Official Title: A Multicenter, Single-Arm, Open-Label Study to Assess the Safety, Pharmacokinetics, and Efficacy of Baloxavir Marboxil in Otherwise Healthy Pediatric Patients From Birth To <1 Year With Influenza-Like Symptoms

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STATISTICAL ANALYSIS PLAN

STUDY TITLE:	**A MULTICENTER, SINGLE-ARM, OPEN-LABEL STUDY TO ASSESS THE SAFETY, PHARMACOKINETICS, AND EFFICACY OF BALOXAVIR MARBOXIL IN OTHERWISE HEALTHY PEDIATRIC PATIENTS FROM BIRTH TO <1 YEAR WITH INFLUENZA-LIKE SYMPTOMS**
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PLAN PREPARED BY:	[redacted], Ph.D. [redacted], MSc
SPONSOR: **LEGAL REGISTERED ADDRESS:**	F. Hoffmann-La Roche Ltd Grenzacherstrasse 124 4070 Basel, Switzerland
APPROVAL	See electronic signature and date stamp on the last page of this document

STATISTICAL ANALYSIS PLAN VERSION HISTORY

This SAP was developed based on Roche SAP model document, dated 28 February 2022.

SAP Version	Approval Date	Based on Protocol (Version, Approval Date)
3	see electronic date stamp on the last page	V 6.0, 27 April 2022
2	see electronic date stamp on the last page	V 6.0, 27 April 2022
1	17 June 2020	V4.0, 01 June 2020

STATISTICAL ANALYSIS PLAN AMENDMENT RATIONALE

Key changes from Version 2 to Version 3, along with the rationales for each change, are summarized below:

Section	Description of Change	Rationale for Change
Section 4.3.2	Clarified that the LLOQ will be used to impute the amount of virus RNA if less than the LLOQ. Added a table to specify the LLOQ for different assay versions.	Different assay versions have been introduced since the study start.
Section 4.4	Specify that if additional sequencing in PB1/PB2 is performed this may be reported in a CSR addendum.	Updated to clarify.
	Additional control virus data has been added for new lots.	New lots have been added since study start.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Term	Description
AE	adverse event
AESI	adverse events of special interest
AUC	area under the concentration–time curve
AUC_{inf}	area under the concentration–time curve from Time 0 to infinity
C_{24}	plasma concentration 24 hours postdose
C_{72}	plasma concentration 72 hours postdose
CARIFS	Canadian Acute Respiratory Illness and Flu Scale
C_{max}	maximum plasma concentration
COVID-19	coronavirus disease 2019
CRF	Case Report Form
eDISH	evaluation of Drug Induced Serious Hepatoxicity
EC_{50}	50% effective concentration
IMC	Internal Monitoring Committee
ICH	International Council for Harmonisation
ITT	intent-to-treat
ITTi	intent-to-treat influenza-infected
MedDRA	Medical Dictionary for Regulatory Activities
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NONMEM	non-linear mixed-effects model
PA	polymerase acidic protein
PK	pharmacokinetic
PCR	polymerase chain reaction
PKEP	pharmacokinetic-evaluable patient
PT	preferred term
RAT	rapid antigen test
RIDT	rapid influenza diagnostic test
RT-PCR	reverse transcriptase-polymerase chain reaction
SAE	serious adverse event
SAP	Statistical Analysis Plan
SOC	system organ class
$t_{1/2}$	half-life
t_{max}	time to maximum plasma concentration
TTAS	time to alleviation of influenza signs and symptoms

Abbreviation or Term	Description
ULN	upper limit of normal
vp	virus particles

1. INTRODUCTION

This Statistical Analysis Plan (SAP) provides details of the planned analyses and statistical methods for the clinical efficacy, clinical safety, and pharmacokinetic (PK) data for Study CP40559.

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

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

1.1 OBJECTIVES AND ENDPOINTS

Specific objectives and corresponding endpoints for Study CP40559 are outlined in Table 1.

Table 1 Objectives and Corresponding Endpoints

Safety (Primary) Objective	**Corresponding Endpoint**
• To evaluate the safety of a single dose of baloxavir marboxil	• Incidence, severity, and timing of adverse events, serious adverse events, vital sign measurements, and clinical laboratory tests
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 cohort • Population PK model derived PK parameters (e.g., AUC_{inf}, C_{max}, T_{max}, $t_{1/2}$ (modelling report)
Efficacy (Secondary) Objective	**Key Corresponding (Secondary) Endpoint**
• To evaluate the efficacy of baloxavir marboxil	• 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 remaining 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 ≤37.2°C)

Table 1 Objectives and Corresponding Endpoints (cont.)

Efficacy (Secondary) Objective	Corresponding Endpoints
• To evaluate the efficacy of baloxavir marboxil	• Duration of fever (time to return to afebrile state [tympanic temperature ≤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	• Time to cessation of viral shedding by virus titer and by reverse transcription-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)
Exploratory Efficacy Objective	**Corresponding Endpoints**
• To evaluate the polymorphic and treatment-emergent amino acid substitutions 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
Exploratory Palatability Objective	**Corresponding Endpoints**
• To evaluate the palatability of the oral suspension in the pediatric population	• Proportion of patients within each response category for Question 1 of the palatability assessment • Proportion of patients with issues with the administration of the full dose

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.

