Official Title: **A Double Blind, Randomized, Stratified, Multi-Center Trial Evaluating Conventional and Double Dose Oseltamivir In The Treatment of Immunocompromised Patients with Influenza**

NCT Number: **NCT00545532**

Document Date: **Statistical Analysis Plan Version 1: 04-Aug-2017**

IDM Audit Trail Report

Report for document SAP NV20234 ((234)) Tamiflu v1, (Post marketing commitment Phase IIIb) 08-Dec-2017

Basic Object Properties:

Document Name:	SAP NV20234 ((234)) Tamiflu v1, (Post marketing commitment Phase IIIb) 08-Dec-2017
Title:	NV20234_SAP_Final.docx
[redacted]	[redacted]
Version number and label:	CURRENT, 1.0, Released
Group:	Statistical Analysis
SubGroup:	DAP
Artifact:	Statistical Analysis Plan
Content item:	SAP Study

Folder Locations:

Folder name	Folder Path	Folder last modified date (UTC)
SAPs	[redacted]	[redacted]

Audit Trail Entries:

User Acknowledgements

Version Number	Outcome	Event Name	Activity	User role of signatory	User Name	Date and time of event (UTC)
0.5	Finalized	Acknowledgement	In Approval	Company Signatory (Clinical)	[redacted]	04-Aug-2017 09:27:21

Change Status

Original Status	New Status	Original Version	New Version	User Name	Date and time of event (UTC)	Reason
Finalized	Released	0.5	1.0	[redacted]	[redacted]	ccak
	Initiated		0.1	[redacted]	[redacted]	

Restricted Property Update

None

Reassign Work

None

Workflow Review Information

None

Terminate Workflow

None

Reclassify

None

Collaboration

Collaboration name	Collaboration type	Participants	Completion date and time (UTC)
Reviewing SAP NV20234 (Post marketing commitment Phase IIb)	Review	[redacted]	03-Aug-2017 15:03:28

Retain Version Flag Update

None

Partner System Transfer

Target System	Document Version	Document Type	Date and time of event (UTC)
[redacted]	[redacted]	[redacted]	[redacted]

STATISTICAL ANALYSIS PLAN

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**
STUDY DRUG:	**Tamiflu® (Oseltamivir Ro 64-0796)**
VERSION NUMBER:	**1**
IND NUMBER:	**53,093**
EUDRACT NUMBER:	**2006-002468-24**
SPONSOR:	F. Hoffmann-La Roche Ltd
PLAN PREPARED BY:	[redacted]
PLAN REVIEWED BY:	
DATE FINAL:	See electronic date stamp below

ABBREVIATIONS

AE	Adverse Event
AUC	Area under the concentration-time curve
AUE	Area under the effect-time curve
AST	Aspartate transaminase
ALT	Alanine aminotransferase
BID	Bis In Die (Latin for Twice A Day)
CARIFS	Canadian Acute Respiratory Infections scale
CBC	Complete Blood Count
CI	Confidence Interval
CRD	Clinically Relevant Difference
CRF	Case Report Form
CSR	Clinical Study Report
C_{trough}	Minimum concentration of a molecule in serum before next dose
EMA	European Medicines Agency
EOT	End Of Trial
F	Bioavailability
FDA	US Food and Drug Administration
HA	Haemagglutinin
HSCT	Hematopoietic stem cell transplant
IC_{50}	50% inhibitory concentration
IC	Immuno-Compromised
IQR	Inter-Quartile Range
ITT	Intent To Treat
ITTi	Intent To Treat infected
IxRS	Interactive voice/web Recognition System
LPLV	Last Patient Last Visit
LRTC	Lower Respiratory Tract Complication
mITTi	Modified Intend To Treat Infected
NA	Neuraminidase
NP-ELISA	Nucleoprotein Enzyme-Linked Immunosorbent Assay
OC	Oseltamivir Carboxylate
OwH	Otherwise healthy
PAC	Post Authorization Commitment
PCR	Polymerase Chain Reaction
PD	Pharmacodynamic
PKEP	Pharmacokinetic Evaluable Population
PK	Pharmacokinetic
PIP	Paediatric Investigation Plan
PMR	Post Marketing Requirement
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOT	Solid Organ Transplant
TAI	Treatment Assignment Information
$TCID_{50}$	50% tissue culture infectious dose
TTR	Time to resolution
vp/mL	virus particles per millilitre

TABLE OF CONTENTS

LIST OF TABLES

1. BACKGROUND

Study NV20234 is a post-authorization commitment (PAC) of Tamiflu® registrations agreed with the European Medicines Agency (EMA) and a post-marketing requirement (PMR) of Tamiflu registrations agreed with the US Food and Drug Administration (FDA). It also forms part of the EMA Paediatric Investigational Plan (PIP).

This Statistical Analysis Plan (SAP) is based on Protocol Version F (18 June 2014).

2. STUDY DESIGN

Study NV20234 is a double-blind, randomized, stratified multi-center trial of conventional and double dose oseltamivir in immuno-compromised (IC) patients. The conventional dose in adults (≥18 years of age) and adolescents (from ≥13 to <18 years of age) is 75 mg twice daily (BID) for 10 days. In children (from 1 to <13 years of age) the conventional doses are 30, 45, or 60 mg BID for 10 days based on body weight. Immunocompromised (IC) patients who develop an influenza-like illness with confirmed influenza from a positive rapid diagnostic test, PCR, or viral culture will be enrolled during the influenza season. Patients will be stratified by:

- Transplant type: hematopoietic stem cell transplant [HSCT] vs. solid organ transplant [SOT] (Protocol Version A and B) or transplant status yes vs. no (Protocol Version C onwards).
- The time between onset of influenza symptoms and treatment start (up to 48 hours) (≤ 24 hours vs. >24 hours) (Protocol Version A and B) or the time between onset of influenza symptoms and treatment start (up to 96 hours) (≤48 hours vs. >48 hours) (Protocol Version C onwards).
- Influenza vaccination status for current influenza season (yes vs. no).
- Age (≤ 12 years vs. >12 years).

See section 4.4.5 for further description of subgroup analyses.

2.1 PROTOCOL SYNOPSIS

The Protocol Synopsis is in Appendix 1. For additional details, see the Schedule of Assessments in Appendix 2.

2.2 OUTCOME MEASURES

All endpoints (safety, resistance, efficacy and PK) are defined in the SAP (descriptions of the endpoints are in Section 2 and the statistical methods used to describe the endpoints are described in Section 4). There are two co-primary endpoints – safety/tolerability and resistance. It is worth noting that the primary endpoint of this study changed during the course of the study. 'Time to Resolution of All Influenza Symptoms' was the primary endpoint in versions A

and B of the protocol. However, this endpoint was relegated to a secondary endpoint to reflect the change in objectives introduced in Protocol Version C.

Baseline is defined for all endpoints as the pre-dose reading on Day 1.

2.2.1 Primary Efficacy Outcome Measures

Not applicable as efficacy is not the primary objective.

2.2.2 Secondary Efficacy Outcome Measures

Secondary efficacy outcome measures are defined below.

2.2.2.1 Symptoms

- Time to Resolution (TTR) of All Symptoms in adolescents and adults is defined as the time from treatment initiation to the start of the 24 hour period in which all seven symptoms have scores ≤ 1 (mild) and remain ≤1 for at least 21.5 hours. Patients with symptom scores ≤ 1 at baseline, which remain ≤1 for at least 21.5 hours thereafter, will have a TTR of all symptoms set to missing. Patients with symptom scores > 1 at baseline followed immediately by scores ≤1 for at least 21.5 hours will have a TTR of all symptoms set to zero. Baseline (time zero) is defined as the time of symptom scores prior to first dose. For any subjects who have baseline scores missing, a subsequent set of symptom scores that is <12 hours post dose will be used. In order to gauge whether the previous action introduces a bias, a sensitivity analysis will exclude patients who do not meet the strict definition of baseline symptoms. Records in which all baseline symptoms are mild, i.e. ≤1, but any rises above 1 after first treatment will be set to missing. Posterior intermittent cycles of relapsing and recovery will be ignored.
- TTR of All Symptoms (children) is defined as the time from treatment initiation to the start of the 24 hour period in which all 18 symptoms items have scores ≤1 (minor or no problem) and remain ≤1 for at least 21.5 hours. If a score of 4 (“do not know” or “not applicable”) occurs at any assessment during the study for any given symptom, the assessment will not be included in the calculation of the resolution of all CARIFS symptoms. Patients with symptom scores ≤1 at baseline which remain ≤1 for at least 21.5 hours thereafter will have a TTR of all symptoms set to missing. Patients with symptom scores ≤1 at baseline followed immediately by scores ≤1 for at least 21.5 hours will have a TTR of all symptoms set to zero. As above, records in which all baseline symptoms are mild, i.e. ≤1, but any rises above 1 after first treatment will be set to missing. Posterior intermittent cycles of relapsing and recovery will be ignored.

