in the Resistance Plan for the development of resistance. The methods described below are an outline of the main planned analyses. For continuous endpoints summary statistics including mean, standard deviation, median, minimum, and maximum will be derived for each treatment group. For categorical data, the number and percentage of events and/or patients for each treatment group will be presented.

8.1 PRIMARY AND SECONDARY STUDY ENDPOINTS

8.1.1 Primary Endpoints

The primary endpoints for safety will be descriptive assessments of:

- Adverse events
- Physical examination
- Vital signs
- Clinical laboratory evaluations
- Tissue rejection and/or graft versus host disease in transplant patients

The primary endpoint of development of resistance will be determined from the genotypic and phenotypic variables measured post baseline and is described further in the Resistance Plan.

8.1.2 Secondary Endpoints

The following endpoints will be analyzed descriptively at relevant timepoints.

The pharmacodynamics assessments based on nose and throat swabs, collected on the days specified in the schedule of assessments:

- Shedding virus by culture and RT-PCR
- Viral load by culture ($log_{10}TCID_{50}$/mL) and quantitative RT-PCR
- Individual symptom scores
- The time (hours) from first dose of study drug until resolution of fever
- Development of secondary illnesses (otitis media, bronchitis, pneumonia, or sinusitis) at any time during the study
- Development of secondary illnesses (otitis media, bronchitis, pneumonia, or sinusitis) at any time during the study that are treated with antibiotics
- Initiation of treatment with antibiotics after randomization
- Hospitalization, and for those who are hospitalized, the duration of hospitalization
- Development of rejection or GVHD

The following are the model-predicted PK secondary endpoints for *oseltamivir and* oseltamivir carboxylate:

- Steady-state area under the concentration–time curve from 0 to 12 hours (AUC_{0-12})
- Maximum plasma concentration (C_{max})
- Trough plasma concentration (C_{trough})

The following model-predicted PK endpoints may be included, if appropriate:

- Elimination half-life ($t_{1/2}$)
- Time to maximum concentration (t_{max})
- Elimination constant (k_e)
- Apparent clearance (CL/F)
- Apparent volume of distribution (Vc/F)
- Apparent total clearance of metabolite (CL_m)
- Last measurable concentration (C_{last}) and time to last measurable concentration (t_{last})

8.2 STATISTICAL AND ANALYTICAL METHODS

8.2.1 Statistical Model

8.2.1.1 Time to Event Variables

A non-parametric model will be assumed with estimation of medians based on Kaplan-Meier methods. Patients without alleviation of symptoms will have their time censored at the last available observation that a complete assessment was made.

8.2.1.2 Dichotomous Variables and Viral Load

For the endpoints defined dichotomously in terms of events or symptoms including the development of resistance, it will be assumed that the number of patients experiencing the event or exhibiting the symptom will have a binomial distribution.

For the continuous endpoints of viral load at Days 1, 2, 6, 8, 11, 15, and 40, no model will be assumed.

8.2.2 Sample Size

The sample size has been chosen to provide an adequate number of patients to estimate the development of resistance with reasonable precision. Assuming that 90% of enrolled patients will have laboratory-confirmed influenza, there will be 75 patients in each treatment arm in the population evaluable for the development of resistance. The following table shows the 95% Pearson-Clopper confidence intervals that would result with a sample size of 75 patients in a treatment arm if certain event rates are observed in the study.

During the study, the number of influenza A virus infected patients and the rate of development resistance will be monitored in a blinded fashion in order to ensure a reasonable precision for the estimation. An additional number of patients may be enrolled if necessary.

Observed Rate (%)	95% Confidence Interval
0.0	0.0%–4.8%
1.3	0.0%–7.2%
2.7	0.3%–9.3%
5.3	1.5%–13.1%
10.7	4.7%–19.9%

A total of 83 patients will be enrolled per arm and will be evaluable for the assessment of safety. This number of patients would provide estimates of adverse event rates with similar precision.

8.2.3 Hypothesis Testing

Formal hypothesis testing will not be performed; any comparisons between groups will be based on comparison of confidence intervals.

8.2.4 Analysis Populations

Three main patient populations will be used for the analysis of data from this study; the Safety Population, the Intent-to treat Population and the Intent-to-Treat Infected Population. Detailed definitions of these populations will be given in the SAP.

