PROTOCOL

TITLE:	**A PHASE II RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE ANTIVIRAL ACTIVITY, SAFETY, PHARMACOKINETICS, AND EFFICACY OF RO7496998 (AT-527) IN NON-HOSPITALIZED ADULT PATIENTS WITH MILD OR MODERATE COVID-19**
PROTOCOL NUMBER:	WV43042
VERSION NUMBER:	6
EUDRACT NUMBER:	2020-005366-34
IND NUMBER:	To be determined
NCT NUMBER:	NCT04709835
TEST PRODUCT:	RO7496998 (AT-527)
MEDICAL MONITOR:	[redacted], M.D.
SPONSORS:	F. Hoffmann-La Roche Ltd (Ex-United States) Atea Pharmaceuticals, Inc. (United States)
APPROVAL DATE:	See electronic date stamp below.

PROTOCOL AMENDMENT APPROVAL

Date	Title	Approver's Name	Signature
14 September 2021	[redacted], Atea Pharmaceuticals, Inc.	[redacted], M.D.	[redacted]

Date and Time (UTC)	Title	Approver's Name
17-Sep-2021 08:24:49	Company Signatory	[redacted]

PROTOCOL HISTORY

Protocol		Associated Country-Specific Protocol		
Version	**Date Final**	**Country**	**Version**	**Date Final**
6	See electronic date stamp on the title page	—	—	—
5	13 May 2021	—	—	—
4	24 February 2021	Ireland	5	21 April 2021
2	10 December 2020	Ireland	3	5 February 2021
1	23 November 2020	—	—	—

PROTOCOL AMENDMENT, VERSION 6: RATIONALE

Protocol WV43042 has been amended primarily to enable further evaluation of the antiviral efficacy and safety of RO7496998 (AT-527) using the dose regimen selected from previous cohorts. Changes to the protocol, along with a rationale for each change, are summarized below:

- In order to perform this evaluation Cohort E has been modified to increase enrollment and the Day 14 telephone visit has been changed to an in-person visit to allow for a nasopharyngeal swab to be collected. Relevant changes have been incorporated throughout the protocol including an updated statistical analysis and a cohort specific schedule of activities (Sections 3.1, 3.2, 4.1, 4.2.1, 4.3.1.1, 6 , Figure 1, and Appendix 3)

Additionally, the following changes have been incorporated

- Additional background has been provided regarding the need for COVID-19 treatment options despite new therapies and availability of vaccines (Section 1.1, 3.3.2, 3.3.3).
- New data from the ongoing Phase 1 and Phase 2 studies have been added and the rationales for dose regimen and patient population have been updated accordingly (Sections 1.2, 1.3, 3.3.1.1 , 3.3.1.2, 3.3.2, 5.1.1, and 6)
- A new secondary efficacy endpoint has been added to evaluate the frequency of all cause hospitalizations and death to capture these important outcomes of COVID-19 (Sections 2 and 6.4.2).
- Based on the data from the most recent drug–drug interaction studies the requirement to supplement hormonal contraceptive methods with a barrier method has been removed (Section 4.1.1).
- It has been clarified that the pregnancy test at screening may be performed from urine or serum sample (Sections 4.1.2, 4.5.5, Appendices 1–3)
- It has been clarified that the Cockcroft-Gault formula will be used to calculate creatine clearance as this is standard practice (Sections 4.1.2, 4.5.5, Appendices 1–3)
- The reference to the introduction of the 275 mg tablets after an approved IMPD has been deleted from Section 4.3.1.1 as an IMPD for this formulation has now been approved and the 275 mg has been introduced.
- It has been clarified that paracetamol and non-steroidal anti-inflammatory drugs taken for other reasons other than for COVID-19 symptoms are permitted (Section 4.4.1)
- The cautionary and prohibited therapy sections have been updated with data from recently completed drug–drug interaction studies (Sections 4.4.2, 4.4.3, and Appendix 6).

Additional minor changes have been made to improve clarity and consistency. Substantive new information appears in italics [note: italics not used in Figure 1 or Appendix 3 for readability]. This amendment represents cumulative changes to the original protocol.

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PROTOCOL AMENDMENT ACCEPTANCE FORM

TITLE:	**A PHASE II RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE ANTIVIRAL ACTIVITY, SAFETY, PHARMACOKINETICS, AND EFFICACY OF RO7496998 (AT-527) IN NON-HOSPITALIZED ADULT PATIENTS WITH MILD OR MODERATE COVID-19**
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I agree to conduct the study in accordance with the current protocol.