- Area Under the Effect-Time Curve (AUE) for all symptoms (adults only). This will be calculated from baseline (symptom score prior to start of study drug or, if missing, before 12 h after starting treatment) to the time at which all symptoms are first alleviated, i.e. ≤1. Totals scores will be tracked over time. The AUE of these average scores is then calculated for each subject using the trapezoidal rule. The median of those AUE will be compared between groups using CI.
- TTR of Individual Symptoms (all patients with baseline symptom scores > 1) is defined as the time from treatment initiation to the start of the 24 hour period at which a specific symptom score is ≤1 and remains ≤1 for at least 21.5 hours. For children, if a score of 4 ("do not know" or "not applicable") occurs at any assessment during the study for any given symptom(s), the assessment will not be included in calculating the TTR. Patients with symptom scores ≤1 at baseline which remain ≤1 for at least 21.5 hours thereafter will have a TTR set to missing. Patients with symptom scores >1 at baseline followed immediately by scores ≤1 for at least 21.5 hours will have a TTR set to zero.
- The AUE for Individual symptoms (adults only) will be calculated from baseline (symptom score prior to start of study drug) to the time at which the individual symptom is first alleviated (≤1). The AUE of the individual symptom score is calculated for each subject using the trapezoidal rule.
- Time to resolution of fever is defined as the time from treatment initiation to the last time at which temperature was ≥37.8^{o} C (all patients). For patients who did not have a temperature ≥37.8^{o}C at baseline and throughout the dosing period, the time to resolution of fever will be missing. For patients who had a temperature of ≥37.8^{o}C at baseline, followed immediately by a temperature of <37.8^{o} C at their first post-baseline assessment, the time to resolution of fever will be zero. Patients who still had a temperature ≥37.8^{o} C at the end of the follow-up period will be censored at that time point. Patients who withdraw prior to resolution of fever will be censored at the time of withdrawal.

Note: As the introduction of the electronic diary removed the requirement for the patient to record their own baseline temperature, the baseline for these patients will be defined as the temperature recording collected at the first site visit. It is recognized that the absence of a true patient recorded temperature, using the same thermometer and body site, will limit interpretation of this endpoint.

2.2.2.2 Viral load

Viral load is the number of viral particles per mL of blood. It is reported in $\log_{10}$ scale and expressed in 1) virus particles/mL ($\log_{10}$ vp/mL) calculated from the quantitative RT-PCR assay (reverse transcriptase polymerase chain reaction) and 2) 50% tissue culture infectious dose ($\log_{10}$ $TCID_{50}$) obtained after viral culture.

- Viral load change from baseline over time expressed in log_{10} vp/mL, where baseline is defined as the pre-dose day 1 result.
- Viral load change from baseline over time expressed in log_{10} $TCID_{50}$/mL, where baseline is defined as pre-dose day 1 result. Log_{10} $TCID_{50}$/mL is calculated as: $TCID_{50} = -\left(X_0 - {}^{d}/_{2} + d\sum_{x_{p=1}}^{x_{min}} p_x\right)$, (Ramakrishnan MA, 2016) where:

  X_0 = positive logarithm of the highest dilution at which all quadruplicate wells are positive in the haemagglutination assay or NP-ELISA,

  $p_x = \frac{r_x}{4}$, where r_x is the number of positive wells (out of 4) at dilution x,

  $x_{p=1}$ is maximum dilution at which all 4 wells are positive,

  x_{min} is the dilution at which all four wells are negative for the first time, and

  d is the log_{10} dilution factor, i.e. $log_{10}10^{-1}$=-1.

  N.B. The data contains two variables: X0 and X1, where $X1 = -\sum_{x_{p=1}}^{x_{min}} p_x$ and X0=-X_0 (see above). Thus, programmatically, $TCID_{50} = X0 - 0.5 + 0.25X1$
- Area under the viral titer curve calculated applying the trapezoidal rule to the log_{10} $TCID_{50}$/mL viral load titer.
- Area under the viral titer curve calculated applying the trapezoidal rule to the log_{10} vp/mL viral load titer.
- Peak viral titer per arm defined as the maximum log_{10} $TCID_{50}$/mL.
- Peak viral titer per arm defined as the maximum lo_{g10} vp/mL.

2.2.2.3 Viral shedding

Time to cessation of viral shedding is defined as the time from treatment initiation to the time of first negative result with no subsequent positive results. Event is applicable only to patients infected at baseline. A value of 0.5 or less log_{10} $TCID_{50}$/mL and a value of 0.26 or less log_{10} vp/mL for Flu A strains and 0.3 or less log_{10} vp/mL for Flu B strains will be interpreted as a negative result. Any subsequent missing values for either RT-PCR or culture will be assumed to be negative. Patients who did not have the right assay to determine viral shedding (Haemagglutination assay or NP-ELISA) will be excluded from this analysis.

- Time to cessation of viral shedding determined by Haemagglutination assay for H1N1 and Flu B strains or NP ELISA (NucleoProtein Enzyme-Linked Immunosorbent Assay) for H3N2 strains after viral culture and expressed in log_{10} $TCID_{50}$/mL (50% Tissue Culture Infectious Dose).
- Time to cessation of viral shedding determined by RT-PCR and expressed in log_{10} virus particles/mL (vp/mL).
- Incidence of patients with viral shedding by RT-PCR by visit.
- Incidence of patients with viral shedding by viral culture by visit.

- Incidence of persistent shedding, defined as <1 $\log_{10}$ vp/mL reduction at end of treatment, compared with baseline.

2.2.2.4 Secondary illness

Note that although the secondary illnesses of interest defined in the protocol do not include the term LRTC this term will be included to ensure other LRTCs not captured as pneumonia or bronchitis will be reported.

- Incidence of any secondary illnesses such as LRTC (otitis media, bronchitis, pneumonia or sinusitis) for all patients at any time during the study.
- Incidence of secondary illnesses, i.e. LRTC, for all patients at any time during the study that are treated with antibiotics. Antibiotic use for secondary illnesses will be selected from the concomitant logs prior to database lock. This selection will be based on a medical decision. Cases will be identified as any antibiotic treatment received for the above secondary illnesses.
- Time to initiation of treatment with antibiotics from randomisation.

  Note: Although this endpoint was defined in the protocol using randomization date and time as the start of the time period, the time from treatment initiation will be used to define the start of the time period. This decision has been made in order to remain consistent with other time to endpoints in this study. The time between randomization and treatment initiation will be checked to ensure time differences across the two treatment groups are balanced.

2.2.2.5 Hospitalisation

- Incidence of hospitalisations for all patients. Given that patients will be in the study for a short period, i.e. ~40 days, we envisage a maximum of 1 hospitalisation per patient. Thus, probabilities of hospitalisation will be compared across treatment arms.
- Duration of hospitalisation (in days), for those who are hospitalized, as recorded directly on the Case Report Form (CRF) for all patients. Given that there may be few hospitalisations, this subgroup analysis will only report median times and ranges by treatment arm.

2.2.3 Exploratory Efficacy Outcome Measures

Not applicable.

2.2.4 Pharmacokinetic Outcome Measures

If estimable, the following model-predicted PK parameters for oseltamivir and oseltamivir carboxylate will be calculated on the basis of the oseltamivir established population PK model.

- Steady-state area under the concentration-time curve from 0 to 12 hours ($AUC_{0\text{-}12}$).
- Maximum plasma concentration C_{max}.

- Trough plasma concentration C_{trough}.

If appropriate, the following model-predicted parameters may also be included.

- 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}).
- Time to last measurable concentration (t_{last}).

2.2.5 Safety Outcome Measures

The endpoints used to support the co-primary objective of safety are the following:

- Incidence and severity of AEs (including influenza-associated complications and abnormal findings from physical examinations), SAEs, and reasons for the discontinuation of any study medication. All SAEs and AEs will be tabulated including their clinical severity.
- Change from baseline in vital signs (weight, temperature, respiratory rate, blood pressure and pulse rate).
- Change from baseline in laboratory parameters (AST, ALT, total bilirubin, urea, creatinine, CBC and differential blood cell count, i.e. neutrophils, lymphocytes, monocytes, eosinophils, basophils).
- Lab abnormalities by baseline and visit status (see SOP COG 3007 for expected normal ranges).
- Incidence of tissue rejection or GVHD (Graft Versus Host Disease) in transplant patients.

Note: Incidence of transplant rejection or GVHD in transplant patients has been defined in the protocol as both a primary and secondary endpoint. It will be reported as a primary safety endpoint.

2.2.6 Resistance Outcome Measures

The following endpoint is used to support the co-primary objective of resistance.

