Official Title: **A MULTICENTER, RANDOMIZED, DOUBLE-BLIND, ACTIVE (OSELTAMIVIR)-CONTROLLED STUDY TO ASSESS THE SAFETY, PHARMACOKINETICS, AND EFFICACY OF BALOXAVIR MARBOXIL IN OTHERWISE HEALTHY PEDIATRIC PATIENTS 1 TO < 12 YEARS OF AGE WITH INFLUENZA-LIKE SYMPTOMS**

NCT Number: NCT03629184

Document Date: SAP Version 2: 24-May-2019

STATISTICAL ANALYSIS PLAN

TITLE: **A MULTICENTER, RANDOMIZED, DOUBLE-BLIND, ACTIVE (OSELTAMIVIR)-CONTROLLED STUDY TO ASSESS THE SAFETY, PHARMACOKINETICS, AND EFFICACY OF BALOXAVIR MARBOXIL IN OTHERWISE HEALTHY PEDIATRIC PATIENTS 1 TO < 12 YEARS OF AGE WITH INFLUENZA-LIKE SYMPTOMS**

PROTOCOL NUMBER: CP40563

STUDY DRUG: Baloxavir Marboxil

VERSION NUMBER: 2

IND NUMBER: 126653

EUDRACT NUMBER: 2018-002169-21

SPONSOR: F. Hoffmann-La Roche Ltd

PLAN PREPARED BY: ███████████████ , B.Sc.

DATE FINAL: See electronic date stamp below

STATISTICAL ANALYSIS PLAN APPROVAL

Name	Reason for Signing	Date and Time (UTC)
████████████████	Company Signatory	24-May-2019 16:46:39

CONFIDENTIAL

This is an F. Hoffmann-La Roche Ltd document that contains confidential information. Nothing herein is to be disclosed without written consent from F. Hoffmann-La Roche Ltd.

STATISTICAL ANALYSIS PLAN AMENDMENT
RATIONALE

This Statistical Analysis Plan (SAP) has been amended to incorporate the following changes:

- Addition of Sparse Pharmacokinetic-Evaluable Population (Section 4.1.6) and Extensive Pharmacokinetic-Evaluable Population (Section 4.1.7).

- Clarification of the baseline definition for Body Temperature (Section 4.5.5).

- Addition of rule for imputation of CARIFS assessment time if the time was not recorded in the correct assessment time period (Section 4.6).

- Clarification of censoring rule in time to event analysis (Section 4.6).

- Clarification on handling rule for missing data in CARIFS assessments.

- Further details on exploratory analysis endpoint Polymorphic and treatment-emergent amino acid substitutions in the PA, PB1, and PB2 genes (Section 4.6.3)

- Addition of subgroups analysis of some secondary endpoints, based on virus type and subtype (Section 4.6.4).

- Additional minor changes have been made to improve clarity and consistency.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICES

1. BACKGROUND

This Statistical Analysis Plan (SAP) provides details of the planned analyses and statistical methods for the clinical efficacy, clinical safety, pharmacokinetic (PK), and pharmacodynamic (PD) data for Study CP40563.

Baloxavir marboxil (also referred to as S-033188, Shionogi Compound Identification Number) is a pro-drug that is converted to an active form (baloxavir, S-033447) in the blood, liver, and small intestine through a metabolic process called hydrolysis.

Baloxavir marboxil acts on cap-dependent endonuclease, an enzyme specific to influenza viruses, and inhibits viral cap-snatching, thereby suppressing the replication of influenza viruses.

2. STUDY DESIGN

This is a multicenter, randomized, double-blind, active-controlled (i.e., oseltamivir) study to assess the safety, PK, and efficacy of baloxavir marboxil compared with oseltamivir in pediatric patients 1 to $<$ 12 years of age with influenza-like symptoms. This study is designed to enroll approximately 120 otherwise healthy male and female pediatric patients with influenza-like symptoms presenting within 48 hours of symptoms onset.

2.1 PROTOCOL SYNOPSIS

The Protocol Synopsis is in Appendix 1 and includes the study objectives, inclusion and exclusion criteria, outcome measures, and statistical methods as stated in the Protocol.

2.2 OUTCOME MEASURES

All assessments and procedures are detailed in the Protocol.

2.2.1 Safety (Primary) Endpoints

Incidence, severity, and timing of:

- Adverse events (AEs)
- Serious adverse events (SAEs)
- Vital sign measurements
- Clinical laboratory test results

2.2.2 Pharmacokinetic Endpoints

- Plasma concentrations of baloxavir marboxil (pro-drug) and S-033447 (active metabolite)
- Population PK model derived parameters (e.g., AUC_{inf}, C_{max}, T_{max}, $t_{1/2}$) (modelling report)

2.2.3   Efficacy (Secondary) Endpoints

- Time to alleviation of influenza signs and symptoms, defined as the length of time taken from the start of treatment to the point at which all of the following criteria are met and remain so for at least 21.5 hours:

- A score of 0 (no problem) or 1 (minor problem) for cough and nasal symptoms (items 14 and 15 of the Canadian Acute Respiratory Illness and Flu Scale (CARIFS) questionnaire (Appendix 4)

- A "yes" response to the following question on the CARIFS: "Since the last assessment has the subject been able to return to day care/school, or resume his or her normal daily activity in the same way as performed prior to developing the flu?"

- First return to afebrile state (tympanic temperature $\leq 37.2°C$)

- Duration of fever (time to return to afebrile state [tympanic temperature $\leq 37.2°C$] and remaining so for at least 21.5 hours)

- Duration of symptoms (alleviation of all symptoms as defined by a score of 0 [no problem] or 1 [minor problem] and remaining so for at least 21.5 hours, for all 18 symptoms specified in the CARIFS questionnaire)

- Time to return to normal health and activity

- Frequency of influenza-related complications (death, hospitalization, radiologically confirmed pneumonia, bronchitis, sinusitis, otitis media, febrile seizures, encephalitis/encephalopathy, myositis)

- Proportion of patients requiring antibiotics

2.2.4   Virology (Secondary) Endpoints

- Time to cessation of viral shedding by virus titer and by reverse transcriptase-polymerase chain reaction (RT-PCR)

- Change from baseline in influenza virus titer and in the amount of virus RNA (RT-PCR) at each time point

- Proportion of patients with positive influenza virus titer and proportion of patients positive by RT-PCR at each time point

- Area under the curve in virus titer and in the amount of virus RNA (RT-PCR)

2.2.5   Exploratory Endpoints

- Polymorphic and treatment-emergent amino acid substitutions in the PA, PB1, and PB2 genes

- Drug susceptibility in patients with evaluable virus

- Non-compartmental PK parameters (intensive PK)

2.3   DETERMINATION OF SAMPLE SIZE

No formal sample-size calculations have been performed. Approximately 120 pediatric patients (80 patients in the baloxavir marboxil treatment group and 40 patients in the oseltamivir treatment group) will be enrolled.