8.2.4.1 Intent to Treat Population

All patients randomized will be included in the intent-to-treat population [Patients will be assigned to treatment groups as randomized for analysis purposes].

8.2.4.2 Intent to Treat Infected Population

All patients randomized and with laboratory confirmation of influenza infection, excluding patients infected with oseltamivir-resistant influenza at baseline, will be included in the intent-to-treat influenza infected population [Patients will be assigned to treatment groups as randomized for analysis purposes].

The ITTI Population will be the primary population for the summary and analysis of the development of resistance and the efficacy variables.

8.2.4.3 Pharmacokinetic Evaluable Patient Population

The Pharmacokinetic evaluable patient population (PKEP) population comprises all patients in the ITT population who have at least one post-dose drug concentration measurement at a scheduled visit timepoint. Patients may be excluded from the PKEP population if they significantly violate the inclusion or exclusion criteria, deviate significantly from the protocol, or have unavailable or incomplete data that may influence the PK analysis.

Decisions on patient exclusion from the PK analysis will be made prior to database closure by the clinical pharmacologist. Excluded patients will be documented along with the reason for exclusion.

8.2.4.4 Subpopulations

A subpopulation of the ITTI population will be defined comprising patients who received their first dose of study drug within 48 hours of influenza symptom onset for the comparison of efficacy endpoints with the age appropriate placebo treated patients (pediatric or adult) from the registration trials.

8.2.5 Safety Data Analysis

The safety analysis population will include all patients who receive at least one dose of study drug and had a safety assessment performed post randomization. All safety variables will be summarized and presented in tables based on this safety population:

- Adverse events
- Physical examination
- Vital signs
- Clinical laboratory evaluations
- Rejection and/or graft versus host disease

8.2.6 Analysis of Resistance

For the summary of the development of resistance for each treatment arm, 95% confidence intervals will be provided with the estimated rates. Further details are available in the Resistance Plan for NV20234. These data will be summarized in a report separate from the final study report.

8.2.7 Efficacy Analysis

For the analysis of time to alleviation of all clinical symptoms, the following two assessments will be made independently of each other and without regard to the outcomes in each case.

- Comparison to placebo control from pivotal registration trials

  From the integrated efficacy database associated with the oseltamivir treatment approval, a population of placebo-treated patients will be established that is comparable to the subpopulation of patients in the current study whose first dose of study drug was within 48 hours of symptom onset. For this population, a median time to resolution of all clinical influenza symptoms will be determined along with its 95% confidence interval.

  The median time to resolution of all influenza symptoms will be determined for each treatment group, along with its 95% confidence interval.

- The two dose groups will be compared to each other by estimating the difference in the median times to alleviation of symptoms and deriving an associated 95% confidence interval.

For the dichotomous endpoints, the proportion of patients experiencing the event or symptom, and its associated 95% confidence interval will be derived for each treatment group. Where possible, the corresponding confidence intervals from the placebo control population will be presented for comparison to those derived for the active treatment groups in this trial.

The continuous secondary endpoint time to resolution of fever will be analyzed as for the time to alleviation of all symptoms.

8.2.7.1 Exclusion of Data from Analysis

Efficacy data collected from any patient who begins treatment with another antiviral for influenza (for example, amantadine, rimantadine, laninamivir, peramivir, or zanamavir), after the other antiviral treatment commences, will be excluded from the primary and secondary efficacy analyses. Such patients will be considered failures to oseltamivir treatment in the analyses. Further details will be provided in the SAP.

8.2.7.2 Interim Analysis

No interim analyses are planned.

8.2.8 Other Analyses

Further exploratory analyses will be detailed in the SAP.

8.3 PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSIS

8.3.1 Pharmacokinetic Analysis

The primary study variables are the model-derived PK parameters: steady-state AUC, C_{max}, and C_{trough} of *oseltamivir and* oseltamivir carboxylate.

Individual and mean plasma concentrations at each sampling timepoint for *oseltamivir and* oseltamivir carboxylate will be presented by listings and descriptive summary statistics, including means, geometric means, medians, ranges, standard deviations, and coefficients of variation. Individual and mean concentration-versus-time profiles will be plotted on linear and semi-logarithmic scales.