Resistance is defined as the presence of oseltamivir resistance in samples identified by sequencing of the neuraminidase (NA) and haemagglutinin (HA) genes (**genotypic resistance**) or by oseltamivir IC_{50} determination by a NA inhibition assay, using statistical outlier rules (**phenotypic resistance**). Resistance (genotypic, phenotypic and pooled) will be summarized per age (adults, paediatrics), dose group, viral type and subtype.

For patients who are infected with both A and B virus types or two Flu A viruses the following algorithm will be applied:

- "A" if RT-PCR A is positive (i.e. not NEGATIVE) and RT-PCR B is NEGATIVE at the 1st timepoint with RT-PCR results.
- "B" if RT-PCR B is positive (i.e. not NEGATIVE) and RT-PCR A is NEGATIVE at the 1st timepoint with RT-PCR results.
- "AB" if RT-PCR A and RT-PCR B are positive (i.e. not NEGATIVE) and Mixed Flu A if both RT-PCRs for Flu A subtyping are positive (i.e. not NEGATIVE) at the 1st time point with RT-PCR results.
- "UNK" otherwise.

For patients with a mixed infection, samples from time points with only one detected Flu type will be included in the overall virology analyses. Entire virology data will be described separately per patient.

2.2.6.1 Genotypic resistance

Baseline and post-baseline genotypic resistance is defined as the presence of oseltamivir resistance mutations in the NA or HA genes identified by sequencing of influenza viruses isolated from nasopharyngeal samples.

Genotypic resistance calculation: Presence of genotypic resistance (yes/no), together with the specific resistance mutations will be identified (see USPI list in Appendix 6) for each nasopharyngeal sample and will be listed.

> Note: a H274Y-specific RT-PCR assay was performed for some H1N1 samples of season 2008-2009 to determine baseline genotypic resistance and results will be also included.

2.2.6.2 Phenotypic Resistance

Baseline and post-baseline phenotypic resistance is defined as the presence of phenotypic resistance identified by oseltamivir IC_{50} determination, using statistical outlier rules and in accordance with the algorithm described in surveillance studies (Sheu TG et al 2008, Okomo-Adhiambo M et al, 2010).

Phenotypic resistance calculation: The mean IC_{50} of the baseline (pre dose Day 1 result) samples of all patients will be calculated separately for each virus subtype. Extreme outliers will be defined as any baseline or post-baseline IC_{50} values > 10-fold the baseline mean IC_{50}. The baseline IC_{50} distribution statistics (i.e. mean, standard deviation [SD], median, 75^{th} percentile [$X_{0.75}$]) will then be re-calculated excluding any baseline extreme outliers previously identified. Outliers will be defined as any baseline or post-baseline IC_{50} values less than 10-fold the baseline mean, but more than three interquartile ranges (IQRs) greater than the

baseline third quartile ($X_{0.75}$) (> [$X_{0.75}$ + {3 × IQR}]). Outliers and extreme outliers will be considered to have phenotypic resistance.

> Note: the phenotypic resistance algorithm assumes that the IC_{50} samples are normally distributed. Appropriate transformations will be considered if this assumption is not met. Furthermore, alternative algorithms (e.g., fold-changes, pooling of NA subtypes) may be used if the number of IC_{50} samples per NA subtype is considered small.

2.3 DETERMINATION OF SAMPLE SIZE

The sample size has been chosen to provide an adequate number of patients to estimate the development of resistance (genotypic and/or phenotypic) 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.

Table 1 Expected confidence intervals associated to specific event rates

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 (see Table 1).

The sample size of 75 patients per group will have 66% power to detect a clinically relevant difference (CRD) between active treatment and historical placebo of 34 hours in time to resolution of symptoms, assuming a common standard deviation (SD) of 98 hours, using a 2-sided 10% alpha. The CRD and SD reflect those used to power the original study (original protocol).This has been calculated in EAST version 6.0, based on a Wilcoxon sign rank test. As no formal hypothesis will be tested in this study, the use of a 2-sided 10% alpha will be reflected by the use of 90% CIs.

2.4 ANALYSIS TIMING

The efficacy and safety data will be reported at the end of the study, following database lock. Database lock will occur once the final SAP has been approved

and the database is clean. The analysis population datasets will then be created and reviewed prior to release of Treatment Allocation Information (TAI).

3. STUDY CONDUCT

3.1 RANDOMIZATION

Patients will be randomized 1:1 to conventional oseltamivir or double dose oseltamivir, stratified according to 4 binary stratification factors. Patients randomized under Protocol versions A and B will be stratified according to transplant (SOT vs. HSCT); time between onset of influenza symptoms and treatment start (≤24 hours vs. >24 hours); influenza vaccination status for current influenza season (yes vs. no), and age (≤12 years vs. >12 years).

Patients randomized under Protocol C onwards will be stratified according to transplant (yes vs. no), 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 vs. no) and by age (≤12 years vs. >12 years). As only transplant patients were enrolled during Protocol A and B, at this time, the randomization was not stratified by transplant status.

The master randomization list is an un-stratified randomization list implemented in the Interactive Voice/Web Recognition System (IxRS) using a dynamic block allocation algorithm to centrally assign patients to the correct strata.

Following the release of TAI, the treatment assignments from the IxRS listing will be checked against the master randomization list to ensure the correct treatments have been assigned. Furthermore, the date and time of randomization will be checked against the date and time of first dose to ensure patients were randomized prior to receiving treatment. Any errors noted will be listed as protocol deviations in the clinical study report (CSR).

Any patients who were assigned to the incorrect strata level at randomization will have the strata information corrected for reporting purposes but the original randomization will remain unaltered.

3.2 INDEPENDENT REVIEW FACILITY

Not applicable.

3.3 DATA MONITORING

Blinded reviews of the data will be performed on an ongoing basis by the study scientist.

4. STATISTICAL METHODS

All analyses, summaries and listings will be performed using SAS® software (version 9.2 or higher) in a UNIX environment. The data will be listed in the CSR.

All endpoints previously defined will be listed and summarized using appropriate descriptive statistics.

4.1 ANALYSIS POPULATIONS

The main analysis populations are defined below.

4.1.1 Intent To Treat (ITT) Population

All patients randomized will be included in the ITT population. Patients will be summarized under the treatment to which they were randomized. This population may be used to summarize efficacy endpoints for the purpose of sensitivity analyses. This population will generate the most conservative effect estimates because there may be allocation errors, non-infected patients and/or non-adherence to treatment.

4.1.2 Intent To Treat infected (ITTi) Population

All patients randomized and with central laboratory confirmation of influenza infection, excluding patients infected with oseltamivir-resistant influenza at baseline, will be included in the ITTi population.

4.1.3 Modified Intent To Treat infected (mITTi) Population

All patients randomized to a particular treatment, regardless of whether they received that treatment or not, and received at least one dose of study drug and with central laboratory confirmation of influenza infection, excluding patients infected with oseltamivir-resistant influenza at baseline, will be included in the mITTi population. This population will also be used to summarize all efficacy and resistance endpoints because it will render least biased effect estimates. This population was not defined in the protocol.

The definition of oseltamivir-resistant influenza at baseline is defined in Section 2.2.6. Decisions on patient exclusion from the ITT, ITTi and mITTi population sets will be made by the statistician and study scientist after database closure. Excluded patients will be documented along with the reason for exclusion.

4.1.4 Pharmacokinetic-Evaluable Patient Population

The Pharmacokinetic Evaluable Patient (PKEP) population comprises all patients in the ITT population have at least one post-dose drug concentration measurement at a scheduled visit time point. 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 PKEP will be made prior to database closure by the clinical pharmacologist based on information supplied by the Data acquisition specialist (whether patients in the ITT population have at least one post-dose drug concentration measurement at a scheduled visit time point). No

PK data will be shared with the team as this would unblind the study. Excluded patients will be documented along with the reason for exclusion.

4.1.5 Safety Population

The safety analysis population will include all patients who receive at least one dose of study drug and have had a safety assessment performed post randomization. All safety variables will be summarized and presented in tables based on this safety population. Any patients who receive a dose that differs from the dose to which they were randomized will be reported under their actual treatment.

4.1.6 Protocol Deviations

Major protocol deviations will be listed in the CSR. Major protocol deviations will include those captured during the study conduct ([redacted] file), as well as any additional deviations found as a result of programmatic checks of the following criteria:

- Patients who received their first study drug dose more than 96 hours from onset of symptoms.

- Patients with randomization errors – randomized and not dosed, dosed prior to randomization.

- Use of antiviral treatment for influenza in the 2 weeks prior to randomization and during the 10 day treatment phase of the study.

- Less than 16 of the 20 planned doses (80%) of study drug.

Sensitivity analyses may be carried out excluding any major protocol deviations.

4.2 ANALYSIS OF STUDY CONDUCT

The following tables will be summarized by treatment group according to current sponsor data standards.