1.2 STUDY DESIGN

This is a multicenter, single-arm, open-label study to assess the safety, pharmacokinetics, and efficacy of baloxavir marboxil in otherwise healthy pediatric patients from birth to <1 year with influenza-like symptoms. This study is designed to enroll approximately 30 otherwise healthy male and female pediatric patients with influenza-like symptoms presenting within 96 hours of symptom onset.

Patients will be screened on Day 1 and assigned to receive a single oral dose of baloxavir marboxil according to body weight and age, as defined below.

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

- **Cohort I:** ≥3 months to <12 months old (minimum 8 patients): 2 mg/kg
- **Cohort II:** ≥4 weeks to <3 months old (minimum 4 patients): 1 mg/kg
- **Cohort III:** birth to <4 weeks old (minimum 3 patients): 1 mg/kg

The study consists of two periods: a 1-day treatment period and a 28-day safety follow-up period. Therefore, the total study duration for each patient will be 29 days.

The Study CP40559 protocol synopsis is provided in Appendix 1. For additional details, see the Schedule of Assessments in Appendix 2 and Appendix 3.

1.2.1 Independent Review Facility

No Independent Review Facility (IRF) is planned for this study.

1.2.2 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. If there is any concern noted in the safety parameters, the Sponsor may suspend or discontinue the entire study, as appropriate.

1.2.3 Analysis Timing

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

2. STATISTICAL HYPOTHESES AND SAMPLE SIZE DETERMINATION

2.1 STATISTICAL HYPOTHESES

The primary objective of this study is to evaluate the safety of baloxavir marboxil in pediatric patients from birth to <1 year with influenza-like symptoms. Therefore, and as it is a single group study, there will be no formal statistical hypothesis testing.

2.2 SAMPLE SIZE DETERMINATION

No formal sample-size calculations have been performed.

A total of approximately 30 pediatric patients will be enrolled in order to estimate the PK parameters and maturation factor which is incorporated in the population PK model with a probability of >90%.

The number of patients in the intent-to-treat influenza-infected (ITTi) set 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 set is considerably smaller than expected.

The number of patients in the ITTi set entering the study with ≤48 hours since symptom onset will also be monitored. Additional patients may be recruited if considerably fewer patients than expected enter the study with ≤48 hours since symptom onset.

3. ANALYSIS SETS

All Patients Set

The 'All Patients' set comprises all patients who were enrolled in the study.

Intent-to-Treat Set

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

Intent-to-Treat Influenza-Infected Patient Set

The ITTi set 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 set is the main efficacy set, unless specified otherwise.

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

Pharmacokinetic-Evaluable Set

The pharmacokinetic-evaluable patient (PKEP) set comprises all patients in the ITT set who have at least one post-dose drug concentration measurement at a scheduled visit time-point. Patients may be excluded from the PKEP set 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 patient exclusion from the PKEP set will be made prior to database lock by the clinical pharmacologist. Excluded patients will be documented, together with the reason for exclusion.

Safety Set

The safety set will be the same as the ITT set (i.e., patients who have received any portion of a single dose) regardless of whether or not they had any follow-up visits.

4. STATISTICAL ANALYSES

The number of patients who enroll, discontinue, or complete the study and the number of patients who discontinue, or complete study treatment will be summarized. Reasons for premature study withdrawal and study treatment discontinuation will be listed and summarized. Major protocol deviations will be listed and summarized and evaluated for their potential effects on the interpretation of study results. A summary of enrollment by country and investigator name will be provided. Exclusion of patients from the analysis sets will be summarized, including the reason for exclusion.

4.1 GENERAL CONSIDERATIONS

Disposition summaries will be based on the 'All Patients' set. Analysis of safety data will be based on the safety set and analysis of PK data will be based on the PKEP set. The analysis set for efficacy and virology will be the ITTi set. Analysis of palatability will be based on those patients in the ITT set who consented to Version 2 (or subsequent versions) of the Study CP40559 protocol.

In safety analyses, all deaths are included, from all sources, regardless of whether a complete death date is available; 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.

Visit Windows

Table 2 presents the acceptable time windows for parameters other than data from the patient diary and PK assessments.

Table 2 Acceptable Time Windows for Parameters other than Data from the Patient Diary and PK Assessments

Time Point (Scheduled Time point)	Acceptable Time Window
Pre-dose at Visit 1 (Day 1) [a]	Before dosing on Day 1
Post-dose at Visit 1 (Day 1) [a]	After dosing on Day 1
Visit 2 (Day 2)	Day 2
Optional Visit 1 (Day 3)	Day 3
Visit 3 (Day 4)	Between Day 4 and Day 5
Visit 4 (Day 6)	Between Day 6 and Day 7
Visit 5 (Day 10)	Between Day 8 and Day 12 as follows: If a patient presents between Day 8 and Day 11 and presents on Day 12 then the visit between Day 8 and Day 11 will be classed as Visit 5 and the Day 12 visit will be classed as Optional Visit 2 If a patient does not present between Day 8 and Day 11 and presents on Day 12 this will be classed as Visit 5
Optional Visit 2 (Day 15)	Between Day 12 and Day 22
Visit 6 (Day 29)	On or after Day 23

[a] If time is missing for data collected on Visit 1 where this data should be collected at 'v1 screening' or 'v1 post-dose' as per the schedule of activities, then 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 AEs, medical history and concomitant medications, all data up to the study completion/discontinuation date will be included.