Plasma concentration data from sparse sampling will be analyzed using an established population PK model to determine key exposure parameters (e.g., C_{max}, C_{trough}, and AUC). For immunocompromised children aged 1 to 18 years, plasma *oseltamivir and* oseltamivir carboxylate concentration will be modeled in NONMEM using a structure similar to a comprehensive population PK model, which was previously developed using plasma data from non-immunocompromised children and adults (ages 1 to 80 years) [36]. The basic structure consists of a 2-compartment model with first-order absorption and direct conversion of oseltamivir to oseltamivir carboxylate, while a 1-compartment model is used to account for the renal elimination of oseltamivir carboxylate from the plasma. Body weight, evaluated using a power function and centered around 70 kg, is a statistically significant predictor of *the* CL/F *for oseltamivir and both CL/F* and apparent volume of distribution (Vc/F) for oseltamivir carboxylate. For oseltamivir carboxylate, CrCl is also a significant predictor of CL/F, while Vc/F decreases linearly with age.

8.3.2 Pharmacokinetic/Pharmacodynamic Analysis

If feasible, exposure-response relationships [43] will be evaluated between independent variables of exposure (e.g., AUC, C_{min}) and dependent variables including continuous (area under the viral titer curve, and peak viral titer) and time-to-event (cessation of viral shedding) virologic endpoints. Exploratory PK/PD analyses will be performed using appropriate methodologies. In order to account for potential non-linearity and non-monotonicity, each continuous independent variable (e.g., AUC) will be evaluated in its original form, as a categorical variable based on quartiles, as two- and three-group categorical variables.

9. DATA QUALITY ASSURANCE

The overall procedures for quality assurance of clinical study data are described in the Roche Standard Operational Procedures.

Accurate and reliable data collection will be assured by verification and cross–check of the CRFs against the investigator's records by the study monitor [source document verification] and the maintenance of a drug–dispensing log by the investigator.

The data collected will be entered into the study database from the working copy of the CRF faxed from the site.

A comprehensive validation check program will verify the data and discrepancy reports will be generated accordingly for resolution by the investigator. As patients complete the study [or prematurely withdraw] and their signed CRFs become available, a second data entry will be performed from the original, signed CRF. A comparison check will be run to identify and resolve any discrepancies between the first and second data entry.

Throughout the study the Study Management Team [SMT] will review data according to the SMT Data Review Plan as described in the Data Quality Plan.

9.1 ASSIGNMENT OF PREFERRED TERMS AND ORIGINAL TERMINOLOGY

For classification purposes, preferred terms will be assigned by the sponsor to the original terms entered on the CRF, using the current version of MedDRA (Medical Dictionary for Regulatory Activities terminology) for adverse events and diseases and the INN (International Non-Proprietary name) drug terms and procedures dictionary for treatments and surgical and medical procedures.

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PART II: ETHICS AND GENERAL STUDY ADMINISTRATION

11. ETHICAL ASPECTS

11.1 LOCAL REGULATIONS/DECLARATION OF HELSINKI

The investigator will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study must fully adhere to the principles outlined in "Guideline for Good Clinical Practice" ICH Tripartite Guideline [January 1997] or with local law if it affords greater protection to the patient. For studies conducted in the European Union/European Economic Area countries, the investigator will ensure compliance with the EU Clinical Trial Directive [2001/20/EC]. For studies conducted in the United States or under U.S. IND, the investigator will additionally ensure that the basic principles of "Good Clinical Practice" as outlined in the current version of 21 CFR, subchapter D, part 312, "Responsibilities of Sponsors and Investigators", part 50, "Protection of Human Subjects", and part 56, "Institutional Review Boards", are adhered to.

In other countries where "Guideline for Good Clinical Practice" exists, Roche and the investigators will strictly ensure adherence to the stated provisions.