_____

Principal Investigator's Name (print)

_____ _____

Principal Investigator's Signature Date

Please retain the signed original of this form for your study files. Please return a copy of the signed form as instructed by your study monitor.

PROTOCOL SYNOPSIS

TITLE:	**A PHASE II RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE ANTIVIRAL ACTIVITY, SAFETY, PHARMACOKINETICS, AND EFFICACY OF RO7496998 (AT-527) IN NON-HOSPITALIZED ADULT PATIENTS WITH MILD OR MODERATE COVID-19**
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EUDRACT NUMBER:	2020-005366-34
IND NUMBER:	To be determined
NCT NUMBER:	NCT04709835
TEST PRODUCT:	RO7496998 (AT-527)
PHASE:	Phase II
INDICATION:	COVID-19
SPONSOR:	F. Hoffmann-La Roche Ltd (Ex-United States) Atea Pharmaceuticals, Inc. (United States)

Objectives and Endpoints

This study will evaluate the antiviral activity, safety, pharmacokinetics, and efficacy of RO7496998 (AT-527) compared with placebo in non-hospitalized adult patients with mild or moderate coronavirus disease 2019 (COVID-19). Specific objectives and corresponding endpoints for the study are outlined are outlined below.

Primary Objective	Corresponding Endpoint
• To evaluate the antiviral activity of AT-527 compared with placebo	• Change from baseline in amount of SARS-CoV-2 virus RNA as measured by RT-PCR at specified timepoints
Secondary Objectives	**Corresponding Endpoints**
• To evaluate the antiviral activity of AT-527 compared with placebo	• Time to cessation of SARS-CoV-2 viral shedding as measured by RT-PCR • Time to sustained non-detectable SARS-CoV-2 virus RNA • Proportion of patients positive for SARS-CoV-2 virus RNA by RT-PCR at specified timepoints • Area under the curve in the amount of SARS-CoV-2 virus RNA as measured by RT-PCR

RT-PCR = reverse- transcription polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2.

Secondary Objectives (cont.)	Corresponding Endpoints
• To evaluate the safety of AT-527 compared with placebo	• Incidence and severity of adverse events, with severity determined according to NCI CTCAE v5.0 • Change from baseline in targeted vital signs, including SpO_2 • Change from baseline in targeted clinical laboratory test results
• To characterize the PK profile of AT-511 (free base form of AT-527) and its major metabolites	• Plasma concentration of AT-511, AT-551, AT-229, and AT-273 (surrogate for the intracellular concentration of the active triphosphate metabolite AT-9010) at specified timepoints
• To evaluate the efficacy of AT-527 compared with placebo	• The time to alleviation or improvement of COVID-19 symptoms (Items 1–12 of the COVID-19 Symptom Diary) maintained for a duration of 21.5 hours, defined as follows: – For new symptoms: time from start of treatment to the alleviation of COVID-19 symptoms (i.e., a score of 0 [none] or 1 [mild] on the COVID-19 Symptom Diary) – For preexisting symptoms: time from start of treatment to when a patient's symptoms have been maintained or improved (Note: Improved requires at least a single category improvement from baseline on the Likert scale.) • The time to alleviation or improvement of COVID-19 symptoms (Items 1–12 of the COVID-19 symptom diary) maintained for a duration of 43 hours, defined as follows: – For new symptoms: time from start of treatment to the alleviation of COVID-19 symptoms (i.e., a score of 0 [none] or 1 [mild] on the COVID-19 Symptom Diary) – For preexisting symptoms: time from start of treatment to when a patient's symptoms have been maintained or improved (Note: Improved requires at least a single category improvement from baseline on the Likert scale.) • Time to alleviation of COVID-19 symptoms, defined as the length of time taken from start of treatment to the point at which the following criterion is met and maintained for at least 21.5 hours: Score of 0 or 1 on Items 1–12 of the COVID-19 Symptom Diary (without consideration for presence of pre-existing symptoms)

COVID-19 = coronavirus disease 2019; NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events; PK = pharmacokinetic;
SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; SpO_2 = peripheral capillary oxygen saturation.