4.2 PRIMARY ENDPOINT ANALYSES

4.2.1 Safety Endpoints

The primary objective of this study is to evaluate the safety of baloxavir marboxil in pediatric patients from birth to <1 year with influenza-like symptoms. The safety set will be used for all safety analyses. There will be no formal statistical hypothesis testing. The statistical analyses of safety will be descriptive.

The safety of baloxavir marboxil will be evaluated based on AEs, vital sign measurements (including parent/caregiver recorded temperature), and clinical laboratory tests. Safety parameters will be summarized or listed for the safety set.

4.2.1.1 Adverse Events

AEs will be classified by system organ class (SOC) and preferred term (PT) using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) at the time of database lock and AE severity will be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 5.0 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 electronic CRF. AEs reported after the initial dose of the study drug will be used for safety analyses.

In summaries by SOC and 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.

AEs 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. AEs will also be tabulated by severity and relationship to the study drug as indicated by the investigator.

A listing of all AEs will be produced, and the following will be summarized:

- All AEs
- Serious AEs
- AEs related to study medication
- AEs by severity
- AEs by outcome

- AEs by onset (Day 1–7, Day 8–14, and Day ≥15)
- AEs leading to withdrawal from study
- AEs leading to withdrawal from study drug
- AEs leading to death
- AEs leading to a dose modification or interruption
- Most frequent AEs

A listing of incorrect doses and overdoses will be provided if they occur during the study and a listing of any accidental overdose or medication error AEs will also be provided.

If there are sufficient number of patients that are ≤27 days old, then summaries of AEs split by age (≤27 days and >27 days) will be produced where required.

If the overall number of AEs is low then listings may be provided instead of the above summaries.

Adverse Events of Special Interest

All adverse events of special interest (AESI) will be presented. AESI include:

- Cases of potential drug-induced liver injury that include an elevated alanine aminotransferase (ALT) or aspartate aminotransferase (AST) in combination with either an elevated bilirubin or clinical jaundice, as defined in the protocol
- Suspected transmission of an infectious agent by the study drug

4.2.1.2 Deaths

Details of any deaths will be presented in an individual patient listing.

4.2.1.3 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 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 for each scheduled time point. Patients with values outside the reference will be listed, with an indication of the direction of the abnormality (High, Low). Additionally, a shift table will present the changes from baseline at each scheduled time point. Additional visual presentations may be produced based on the data accumulated during the study.

A summary of categorized liver lab data and a summary of Grade 1 to 4 of chemistry and hematology data over time (per NCI CTCAE v5.0) will be provided, if there is sufficient data available.

An evaluation of Drug Induced Serious Hepatoxicity (eDISH) plot will be produced with the patients in each International Council for Harmonisation (ICH) age category indicated by different symbols (Merz et al. 2014).

The number and proportion of patients who meet the pre-specified criteria, shown in Table 3, will be presented 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 >3 × ULN or ALT >3 × ULN Total bilirubin value >2 × ULN at same timepoint

ALT=alanine aminotransferase; AST=aspartate aminotransferase; ULN=upper limit of normal.

4.2.1.4 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 for each scheduled time point.

Baseline is defined as the last assessment prior to treatment.

4.2.1.5 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 for each recorded time point.

Baseline is defined as the first recorded assessment on Day 1 after treatment. Patients who do not have body temperature recorded on Day 1 after the start of treatment will have their baseline body temperature set to missing.

4.2.1.6 Exposure of Study Medication

Study drug exposure will be summarized descriptively.

4.3 SECONDARY ENDPOINT ANALYSES

4.3.1 Efficacy Analyses

The statistical analyses of efficacy endpoints will be descriptive. The ITTi set will be used for all efficacy analyses.

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.

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

Table 4 CARIFS Questionnaire Data Recording Time Periods

Period	Time [a]
Morning	05.00–10.59
Noon	11.00–14.59
Evening	15.00–18.59
Bedtime	19.00–23.59

[a] data should not be recorded between 00.00 and 04.59.

In the case where the device was activated after Day 1, the actual day will be used as the assessment day rather than the device-labelled day. In the case where assessments should be recorded twice daily (morning and evening) but are recorded for more than two time periods, only the morning and evening assessments will be presented in summaries over time. In the case where assessments should be recorded once daily but are recorded for more than one time period, a conservative approach will be taken to select the data points to be included in the summaries over time.

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 04.59 (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 possible scores for the 18 symptoms specified in the CARIFS questionnaire (see Appendix 4) are:

- 0 (“No Problem”)
- 1 (“Minor Problem”)
- 2 (“Moderate Problem”)
- 3 (“Major Problem”)
- 4 (“Don’t Know/Not Applicable”)

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

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

If the number of patients in the ITTi set is small, then listings may be provided.

Time to Alleviation of Influenza Signs and Symptoms

Time to alleviation of influenza signs and symptoms (TTAS) in hours, will be summarized using descriptive statistics, 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?"
- Return to afebrile state (tympanic temperature $\leq$37.2°C).

If a score of 4 ("Don't Know/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 $\leq$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?" simultaneously at baseline will be excluded from the analysis.