To detect adverse events with a 3% incidence for at least 1 patient with a probability of $\geq 90\%$, the study will require 80 patients exposed to baloxavir marboxil. The given sample size provides a probability of at least 1 patient experiencing an adverse event with a true incidence rate of from 1% to 5% as shown in Table 1.

Table 1   Probability of Adverse Events by Incidence Rates

	True incidence rate of an AE				
	1%	2%	3%	4%	5
Probability of at least one patient experiencing an AE	55.2%	80.1%	91.3%	96.2%	98.3%

AE = adverse event.

The number of patients in the intention to treat influenza infected (ITTi) population will be monitored on an ongoing basis in order to ensure that an adequate number of influenza-infected patients are recruited. Additional patients may be recruited if the ITTi population is considerably smaller than expected.

2.4   ANALYSIS TIMING

The primary study analysis will occur when the last patient has either withdrawn or completed his or her Day 29 visit, and will be based on all available data for all patients up to and inclusive of their Day 29 assessment date, as well as safety follow-up data for patients that withdrew prior to Day 29.

3.   STUDY CONDUCT

3.1   RANDOMIZATION

Following completion of the screening period and after all patient eligibility requirements are confirmed, patients will be assigned a patient number (a different number from the screening number) on Day 1 and will undergo randomization in a 2:1 ratio to baloxavir marboxil or oseltamivir respectively. All patients will receive one of two active treatments: a single oral dose of baloxavir marboxil based on body weight (2 mg/kg for patients weighing <20 kg or 40 mg for patients weighing ≥ 20 kg) or oseltamivir (dose based on body weight) for 5 days. Both groups will receive matching placebo for the alternate treatment. A permuted block randomization method will be used to obtain an approximate 2:1 ratio of baloxavir marboxil to oseltamivir.

3.2   DATA MONITORING

To facilitate early and close monitoring of safety parameters over time, an Internal Monitoring Committee (IMC) will review aggregate safety data on an ongoing basis as defined in the IMC charter and according to the process defined in the Blinding/Unblinding Process document. If there is any concern noted in the safety

parameters, the Sponsor may suspend or discontinue a treatment arm or the entire study, as appropriate.

4. STATISTICAL METHODS

4.1 ANALYSIS POPULATIONS

Disposition summaries will be based on the Randomized Population. Analysis of safety data will be based on the safety population. The primary analysis population for efficacy will be the ITTi population.

4.1.1 Randomized Population

The randomized population will include all patients randomized in the study.

4.1.2 Per Protocol Population

Not applicable.

4.1.3 Intent-to-Treat Population

The intent-to-treat (ITT) population comprises all patients who received at least one dose of treatment regardless of whether they have any follow-up assessments. Patients will be grouped on the basis of randomized treatment. If there is any doubt whether a patient was treated, that patient will be assumed to have been treated for the purpose of analysis.

4.1.4 Intent-to-Treat Influenza-Infected Population

The ITTi population is a subset of ITT patients who have had a laboratory confirmation of influenza infection (polymerase chain reaction [PCR] result) from any swab sample collected at baseline or during the study. The ITTi population is the primary efficacy population, unless specified otherwise. Patients will be grouped based on randomized treatment.

Decisions on patient exclusion from the ITTi population will be made prior to database closure. Excluded patients will be documented, together with the reason for exclusion.

4.1.5 Pharmacokinetic-Evaluable Population

The pharmacokinetic-evaluable patient (PKEP) or PK 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 or deviate significantly from the protocol, or if data are unavailable or incomplete which may influence the PK analysis, such as the absence of properly documented date and time for the baloxavir marboxil administration and PK sample collection.

Decisions on exclusion from the PKEP population may be made prior to database closure by the clinical pharmacologist. Excluded patients will be documented, together with the reason for exclusion.

4.1.6 <u>Sparse Pharmacokinetic-Evaluable Population</u>

The sparse PKEP or sparse PK population comprises all patients in the PK population who did not provided informed consent to intensive PK sampling.

4.1.7 <u>Extensive Pharmacokinetic-Evaluable Population</u>

The extensive PK population comprises all patients in the PK population who provided informed consent to intensive PK sampling.

4.1.8 <u>Safety Population</u>

The safety-evaluable patient population comprises all patients who received at least one dose of treatment regardless of whether they have any follow-up assessments. Patients will be classified according to treatment actually received.

4.2 ANALYSIS OF STUDY CONDUCT

The number of patients who enroll, discontinue, or complete the study will be summarized by treatment arm. Reasons for premature study withdrawal will be listed and summarized by treatment arm. Major protocol deviations will be listed and evaluated for their potential effects on the interpretation of study results.

4.3 ANALYSIS OF TREATMENT GROUP COMPARABILITY

Demographic and baseline characteristics (including age, sex, race/ethnicity, height, weight, BMI and vaccination status) will be summarized using means, standard deviations, medians, and ranges for continuous variables and proportions for categorical variables, as appropriate. Summaries will be presented overall and by treatment received for the safety population. If it is found that there is a large difference in the number of patients in the safety population compared to the ITTi population, additional summaries will be produced based on randomized treatment.

Medical history data, including surgery and procedures and baseline conditions, will be summarized descriptively by treatment group using the safety population. Descriptive summaries of any previous and concomitant treatment will be produced by treatment group.

4.4   Visit Windows

Table 2   Acceptable Time Windows for Parameters Other Than Data from the Patient Diary

Time Point (Scheduled Time Point)	Acceptable Time Window
Pre-dose at Visit 1 (Day 1)'	Before dosing on Day 1
Post-dose at Visit 1 (Day 1)'	Between 0.5 and 4 hours after dosing (on Day 1)
Visit 2 (Day 2)	Day 2
Optional Visit 1 (Day 3)	Day 3
Visit 3 (Day 4)	Day 4
Visit 4 (Day 6)	Between Day 5 and Day 7 as follows: -if patient presents on Day 5 with no Day 4, and no Day 6 this will be classed as Visit 4 -if patient presents on Day 5 with no Day 4, and Day 6 this will be classed as Visit 3. -if patient presents on Day 5 with Day 4 and no Day 6 this will be classed as Visit 4.
Visit 5 (Day 10)	Between Day 8 and Day 12
Optional Visit2 (Day 15)	Between Day 13 and Day 22
Visit 6 (Day 29)	On or after Day 23
Withdrawal	Date of withdrawal + 14 days

* if time is missing for data collected on Visit 1 where, per the schedule of activities, this data should be collected at 'v1 screening' or 'v1 post-dose' it is defined as 'Pre-dose at Visit 1 (Day 1)' or 'Post-dose at Visit 1 (Day 1)' respectively.