Secondary Objectives (cont.)	Corresponding Endpoints
• To evaluate the efficacy of AT-527 compared with placebo (cont.)	• Time to alleviation of COVID-19 symptoms, defined as the length of time taken from start of treatment to the point at which the following criterion is met and maintained for at least 43 hours: Score of 0 or 1 on Items 1–12 of the COVID-19 Symptom Diary (without consideration for presence of pre-existing symptoms) • Duration of fever, defined as the time to return to an afebrile state (temperature ≤ 37.5°C) maintained for at least 21.5 hours • Frequency of COVID-19–related complications (death, hospitalization, radiologically confirmed pneumonia, acute respiratory failure, sepsis, coagulopathy, pericarditis, myocarditis, cardiac failure) • *Frequency of hospitalizations and death (all cause)* • Time to alleviation of an individual symptom, defined as the time taken from the start of treatment to the point at which the following criterion is met and maintained (for each individual symptom) for at least 21.5 hours: – Score of 0 or 1 for Items 1–12 of the COVID-19 Symptom Diary – Score of 0 for Items 13 and 14 of the COVID-19 Symptom Diary
Exploratory Objectives	**Corresponding Endpoints**
• To evaluate the antiviral activity of AT-527 compared with placebo	• Treatment-emergent amino acid substitutions in SARS-CoV-2 viral genes (*nsp12* and potentially other genes) • Anti–SARS-CoV-2 antibody status/titer at specified timepoints • Change from baseline in SARS-CoV-2 virus titer at specified timepoints • Time to cessation of SARS-CoV-2 viral shedding as measured by virus titer • Proportion of patients with positive SARS-CoV-2 virus titer at specified timepoints • Area under the curve of SARS-CoV-2 virus titer • Drug susceptibility in patients with evaluable virus at specified timepoints
• To evaluate the relationship between drug exposure and antiviral activity of AT-527	• Relationship between plasma concentration of AT-273 and anti-viral activity.
• To evaluate patient-reported COVID-19 symptom severity	• Patient's global impression of severity of COVID-19 symptoms as assessed through the use of PGIS at Day 14 and Day 28

COVID-19 = coronavirus disease 2019; PGIS = Patient Global Impression of Severity;
PK = pharmacokinetic;
SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2.

Exploratory Objectives (cont)	Corresponding Endpoints
• To identify and/or evaluate biomarkers that are predictive of response to AT-527 (i.e., predictive biomarkers), are early surrogates of efficacy, are associated with progression to a more severe disease state (i.e., prognostic biomarkers), can provide evidence of AT-527 activity (i.e., pharmacodynamic biomarkers), or can increase the knowledge and understanding of disease biology and drug safety	• Relationship between biomarkers in blood, saliva, nasopharyngeal swab, and nasosorption samples and efficacy, safety, PK, or other biomarker endpoints

PK = pharmacokinetic.

Study Design

Description of Study

This is a Phase II, randomized, double-blind, placebo-controlled study to evaluate the antiviral activity, safety, pharmacokinetics, and efficacy of selected dose regimens of AT-527 compared with placebo in non-hospitalized adult patients with mild or moderate COVID-19. If all potential cohorts are fully enrolled, the study will enroll up to approximately *404* patients globally.

Patients will be screened up to 72 hours prior to randomization. Patients who do not meet the criteria for participation in this study (screen failure) cannot be re-screened. The investigator will record reasons for screen failure in the screening log.

Eligible patients will be enrolled sequentially into one of up to five possible treatment cohorts (Cohorts A–E). Sixty patients will be enrolled into Cohort A; Cohorts B–*D* will enroll 40 patients each, *and Cohort E will enroll approximately 224 patients*.

Patients who withdraw from the study or who discontinue study treatment will not be replaced. However, patients who withdraw from the study after screening, but before receiving the first dose, will be replaced.

In Cohort A, patients will be randomized in a 1:1 ratio to receive either AT-527 550 mg (or placebo) twice a day (BID) on Days 1–5, with assessment visits on Days 3, 5, and 7. After 30 patients (50% enrollment) in Cohort A have completed assessments through Day 10, an interim review of safety data will be conducted to determine the AT-527 dose and regimen for Cohort B.