11.2 INFORMED CONSENT

It is the responsibility of the investigator, or a person designated by the investigator [if acceptable by local regulations], to obtain written informed consent from each patient participating in this study after adequate explanation of the aims, methods, anticipated benefits, and the potential hazards of the study. For patients not qualified or incapable of giving legal consent, written consent must be obtained from the legally acceptable representative. In the case where both the patient and his/her legally acceptable representative are unable to read, an impartial witness should be present during the entire informed consent discussion. After the patient and representative have orally consented to participation in the trial, the witness' signature on the form will attest that the information in the consent form was accurately explained and understood. The investigator or designee must also explain that the patients are completely free to refuse to enter the study or to withdraw from it at any time, for any reason. The Case Report Forms [CRFs] for this study contain a section for documenting informed patient consent, and this must be completed appropriately. If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All patients [including those already being treated] should be informed of the new information, given a copy of the revised form, and give their consent to continue in the study.

If children are old enough to understand the risks and benefits of the study, they should also be informed and should also provide their written consent.

11.3 INDEPENDENT ETHICS COMMITTEES/INSTITUTIONAL REVIEW BOARD

Independent Ethics Committees [non-U.S.]: This protocol and any accompanying material provided to the patient [such as patient information sheets or descriptions of the study used to obtain informed consent] as well as any advertising or compensation given to the patient, will be submitted by the investigator to an Independent Ethics Committee. Approval from the committee must be obtained before starting the study and should be documented in a letter to the investigator specifying the date on which the committee met and granted the approval.

Any modifications made to the protocol after receipt of the Independent Ethics Committee approval must also be submitted by the investigator to the Committee in accordance with local procedures and regulatory requirements.

When no local review board exists, the investigator is expected to submit the protocol to a regional committee. If no regional committee exists, Roche will assist the investigator in submitting the protocol to the European Ethics Review Committee.

Institutional Review Board [U.S.]: It is the understanding of the sponsor that this protocol [and any modifications] as well as appropriate consent procedures, will be reviewed and approved by an Institutional Review Board. This board must operate in accordance with the current Federal Regulations. A letter or certificate of approval will be sent by the investigator to the Sponsor or designee prior to initiation of the study, and also whenever subsequent modifications to the protocol are made.

12. CONDITIONS FOR MODIFYING THE PROTOCOL

Protocol modifications to ongoing studies must be made only after consultation between an appropriate representative of the sponsor and the investigator [investigator representative[s] in the case of a multicenter trial]. Protocol modifications must be prepared by a representative of the sponsor and initially reviewed and approved by the Clinical Science Leader/Clinical Pharmacologist and Biostatistician.

All protocol modifications must be submitted to the appropriate Independent Ethics Committee or Institutional Review Board for information and approval in accordance with local requirements, and to Regulatory Agencies if required. Approval must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial patients, or when the change[s] involves only logistical or administrative aspects of the trial [e.g., change in monitor[s], change of telephone number[s].

13. CONDITIONS FOR TERMINATING THE STUDY

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange the procedures on an individual study

basis after review and consultation. In terminating the study, Roche and the investigator will assure that adequate consideration is given to the protection of the patient's interests.

14. STUDY DOCUMENTATION, CRFS, AND RECORD KEEPING

14.1 INVESTIGATOR'S FILES/RETENTION OF DOCUMENTS

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two different separate categories [1] Investigator's Study File, and [2] patient clinical source documents.

The Investigator's Study File will contain the protocol/amendments, Case Report and Query Forms, Independent Ethics Committee/Institutional Review Board and governmental approval with correspondence, sample informed consent, drug records, staff curriculum vitae and authorization forms, and other appropriate documents/correspondence, etc.

Patient clinical source documents [usually defined by the project in advance to record key efficacy/safety parameters independent of the CRFs] would include patient hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, EEG, X-ray, pathology and special assessment reports, signed informed consent forms, consultant letters, and patient screening and enrollment logs. The Investigator must keep these two categories of documents on file for at least 15 years after completion or discontinuation of the study. After that period of time the documents may be destroyed, subject to local regulations.

Should the Investigator wish to assign the study records to another party or move them to another location, Roche must be notified in advance.

If the Investigator cannot guarantee this archiving requirement at the investigational site for any or all of the documents, special arrangements must be made between the Investigator and Roche to store these in a sealed container[s] outside of the site so that they can be returned sealed to the Investigator in case of a regulatory audit. Where source documents are required for the continued care of the patient, appropriate copies should be made for storing outside of the site.