- Summary of treatment.
- Summary of disposition of patients in the Safety, ITT, ITTi, mITTi and PKEP populations.
- Summary of patients excluded and reasons for exclusions from each of the analysis populations (Safety, ITT, PKEP, ITTi, mITTi).
- Summary of withdrawals, including the reason for withdrawal (Safety, ITT, ITTi, mITTi).
- Summary of concomitant medications split by: immunosuppressive treatment; treatment for influenza-like symptoms; treatment for AEs; other treatments.

Protocol deviations, as reported directly by sites or captured through programmatic checks of the database of deviations that occur during study

conduct, will be listed and summarized by randomized treatment accordingly, as per Section 4.1.6.

4.3 ANALYSIS OF TREATMENT GROUP COMPARABILITY

Baseline patient characteristics will be summarized by treatment group. The main population for these baseline summaries will be the mITTi. However, summaries based on the Safety, ITT and ITTi populations will also be included in the CSR.

All baseline patient characteristics data will be listed. To assess comparability, descriptive statistics will be presented by treatment group as follows:

- Demography including age, gender, race and ethnicity.
- Immunodeficiency conditions at baseline.
- Transplant history.
- Historical CD4 count.
- Influenza vaccination status.
- Previous or current diseases at baseline (Medical History).
- Previous treatments, split by immunosuppressive treatment, treatment for influenza-like symptoms, and others.
- Influenza infection status: Flu Type (A or B) and Flu A subtype (H1N1, H3N2) (mITTi only).

No statistical comparisons will be made, as no formal hypothesis is being tested.

4.4 EFFICACY ANALYSIS

All efficacy endpoints are considered secondary or exploratory. As no formal efficacy hypothesis is being tested, treatment differences presented in any efficacy analyses will be reported with associated confidence intervals (CIs) only. Efficacy endpoints will be summarized by age group. All summaries and analyses are described below and listed in Appendix 5. All efficacy analyses will be based on the mITTi and ITTi populations. ITT will be presented for the main efficacy TTR summary tables (all symptoms and temperature only) to investigate the effect of treatment in a “real world” scenario, i.e. unknown infection status and/or adherence. N.B. Regulatory bodies have asked for these outputs in pivotal studies.

All efficacy summaries and analyses will be carried out separately for adults (patients ≥ 18 years) and paediatrics (patients <13 years old). Adolescent data (patients aged between ≥13 and <18 years old) will be listed as appropriate.

As the symptom data are recorded on patient diaries, data errors cannot be queried. Obvious data errors, i.e. temperature 100^{o}C, will be excluded and documented. For duplicated data, the worst evaluation will be used to obtain conservative estimates.

The endpoints as described in the protocol have been expanded to give sufficient information of the derivations required, e.g. *symptom scores* was the endpoint defined in the protocol but *Time To Resolution of symptom score*s is how it is described in the SAP.

4.4.1 Primary Efficacy Endpoint

Not applicable.

4.4.2 Secondary Efficacy Endpoints

4.4.2.1 Time to Event Endpoints

Time to event endpoints include the following: TTR of all symptoms, TTR of individual symptoms, time to cessation of viral shedding and time to resolution of fever. These endpoints will be summarized using Kaplan-Meier tables and graphs. Viral shedding will be studied also per virus strain. The median TTR and associated 95% CIs will be estimated for each of the two treatment groups. The exact CIs of the median differences of Hodges-Lehmann (HL) estimators will be derived using the PROC NPAR1WAY in SAS. The protocol mentioned bootstrapping, however in SAS, bootstrapping calculates differences between medians, when the correct estimator in skewed distributions is the median of differences (or HL estimator). Asymptotic theory exists to obtain those CI (aka Moses CI).

Patients who have not experienced the event, e.g. resolution of all symptoms, by the last day of contact will be censored at this time. Patients who withdraw from the study without experiencing the event will be censored at the time of withdrawal (see Appendix 7).

4.4.2.2 Comparison of TTR of All Symptoms to Historical Control

It is unethical to treat IC patients with placebo. Nevertheless, it is important to ascertain whether treating IC patients for influenza is still better than not treating them. Given the ethical constraint, the next best possible comparison available is against historical placebo controls. Thus, any difference between these two groups will be due to both IC status (yes/no) and oseltamivir effect. It will not be possible to differentiate both effects statistically. Historical controls will come first and foremost from pivotal historical oseltamivir studies where otherwise healthy (OwH) were enrolled. The main comparison of active treatment with historical control placebo will be based on a 1:1 matching of all adult NV20234 patients with OwH placebo patients from the pivotal studies. All patients in the adult mITTi population will be matched to historical patients (from the pivotal studies) that received a placebo.

There are three types of matches in order of priority:

1. Patients will be matched 1:1 within viral strain and then by age with ± 5 years caliper (the lowest limit being 18) and gender. Any remaining unmatched individual will be matched by age and gender alone. Only

pivotal studies will be used. Outputs in appendix 5 refer to this matching rule.
2. Patients will be strictly matched 1:1 by age as above, gender and influenza strain. Only pivotal studies will be used. There will probably leave more unmatched patients but those matched will be more similar than in 1.
3. Patients will be matched 1:>1 as in 1 above using pivotal and non-pivotal but relevant historical studies. Additional matching by time between symptoms onset and first treatment (possibly with a caliper) will be used if closer matching is required. This is a sensitivity analysis captured in section 4.4.4.

In cases where those matching rules are not sufficient, i.e. more than one patient in the 1:1 placebo matching, an additional rule will be the elapsed time from first symptoms to first dose. If several matches are still available in the 1:1 ratio, one match will be chosen at random (the seed of the random number generator will be kept for reproducibility).

Any patient from study NV20234 who has not been matched will not be included in these analyses. Matches will be done independently for historical placebo and historical treated. Modifications of the matching rules above may be permitted if scientifically required, e.g. matching by time in 3 above requires a window. The ITTi population in historical studies is equivalent to the current mITTi population.

The pivotal studies to be used are: WV15671 (18-65y, OwH, USA, placebo-conventional-double) and WV15670 (18-65y, OwH, Europe, Canada, Hong-Kong, placebo-conventional-double). Potential additional studies include: WV15730 (18-65y, OwH), M76001 (13-80y, OwH, placebo-conventional) and WV15758 (1-12y, OwH, placebo-conventional).

A similar comparison may be done between current IC patients and historical patients defined as at risk. There may be a 1:1 and 1:>1 matching ratios. The historical studies with patients at risk include WV15812 (≥13y, placebo-conventional) and WV15872 (≥12y, placebo-conventional). Additional studies in elderly (>65 years of age) are: WV15819, WV15876, WV15978 and WV15707 (see Appendix 4).

The following median differences in TTR of all symptoms (90% and 95% CIs) will be reported:

1. Comparison of conventional dose with matched placebo.
2. Comparison of double dose with matched placebo.
3. Comparison of combined conventional and double dose with combined matched historical placebo (if the results of the NV20234 dose group comparisons suggest responses are similar).

To aid interpretation of the results, the median TTR of all symptoms and associated 90% CIs will be plotted against historical placebo responses for a) NV20234 conventional dose b) NV20234 double dose c) NV20234 combined dose groups.

In addition to the comparison of NV20234 active with placebo patients from the pivotal studies, a *treatment* historical control dataset will be created from the same adult treatment studies, matching NV20234 patients randomized to the conventional dosing arm with the conventional dose from the adult treatment studies. This will enable an informal comparison of the treatment response of IC patients with the treatment response in otherwise healthy patients. Patients will be matched using the same rules as those for historical control.

The full specifications for historical control groups can be found in Appendix 4. The final set of historical placebo patients will be confirmed prior to treatment unblinding and database lock.

4.4.2.3 Continuous Endpoints

Continuous endpoints will be summarized by treatment group using descriptive statistics N, mean (geometric mean where appropriate), SD, median, and range.

This will apply to: duration of hospitalisation; absolute and change from baseline over time in $\log_{10}$ viral load from quantitative RT-PCR (vp/mL), $\log_{10}$ viral load titer from culture ($\log_{10}$ $TCID_{50}$/mL), AUC viral load titer, peak viral load titer, AUE total symptom score, and AUE individual symptom scores.

4.4.2.4 Binary Endpoints (Incidences)

The proportion of patients experiencing the event will be presented for each of the two treatment groups. This will apply to the patients still shedding virus per planned visit, all secondary illnesses endpoints, and hospitalisations (for viral shedding it will be presented by treatment group, age cohort and flu type).

- Incidence of any secondary illnesses: each patient can have several LRTCs, therefore a table of frequencies of 0 to 4 LRTC will be obtained per treatment arm.
- Incidence of secondary illnesses for patients treated with antibiotics: as above, the frequency of LRTC will be obtained by treatment arm.
- Incidence of initiation of treatment with antibiotics: The probability of antibiotic use will be compared between treatment arms using probability differences and confidence intervals of those differences.
- Incidence of hospitalisations: probabilities of hospitalisation per treatment arm and their CI's will be provided.
- Duration of hospitalisation: given that there may be few hospitalisations, this subgroup analysis will only report median times and ranges by treatment arm.