In Cohorts B–*D*, patients will be randomized in a 3:1 ratio to receive either AT-527 or placebo on Days 1–5, with assessment visits on Days 3, 5 and 7. Dose regimen decisions for Cohorts C–*D* will be made by the Sponsor study team following unblinded review of safety data by an Internal Monitoring Committee (IMC) after 20 patients (approximately 15 active and 5 placebo) enrolled in the previous cohort have completed assessments through Day 10. The maximum dose to be tested in the study will not exceed AT-527 1100 mg BID for 5 days. Depending on emerging data, the Sponsor may also evaluate regimens with doses of AT-527 lower than 550 mg BID. *Cohorts that evaluate regimens with AT-527 doses lower than a previously tested dose regimen will not require IMC oversight.*

In Cohort E, patients will be randomized in a 1:1 ratio to receive either AT-527 (or placebo) BID on Days 1–5, with assessment visits on Days 3, 5, 7 and 14. Cohort E may be initiated prior to the completion of all four prior cohorts, and will utilize a dose regimen selected from a previous cohort. As Cohort E dose regimen will have been previously tested, this cohort does not require an IMC.

For all cohorts, visits may be conducted in clinic or by mobile nursing. Where local policies and local site logistics permit, patients may also be confined in clinic during Days 1–7. Where treatment occurs in the inpatient setting, the purpose of hospitalization must not be for severe COVID-19 requiring inpatient treatment. An end of study safety follow-up telephone call on Day 33. The total study duration for each patient will be 33 days.

For all cohorts, a selection of different samples will be collected to assess severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) viral status at various timepoints. To evaluate the PK properties of AT-527, PK samples will be collected at various timepoints. Patients will be closely monitored for COVID-19 signs and symptoms and adverse events; adverse events will be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events v5.0.

During Cohorts B–D, an IMC will provide additional safety oversight for cohorts evaluating regimens with AT-527 doses higher than 550 mg BID. The IMC will include representatives from Clinical Science and Clinical Safety who are independent of the Sponsor study team and a Biostatistician who does not have regular contact with the sites. In addition to the ongoing assessment of the incidence, nature, and severity of adverse events, serious adverse events, deaths, and vital sign and laboratory abnormalities performed by the Medical Monitor, the IMC will review safety data through Day 10 for the first 20 patients enrolled in those cohorts.

The IMC will pause further enrollment in the study to conduct a comprehensive safety assessment of all cumulative data if any of the following criteria are met.

- Death in any patient for which the cause of death is judged by the investigator to have a reasonable possibility of relatedness to the study drug
- Occurrence in any patient of a life-threatening serious adverse event for which the causal relationship is judged by the investigator to have a reasonable possibility of relatedness to the study drug
- $>$ 20% of the enrolled patients in any cohort experience clinically significant Grade 3 or 4 adverse events judged by the investigator to have a reasonable possibility of relatedness to the study drug

The IMC will make recommendations as to the further conduct of the study as outlined in the IMC Charter. Other specific operational details such as the IMC's composition, frequency and timing of meetings, and members' roles and responsibilities will be detailed in the IMC Charter. *Cohorts evaluating regimens with AT-527 doses lower than a previously tested dose regimen will not require additional IMC oversight..*

Number of Patients

Up to approximately *404* non-hospitalized adult patients with mild or moderate COVID-19 will be enrolled in this study.

Target Population

Inclusion Criteria

Patients must meet the following criteria for study entry:

- Signed Informed Consent Form
- Age ≥18 years at time of signing Informed Consent Form
- Willing and able to comply with the study protocol and all study procedures, in the opinion of the investigator
- Positive SARS-CoV-2 diagnostic test (reverse transcription polymerase chain reaction [RT-PCR] or rapid antigen test) at screening
- Has symptoms consistent with mild or moderate COVID-19, as determined by the investigator, with onset ≤ 5 days prior to randomization
- For women of childbearing potential: agreement to remain abstinent (refrain from heterosexual intercourse) or use adequate contraception during the treatment period and for 30 days after the final dose of study drug.

    A woman is considered to be of childbearing potential if she is postmenarchal, has not reached a postmenopausal state (≥ 12 continuous months of amenorrhea with no

identified cause other than menopause), and is not permanently infertile due to surgery (i.e., removal of ovaries, fallopian tubes, and/or uterus) or another cause as determined by the investigator (e.g., Müllerian agenesis). The definition of childbearing potential may be adapted for alignment with local guidelines or regulations. Women of childbearing potential must be established on their chosen method of contraception at screening.

The following are examples of adequate contraceptive methods: with a bilateral tubal ligation, male sterilization, hormonal contraceptives, hormone-releasing intrauterine devices, and copper intrauterine devices; male or female condom with or without spermicide; and cap, diaphragm, or sponge with spermicide.