Measurements collected within the acceptable time window for each scheduled assessment time point, including data obtained at the time of withdrawal, will be used for the analyses of all endpoints at each assessment time point. For all patients with multiple values within a visit window, the value obtained closest to the target time point will be used. If two measurements collected with the same time deviation exist before and after the target time point, the measurement obtained before the target time point will be adopted for analysis. The assessment time point having no measurements within the corresponding acceptable time window will be considered as missing.

In the assessment of data from sources other than the patient diary, if there are multiple values which may be adopted for a scheduled assessment time point (even though the above rules have been strictly followed), the measurements at the time point entered in the Case Report Form (CRF) will be adopted.

For summaries of data not collected by visit, such as adverse events, medical history and concomitant medications, all data up to the last exposure date will be included.

4.5   SAFETY ANALYSES

Safety Endpoints (Primary Objective)

The primary objective for this study is to compare the safety of a single dose of baloxavir marboxil with 5 days of oseltamivir administered twice daily. The safety population will be used for all safety analyses. Patients will be analyzed according to the treatment they actually received.

The safety of baloxavir marboxil will be evaluated for adverse events, vital signs measurements, and clinical laboratory tests. Safety parameters will be summarized or listed for the safety population.

4.5.1   <u>Adverse Events</u>

AEs will be classified by system organ class (SOC) and preferred term using Medical Dictionary for Regulatory Activities (MedDRA) Version 22.0 and adverse event severity will be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v5.0 or the Adverse Event Severity Grading Scale for events not specifically listed in NCI CTCAE toxicity grading scale (Table 2 of the protocol) on the eCRF, AEs reported after the initial dose of the study drug will be used for safety analyses.

In summaries by SOC and preferred term (PT), AEs will be sorted by overall decreasing frequency within each SOC and PT.

All Adverse Events

Only treatment-emergent AEs will be summarized. Treatment-emergent events are defined as those AEs with observed or imputed onset date on or after the start date of trial treatment. Only where the most extreme intensity is greater than the initial intensity (or if most extreme intensity is not missing and initial intensity is missing) will events with an onset date prior to the start of study treatment be considered treatment-emergent. An AE with a completely missing start date will be assumed to be treatment-emergent unless the AE has a complete non-imputed end date that is prior to study Day 1.

Adverse events will be coded and tabulated by SOC and/or PT. In tabulations, PTs and their associated SOC will be presented in order of descending frequency summed across the treatment arms. Adverse events will also be tabulated by severity and relationship to the study drug as indicated by the investigator. Adverse events leading to withdrawal, AEs leading to death, and AEs leading to a dose modification or interruption will be summarized.

The following will also be summarized by treatment, and listings produced where required:

- Serious AEs
- AEs leading to withdrawal
- AEs leading to death
- AEs leading to a dose modification or interruption
- Non-serious AEs occurring in $\geq$ 5% of patients in at least one treatment group
- Non-serious AEs occurring in $\geq$ 1% of patients in at least one treatment group

Adverse Events of Special Interest

All adverse events of special interest (AESI) will be presented by treatment group. AESI include but are not limited to the following:

- Case of an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined in protocol
- Suspected transmission of an infectious agent by the study drug

4.5.2   Deaths

Details of any deaths will be presented in the form of an individual patient listing, as well as a frequency table by treatment.

4.5.3   Laboratory Data

For each of the hematological and biochemical test parameters, summary statistics of observed and change from baseline values at each time point will be presented by treatment group for each scheduled time point. For laboratory parameters, post-dose observations on Day 1 will be classed as baseline.

Each observed value will be classified into three categories: Normal (for values within the normal range), High (for values higher than normal) and Low (for values lower than normal). The frequency of each category will be summarized by treatment group for each scheduled time point. Additionally, a shift table will present the changes from baseline at each scheduled time point.

The number and proportion of patients who meet the pre-specified criteria shown in Table 3, will be presented by treatment group during the study.

Table 3   Pre-specified Abnormal Laboratory Criteria

Term	Criteria
AST (U/L) or ALT (U/L) and Total bilirubin (mg/dL)	Meet all of the following criteria: • AST $\geq 3 \times$ ULN or ALT $\geq 3 \times$ ULN • Total bilirubin Value $\geq 2 \times$ ULN at same timepoint

ULN $=$ upper limit of normal.

4.5.4   Vital Signs

For each of the vital signs (diastolic blood pressures, systolic blood pressures, respiratory rate and pulse rate), summary statistics of observation and the change from baseline will be presented by treatment group for each scheduled time point.

Baseline is defined as the last assessment prior to treatment.

4.5.5   Body Temperature

Body temperature is collected in the patient diary 4 times daily for Day $1-3$, twice daily for Day $4-9$ and once daily thereafter. Summary statistics of observation and the change from baseline in body temperature will be presented by treatment group for each recorded time point.

Baseline is defined as the first recorded assessment after treatment on Day 1.

4.5.6   Exposure of Study Medication

The duration of treatment exposure will be summarized with descriptive statistics by treatment group for the safety population.

The duration of treatment exposure [days] in the oseltamivir group is defined as the dosing period during which a patient takes medication as follows:

$$\{(\text{final dose date}) - (\text{initial dose date}) + 1\}$$

For the baloxavir marboxil group, duration of exposure is defined as 1 day if actual baloxavir marboxil granules for oral suspension are dosed.

The treatment compliance rate will be summarized with descriptive statistics by the treatment group for the safety population. In addition, the frequency and percentage of patients with compliance $< 80\%$ and $\geq 80\%$ will be presented.

The treatment compliance rate [%] in the oseltamivir group is defined as:

$$\frac{\text{actual frequency of treatment exposure}}{\text{expected frequency of treatment exposure}} * 100$$

For baloxavir marboxil group treatment compliance is defined as 100% if patients are dosed per protocol.

4.6 EFFICACY ANALYSIS

The statistical analyses of efficacy endpoints will be descriptive. The ITTi population will be used for all analyses. Additional analysis of efficacy analysis presenting the ITT population will not be completed.