4.4.3 Exploratory Efficacy Endpoints

Descriptive statistics may be used to further investigate symptom scoring in adults and children considering that symptoms of the patient's underlying immune status and baseline conditions could overlap with influenza-like illness symptoms in this patient population.

4.4.4 Sensitivity Analyses

4.4.4.1 Efficacy Analyses using ITTi

All analyses relating to TTR of all symptoms and TTR of individual symptoms, described in Section 4.4.2.1, will be repeated using the ITTi population.

4.4.4.2 Efficacy Analyses excluding other antiviral patient data

The TTR (mITTi) of all symptoms analyses described in Section 4.4.2.1 will be rerun excluding any follow-up data collected from those patients who began treatment with another antiviral for influenza, on or after discontinuation of study drug. Note that although Section 8.2.7.1 of the protocol indicates that such exclusions should be made for all efficacy analyses, this statement contradicts the definition of the ITT, ITTi and mITTi populations, so the exclusions of antiviral patient data will be regarded as a sensitivity analysis rather than the main analysis. Non-immunocompromised patients will be excluded.

4.4.4.3 Additional efficacy analyses using alternative historical controls

Further sensitivity analyses of TTR of all symptoms may be carried out using alternative historical control datasets as follows:

1. 1: >1 mapping with OwH patients.

2. 1: >1 mapping with At Risk patients.

The purpose of the alternative historical control datasets is two-fold:

1) Increase the precision on the historical median response by increasing the number of patients contributing to this sample using a 1 :> 1 mapping approach.

2) Provide a range of placebo responses that may be considered plausible surrogate responses for an IC untreated population. This is based on the evidence of an observed difference in placebo responses for otherwise healthy patients compared with at risk and elderly patients in the oseltamivir adult treatment database.

To allow for potential exploratory analyses of other efficacy endpoints, the historical control dataset will also include the patient level variables where readily available: baseline creatinine clearance, age, gender, virus type and subtype,

time to cessation of viral shedding, AUE of symptom scores, AUC of viral titer, time to resolution of individual symptoms.

4.4.4.4 Comparison of TTR of All Symptoms to Historical Control

The following Kaplan-Meier analyses will be carried out using the mITTi population to support the comparison of Study NV20234 treatment response with the historical placebo response with both 1:1 and 1:>1 ratios:

- The TTR of all symptoms for adults using only follow-up symptom data recorded up to and including 25 days compared with historical placebo data. This is to investigate the impact of differences in the follow-up observation periods between study NV20234 and the oseltamivir treatment database. Adult patients in Study NV20234 who have not experienced resolution of symptoms by day 25 will be censored on day 25. In the historical studies (see section 4.4.2.2 above and appendix 4) the final assessment was between days 17 and 25 post onset. A sensitivity analysis will be carried out comparing results from the previous analyses against an analysis without censoring at 25 days, i.e. using the current final assessment ~40 days after onset of symptoms.
- The TTR of all symptoms for patients who had symptoms onset ≤48 hours before first dose, for each active treatment group and combined, compared with matched historical control patients.

4.4.4.5 Additional Secondary Illnesses Analysis

The incidence of specified secondary illnesses summaries will be repeated, sub-setting on any LRTC starting on or after study day 3 or at least 48 hours after first study drug intake and treated with antibiotics. This should exclude pre-existing secondary illnesses that may not be linked to the treatment. These incidences will be repeated for LRTCs regardless of antibiotic use. Antibiotic use for secondary illnesses will be selected from the concomitant medications log prior to database lock. These will be identified as antibiotic treatment received for the above secondary illnesses.

4.4.5 Subgroup Analyses

To check for comparability of treatment groups across the stratification levels used to randomize the study, the TTR of All Symptoms in the mITTi population will be summarized by treatment group split by:

- Vaccination status (yes vs. no).
- Transplant status (yes vs. no).
- Time from symptom onset to time of first dose (≤48 hours vs. >48 hours).
- Age (≤12 vs. >12 years).

The stratification by Transplant status was added in Protocol version C.

A further subgroup analysis of TTR of All Symptoms (mITTi) will be carried out by geographical location. The 5 regions will be North America (USA and Mexico), South America (Brazil, Colombia and Guatemala), Eastern Europe (Bulgaria,

Estonia, Hungary, Latvia, Lithuania, Poland and Ukraine), Western Europe (Belgium, Italy, Spain and Israel) and South Africa. A single Kaplan-Meier analysis will be done by geographic region.

4.4.6 Additional analyses not specified in the protocol

Two additional analyses for efficacy (TTR and temperature) that were not in the original protocol have been introduced in the SAP.

4.4.6.1 Use of 90% Cis

In addition to 95% CI, 90% CI will be also shown when comparing current data against historical controls. This was agreed jointly by PDCO, CHMP and Roche in a briefing document dated [redacted] where the possibility of establishing a new dose recommendation/regimen was discussed.

4.4.6.2 Removal of >48 hour symptom onset to dose restriction

Although the protocol originally specified this comparison would be restricted to those patients who received treatment within 48 hours (≤48 hours), such analysis will be conducted as a sensitivity analysis only. The rationale for the additional analyses is that there is no direct evidence that dosing more than 48 hours after the start of symptoms will not be effective. Whilst evidence from a large observational study suggests an increased benefit for patients if dosed within 48 hours, compared with patients dosed after 48 hours, there is still evidence of benefit if dosed more than 48 hours after symptom onset. In addition, IC patients can have prolonged viral shedding compared to immune-competent patients, suggesting that administration of drug after 96 hours after symptom onset may still provide benefit to these patients by limiting duration of their influenza.

4.5 PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSES

Individual and mean plasma concentrations from the oseltamivir population PK model at each sampling time-point 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 analysed by the clinical pharmacologist using an established population PK model to determine key exposure parameters defined in Section 2.2.4. Note: For IC children (≤ 12 years) and adolescents (> 12 - ≤ 17 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 oseltamivir and oseltamivir carboxylate plasma concentration data from non-IC children and adults (ages 1 to 80 years).
The PK parameters will be listed and summarized by treatment group using descriptive summary statistics including means, geometric means, medians, ranges, standard deviations and coefficients of variation.

Exposure-response relationships will be presented in the CSR through the use of scatter plots, plotting each of the estimated PK parameters separately against the area under the viral titer curve; the peak viral titer, and the time to cessation of viral shedding. Different symbols will be used to differentiate treatment groups.
All listings and summaries of plasma concentrations and estimable PK parameters will be based on the PKEP population.

Further PK and PK/PD analyses may be carried out by the clinical pharmacology group. This will be reported in a separate report when appropriate.

4.6 SAFETY ANALYSES

No formal hypothesis is being tested to support the co-primary objective of safety. Therefore, there will be no formal statistical comparisons between the two treatment groups. Summaries will be based on the Safety Population and will be provided by treatment and split by age: adults (≥18 years) vs. pool of adolescents and children (<18 years). Only tables for adverse event (AE) and serious adverse event (SAE) [summary and incidence by System Organ Class (SOC) and Preferred Term (PT)] will be provided separately for adolescents (≥13 to <18 years) and children (<13 years).

All continuous safety outcomes will be given as medians or medians and 95% confidence intervals per treatment arm. All categorical safety outcomes will be shown as frequencies by treatment arm.

4.6.1 Exposure of Study Medication

All treatment data will be listed as collected. The total treatment duration, number of doses, total cumulative doses, missed doses and dose modifications will be summarized by treatment group with the use of descriptive statistics (N, mean, median, SD, and minimum and maximum values).

4.6.2 Adverse Events

Adverse Events (AEs) will be mapped to the Medical Dictionary for Regulatory Activities (MedDRA) preferred terms using the MedDRA version current at the time of reporting, prior to the database lock and will be summarized for the Safety Population according to current sponsor safety standards. Summaries to be presented:

- Overall Incidence of AEs.
- Summary of AEs by relationship to study drug.
- Summary of AEs by body system and Intensity.
- Summary of Serious Adverse Events (SAEs).
- Summary of SAEs by relationship to study drug.

- Summary of SAEs by body system and Intensity.
- Summary of Treatment Emergent AEs resulting in death.
- Summary of AEs leading to study or study drug withdrawal.

As well as summarizing all AEs overall, summaries by treatment phase (on treatment, off treatment) will be presented, where on treatment is defined as an AE starting during treatment or up to 2 days after the last dose, and off treatment is defined as any AE starting more than 2 days after the last dose.

Multiple reports of an event by a subject will only be counted once for tabulations of adverse event incidences, with the most severe intensity being used. All AEs reported on treatment will be considered to be treatment emergent.