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 on Day 1 after the start of treatment. Patients who do not have a CARIFS assessment on Day 1 after the start of treatment will have their baseline CARIFS assessment set to missing.

The number of patients with completed CARIFS as well as the number with missing CARIFS per assessment will be presented for the ITT and ITTi sets. Additionally, the number and percentage of those with "Don't Know/ Not Applicable" per question per assessment will be presented for the ITT and ITTi sets.

In the main analysis of the TTAS, only complete assessments (i.e., assessments that have responses for items 14 and 15 of the CARIFS questionnaire, the normal daily activity question and temperature) that do not have scores of 4 for items 14 and 15 are considered in the calculation of the TTAS. A sensitivity analysis will be conducted that considers all assessments in the calculation of the TTAS, where a score of 4 for items 14 or 15 does not break the incidence of alleviation.

In addition, a sensitivity analysis will be conducted in which the following criterion will be removed from the calculation of the TTAS:

- 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?"

Duration of Fever

Duration of fever will be summarized using descriptive statistics, as well as Kaplan-Meier plots. It is defined as the time to return to afebrile state (tympanic temperature ≤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.

Patients who are afebrile at baseline (tympanic temperature ≤37.2°C) or whose body temperature is not collected will be excluded from the analysis.

Duration of Symptoms

Duration of symptoms will be summarized using descriptive statistics, 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 ("Don't know/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 duration 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.

All patients will be included in the analysis, regardless of their symptoms at baseline.

In addition, duration of individual symptoms will be summarized using descriptive statistics, as supportive analysis, with the exception of items 10, 11, and 12. If a score of 4 ("Don't know/Not Applicable") occurs for the individual symptom at any assessment during the study the assessment will not be included in the calculation of the duration of that individual symptom. Patients who have an individual symptom score ≤1 at baseline will have the duration of that symptom set to missing.

In the main analysis of the duration of symptoms, only complete assessments (i.e., assessments that have responses for all 18 symptoms in the CARIFS questionnaire) that do not contain a score of 4 for any items, with the exception of items 10, 11, and 12, are considered. A sensitivity analysis will be conducted that considers all assessments in the calculation of the duration of symptoms, where a score of 4 does not break the

incidence of alleviation for items other than 10, 11, and 12, and a score of 4 is considered alleviation for items 10, 11, and 12.

Time to Return to Normal Health and Activity

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

Time to return to normal health and activity as defined 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?" and remaining so for at least 21.5 hours.

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. 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 influenza-related complications will be presented.

4.3.1.1 Subgroup Analyses

Subgroup analyses of the TTAS, based on virus type and subtype, may be conducted if there are a sufficient number of patients in each subgroup.

4.3.2 Virology (Secondary) Endpoints

The ITTi set will be used for all virology analyses.

For all virology analyses, baseline is defined as the last pre-dose assessment and where no pre-dose assessment is available baseline will be defined as the first post-dose assessment on Day 1. For nasal swab influenza hemagglutinin subtype, baseline is defined as the last pre-dose assessment and where no pre-dose assessment is available, baseline will be defined as the first post-dose assessment on Days 1–3.

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 (0.75 $\log_{10}$ $TCID_{50}$/mL). 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, 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 study treatment and first time when the virus RNA by RT-PCR qualitative result is negative (no cycle threshold [Ct]-value detectable). Patients who do not have a negative result 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 qualitative result is negative for all virus types. One day is converted into 24 hours.

Patients with a positive qualitative result on Day 1 will be included in this analysis.

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

Change from Baseline in Influenza Virus Titer at Each Timepoint

Change from baseline in influenza virus titer ($log_{10}TCID_{50}$/mL) will be presented 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 Timepoint

Change from baseline in the amount of virus RNA (unit: log_{10} vp/mL) will be presented 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 the relevant LLOQ (log_{10} vp/mL). If a patient is infected with multiple virus types, the sum of virus RNA (log_{10} vp/mL) will be used for analysis.

Table 5 LLOQ for Different Assay Versions

Influenza Type	Assay Version	LLOQ (log10 vp/ml)
Influenza A	Version 1 50µl, 2014	2.18
	Version 1 20µl, 2019	2.48
	Version 2 20µl, 2023	2.79
Influenza B	Version 1 50µl, 2014	2.93
	Version 1 20µl, 2019	2.63

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 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 Timepoint

Proportion of patients positive by RT-PCR will be presented at each visit and is defined as the percentage of patients with a positive qualitative result among those assessed by RT-PCR on Days 2, 4, 6, and 10.

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

Area under the Curve in Virus Titer

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

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

where t_k (hours) represents the date of the kth viral titer assessment ($k = 0, \ldots, K$) and y_k represents the $\log_{10}$ value of the kth viral titer assessment (TCID$_{50}$/mL).
Twenty-four hours of time will be converted into one day. Patients with a positive virus titer on Day 1 will be included in this analysis. The lower limit of quantification and lower limit of detection is defined as 0.75 $\log_{10}$TCID$_{50}$/mL for flu A and 0.75 $\log_{10}$TCID$_{50}$/mL for flu B. If a patient is infected with multiple virus types, the sum of those virus titers will be used for analysis.