The data will be summarized using means, standard deviations, medians, and ranges for continuous variables and proportions for categorical variables, as appropriate. Time-to-event endpoints and duration endpoints will be summarized using Kaplan-Meier plots and summaries of the median survival time. Summaries will be presented by treatment group.

Efficacy endpoints are based on the CARIFS questionnaire data which is recorded by parent/ caregiver using handheld device. This assessment data should be recorded for the following time periods:

Period	Time
Morning	0.500-10.59
Noon	11.00-14.59
Evening	15.00-18.59
Bedtime	19.00-23.59

*data should not be recorded between 00.00 and 4.59.

In the case where the recorded time of an assessment is outside the period time window for that assessment, the end time of the period should be imputed as the assessment time.

In the case where the assessment time falls into the period between 00.00 and 4.59 (due to technical error) the assessment date will be imputed to the date prior to recorded assessment date and the assessment time will be imputed to the end time of the period time window.

The imputation method used in the derivation of time to alleviation of influenza signs and symptoms is described in Section 4.6.1. No further imputation methods will be used.

In all time to event analysis, patients who withdraw prior to event of interest will be censored at the last observation timepoint.

4.6.1   Efficacy Endpoints (Secondary Objectives)

Time to alleviation of influenza signs and symptoms

Time to alleviation (TTAS) of influenza signs and symptoms in hours, will be summarized using descriptive statistics by treatment, as well as Kaplan-Meier plots. It is defined as the length of time taken from the start of treatment to the point at which all of the following criteria are met and remain so for at least 21.5 hours:

–   A score of 0 (no problem) or 1 (minor problem) for cough and nasal symptoms (items 14 and 15 of the CARIFS questionnaire - see Appendix 4)

–   A "yes" response to the following question on the CARIFS: "Since the last assessment has the subject been able to return to day care/school, or resume his or her normal daily activity in the same way as performed prior to developing the flu?"

–   First return to afebrile state (tympanic temperature $\leq 37.2°C$).

If a score of 4 ("do not know" or "not applicable") occurs at any assessment during the study for items 14 or 15, the assessment will not be included in the calculation of the alleviation of symptoms because the assessment was unobservable.

Patients with symptom scores ≤ 1 for both items 14 and 15, as well as tympanic temperature $\leq 37.2°C$ and a "yes" response to the question 'Since the last assessment has the subject been able to return to day care/school, or resume his or her normal daily activity in the same way as performed prior to developing the flu?' at baseline will have a TTAS of all symptoms set to missing.

Patients who do not experience alleviation of symptoms prior to completion or withdrawal from study will be censored at the last observation time point.

Baseline is defined as the first CARIFS assessment after start of treatment on Day 1.

The number of patients with completed CARIFS as well as the number with missing CARIFS per assessment, will be presented by treatment. Additionally, the number and percentage of those with 'Don't Know/ Not Applicable' per Question per assessment will be presented by treatment.

Duration of fever

Duration of fever will be summarized using descriptive statistics by treatment, as well as Kaplan-Meier plots. It is defined as the time to return to afebrile state [tympanic temperature $\leq 37.2°C$] and remaining so for at least 21.5 hours.

Patients who do not return to afebrile state will be censored at the last observation time point.

Duration of symptoms

Duration of symptoms will be summarized using descriptive statistics by treatment, as well as Kaplan-Meier plots. Duration of symptoms is defined as alleviation of all symptoms as defined by a score of 0 [no problem] or 1 [minor problem] and remaining so for at least 21.5 hours, for all 18 symptoms specified in the CARIFS questionnaire.

If a score of 4 ("do not know" or "not applicable") occurs at any assessment during the study for any item, with the exception of items 10, 11 and 12 the assessment will not be included in the calculation of the alleviation of symptoms because the assessment was unobservable.

However, if a score of 4 occurs for items 10, 11 or 12, the assessment will be included, with a score of missing for these items.

In addition, duration of individual symptoms will be summarized using descriptive statistics by treatment, as supportive analysis. Patients who have an individual symptom score ≤ 1 at baseline will have the duration of that symptom set to missing.

Time to return to normal health and activity

Time to return to normal health and activity will be summarized using descriptive statistics by treatment, as well as Kaplan-Meier plots.

'Normal health and activity' is identified by a 'Yes' response to the following question on the CARIFS: "Since the last assessment has the patient been able to return to day care/school, or resume his or her normal daily activity in the same way as performed prior to developing the flu?"

Patients who do not return to normal health and activity will be censored at the last observation time point.

Frequency of influenza-related complications

The number of influenza-related complications, as well as the number and percentage of patient with these complications, after treatment, will be presented by treatment. Influenza-related complications include death, hospitalization, radiologically-confirmed pneumonia, bronchitis, sinusitis, otitis media, encephalitis/encephalopathy, febrile seizures and myositis, as recorded on the AE pages of eCRF.

Proportion of patients requiring antibiotics

The number and percentage of patients requiring antibiotics for any reason will be presented by treatment group.

4.6.2   Virology (Secondary) Endpoints

Time to cessation of viral shedding by virus titer

Time to cessation of viral shedding by virus titer is defined as the time, in hours, between the initiation of any study treatment and first time when the influenza virus titer is below the limit of detection. Patients whose virus titers have not reached the limit by the last observation time point will be treated as censored at that time point. One day is converted into 24 hours.

Patients with a positive virus titer on Day 1 will be included in this analysis.

This endpoint will be summarized using descriptive statistics by treatment, as well as Kaplan-Meier plots.

Time to cessation of viral shedding by RT-PCR

Time to cessation of viral shedding by RT-PCR, in hours, is defined as the time between the initiation of any study treatment and first time when the virus RNA by RT-PCR is below the limit of detection. Patients whose virus RNA have not reached the limit by the last observation time point will be treated as censored at that time point. For the patients with multiple virus types, this endpoint is defined as the time between the initiation of the study treatment and first time when the virus RNA by RT-PCR is below the limit of detection for all virus types. One day is converted into 24 hours.

Patients with a positive virus RNA on Day 1 will be included in this analysis.

This endpoint will be summarized using descriptive statistics by treatment, as well as Kaplan-Meier plots.

Change from baseline in influenza virus titer at each time point

Change from baseline in influenza virus titer ($\log_{10}TCID_{50}/ML$) will be presented by treatment and is defined as the change from baseline in influenza virus titer on Days 2, 4, 6, and 10. If influenza virus titer is less than the lower limit of quantification, the virus titer will be imputed as 0.749 ($\log_{10}TCID_{50}/mL$).