4.6.3 Laboratory Data

Blood samples for planned central laboratory tests for safety evaluation of clinical chemistry and haematology are collected at baseline (pre-dose Day 1) and Day 11/End of Treatment. Results will be listed and summarized for the Safety Population, Adults (≥ 18 years) separately from adolescents and children (≤17 years). Summaries to be presented:

- Change in baseline to Day 11 will be summarized using descriptive statistics (N, Mean (STD), Median, Min, Max)
- Incidence of marked laboratory abnormalities will be summarized for adults only as per Roche [redacted]
- Unscheduled laboratory results will not be included in any of the summaries. If a test was repeated on the same day, the average result will be used in the summary tables. If the scheduled Day 11 laboratory test was taken after the end of treatment, these off treatment results will still be presented in the summaries.

Historical and Day 1 (Protocol versions A and B only) creatinine clearance, CD4 counts and white blood cell (WBC) counts will be listed, where available. CD4 counts will be summarized separately using descriptive summaries for patients only with the inclusion criteria “HIV with a most recent CD4 count <500/mm^3 (or <25% in children ≤ 5years old) within the last 6 months and, in the investigator’s opinion, considered immunocompromised”.

4.6.4 Vital Signs

Vital sign measurements including weight, temperature, respiratory rate, blood pressure (position not specified) and pulse rate are planned on Days 1, 2 or 3, 6, 8 and Days 11, 15 and 40. These will be listed and summarized for the Safety Population (adults will be summarized separately from adolescent and children) according to current sponsor safety standards.

4.6.5 Tissue rejection and/or GVHD in transplant patients

The proportion of transplant patients in the safety population who experience tissue rejection and/or GVHD will be summarized by treatment group and age

4.7 RESISTANCE

To support the co-primary objective of this study, the proportion and Clopper-Pearson 95% CIs of patients with post-baseline genotypic or phenotypic resistance among all patients will be summarized by treatment group. The mITTi population will be used whenever possible.

In addition, the following outputs will be included:

- Incidence of overall post-baseline resistance (i.e. developed after first visit) by age (≥18 years vs <18 years old) and treatment group, split by flu type and subtype.
- Incidence of overall baseline resistance (i.e. observed at first visit) by age and treatment group, split by flu type and subtype in ITT population. N.B. Patients with baseline resistance are excluded from both ITTi and mITTi populations.
- Profiles (listings) of viral load in $\log_{10}$ vp/mL for patients with phenotypic or genotypic resistance at baseline. Also listings of genotypic resistance and phenotypic outlier samples.
- Incidence of known OC resistance mutations in phenotypic outlier samples compared with phenotypically OC sensitive samples.
- Incidence (in %) of post-baseline resistance in patients with detectable viral shedding (in $\log_{10}$ vp/mL) at EOT and during follow-up period, by treatment group, age (≥18 years vs <18 years old) and flu type and subtype.
- Summary (median and CI) of the TTR by treatment group, split by post-baseline resistance status.
- Summary (median and CI) of the TTR by treatment group in patients with post-baseline resistance, split by flu type.
- Summary of viral load in $\log_{10}$ vp/mL for patients with phenotypic or genotypic resistance at baseline.

4.8 VIROLOGY

The main virology outputs are:

- Incidence of persistent shedding (%), defined as < 1 $\log_{10}$ viral load (vp/mL) reduction at end of treatment compared with baseline.
- Summary of IC_{50} at baseline and fold-change over time by treatment group split by flu type and subtype using descriptive statistics: mean, SD, median, IQR, range.

- Summary of viral load in log_{10} vp/mL for patients with resistant phenotype or genotype at baseline (ITT).
- Summary of viral load change from baseline over time in log_{10} $TCID_{50}$ (viral culture) (adults mITTi).
- Summary of viral load change from baseline over time in log_{10} $TCID_{50}$ (viral culture) (paediatrics mITTi).
- Summary of viral load change from baseline over time in log_{10} vp/mL (RT-PCR) (adults mITTi).
- Summary of viral load change from baseline over time in log_{10} vp/mL (RT-PCR) (paediatrics mITTi).
- Incidence of patients with viral shedding in log_{10} $TCID_{50}$/mL and in log_{10} vp/mL by visit (adults and adolescents mITTi)
- Incidence of patients with viral shedding in log_{10} $TCID_{50}$/mL and in log_{10} vp/mL by visit (paediatrics mITTi).

4.9 MISSING DATA

As no formal statistical analyses will be carried out, imputation methods for missing data for the purposes of statistical model fitting are not required.

For the derivation of time to resolution of symptoms endpoints, a missing symptom score will be estimated by linear interpolation between the previously available assessment and the subsequent available assessment. (More details are provided in Appendix 7).

For the derivation of time to resolution of fever, a missing temperature entry will be estimated by linear interpolation between the previously available assessment and the subsequent available assessment.

4.10 INTERIM ANALYSES

No efficacy interim analyses are planned.

5. REFERENCES

Okomo-Adhiambo M, Sleeman K, Ballenger K, et al. Neuraminidase inhibitor susceptibility testing in human influenza viruses: a laboratory surveillance perspective. Viruses 2010;2:2269-2289.

Ramakrishnan MA. Determination of 50% endpoint titer using a simple formula. World Journal of Virology 2016;5:85-86

[redacted]

Sheu TG, Deyde VM, Okomo-Adhiambo M, et al. Surveillance for neuraminidase inhibitor resistance among human influenza A and B viruses circulating worldwide from 2004 to 2008. Antimicrob Agents Chemother 2008;52:3284-92.

6. APPENDIX 1: 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 IC patients under standard and double dose, and characterize the effects of oseltamivir in IC patients on the development of resistant influenza virus (see 4.6).

Secondary

To evaluate the effects of conventional and double dose oseltamivir in IC patients on:

- The population pharmacokinetics of *both oseltamivir and* oseltamivir carboxylate in IC 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 IC patients. Patients will be stratified by age (<13 vs. >12 years), transplant status (yes vs. no), time since onset of flu symptoms and treatment start (up to 96 hours) ($\leq$48 vs. >48 hours) and vaccination status (yes vs. 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 paediatric 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 paediatric 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 viral 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.

Table A1.1. Confidence interval given the rate of events

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}.

7. APPENDIX 2: SCHEDULE OF ASSESSMENTS

Table A2.1. Schedule of Assessments and Procedures

	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, respiratory rate, 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.

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.

i. 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.

j. 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).

k. 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.

l. 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.

m. 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.

n. 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).

8. APPENDIX 3: PATIENT DIARY DATA AND SYMPTOM RECORD FOR ADULTS AND CHILDREN

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 mln)

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.

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.

9. APPENDIX 4: HISTORICAL CONTROL SPECIFICATION FOR EFFICACY

Table A4.1. Studies from which historical controls (both placebo and treated) may be selected from.

*Tables and plots from Statistical Programmer Analysts (SPA)

#	Label	Protocols to include	Specifications	Comments
1	1:1 Otherwise Healthy Placebo Adults ITTi	WV15670, WV15671	Match each NV20234 adult mITTi patient with 1 ITTi placebo-treated patient who have same age and gender	Potential additional studies: M76001, WV15730, WV15758
2	1:>1 Otherwise Healthy Placebo Adults ITTi	WV15670, WV15671	Match each NV20234 adult mITTi patient with ITTi placebo-treated patients who have same age and gender.	Potential additional studies: M76001, WV15730, WV15758
3	1:1 At risk Placebo Adults ITTi	WV15812, WV15872	Match each NV20234 adult mITTi patient with ITTi placebo-treated patient who have same age and gender.	Potential additional studies: WV15819, WV15876, WV15978 and WV15707
4	1:>1 At risk Placebo Adults ITTi	WV15812, WV15872	Match each NV20234 adult mITTi patient with ITTi placebo-treated patient who have same age and gender.	Potential additional studies: WV15819, WV15876, WV15978 and WV15707
5	ITTi treated adult datasets		Repeat datasets 1-2 matching NV20234 patients with historical treated patients by randomised treatment.	

N.B. NV20234 will focus on the mITTi population. In historical studies, the definition of ITTi population was identical to the current mITTi population. Historical studies contain a standard population with a more strict definition i.e. “randomised, infected, no major violations and who received at least the first six scheduled doses of treatment within 72 hours or who received the first five doses within 72 hours but then went on to take nine out of ten doses”, however this population is not present in the current study. Adolescents will only be listed for efficacy.

Variables required for each dataset:

Variables required in each of the datasets, where available: NV20234 patient ID, centre ID number, country, age, gender, vaccination status, primary flu type, primary flu A subtype, randomised treatment, time from onset of symptoms to start of drug, time to alleviation of all symptoms, time to alleviation of individual symptoms, time to cessation of viral shedding, AUC viral titer, peak viral titer, AUC total symptom score, AUC individual symptom score, time to alleviation of fever.