Additionally, the AUC in virus titer adjusted for baseline will be presented and will be calculated using the trapezoidal method. AUC of change from baseline 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 kth viral titer assessment ($k = 0, \ldots, K$) and y_k represents the $\log_{10}$ value of the kth viral titer assessment ($TCID_{50}$/mL).

Twenty-four hours 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. If a patient is infected with multiple virus types, the sum of virus titers ($TCID_{50}$/mL) will be used for analysis.

Area under the Curve in the Amount of Virus RNA (RT-PCR)

Area under the concentration–time curve (AUC) in virus RNA (RT-PCR) will be presented and is defined as AUC of change from baseline in the amount of virus RNA (RT-PCR). 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 ($\log_{10}$ vp/mL). If a patient is infected with multiple virus types, the sum of those the amount of virus RNA will be used for analysis.

4.3.2.1 Subgroup Analyses

Subgroup analyses of the following endpoints, based on virus type and subtype, may be conducted if the numbers of patients in the relevant subgroups of the ITTi set are sufficient:

- Time to cessation of viral shedding by virus titer
- Time to cessation of viral shedding by RT-PCR

4.4 EXPLORATORY ANALYSES

Exploratory efficacy analyses will be based on the ITTi set. Exploratory palatability analyses will be based patients in the ITT set who consented to Version 2 (or subsequent versions) of the Study CP40559 protocol.

Polymorphic and Treatment-Emergent Amino Acid Substitutions in the *PA*, *PB1*, and *PB2* Genes

Sanger sequencing of the influenza *PA* gene will be performed to evaluate the incidence of polymorphic (baseline vs. reference) and treatment-emergent (post-baseline vs. baseline) amino acid substitutions in treated subjects with evaluable virus. Sequencing will be performed for samples taken at baseline (Screening) and at the last evaluable timepoint (RNA titer >4 log10 vp/mL). The frequency of polymorphic substitutions will be reported in percent of total baseline samples, and the frequency of treatment-emergent

substitutions will be reported in percent of total paired samples (baseline and post-baseline sequence pair). If a RAS (resistance associated substitution) is detected, additional intermediate timepoint samples may be sequenced. Sequencing of *PB1* and *PB2* will be done for the points below according to the criteria per Table 6:

- 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). A listing of EC_{50} fold-change will be provided.
- viruses (baseline and last evaluable timepoint) without amino acid substitution in *PA*, but with reduced response to treatment or with virus rebound.

Patients meeting a criteria per Table 6 will be listed and the number and proportion of patients meeting the criteria over time will be presented. PA data will be listed for all patients with an amino acid substitution. If additional sequencing in PB1/PB2 is performed, a listing is produced for any patient with a substitution in PB1/PB2, which may be reported in a CSR addendum.

Table 6 *PB1* and *PB2* Gene Sequencing

	Criteria
1) Subjects who exhibit viral titer rebound:	Viral titer at [(a certain time point) – (just before time point)] ≥2 × SD value of viral titer of control virus in assay [a]. [a] Viroclinics database; Influenza A - control virus data generated from 09 May 2017 until 30 November 2022 – Lot#: VC-170180018: • A/PR/8/1934: 3.82 + 0.36 log10TCID50/mL (mean +/- SD) (n = 521); 2 x SD = 0.72 control virus data generated from 26 July 2022 until 04 May 2023 - Lot#: VC-220180034: • A/PR/8/1934: 2.19 + 0.49 log10TCID50/mL (mean +/- SD) (n = 90); 2 x SD = 0.98 Mean SD of both lots to be used, therefore 2 x SD = 2 x ((0.36 + 0.49) / 2) = 0.85 Influenza B - control virus data generated from 09 May 2017 until 04 May 2023: • B/Lee/1940: 5.33 + 0.45 log10TCID50/mL (mean +/- SD) (n = 516); 2 x SD = 0.90

2) Subjects who continue to shed virus at Day 6 and beyond:	Viral titer (log_{10}TCID50/mL) at Day 6 and beyond >1.5
3) Subjects who do not show a reduction in virus titer:	Viral titer (log_{10}TCID50/mL) at a certain time point: A >1.5 Viral titer (log_{10}TCID50/mL) at just before time point: B >1.5 AND Viral titer (log_{10}TCID50/mL) at ([a certain time point: A]–[just before time point: B]) ≥0

SD=standard deviation; $TCID_{50}$=50% tissue culture infectious dose.

Drug Susceptibility in Patients with Evaluable Virus

As assessments of the drug susceptibility of the influenza virus, the 50% effective concentration (EC_{50}) of baloxavir will be measured by the ViroSpot™ assay using baseline swab samples. Values will be compared with values of reference strains and respective ratio (EC_{50} / EC_{50} reference) will be reported. The following influenza virus vaccine strains from 2018–2019 season will be used as reference for the ViroSpot™ assay: A/Michigan/45/2015 (H1N1)pdm09 for A/H1N1 samples, A/Singapore/INFIMH-16-0019/2016 for A/H3N2 samples from Northern hemisphere, A/Switzerland/8060/2017 for A/H3N2 samples from Southern hemisphere, and B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage) and B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage) for influenza type B samples. Mean EC_{50} value of the two B reference strain EC_{50} values will be used to calculate EC_{50} ratio.