Only patients with a positive virus titer on Day 1 will be included in this analysis.

Change from baseline in the amount of virus RNA (RT-PCR) at each time point

Change from baseline in the amount of virus RNA (unit: $\log_{10}$ virus particles/mL) will be presented by treatment and is defined as the change from baseline in the amount of virus RNA on Days 2, 4, 6, and 10.

If the amount of virus RNA is less than the lower limit of quantification, the amount of virus RNA will be imputed as 2.18 for flu A and 2.93 for flu B ($\log_{10}$ virus particles/mL). If a patient is infected with multiple virus types, the sum of those amounts of virus RNA will be used for analysis.

Patients with a positive by RT-PCR on Day 1 will be included in this analysis.

Proportion of patients with positive influenza virus titer at each timepoint

Proportion of patients positive for influenza virus titer will be presented by treatment at each visit and is defined as the percentage of patients whose influenza virus titer is not less than the lower limit of quantification ($0.75 \log_{10} TCID_{50}/mL$) or positive among those assessed for influenza virus titer on Days 2, 4, 6, and 10.

Patients with a positive influenza virus titer on Day 1 will be included in this analysis.

Proportion of patients positive by RT-PCR at each time point

Proportion of patients positive by RT-PCR will be presented by treatment at each visit is defined as the percentage of patients with detectable virus RNA (2.05 for flu A and 2.83 for flu B log10 virus particles/mL) among those assessed by RT-PCR on Days 2, 4, 6, and 10.

Patients with a positive by RT-PCR on Day 1 will be included in this analysis.

Area under the curve in virus titer

Area under the curve (AUC) in virus titer will be presented by treatment group and will be calculated using the trapezoidal method. AUC of change from time 0 (t_0) to time K (t_k) is given by the formula

$$\sum_{k=1}^{K} \frac{(y_k + y_{k-1} - 2y_0)(t_k - t_{k-1})}{2}$$

where t_k (hours) represents the date of the k^h viral titer assessment (k = 0, ..., K) and y_k represents the $\log_{10}$ value of the kth viral titer assessment ($TCID_{50}/mL$).

24-hours of time will be converted into one day. Patients with a positive virus titer on Day 1 will be subjected to this analysis. The lower limit is defined as $0.75 \log_{10}$ $TCID_{50}/mL$ for flu A and $0.75 \log_{10}$ $TCID_{50}/mL$ for flu B ($TCID_{50}/mL$). If a patient is infected with multiple virus types, the sum of those virus titers will be used for analysis.

Area under the curve in the amount of virus RNA (RT-PCR)

AUC in virus RNA (RT-PCR) will be presented by treatment group and is defined as AUC of change from baseline in the amount of virus RNA (RT-PCR) from Day 1 to Day 10. AUC is calculated using the trapezoidal method similar to AUC in virus titer. Patients with a positive RT-PCR result on Day 1 will be subjected to this analysis. The lower limit of quantification is defined as 2.18 for flu A and 2.93 for flu B ($\log_{10}$ virus particles/mL). If a patient is infected with multiple virus types, the sum of those the amount of virus RNA will be used for analysis.

4.6.3 <u>Exploratory Endpoints</u>

Polymorphic and treatment-emergent amino acid substitutions in the PA, PB1, and PB2 genes

Sequencing of PB1 and PB2 will be done for:

- baseline viruses with reduced susceptibility to baloxavir (fold-change in EC_{50} > 10 for type A viruses and > 5 for type B viruses compared to reference virus in phenotypic assay).

- viruses (baseline and last evaluable timepoint) without amino acid substitution in PA, but with reduced response to treatment or with virus rebound.

These will be defined according to the following criteria below. If an amino acid substitution is detected, an additional intermediate timepoint sample will be sequenced.

	Criteria
1) Subjects who exhibit viral titer rebound:	Viral titer at [(a certain time point) – (just before time point)] $\geqslant$ 2 x SD value of viral titer of control virus in assay * * ██████████████████████████████████████ ████████████ █████████████████████████████████ ███████ ████████████████████████ ███████
2) Subjects who continue to shed virus at Day 6 and beyond:	Viral titer (log10TCID50/mL) at Day 6 and beyond > 1.5
3) Subjects who do not show a reduction in virus titer:	Viral titer (log10TCID50/mL) at a certain time point: A > 1.5 Viral titer (log10TCID50/mL) at just before time point: B > 1.5 AND Viral titer (log10TCID50/mL) at [(a certain time point: A) - (just before time point: B)] >= 0

Drug susceptibility in patients with evaluable virus

Non-compartmental PK parameters (intensive PK)

Analysis methods for exploratory analysis will be described in a separate PK plan/report.

4.6.4   Subgroup Analyses

Subgroup analysis of the following secondary endpoints, based on virus type and subtype, will be conducted:

- Time to alleviation of influenza signs and symptoms
- Time to cessation of viral shedding by RT-PCR
- Time to cessation of viral shedding by virus titer

4.7   PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSES

The PKEP population will be used for all analyses.

For patients with blood samples collected, individual plasma baloxavir marboxil and S-033447 concentrations will be tabulated by patient and by timepoint. Individual and mean (if appropriate) plasma baloxavir marboxil and S-033447 concentrations versus time data will be plotted by defined body-weight groups (e.g., $5-10$ kg, $10-15$ kg, etc.). The C_{24} and C_{72} of S-033447 will be listed and summarized (mean, standard deviation, coefficient of variation, median, minimum, and maximum) by defined body-weight group.

Additional PK analyses may be conducted as appropriate. For example, for patients undergoing intensive PK assessments (Appendix 3), individual PK parameters of baloxavir marboxil and S-033447 (AUC, C_{max}, time to maximum plasma concentration, and terminal half-life) may be calculated using model-independent methods, as applicable (if sufficient data available for reliable AUC estimates). Parameters will be listed and if deemed useful, summarized (mean, standard deviation, coefficient of variation, median, minimum, and maximum) by defined body-weight group.

In addition, non-linear mixed-effects model (NONMEM, Version 7.3 or higher) will be used to analyze the PK data. The population typical PK parameters (e.g., apparent clearance, apparent volume of distribution for the central compartment) and their covariates will be estimated. Individual PK metrics, such as area under the concentration-time curve from Time 0 to infinity (AUC_{inf}), C_{24}, C_{72}, and C_{max}, will be also calculated using post hoc Bayesian estimation for each patient, and plotted against significant covariates identified (e.g., body weight, age). The derived individual values will be plotted against body weight and age. PK parameters will be tabulated and summarized (i.e., by mean, standard deviation, coefficient of variation, median, and minimum and maximum) by age group. PK exposure and response (e.g., virus titers) relationships will be explored. Details related to the Population PK analyses and downstream PK exposure-response analyses will be provided in a dedicated modelling and simulation plan, and results obtained in dedicated M&S reports.