10. APPENDIX 5: SUMMARY OF MAIN EFFICACY ANALYSES

Table A5.1. List of main efficacy analyses
*Tables and plots from Statistical Programmer Analysts (SPA). Suffixes: AD = adults (age ≥ 18), ADOL = adolescents (13 ≤ age < 18), CH = children (1 ≤ age < 18), PED = paediatric (1 ≤ age <13).
**Benchmarking plots contain median TTR and 90% CI for standard dose, double dose, combined dose, historical control and historical treated (separate plots will contain 95% CI)
N.B. Median differences will be obtained using the Hodges-Lehmann estimator and associated Moses CI. TTR of symptoms and temperature will be assessed in adults (≥18), in adults and adolescents (≥13), and paediatrics (<13) separately; for all other efficacy, virology and safety data adults will be separated from children (<18).

Endpoint	Analysis	Comparisons	Population	Purpose	Age groups	SPA output*
TTR all symptoms	Kaplan-Meier median and 95%CI	Conventional- double	mITTi , ITTi, ITT	Main dose-response comparison	Adults Adults + Adolescents Paediatric	t_ef_ttr_ALLEVAS_MITI_AD_95 g_km_ttr_LLEVAS_MITI_AD_95 t_ef_ttr_ALLEVAS_ITI_AD_95 g_km_ttr_LLEVAS_ITI_AD_95 t_ef_ttr_ALLEVAS_IT_AD_95 g_km_ttr_ALLEVAS_IT_AD_95

TTR all symptoms	Kaplan-Meier median and 90%CI	a. conventional b. double c. combined d. historical placebo e. historical treated	mITTi	Main comparison with historical control: NV20234 treatment against Historical Otherwise Healthy 1:1	Adults	t_ttr_hc_5g_1to1_ALLEVAS_MITI_AD_90 g_km_ttr_5g_1to1_ALLEVAS_MITI_AD_90
TTR all symptoms	Kaplan-Meier	a. conventional b. double c. combined d. historical placebo e. historical treated	mITTi	NV20234 treatment against Historical Otherwise Healthy 1:>1	Adults	t_ttr_hc_5g_1tom_ALLEVAS_MITI_AD_90 g_km_ttr_5g_1tom_ALLEVAS_MITI_AD_90
TTR all symptoms	Kaplan-Meier	a. conventional b. double c. combined d. historical placebo e. historical treated	mITTi	NV20234 treatment against at risk 1:>1	Adults	t_ttr_hc_5g_atrisk_ALLEVAS_MITI_AD_90 g_km_ttr_5g_atrisk_ALLEVAS_MITI_AD_90
TTR all symptoms	Kaplan-Meier	a. conventional b. double c. combined d. historical placebo e. historical treated	mITTi	NV20234 – Historical control Otherwise Healthy 1:1 Sensitivity for follow-up observational period : only including symptom scores recorded ≤25 days	Adults	t_ttr_hc_5g_25d_ALLEVAS_MITI_AD_90 g_km_ttr_5g_25d_ALLEVAS_MITI_AD_90
TTR all symptoms	Kaplan-Meier	a. conventional b. double c. combined d. historical placebo e. historical treated	mITTi	NV20234 – Historical Otherwise Healthy 1:1 Sensitivity analysis: patients who were dosed no more than 48 hours after symptom onset	Adults	t_ttr_hc_5g_48h_ALLEVAS_MITI_AD_90 g_km_ttr_5g_48h_ALLEVAS_MITI_AD_90
TTR all symptoms	Kaplan-Meier	a. conventional b. double	mITTi	Subgroup analysis ● By vaccination status (yes/no) ● By transplant status (yes/no) ● By ≤ 48, >48 h from symptoms onset ● By geographical region	Adults	t ef ttaas vac MITI_AD t ef ttaas trans MITI AD t ef ttaas onset MITI AD t ef ttaas reg MITI AD

TTR individual symptoms	Kaplan-Meier	Treatment Responses by treatment arms	mITTi	Main Summary for this endpoint	Adults Children	t_ef_ttais_MITI_AD t_ef_ttais_MITI_CH
Time to cessation viral shedding	Kaplan-Meier	Treatment Responses by treatment arms	mITTi	Dose-Response main analysis for this endpoint	Adults Children	t_ef_ttr_CESCLT_MITI_AD t_ef_ttr_CESPCR_MITI_AD g_km_ttr_CESCLT_MITI_AD g_km_ttr_CESPCR_MITI_AD t_ef_ttr_CESCLT_MITI_CH t_ef_ttr_CESPCR_MITI_CH g_km_ttr_CESCLT_MITI_CH g_km_ttr_CESPCR_MITI_CH
Time to cessation viral shedding	Kaplan-Meier	Treatment median [90% CI] Responses for : NV20234 conventional, NV20234 double, NV20234 pooled, historical treatment conventional, historical treatment double, historical placebo for conventional, historical placebo for double, historical placebo for pooled.	mITTi	Benchmarking plot	Adults All CI's on one plot but also split by a) pooled b) conventional c) double	g_km_t1_t2_histplac_treat_TTCVS_MITI_AD_95 g_km_t1&2_histplac_treat_TTCVS_MITI_AD_95 Repeated 1:1 and 1:>1 matches
TTR fever	Kaplan-Meier	Treatment Responses by treatment arms	ITT, ITTi ,mITTi	Dose-Response main analysis for this endpoint	1) Adults 2) Adults+Adolescents 3) Paediatric	g_ef_ttr_RESF_ITT_AD g_ef_ttr_RESF_ITT_PE

						g_ef_ttr_RESF_ITT_AD_ADOL t_ef_ttr_RESFBL_ITT_AD t_ef_ttr_RESFBL_ITT_PE t_ef_ttr_RESFBL_ITT_AD_ADOL g_ef_ttr_RESF_ITI_AD g_ef_ttr_RESF_ITI_PE g_ef_ttr_RESF_ITI_AD_ADOL t_ef_ttr_RESFBL_ITI_AD t_ef_ttr_RESFBL_ITI_PE t_ef_ttr_RESFBL_ITI_AD_ADOL g_ef_ttr_RESF_MITI_AD g_ef_ttr_RESF_MITI_PE g_ef_ttr_RESF_MITI_AD_ADOL t_ef_ttr_RESFBL_MITI_AD t_ef_ttr_RESFBL_MITI_PE

						t_ef_ttr_RESFBL_MITI_AD_ADOL
TTR fever	Kaplan-Meier	Treatment median [90% CI] Responses for : NV20234 conventional, NV20234 double, NV20234 pooled, historical treatment conventional, historical treatment double, historical placebo for conventional, historical placebo for double, historical placebo for pooled.	mITTi	Benchmarking plot**	1) Overall 2) Age cohort (>= 18 Y, < 18 Y) All CI's on one plot but also split by a) pooled b) conventional c) double	g_km_t1_t2_histplac_treat_RESF_MITI_AD_90 g_km_t1&2_histplac_treat_RESF_MITI_AD_90 Repeated 1:1 and 1:>1 matches
AUC total symptom scores	Median, IQR	Treatment Responses by treatment arms	mITTi, ITT, ITTi	Dose-Response main summary for this endpoint	Adults only	g_auc_ALLEVAS_AD t_auc_ALLEVAS_AD
AUC total symptom scores	Median, IQR	Treatment median [IQR] for: NV20234 conventional, NV20234 double, NV20234 pooled, historical treatment conventional, historical treatment double, historical placebo for conventional, historical placebo for double, historical placebo for pooled.	mITTi	Benchmarking plot	For adults only : All plots on one plot but also split by a) pooled b) conventional dose c) double dose	g_km_t1_t2_histplac_treat_AUCALLEVAS_MITI_AD_90 g_km_t1&2_histplac_treat_ALLEVAS_MITI_AD_90 Repeated 1:1 and 1:>1 matches
AUC individual symptom score	Median, IQR	Treatment Responses by treatment arm	mITTi	Dose-Response main summary for this endpoint	Adults	g_auc_ALLEVIS_AD t_auc_ALLEVIS_AD
AUC viral titer	Median, IQR	Treatment Responses by treatment arm	mITTi	Dose-Response main summary for this endpoint	Adults Children	g_ef_auc_AUCCLT_MITI_AD t_ef_auc_AUCCLT_MITI_AD

						t_ef_auc_AUCCLT_MITI_CH t_ef_auc_AUCPCR_MITI_CH t_ef_auc_AUCPCR_MITI_CH
AUC viral titer	Plot Median, IQR	Treatment median [IQR] Responses for: NV20234 conventional, NV20234 double, NV20234 pooled, historical treatment conventional, historical treatment double, historical placebo for conventional, historical placebo for double, historical placebo for pooled.	mITTi	Benchmarking plot	All plots on one plot but also split by a) pooled b) conventional c) double	g_km_t1_t2_histplac_treat_AUCCLT_MITI_AD_90 g_km_t1&2_histplac_treat_AUCCLT_MITI_AD_90 Repeated 1:1 and 1:>1 matches
Peak viral titer	Median, IQR	Treatment Responses by treatment arms	mITTi, ITT, ITTi	Dose-Response main summary for this endpoint	a) Adults b) Children	t_ef_auc_PEAKCLT_MITI_AD t_ef_auc_PEAKCLT_MITI_PE t_ef_auc_PEAKPCR_MITI_AD t_ef_auc_PEAKPCR_MITI_PE
Virus shedding	Proportions	Percentage of patients with viral shedding by baseline and planned visit.	mITTi	Main summary table	a) Adults b) children	t_ef_shed_CLTR_MITI_AD t_ef_shed_CLTR_MITI_CH t_ef_shed_PCR_MITI_AD t_ef_shed_PCR_MITI_CH