14.2 SOURCE DOCUMENTS AND BACKGROUND DATA

The investigator shall supply the sponsor or designee on request with any required background data from the study documentation or clinic records. This is particularly important when CRFs are illegible or when errors in data transcription are suspected. In case of special problems and/or governmental queries or requests for audit inspections, it is also necessary to have access to the complete study records, provided that patient confidentiality is protected.

14.3 AUDITS AND INSPECTIONS

The investigator should understand that source documents for this trial should be made available to appropriately qualified personnel from the Roche Pharma Development Quality Assurance Unit or its designees or to health authority inspectors after appropriate notification. The verification of the CRF data must be by direct inspection of source documents.

14.4 CASE REPORT FORMS

For each patient enrolled, a CRF must be completed and signed by the principal investigator or authorized delegate from the study staff. This also applies to records for those patients who fail to complete the study [even during a pre-randomization screening period if a CRF was initiated]. If a patient withdraws from the study, the reason must be noted on the CRF. If a patient is withdrawn from the study because of a treatment-limiting AE, thorough efforts should be made to clearly document the outcome.

All forms should be typed or filled out using indelible ink and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or his/her authorized delegate. The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor or designee in the CRFs and in all required reports.

15. MONITORING THE STUDY

It is understood that the responsible Roche monitor [or designee] will contact and visit the investigator regularly and will be allowed, on request, to inspect the various records of the trial [CRFs and other pertinent data] provided that patient confidentiality is maintained in accord with local requirements.

It will be the monitor's responsibility to inspect the CRFs at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitor should have access to laboratory test reports and other patient records needed to verify the entries on the CRF. The investigator [or his/her deputy] agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

16. CONFIDENTIALITY OF TRIAL DOCUMENTS AND PATIENT RECORDS

The investigator must assure that patients' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents *(both paper and electronic)* submitted to the sponsor or designee, patients should not be identified by their names but by an identification code. The investigator should keep a patient enrollment log showing codes, names, and addresses. The investigator should

maintain documents not for submission to Roche, e.g., patients' written consent forms, in strict confidence.

17. PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to Roche prior to submission. This allows the sponsor or designee to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the investigator.

In accord with standard editorial and ethical practice, Roche will generally support publication of multicenter trials only in their entirety and not as individual center data. In this case, a coordinating investigator will be designated by mutual agreement.

Any formal publication of the study in which input of Roche personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate Roche personnel. Authorship will be determined by mutual agreement.

Appendix 1 Calculation of Creatinine Clearance and Grading of Chronic Extensive GVHD

Calculation of Creatinine Clearance

Conversion Factor for Serum Creatinine:
Conventional Units (mg/dL) = SI Units (μmol/L) ÷ 88.4
Age = number of years (12 years 11 months = 12 years)

Estimated Creatinine Clearance according to Cockcroft-Gault [13]

(for patients ≥ 18 years):

- **Males**

Creatinine Clearance (mL/min) = **[(140 – age) × Body Weight (kg)] ÷ [72 × Serum Creatinine (mg/dL)]**

- **Females**

Creatinine Clearance = above equation × 0.85

Estimated Creatinine Clearance according to Schwartz equation [14]
(for patients < 18 years):

Creatinine Clearance
(mL/min/1.73 M^2) = k × Height (cms) ÷ **Serum Creatinine** (mg/dL)

Value for k:

k	Age (years)
0.45	< 2
0.55	≥ 2 to < 13
0.7	≥ 13 to < 18 (males)
0.55	**≥ 13 to < 18 (females)**

Appendix 1 Calculation of Creatinine Clearance and Grading of Chronic Extensive GVHD (cont.)

Grading of Chronic GVHD [15]

Type of Disease	Extent of Disease
Limited	Localized skin involvement, liver dysfunction or both
Extensive	Generalized skin involvement
	Localized skin involvement or liver dysfunction plus any one of the following: 1. Chronic aggressive hepatitis, bridging necrosis or cirrhosis 2. Eye involvement (Schirmer's test, $<$5 mm) 3. Involvement of mucosalivary glands 4. Mucosal involvement (on lip biopsy) 5. Involvement of other target organs

Appendix 2 Patient Diary Data and Symptom Record for Adults

The purpose of the patient diary is for all adults (13 years and older) to record any symptoms of influenza-like illness for the duration of the study. Temperature and date and time of drug administration should also be recorded on the patient diary.