Pediatric exposure matching to adults will use C_{24} (observed, as well as Bayesian post hoc) as a primary PK parameter. Key secondary PK metrics, such as C_{72} and AUC_{inf} (derived as Bayesian post hoc), will assess whether exposure is sustained similarly as in adults, and to explore whether or not viral rebound, if any, may be associated with lower C_{72} values. For completeness, C_{max} will be calculated as secondary PK parameter. Assessment of pediatric exposure and comparison to adults will use all PK data (sparse and intensive PK). Results may be described within the population PK report or in a dedicated extrapolation report.

4.8   MISSING DATA

In safety analyses, all deaths are included, from all sources, regardless of completeness of death date; patients who died with only a partial death date available will be included. Partial dates for AEs, concomitant medications, laboratory assessments, and medical history will be imputed following a conservative approach.

In efficacy analyses, a death is considered an event if and only if a complete death date is available; patients who died with only a partial death date available will be censored.

4.9   INTERIM ANALYSES

An interim PK analysis is planned in this study. The PK samples, up to and including Visit 4/5 of first 12 patients dosed, will be batched for an early shipment. This will enable a timely review of the systemic plasma concentrations, in particular the concentration at 24 hours and 72 hours post dosing. This is in order to verify, in a timely manner, that exposures at the proposed dose are in reasonable agreement with the corresponding values seen in adults.

The estimated turnaround time between last sample collected and data review is estimated to be approximately 2 to 3 weeks and recruitment will continue during this time. Data review of blinded PK samples (dummy ID) will be performed by members of the Sponsor study team.

No other interim analyses are planned in this study.

5. REFERENCES

Not applicable

Appendix 1
Protocol Synopsis

TITLE:	A MULTICENTER, RANDOMIZED, DOUBLE-BLIND, ACTIVE (OSELTAMIVIR)-CONTROLLED STUDY TO ASSESS THE SAFETY, PHARMACOKINETICS, AND EFFICACY OF BALOXAVIR MARBOXIL IN OTHERWISE HEALTHY PEDIATRIC PATIENTS 1 TO < 12 YEARS OF AGE WITH INFLUENZA-LIKE SYMPTOMS
PROTOCOL NUMBER:	CP40563
VERSION NUMBER:	1
IND NUMBER:	126653
EudraCT NUMBER:	2018-002169-21
TEST PRODUCT:	Baloxavir marboxil (RO7191686)
PHASE:	III
INDICATION:	Influenza
SPONSOR:	F. Hoffmann-La Roche Ltd

Objectives and Endpoints

This study will evaluate the safety, pharmacokinetics, and efficacy of baloxavir marboxil compared with oseltamivir in a single influenza episode in otherwise healthy pediatric patients (i.e., 1 to < 12 years of age) with influenza-like symptoms. Specific objectives and corresponding endpoints for the study are outlined below.

Safety (Primary) Objective	Corresponding Endpoints
• To compare the safety of a single dose of baloxavir marboxil with 5 days of oseltamivir administered twice daily	• Incidence, severity, and timing of adverse events, serious adverse events, vital sign measurements, and clinical laboratory test results
Pharmacokinetic Objective	**Corresponding Endpoints**
• To evaluate the PK of baloxavir marboxil after single-dose administration	• Plasma concentrations of baloxavir marboxil (pro-drug) and S-033447 (active metabolite) will be summarized by time (C_{24} and C_{72}) and body weight • Population PK model derived parameters (e.g., AUC_{inf}, C_{max}, T_{max}, $t_{1/2}$) (modelling report)
Efficacy (Secondary) Objectives	**Key Corresponding Secondary Endpoint**
• To evaluate the clinical efficacy of baloxavir marboxil compared with oseltamivir	• Time to alleviation of influenza signs and symptoms, defined as the length of time taken from the start of treatment to the point at which all of the following criteria are met and remain so for at least 21.5 hours: – A score of 0 (no problem) or 1 (minor problem) for cough and nasal symptoms (items 14 and 15 of the CARIFS)

	– A "yes" response to the following question on the CARIFS: "Since the last assessment has the subject been able to return to day care/school, or resume his or her normal daily activity in the same way as performed prior to developing the flu?" – First return to afebrile state (tympanic temperature $\leq 37.2°C$)
Efficacy (Secondary) Objectives	**Corresponding Endpoints**
• To evaluate the clinical efficacy of baloxavir marboxil compared with oseltamivir	• Duration of fever (time to return to afebrile state [tympanic temperature $\leq 37.2°C$] and remaining so for at least 21.5 hours) • Duration of symptoms (alleviation of all symptoms as defined by a score of 0 [no problem] or 1 [minor problem] and remaining so for at least 21.5 hours, for all 18 symptoms specified in the CARIFS questionnaire) • Time to return to normal health and activity • Frequency of influenza-related complications (death, hospitalization, radiologically confirmed pneumonia, bronchitis, sinusitis, otitis media, encephalitis/encephalopathy, febrile seizures, myositis) • Proportion of patients requiring antibiotics
Secondary Virology Objective	**Corresponding Endpoints**
• To evaluate the virological activity of baloxavir marboxil compared with oseltamivir	• Time to cessation of viral shedding by virus titer and by RT-PCR • Change from baseline in influenza virus titer and in the amount of virus RNA (RT-PCR) at each time point • Proportion of patients with positive influenza virus titer and proportion of patients positive by RT-PCR at each time point • Area under the curve in virus titer and in the amount of virus RNA (RT-PCR)

Exploratory Objectives	Corresponding Endpoints
• To evaluate the polymorphic and treatment-emergent amino acid substitutions in the PA, PB1, and PB2 genes and drug susceptibility in patients with evaluable virus	• Polymorphic and treatment-emergent amino acid substitutions in the PA, PB1, and PB2 genes • Drug susceptibility in patients with evaluable virus
• To evaluate extended PK parameters of baloxavir marboxil after single-dose administration	• Non-compartmental PK parameters (intensive PK)

AUC_{inf} = area under the concentration–time curve from Time 0 to infinity; C_{24} = plasma concentration 24 hours postdose (acceptable time window: 20 to 28 hours); C_{72} = plasma concentration 72 hours postdose; CARIFS = Canadian Acute Respiratory Illness and Flu Scale; C_{max} = maximum plasma concentration; PA = polymerase acidic protein; PK = pharmacokinetic; RT-PCR = reverse transcriptase-polymerase chain reaction; $t_{1/2}$ = half-life; T_{max} = time to maximum plasma concentration.