Viral Load RT-PCR	Change from baseline in viral load RT-PCR	Summary Statistics (Mean, SD, Median, Range)	mITTi	Main summary table	Adults Children	t_ef_cfb_PCR_MITI_AD t_ef_cfb_PCR_MITI_CH
Viral Load Culture	Change from baseline in viral load from culture	Summary Statistics (Mean, SD, Median, Range)	mITTi	Main summary table	Adults Children	t_ef_cfb_CLTR_MITI_AD t_ef_cfb_CLTR_MITI_CH
Secondary Illnesses (SI)	summary only	N(%) by treatment group	Safety	Main summary table	1) Overall 2) Adult 3) Children Split by : a) any SI starting at any time b) any SI starting at any time that is treated with ant biotics c) SI started >=48 hours after start of study treatment that is treated with ant biotics	t_ae_secil_MITI t_ae_secil_ANTIB_SE

11. APPENDIX 6: VIRAL MUTATIONS ASSOCIATED WITH RESISTANCE

Table A6.1. USPI table with Neuraminidase (NA) Amino Acid Substitutions Associated with Reduced Susceptibility to Oseltamivir*

Influenza A N1 (N1 numbering in brackets)	
I117V (I117V)	E119V (E119V)
R152K (R152K)	Y155H (Y155H)
F173V (F174V)	D198G/N (D199G/N)
I222K/R/T/V (I223K/R/T/V)	S246N (S247N)
G248R+I266V (G249R+I267V)	H274Y (H275Y)
N294S (N295S)	Q312R+I427T (Q313R+I427T)
N325K (N325K)	R371K (R368K)
Influenza A N2	
E41G	
E119I/V	D151V
I222L/V	Q226H
SASG245-248	S247P
R292K	N294S
Influenza B (B numbering in brackets)	
E119A (E117A)	
P141S (P139S)	G142R (G140R)
R152K (R150K)	D198E/N/Y (D197E/N/Y)
I222L/T/V (I221L/T/V)	A246D/S/T (A245D/S/T)
H274Y (H273Y)	N294S (N294S)
R371K (R374K)	G402S (G407S)

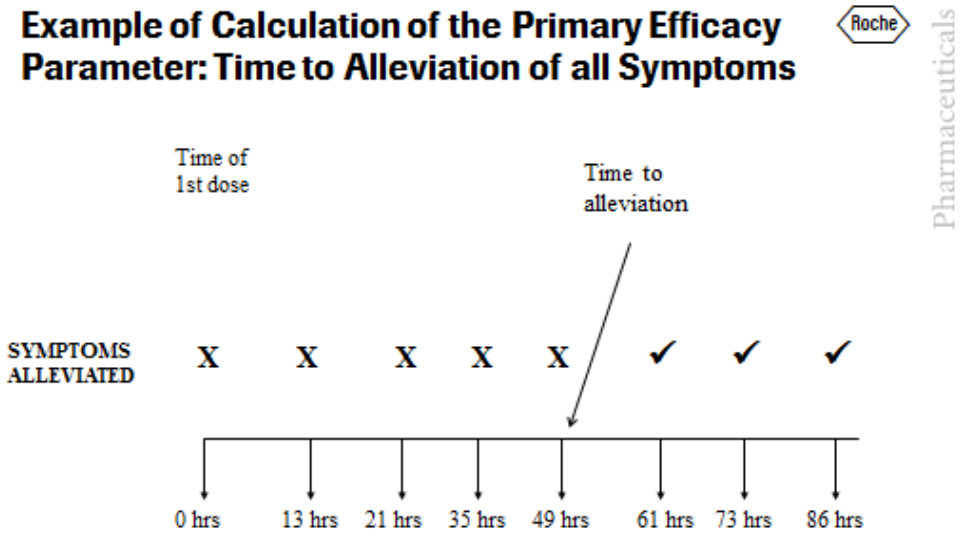
*All numbering is N2, except where indicated

Haemagglutinin (HA) Amino Acid Substitutions Associated with Reduced Susceptibility to Oseltamivir

According to the current Tamiflu label, HA mutations should include A28T and R124M in influenza A H3N2 and H154Q in H1N9.
Per 2016 FDA feedback: *'the previously-listed substitutions A28T and R124M are now referred to as A11T and R453M, respectively, consistent with the convention of numbering from the beginning of the processed HA (without the signal peptide). We removed previously listed substitutions that were identified in strains not currently relevant to humans (H154Q in H1N9*)." In summary, per FDA communication the HA residues to be included on the resistance list (renamed nomenclature, taking into account only the mature protein, w/o the signal peptide) are A11T, K173G, and R453M in H3N2; and H99Q in influenza B virus (no HA mutations listed for H1N1)

12. APPENDIX 7: ALGORITHMS USED FOR DERIVATION AND SUMMARIES OF TIME TO ALLEVIATION OF ALL SYMPTOMS

The purpose of this document is to describe the algorithm used to derive and analyse the Time To Resolution of all symptoms in the Tamiflu program (adults + adolescents).
Time to alleviation (TTA) of all symptoms (Efficacy VAD variable TALLSYMP), was calculated as the time **from start of study drug (Time=0) to the start of the first 24 hour period in which** all 7 symptoms were less than or equal to 1 and they remained at one or less for at least 21.5 hours, i.e., up to the end of the 21.5 hour period.



For subjects with missing data or who withdrew or ceased recording data prior to alleviation, data was imputed according to the following rules:
At the individual symptom level, a missing value was estimated by linear interpolation between the previously available assessment and the subsequently available assessment.
If the interpolated value was ≤ 1 for the first time (e.g. interpolation between a score of 2 and 0 gave 1) then the interpolated time point was not considered as the beginning of the 24 hour period that determined whether alleviation of that symptom had occurred, instead, the beginning of the 24 hour period started at the next observed time point at which the value was ≤1. The VAD variables which denote missing data rules are TESTTYPE AND DATETYPE (refer to the efficacy VAD specifications for details).
If symptoms were missing, 0 or 1 at baseline and were subsequently recorded as missing, 0 or 1 for at least 21.5 hours, then the TTA of all symptoms was set to

missing. [Corresponding Efficacy VAD variables TALLSYMP=. And CENSORED=0.]
If all non-missing baselines symptoms were > 1 at baseline and recovered (<=1) immediately and remained <=1 for at least 21.5 hours OR if all non-missing baselines symptoms were > 1 at baseline and no further recordings of symptom scores had been recorded, then the Time to Alleviation of All symptoms was 0. [Corresponding Efficacy VAD variables TALLSYMP=0 and CENSORED=0]
For Paediatric studies, should a subject only have No/mild symptoms from baseline onwards, and subsequent visits satisfy the 21.5 hour rule, then set TALLSYMP=0 and CENSORED=0.
Subjects withdrawing prior to alleviation of symptoms were **censored** at the time of withdrawal. [denoted in the efficacy VAD as CENSORED=1]
The primary endpoint was compared between groups using statistical methodology appropriate for survival data since censored data is present. The within-treatment group summary measures of median duration, was estimated from the Kaplan-Meier curve. A weighted Mantel-Haenszel test was used to formally test for a between-treatment group effect since the proportional hazards assumption was considered unlikely to hold *a priori*.
The weighting scheme used was the generalised Gehan Wilcoxon weights in which the weighting is equal to the number in the risk set**.**
Kaplan-Meier graphs of duration of symptoms according to treatment group were provided. Estimates of the difference between median durations were provided by subtracting the observed treatment group medians obtained from the Kaplan-Meier curve and estimating between group variability through bootstrapping. The dataset was sampled 2000 times in order to provide 95% confidence intervals using the percentile method (Efron 1993).
The bootstrap methodology had previously been applied within the influenza clinical trial field by the MIST group (MIST 1998) when comparing time to alleviation of illness in subjects treated with inhaled Zanamivir or placebo.