Respiratory Coinfections (BioFire)

Baseline samples taken as part of Study CP40559 Protocol Version 2 (or subsequent versions) will be tested for respiratory pathogens using the qualitative PCR-based FDA cleared BioFire FilmArray® Respiratory Panel 2 assay. This assay detects 17 respiratory viral or bacterial pathogens in addition to influenza A and B. Frequency and proportions for each co-infection and the proportion of patients with at least one coinfection will be presented.

Proportion of Patients within each Response Category for Question 1 of the Palatability Assessment

The palatability endpoint for patients who receive the study treatment will be summarized descriptively.

The number and proportion of patients within each response category for Question 1 of the palatability assessment will be presented.

Proportion of Patients with Issues with the Administration of the Full Dose

The number and proportion of patients with issues with the administration of the full dose will be presented.

4.5 OTHER ANALYSIS

4.5.1 Summaries of Demographics and Baseline Characteristics

Demographic and baseline characteristics (including age, sex, race/ethnicity, weight, influenza subtype, vaccination status, and age category [per ICH]) will be summarized using means, standard deviations, medians, and ranges for continuous variables and proportions for categorical variables, as appropriate. Summaries will be presented for the safety set and for the ITTi set.

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

A listing of patients in the ITTi set who are negative by RT-PCR at baseline will be provided.

4.5.2 Pharmacokinetic Analyses

The PKEP set will be used for all PK analyses. The statistical analyses of PK will be descriptive.

For patients with blood samples collected, individual plasma baloxavir marboxil, and baloxavir concentrations will be tabulated by patient and by timepoint. Individual and mean (if appropriate) plasma baloxavir marboxil and baloxavir concentrations versus time data will be plotted by age group.

As this study uses an optimized sparse PK regimen, individual PK parameters of baloxavir marboxil and baloxavir (AUC, maximum plasma concentration [C_{max}], time to maximum concentration [t_{max}], and terminal half-life [$t_{1/2}$]) will not be determined using model-independent methods. However, individual drug concentrations determined plasma concentration 24 and 72 hours postdose (C_{24} and C_{72}, respectively) of baloxavir will be listed and summarized (mean, standard deviation, coefficient of variation, median, minimum, and maximum) by age group.

In addition, a non-linear mixed-effects model (NONMEM, Version 7.3 or higher) will be used to analyze the PK data in combination with PK data from other studies to estimate the typical population PK parameters (e.g., apparent clearance [CL/F], apparent volume of distribution for the central compartment [V_c/F]) in the pediatric population, to assess the inter-subject variability and to evaluate the impact of predefined covariates such as race and body-weight on baloxavir pharmacokinetics. 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 calculated based on individual posthoc Bayesian estimates for each patient and the impact of identified covariates will be assessed graphically and numerically. PK parameters will be tabulated and summarized (i.e., by mean, standard deviation, coefficient of variation, median, and minimum and maximum) by age group.

The population PK analysis will be described in a specific analysis plan and the population PK analysis will be reported in a dedicated modelling and simulation report.

Additional PK analyses will be conducted as appropriate.

Pediatric exposure matching to adults will use different PK metrics, such as C_{24} (observed as well as Bayesian post hoc) C_{72} and AUC_{inf} (derived as Bayesian post hoc), to 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. Results may be described within the population PK report or in a dedicated extrapolation report.

4.6 INTERIM ANALYSES

An interim PK analysis is planned for this study. PK samples, up to and including those collected at Visit 4/5 of first 6 patients dosed, will be batched for an early shipment.

This will enable a timely review of the systemic plasma concentrations, in particular the C_{24} and C_{72}. This is in order to verify, in a timely manner, that exposures at the proposed doses 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 will be performed by members of the Sponsor study team.

No other interim analyses are planned for this study. However, given there is no formal statistical hypothesis testing in this study, the Sponsor may choose to conduct optional interim analyses as appropriate. 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 will be performed and interpreted by members of the Sponsor study team and appropriate senior management personnel.

5. SUPPORTING DOCUMENTATION

The Study CP40559 protocol synopsis is provided in Appendix 1. For additional details, see the Schedule of Assessments in Appendix 2 and Appendix 3.

Appendix 1 Protocol Synopsis

TITLE:	**A MULTICENTER, SINGLE-ARM, OPEN-LABEL STUDY TO ASSESS THE SAFETY, PHARMACOKINETICS, AND EFFICACY OF BALOXAVIR MARBOXIL IN OTHERWISE HEALTHY PEDIATRIC PATIENTS FROM BIRTH TO <1 YEAR WITH INFLUENZA-LIKE SYMPTOMS**
PROTOCOL NUMBER:	CP40559
VERSION NUMBER:	6
IND NUMBER:	126653
EUDRACT NUMBER:	2018-002154-70
TEST PRODUCT:	Baloxavir marboxil (RO7191686)
PHASE:	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 in otherwise healthy patients from birth to <1 year of age with influenza-like symptoms. Specific objectives and corresponding endpoints for the study are outlined below.