Date of assessments ⌊ ⌋⌊ ⌋⌊ ⌋ (dd mm yy) Time of assessments (24 hr clock) ⌊ ⌋⌊ ⌋ (h min)

Vital signs

Temperature ⌊ ⌋■⌊ ⌋ °C

Symptoms of influenza-like illness

Please record the worst you have felt since the last assessment.
Please mark one box only per symptom.
The information you provide is very important and will remain strictly confidential.

	absent 0	mild 1	moderate 2	severe 3
1. Nasal congestion	☐	☐	☐	☐
2. Sore throat	☐	☐	☐	☐
3. Cough	☐	☐	☐	☐
4. Aches and pains	☐	☐	☐	☐
5. Fatigue (tiredness)	☐	☐	☐	☐
6. Headache	☐	☐	☐	☐
7. Chills/sweats (feverish)	☐	☐	☐	☐

Please inform study staff at your next visit of any medications that you have taken today or any medical problems that you have experienced.

Appendix 3 Patient Diary Data and Symptom Record for Children

Temperature and date and time of drug administration will also be recorded on the patient diary.

Study medication administration (if applicable)

Date of dose ___ ___ ___ (dd mm yy)

Time of dose (24 hr clock) ___ ___ : ___ ___ (h min)

Bottle A amount mg ___

Bottle B amount mg ___

Date of assessments ___ ___ ___ (dd mm yy)

Time of assessments (24 hr clock) ___ ___ : ___ ___ (h min)

Vital signs

Temperature ___ . ___ °C

Symptoms of influenza-like illness

Please record the worst you have felt since the last assessment.
Please mark one box only per symptom.
The information you provide is very important and will remain strictly confidential.

	No Problem 0	Minor Problem 1	Moderate Problem 2	Major Problem 3	Don't Know or not Applicable 4
1. Poor appetite	☐	☐	☐	☐	☐
2. Not sleeping well	☐	☐	☐	☐	☐
3. Irritable, cranky, fussy	☐	☐	☐	☐	☐
4. Feels unwell	☐	☐	☐	☐	☐
5. Low energy, tired	☐	☐	☐	☐	☐
6. Not playing well	☐	☐	☐	☐	☐
7. Crying more than usual	☐	☐	☐	☐	☐
8. Needing extra care	☐	☐	☐	☐	☐
9. Clinginess	☐	☐	☐	☐	☐
10. Headache	☐	☐	☐	☐	☐
11. Sore throat	☐	☐	☐	☐	☐
12. Muscle aches or pains	☐	☐	☐	☐	☐
13. Fever	☐	☐	☐	☐	☐
14. Cough	☐	☐	☐	☐	☐
15. Nasal congestion, runny nose	☐	☐	☐	☐	☐
16. Vomiting	☐	☐	☐	☐	☐
17. Not interested in what's going on	☐	☐	☐	☐	☐
18. Unable to get out of bed	☐	☐	☐	☐	☐

This form was filled out by:

1 ☐ Parent
2 ☐ Other relative
3 ☐ Nanny
4 ☐ Subject
5 ☐ Other *specify* _____

Please inform study staff at your/your child's next visit of any medications that you/your child have taken today or any medical problems that you/your child have experienced.

Appendix 4 Adverse Event [AE] Categories for Determining Relationship to Test Drug

PROBABLE [must have first three]

This category applies to those AEs that are considered, with a high degree of certainty, to be related to the test drug. An AE may be considered probable, if:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It cannot be reasonably explained by the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
3. It disappears or decreases on cessation or reduction in dose. [There are important exceptions when an AE does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists; e.g., [1] bone marrow depression, [2] tardive dyskinesias].
4. It follows a known pattern of response to the suspected drug.
5. It reappears upon rechallenge.

POSSIBLE [must have first two]

1. This category applies to those AEs in which the connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possible if or when:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It may have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
3. It follows a known pattern of response to the suspected drug.

REMOTE [must have first two]

1. In general, this category is applicable to an AE that meets the following criteria:
2. It does <u>not</u> follow a reasonable temporal sequence from administration of the drug.
3. It may readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
4. It does not follow a known pattern of response to the suspected drug.
5. It does not reappear or worsen when the drug is re-administered.