Study Design

Description of Study

This is a multicenter, randomized, double-blind, active-controlled (i.e., oseltamivir) study to assess the safety, pharmacokinetics, and efficacy of baloxavir marboxil compared with oseltamivir in pediatric patients 1 to < 12 years of age with influenza-like symptoms.

The approved dose for oseltamivir in pediatric patients 1 to 12 years of age is twice daily (BID) for 5 days, based on body weight. Baloxavir marboxil will be given as a single-dose, based on body weight at screening. Either drug, along with the corresponding placebo of its comparator in this trial, will be started at the time of randomization.

Patients will be screened and randomized on Day 1 and assigned in a 2:1 ratio to receive a single oral dose of baloxavir marboxil based on body weight (2 mg/kg for patients weighing < 20 kg or 40 mg for patients weighing ≥ 20 kg) or oseltamivir (dose based on body weight) for 5 days. Re-screening of patients who fail to meet the inclusion and exclusion criteria will not be permitted for the same illness episode.

The study consists of two periods: a 5-day treatment period (mandatory visits on Days 1, 2, and 4) and a 24-day safety follow-up period (mandatory visits on Days 6, 10, and 29). Therefore, the total study duration for each patient will be 29 days.

During the 5-day treatment period, each patient randomized to baloxavir marboxil will receive the oseltamivir matching placebo BID for 5 days, and those patients randomized to oseltamivir will receive one dose of baloxavir marboxil matching placebo on Day 1.

The following assessments will be conducted at clinic visits: physical examination, vital signs, adverse events, concomitant therapies, clinical laboratory tests, and nasal/throat swabs.

Throughout the treatment and safety follow-up periods, the parents/caregivers will maintain a patient diary for each patient in order to record body temperatures (tympanic assessment), influenza symptoms, and acetaminophen use. Temperatures will be recorded four times daily (morning, noon, evening, and bedtime) on Days 1 to 3; BID (morning and evening) on Days 4 to 9; and once daily on Days 10 to 15. Influenza symptoms will be recorded BID (morning and evening) on Days 1 to 9 and once daily on Days 10 to 15.

This study will be conducted at sites in the United States and globally.

To facilitate early and close monitoring of safety parameters over time, an Internal Monitoring Committee (IMC) will review aggregate safety data on an ongoing basis as defined in the IMC charter. If there is any concern noted in the safety parameters, the Sponsor may suspend or discontinue a treatment arm or the entire study, as appropriate.

Number of Patients

This study is designed to enroll approximately 120 otherwise healthy male and female pediatric patients with influenza-like symptoms presenting within 48 hours of symptom onset.

Patients will be recruited in parallel to the following two cohorts:

- 5 to < 12 years of age (minimum 40 patients)
- 1 to < 5 years of age (minimum 20 patients)

Target Population

Inclusion Criteria

Patients must meet the following criteria for study entry:

- Written informed consent/assent for study participation obtained from patient's parents or legal guardian, with assent as appropriate by the patient, depending on the patient's level of understanding
- Aged 1 to < 12 years at randomization (Day 1)
- Parent/guardian willing and able to comply with study requirements, in the investigator's judgment
- Patient able to comply with study requirements, depending on the patient's level of understanding
- Patient with a diagnosis of influenza virus infection confirmed by the presence of all of the following:
    - Fever $\geq 38°C$ (tympanic temperature) at screening
    - At least one respiratory symptom (either cough or nasal congestion)
- The time interval between the onset of symptoms and screening is ≤ 48 hours (the onset of symptoms is defined as the time when body temperature first exceeded 37.5°C if known, or the time when the first symptom was noticed by patient, parent or caregiver)

Exclusion Criteria

Patients who meet any of the following criteria will be excluded from study entry:

- Severe symptoms of influenza virus infection requiring inpatient treatment
- Concurrent infections requiring systemic antiviral therapy at screening
- Require, in the opinion of the investigator, any of the prohibited medication during the study
- Previous treatment with peramivir, laninamivir, oseltamivir, zanamivir, or amantadine within 2 weeks prior to screening
- Immunization with a live/attenuated influenza vaccine in the 2 weeks prior to randomization
- Concomitant treatment with steroids or other immuno-suppressant therapy
- Known HIV infection or other immunosuppressive disorder
- Uncontrolled renal, vascular, neurologic, or metabolic disease (e.g., diabetes, thyroid disorders, adrenal disease), hepatitis, cirrhosis, or pulmonary disease or patients with known chronic renal failure
- Active cancer at any site
- History of organ transplantation
- Known allergy to either study drug (i.e., baloxavir marboxil and oseltamivir) or to acetaminophen
- Females who have commenced menarche (i.e., child-bearing potential)
- Participation in a clinical trial within 4 weeks or 5 half-lives of exposure to an investigational drug prior to screening, whichever is longer

End of Study

The end of this study is defined as the date when the last patient, last visit (LPLV) occurs or the date at which the last data point required for statistical analysis or safety follow-up is received from the last patient, whichever occurs later. The end of the study is expected to occur 29 days after the last patient has been enrolled.

Length of Study

The total length of the study, from screening of the first patient to the end of the study, is expected to be approximately 9 months. Given the seasonal variability of incidence and severity of influenza, the total length of the study may be extended to complete enrollment.

In addition, the Sponsor may decide to terminate the study at any time.

Investigational Medicinal Products

Test Product (Investigational Drug)

The investigational medicinal product (IMP) for this study is baloxavir marboxil. Each treatment will be administered in combination with the matching placebo for the alternate treatment.

Comparator

Oseltamivir will be administered as an active control. Each treatment will be administered in combination with the matching placebo for the alternate treatment.

Non-Investigational Medicinal Products

Not applicable.

Statistical Methods

Primary Analysis

The safety population will be used for all analyses.

All verbatim adverse event terms will be mapped to MedDRA thesaurus terms, and adverse event severity will be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events, Version 5.0 (NCI CTCAE v5.0).

The safety of baloxavir marboxil will be evaluated from adverse events, vital sign measurements, and clinical laboratory tests. Safety parameters will be summarized or listed for the safety population.