Safety (Primary) Objective	Corresponding Endpoint
• To evaluate the safety of a single dose of baloxavir marboxil	• Incidence, severity, and timing of adverse events, serious adverse events, vital sign measurements, and clinical laboratory tests
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 cohort • Population PK model derived PK parameters (e.g., AUC_{inf}, C_{max}, T_{max}, $t_{1/2}$ (modelling report)
Efficacy (Secondary) Objectives	**Key Corresponding (Secondary) Endpoint**
• To evaluate the efficacy of baloxavir marboxil	• 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 remaining 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 ≤37.2°C)

Efficacy (Secondary) Objectives (cont.)	Corresponding Endpoint (cont.)
• To evaluate the efficacy of baloxavir marboxil	• Duration of fever (time to return to afebrile state [tympanic temperature ≤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	• 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 Efficacy Objective	**Corresponding Endpoints**
• To evaluate the polymorphic and treatment-emergent amino acid substitutions 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
Exploratory Palatability Objective	**Corresponding Endpoints**
• To evaluate the palatability of the oral suspension in the pediatric population	• Proportion of patients within each response category for Question 1 of the palatability assessment • Proportion of patients with issues with the administration of the full dose

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, single-arm, open-label study to assess the safety, pharmacokinetics, and efficacy of baloxavir marboxil in otherwise healthy pediatric patients from birth to <1 year with influenza-like symptoms.

Potential patients will be prescreened using an influenza test (rapid influenza diagnostic test [RIDT] or polymerase chain reaction [PCR]) and a coronavirus disease 2019 (COVID-19) test (approved rapid antigen test [RAT] or PCR). This is to ensure that patients with a high probability of having influenza infection are enrolled and to minimize the likelihood of enrolling a COVID-19 patient in the study. Patients will be screened on Day 1 and assigned to one of three cohorts, based upon their age, to receive a single oral dose of baloxavir marboxil based on body weight and age. Re-screening of patients who fail to meet the inclusion and exclusion criteria will not be permitted, as the time from symptom onset to treatment window is limited to 96 hours.

The study consists of two periods: a 1-day treatment period (screening and study drug administration on Day 1) and a 28-day safety follow-up period (mandatory visits on Days 2, 4, 6, 10, and 29). Therefore, the total study duration for each patient will be 29 days.

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

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; twice daily (morning and evening) on Days 4 to 9; and once daily on Days 10 to 15. Influenza symptoms will be recorded twice daily (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 the entire study, as appropriate.

NUMBER OF PATIENTS

This study is designed to enroll approximately 30 otherwise healthy male and female pediatric patients with influenza-like symptoms. Patients will be recruited in parallel to the following three cohorts:

- **Cohort I**: ≥3 months to <12 months old (minimum 8 patients)
- **Cohort II**: ≥4 weeks to <3 months old (minimum 4 patients)
- **Cohort III**: birth to <4 weeks old (minimum 3 patients)

TARGET POPULATION

Inclusion Criteria

Patients must meet the following criteria for study entry:

- Written informed consent for study participation obtained from patient's parents or legal guardian
- Age from birth to <1 year at screening
- Parent/guardian willing and able to comply with study requirements, in the investigator's judgment
- Patients with a diagnosis of influenza virus infection confirmed by the presence of all of the following:
  - In the investigator's judgement there is a clinical suspicion of influenza
  - At least one respiratory symptom (either cough or coryza)

- Positive prescreening influenza test (RIDT or PCR) performed within 24 hours of screening

    Results from local testing as part of standard of care are acceptable if samples are collected within 48 hours of screening and recorded in the patients' medical records.

- Patients with a negative prescreening COVID-19 test (RAT or PCR) performed within 48 hours of screening

    Results from local testing as part of standard of care are acceptable if samples are collected within 48 hours of screening and recorded in the patients' medical records.

- The time interval between the onset of symptoms and screening is ≤96 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 the parent or caregiver)

Exclusion Criteria

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

- Hospitalized for complications of influenza or significant comorbidities

    Patients hospitalized for observation, monitoring or precautionary supportive care may still be acceptable for enrollment provided all eligibility criteria are met.

- Concurrent infections requiring systemic antiviral therapy at screening
- Require, in the opinion of the investigator, any of the prohibited medication during the study
- Preterm neonates (born at <37 weeks gestation)
- Weigh <2.5 kg at screening

    Only children >4.5 kg will be eligible to take part in countries that have volume limits for blood draws set at 1% of the total blood volume (maximum over 24-hour period) and 3% of the total blood volume (maximum over 30-day period).

- Previous treatment with peramivir, laninamivir, oseltamivir, zanamivir, or amantadine within 2 weeks prior to screening
- Immunization with a live/attenuated influenza vaccine during the 2 weeks prior to screening
- 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 transplant
- Known hypersensitivity to study drug (i.e., baloxavir marboxil) or the drug product excipients
- Known hypersensitivity to acetaminophen
- Participation in a clinical trial with an investigational drug within 4 weeks or five 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.

NON-INVESTIGATIONAL MEDICINAL PRODUCTS

Not applicable.

STATISTICAL METHODS

PRIMARY ANALYSIS

The safety evaluable 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 of 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 30 pediatric patients will be enrolled in order to estimate the pharmacokinetic (PK) parameters and maturation factor which is incorporated in the population PK model with a probability of >90%.

INTERIM ANALYSES

An interim PK analysis is planned for this study. The PK samples, up to and including those collected at Visit 4/5 of first 6 patients dosed, will be batched for an early shipment. This will enable a timely review of the systemic plasma concentrations, in particular the concentrations at 24 hours and 72 hours after dosing. This is in order to verify, in a timely manner, that exposures at the proposed doses 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 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 optional interim analyses as appropriate. 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 will be performed and interpreted by members of the Sponsor study team and appropriate senior management personnel.