UNRELATED

This category is applicable to those AEs that are judged to be clearly and incontrovertibly due only to extraneous causes [disease, environment, etc.] and do not meet the criteria for drug relationship listed under remote, possible, or probable.

Appendix 4 Adverse Event [AE] Categories for Determining Relationship to Test Drug (cont.)

	Probable	Possible	Remote	Unrelated
Clearly due to extraneous causes	–	–	–	+
Reasonable temporal association with drug administration	+	+	–	–
May be produced by patient clinical state, etc.	–	+	+	+
Known response pattern to suspected drug	+	+	–	–
Disappears or decreases on cessation or reduction in dose	+	–	–	–
Reappears on rechallenge	+	–	–	–

Appendix 5 ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect, or precaution. It is any AE that at any dose fulfills at least one of the following criteria:

- is fatal; [results in death] [NOTE: death is an outcome, not an event].
- is Life-Threatening [NOTE: the term "Life-Threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe].
- required in-patient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is medically significant or requires intervention to prevent one or other of the outcomes listed above.

Medical and scientific judgment should be exercised in deciding whether expedited reporting to the sponsor or designee is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definitions above. These situations should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

An unexpected AE is one, the nature or severity of which is not consistent with the applicable product information.

Causality is initially assessed by the investigator. For Serious Adverse Events, possible causes of the event is indicated by selecting one or more options. (Check all that apply)

- Pre-existing/Underlying disease - specify
- Study treatment – specify the drug(s) related to the event
- Other treatment (concomitant or previous) – specify
- Protocol-related procedure
- Other (e.g., accident, new or intercurrent illness) - specify

The term severe is a measure of intensity, thus a severe AE is not necessarily serious. For example, nausea of several hours' duration may be rated as severe, but may not be clinically serious.

Appendix 5 ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 (cont.)

A serious adverse event occurring during the study or which comes to the attention of the investigator within 15 days after stopping the treatment or during the protocol-defined follow-up period, if this is longer, whether considered treatment-related or not, must be reported. In addition, a serious adverse event that occurs after this time, if considered related to test "drug", should be reported.

Such preliminary reports will be followed by detailed descriptions later, which will include copies of hospital case reports, autopsy reports, and other documents when requested and applicable.

For serious adverse events, the following must be assessed and recorded on the AEs page of the CRF: intensity, relationship to test substance, action taken, and outcome to date.

The investigator must notify the Ethics Review Committee/Institutional Review Board of a serious adverse event in writing as soon as is practical and in accordance with international and local laws and regulations.

ROCHE LOCAL COUNTRY CONTACT for SAEs: Local Monitor

ROCHE HEADQUARTERS CONTACT for SAEs: Clinical Operations/Clinical Science

To ensure the safety of study patients, an Emergency Medical Call Center Help Desk will access the Roche Medical Emergency List, escalate emergency medical calls, provide medical translation service (if necessary), connect the investigator with a Roche Medical Monitor, and track all calls. The Emergency Medical Call Center Help Desk will be available 24 hours per day, 7 days per week. Toll-free numbers for the Help Desk and Medical Monitor contact information will be distributed to all investigators.

Appendix 6 Primary Immunodeficiency Conditions

Category	Conditions
Severe Combined Immunodeficiency (SCID)	Adenosine deaminse (ADA) deficiency
	Artemis deficiency (SCIDA)
	CD45 deficiency
	Cernunnos deficiency
	DNA ligase IV (LIG4) deficiency
	Interleukin receptor γ chain deficiency (X-linked SCID)
	Janus-associated kinase 3 (JAK3) deficiency
	Recombinase activating gene (RAG 1/2) deficiency
	Reticular dysgenesis
	TAP- 1 or TAP- 2 deficiency (MHC class I deficiency)
Primary T cell Deficiency	CD8 deficiency
	diGeorge syndrome
	Interleukin 7 receptor α (IL7RA) deficiency
	MHC class II deficiency
	LCK deficiency
	Orai 1 deficiency
	Nude syndrome (wing helix nude deficiency)
	Purine nucleotide phosphorylase (PNP) deficiency
	T cell receptor deficiency (CD 3γ, δ, ε, and ζ deficiencies)
	Zap 70 tyrosine kinase deficiency
Predominantly Antibody Deficiency	X-Linked CD40 ligand deficiency
	X- Linked IKK-γ (NEMO) deficiency
	CD40 deficiency
Other Well-Defined immunodeficiency Syndromes	Interferon γ receptor deficiency
	X-Linked lymphoproliferative syndrome

Adapted from Table 310-2: p2056 reference 16.