Determination of Sample Size

No formal sample-size calculations have been performed. Approximately 120 pediatric patients (80 patients in the baloxavir marboxil treatment group and 40 patients in the oseltamivir treatment group) will be enrolled.

Interim Analyses

An interim pharmacokinetic (PK) analysis is planned in this study. The PK samples, up to and including Visit 4/5 of first 12 patients dosed, will be batched for an early shipment. This will enable a timely review of the systemic plasma concentrations, in particular the concentration at 24 hours and 72 hours post dosing. This is in order to verify, in a timely manner, that exposures at the proposed dose are in reasonable agreement with the corresponding values seen in adults. The estimated turnaround time between last sample collected and data review is estimated to be approximately 2 to 3 weeks and recruitment will continue during this time. Data review of blinded PK samples (dummy ID) will be performed by members of the Sponsor study team.

No other interim analyses are planned in this study. However, given there is no formal statistical hypothesis testing in this study, the Sponsor may choose to conduct up to two interim analyses. The decision to conduct an optional interim analysis and the timing of the analysis will be documented in the Sponsor's trial master file prior to the conduct of the interim analysis. The interim analysis would be performed and interpreted by members of the Sponsor study team and appropriate senior management personnel. Access to treatment assignment information would follow the Sponsor's standard procedures.

Appendix 2
Schedule of Assessments

	Treatment Period						Follow-Up Period				
Day(s)	D1		D2	D3	D4	D5	D6	D7-9	D10	D15	D29 or ET
Visits	V1 Screening	V1 Post-dose	V2	OpV1 [a]	V3		V4		V5	OpV2 [a]	V6
Visit Window (days)					+1		±1		±2	±3	±3
Written informed consent/assent	x										
Inclusion/exclusion criteria	x										
Demographics	x										
Medical history [b]	x										
Randomization	x										
Study drug administration [c]		x	x	x	x	x					
Patient diary:											
Body temperature measurement [d]	Four times daily				Twice daily				Once daily		
Assessment of influenza symptoms [e]	Twice daily								Once daily		
Physical examination	x[f]		x[f]	(x)[f]	x[f]		x		x	(x[g])	x[f]
Vital signs measurement	x		x	(x)	x		x		x	(x[g])	x[h]
Adverse event assessment		x	x	(x)	x		x		x	(x[g])	x
Concomitant therapies assessment	x		x	(x)	x		x		x	(x[g])	x
Interview for meal consumption	x	x									
Clinical laboratory tests		x					x			(x[g])	(x[g])
Nasal/nasopharyngeal swabs (virology test) [i]	x		x		x		x		x	(x[g])	(x[g])
Sparse PK samples [j]		x	(x)[j]		(x)[i]		x[i]				
Intensive PK samples [k]		x	x		x		x[k]				

CARIFS = Canadian Acute Respiratory Illness and Flu Scale; D = Day; ET = early termination; OpV = optional visit; PK = pharmacokinetic; V = visit.

[a] OpV1 and OpV2 are optional visits to be performed at the discretion of the investigator or parent/caregiver, for example, to obtain intensive PK analyses, or in the event of persistent influenza symptoms.

[b] Prior therapies will also be reviewed.

[c] Oseltamivir and matching placebo will be given orally, 12 hours apart. If the first dose is taken after 4 pm (16:00 hours) on Day 1, the next dose will be taken in the morning of Day 2. For these patients, the 10th dose will be taken on the morning of study Day 6. If the first dose is taken prior to 4 pm (16:00 hours) on Day 1, the next dose 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 10th dose will be taken in the evening of study Day 5.

[d] The parent/caregiver will assess and record in the patient diary, body temperature (tympanic assessment) four times daily (morning, noon, evening, and bedtime) from Days 1 to 3; twice daily (morning and evening) on Days 4 to 9; once daily on Days 10 to 15.

[e] The parent/caregiver will complete the CARIFS questionnaire twice daily (morning and evening) on Days 1 to 9 and then once daily on Days 10 to 15.

[f] Body weight and height will be measured on Days 1 and 29, only. Physical examinations on Days 2, 3, and 4 will be limited symptom-directed physical examinations and may be performed by a mobile nursing professional.

[g] Conduct assessment if the investigator determines that influenza symptoms or viral shedding are persisting or at the investigator's discretion.

[h] Vital signs will be recorded if abnormal physical examination findings or adverse events are recorded since last visit or at investigator's discretion on Day 29.

[i] At least two nasal and/or nasopharyngeal swabs (one for each nostril) to be taken at each visit.

Appendix 3
Schedule of Pharmacokinetic Samples

Visit	Timepoint	Sample Type
Sparse PK Sampling (all patients) [a]		
Visit 1 (Day 1)	One sample between 0.5 and 2 hours post-dose	Drug PK (plasma)
Visit 2 (Day 2) [b]	24 hours post-dose	Drug PK (plasma)
Visit 3 (Day 4) [b]	72 hours post-dose	Drug PK (plasma)
Visits 4–5 (Days 6–10)	Only one sample is to be collected during the follow-up period; this sample may be collected at follow-up Visit 4 or 5	Drug PK (plasma)
Intensive PK samples [c]		
Visit 1 (Day 1)	One sample between 0.5 and 2 hours, one sample at 4 hours, and one sample at 6 hours	Drug PK (plasma)
Visit 2 (Day 2) [b]	24 hours post-dose	Drug PK (plasma)
Visit 3 (Day 4) [b]	72 hours post-dose	Drug PK (plasma)
Visits 4–5 (Days 6–10)	Only one sample is to be collected during the follow-up period; this sample may be collected at follow-up Visit 4 or 5	Drug PK (plasma)

PK=pharmacokinetic.

[a] Samples will be used for S-033447 determination in patients aged ≥2 years; baloxavir marboxil and S-033447 determination in patients aged <2 years.

[b] Patients will be assigned to have either the Day 2 or the Day 4 sample.

[c] Patients undergoing intensive PK assessments are expected to account for 15%–30% of the total patient population and will be distributed approximately equally among age groups. Samples will be used for baloxavir marboxil and S-033447 determination.

Appendix 4
Canadian Acute Respiratory Illness and Flu Scale
(CARIFS) Questionnaire

Item	No Problem	Minor Problem	Moderate Problem	Major Problem	Don't Know/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 out of bed	☐	☐	☐	☐	☐

Since the last assessment has the subject been able to return to day care/school, or resume their normal daily activity in the same way as performed prior to developing the flu? ☐ Yes   ☐ No

This form was filled out by:

☐ Parent

☐ Carer

☐ Other