Appendix 2 Schedule of Assessments

	Prescreening [a]	Treatment Period		Follow-Up Period										
Day(s)	D1	D1		D2	D3	D4	D5	D6	D7	D8	D9	D10	D15	D29 or ET
Visits	NA	V1 Screening	V1 Post-dose	V2	OpV1 [b]	V3		V4				V5	OpV2 [b]	V6
Visit Window (days)	-1					+1		+1				±2	±3	±3
Prescreening influenza test	x [c]													
Prescreening COVID-19 test	x [d]													
Informed consents	x	x												
Inclusion/exclusion criteria		x												
Demographics		x												
Medical history [e]		x												
Study drug administration			x											
Patient diary:														
Body temperature measurement [f]			Four times daily			Twice daily						Once daily		
Assessment of influenza symptoms [g]			Twice daily									Once daily		
Physical examination		x [h]		x [h]	(x) [h]	x [h]		x [h]				x [h]	(x [i])	x [h]
Vital signs measurement		x		x	(x)	x		x				x	(x [i])	(x [j])
Adverse events assessment			x	x	(x)	x		x				x	(x [i])	x
Concomitant therapies assessment		x		x	(x)	x		x				x	(x [i])	x

Appendix 2 Schedule of Activities (cont.)

	Prescreening [a]	Treatment Period		Follow-Up Period										
Day(s)		D1		D2	D3	D4	D5	D6	D7	D8	D9	D10	D15	D29 or ET
Visits	NA	V1 Screening	V1 Post-dose	V2	OpV1 [b]	V3		V4				V5	OpV2 [b]	V6
Visit Window (days)	NA					+1		+1				±2	±3	±3
Interview for meal consumption		x	x											
Clinical laboratory tests			x					x					(x [i])	
Nasopharyngeal swabs [k]		x		x		x		x				x	(x [i])	(x [i])
Pharmacokinetic samples [l]			x	(x) [l]		(x) [l]		x [l]						
Palatability assessment [m]			x											

CARIFS=Canadian Acute Respiratory Illness and Flu Scale; COVID-19 = coronavirus disease 2019; D=Day; ET=early termination; NA=not applicable; OpV=optional visit; PK=pharmacokinetic; V=visit.

[a] Pre-screening assessment is not required if the patient has a negative COVID-19 test result and positive influenza test performed as SOC within 48 hours of screening recorded in their medical records.

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

[c] Test is not required if the patient has a positive influenza test result performed as SOC within 48 hours of screening recorded in their medical records.

[d] Test is not required if the patient has negative COVID-19 test results performed as SOC within 48 hours of screening recorded in their medical records.

[e] Prior therapies will also be reviewed.

[f] The parent/caregiver will assess and record in the patient diary, body temperature (tympanic assessment) four times daily (morning, noon, evening, and bedtime) on Days 1 to 3; twice daily (morning and evening) on Days 4 to 9; once daily on Days 10 to 15. The first temperature recorded by the parent/caregiver will be measured post-dose.

[g] The parent/caregiver will complete the CARIFS questionnaire twice (morning and evening) on Days 1 to 9 and then once daily on Days 10 to 15. The first CARIFS assessment will occur post-dose.

[h] Body weight and height will be measured on Days 1 and 29 only. Physical examinations on Days 2, 3, 4, 6, and 10 will be limited symptom – directed physical examinations and may be performed by a mobile nursing professional.

Appendix 2 Schedule of Activities (cont.)

[i] Perform if the investigator determines that influenza symptoms are persisting or at the investigator's discretion.

[j] Record vital sign measurements if abnormal physical examination, adverse events recorded since last visit, or at investigator's discretion on Day 29.

[k] At least two nasopharyngeal swabs (one for each nostril) will be taken at each visit.

[l] PK samples (all patients): See Appendix 3 for collection timepoints for baloxavir marboxil and S-033447 concentration samples. Patients will be assigned to have either the Day 2 or the Day 4 sample. Only one sample from Days 6 to 10 is required and can be collected at either Visit 4 or Visit 5.

[m] The palatability assessment should be completed by the study investigator (or delegate) who administers baloxavir marboxil to the patient.

Appendix 3 Schedule of Pharmacokinetic Samples

Visit	Timepoint	Sample Type
Sparse PK Sampling [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)

PK = pharmacokinetic.

[a] Samples will be used for baloxavir marboxil and S-033447 determination.

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

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

Note: The term "Carer" in this questionnaire corresponds to the term "caregiver" used throughout the protocol.

Appendix 5 Palatability Assessment

Palatability Assessment

1. On the basis of the reaction / facial expression of your patient, do you think that the medication has a:
   a) very pleasant taste
   b) slightly pleasant taste
   c) neutral taste or no reaction of patients to taste
   d) slightly unpleasant taste
   e) very unpleasant taste
2. Did you have any problems administering the full dose to the patient?
   a) yes
   b) no
3. If you answered “yes” for Question 2, what is the main reason for this problem?

4. Other comments or problems encountered administering the drug:

6. REFERENCES

Merz M, Lee KR, Kullak-Ublick G.A. et al. Methodology to assess clinical liver safety data. Drug Saf 2014;37, 33–45.

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