Appendix 7 Hematologic Malignancies and their Effect on the Immune System

Malignancy	Effect on immunity
ALL, lymphomas	suppression of hematopoiesis, neutropenia, lymphocyte dysfunction
CLL, small lymphocytic lymphoma	hypogammaglobulinemia, increased susceptibility to infections, autoimmune anemia or thrombocytopenia
Hairy cell leukemia, myelodysplastic syndromes	pancytopenia
peripheral T cell and NK neoplasms	lymphocyte dysfunction, increase in immature cells
Hodgkin's disease	suppression of cell-mediated immunity
AML	neutropenia, anemia, thrombocytopenia, predisposed to infections, lymphocyte dysfunction
CML	anemia, granulocyte dysfunction in some patients in early phase and in most patients in blast phase

Adapted from reference 16.

Appendix 8 Immunosuppressive Medications

Class	Category	Drugs
Corticosteroids	-	Glucocorticoids (oral, sc, im, iv)
Cytotoxic agents[16]	Alkylating agents	cyclophosphamide, busulfan, mechlorethamine, chlorambucil, melphalan, carmustine, lomustine, ifosfamide, procarbazine, dacarbazine, temozolomide, cisplatin, carboplatin, oxaliplatin
	Anti-metabolites	methotrexate, 6-mercaptopurine, azathioprine
	Anti-tumor antibiotics	bleomycin, actinomycin D, mitomycin C, etoposide, topotecan, irinotecan, doxorubicin, daunorubicin, idarubicin, epirubicin, mitoxantrone
	Anti-mitotic agents	vincristine, vinblastine, vinorelbine, paclitaxel, docetaxel, estramustine phosphate, NAB-paclitaxel
	Molecularly targeted agents	imatinib, tretinoin, bexarotene, denileukin, diftitox, gefitinib, erlotinib, dasatinib, sorafenib, sunitinib
Calcineurin inhibitors	-	cyclosporine, tacrolimus
mTOR inhibitors (proliferation-signal inhibitors)	-	sirolimus, everolimus
Immunosuppressive antibodies	-	anti lymphocyte and antithymocyte globulins (ALG and ATG)
Monoclonal antibodies [38, 39]	Inhibitors of pro inflammatory cytokines	Adalimumab
		Infliximab
		Cetrolizumab
		Etarnecept
		Basiliximab
		Daclizumab
	Adhesion cell modulators	Natalizumab
	T-cell inhibitors	Abatacept
		Alefacept
		Muromonab
	B-cell inhibitors	Rituximab
		^{90}Y-Ibritumomab
		^{131}I-Tositumomab
	Anti-CD33	Gemtuzumab
	Anti- CD52	Alemtuzumab
Others	-	mycophenolate mofetil, thalidomide

Compiled from Table 81-2: p521-24 reference 16 and references 38, 39.

Appendix 9 Child-Pugh Classification of Severity of Liver Disease

Child–Pugh Scoring on Select Parameters

	Points Scored for Increasing Abnormality		
Clinical and biochemical measurements	**1**	**2**	**3**
Encephalopathy (grade) [a]	None	1 or 2	3 or 4
Ascites [b]	Absent	Slight	Moderate
Bilirubin (mg/100 mL)	< 2	2–3	> 3
Albumin (g/100 mL)	> 3.5	2.8–3.5	< 2.8
Prothrombin time (International Normalized Ratio) [c]	< 1.7	1.7–2.3	> 2.3

Note: With increasing abnormality of each of the five parameters measured, 1, 2, or 3 points are scored. Grade A: 5 or 6 points. Grade B: 7–9 points. Grade C: 10–15 points.

[a] According to grading of Trey, Burns, and Saunders (1966).

[b] As determined by physical examination alone.

[c] Prothrombin time results should be reported and used for calculations only as International Normalized Ratios because of variations in the methods used and reference ranges for controls (expressed in seconds).