The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not adequate methods of contraception. If required per local guidelines or regulations, locally recognized adequate methods of contraception and information about the reliability of abstinence will be described in the local Informed Consent Form.

Exclusion Criteria

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

- Clinical signs indicative of COVID-19 illness requiring hospitalization, defined as any of the following: shortness of breath at rest, respiratory rate ≥ 30, heart rate ≥ 125, peripheral capillary oxygen saturation $\leq 93\%$ on room air
- Treatment with a therapeutic agent against SARS-CoV-2 including, but not limited to, other direct-acting antivirals, convalescent plasma, monoclonal antibodies against SARS-CoV-2, or intravenous immunoglobulin within 3 months or less than 5 drug-elimination half-lives (whichever is longer) prior to screening
- Requirement, in the opinion of the investigator, for any of the prohibited medications during the study
- Use of hydroxychloroquine or amiodarone within 3 months of screening
- Pregnant or breastfeeding, or intending to become pregnant during the study or within 30 days after the final dose of study drug. Women of childbearing potential must have a negative pregnancy test result at screening
- Abnormal laboratory test results at screening, defined as meeting any of the following sets of criteria:
  - ALT or AST $> 5 \times$ upper limit of normal (ULN)
  - Total bilirubin $> 1.5 \times$ ULN, unless the patient has known Gilbert disease
  - Creatinine clearance < 60 mL/min (*Cockcroft-Gault formula*)
  - Total WBC $<$ 2,500/mm3 or ANC $<$ 800/mm3
  - Platelet count $< 80 \times 109$/L
- Clinically significant abnormal ECG, as determined by the Investigator, at screening
- Planned procedure or surgery during the study
- Known allergy or hypersensitivity to study drug or drug product excipients
- Substance abuse, as determined by the investigator, within 12 months prior to screening
- Poor peripheral venous access
- Malabsorption syndrome or other condition that would interfere with enteral absorption
- Any clinically significant history of epistaxis within the last 3 months and/or history of being hospitalized due to epistaxis of any previous occasion
- History of anaphylaxis

- Any uncontrolled serious medical condition or other clinically significant abnormality in clinical laboratory tests that, in the investigator's judgment, precludes the patient's safe participation in and completion of the study

End of Study

The end of this study is defined as the date when the last patient, last visit (i.e., safety follow-up telephone call), 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 33 days after the last patient has been enrolled.

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

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 *12* months

Investigational Medicinal Products

The investigational medicinal product for this study is RO7496998 (AT-527) or matching placebo

Test Product (Investigational Drug)

In Cohort A, patients randomized to receive study drug will receive AT-527 550 mg BID on Days 1–5. AT-527 dose and regimen for Cohort B will be determined following an interim safety review after 30 patients (50% enrollment) in Cohort A have completed assessments through Day 10. Dose regimen decisions for Cohorts C–*D* will be made by the Sponsor study team following unblinded review of safety data by the IMC after 20 patients approximately 15 active and 5 placebo) enrolled in the previous cohort have completed assessments through Day 10. *Cohort E may be initiated prior to the completion of all four prior cohorts, and will utilize a dose regimen selected from a previous cohort.*

Comparator

For patients randomized to receive placebo, placebo will be administered as aligned with the applicable study drug regimen for each cohort.

Non-Investigational Medicinal Products

There are no non-investigational medicinal products for this study.

Statistical Methods

Primary Analysis

The primary objective of this study is to evaluate the antiviral activity of AT-527 compared with placebo on the basis of the change from baseline in amount of SARS-CoV-2 virus RNA as measured by RT-PCR and based on the log-10 scale at specified timepoints.

The change from baseline in the amount of SARS-CoV-2 virus RNA will be compared between each AT-527 group and a combined placebo group at Days 3, 5, and 7 using Analysis of Covariance (ANCOVA) with baseline virus RNA as a covariate. The treatment group least squares mean (LSM) changes, difference in LSM changes, 80% CI and p-value will be presented for each comparison. Additionally, descriptive summaries for the change from baseline will also be presented by treatment group.

If the data are not normally distributed, a non-parametric alternative method will be used (e.g., Mann-Whitney U test) to compare distributions.

Determination of Sample Size

Approximately 60 patients will be randomized in a 1:1 ratio in Cohort A to receive AT-527 or placebo. Cohorts B–*D* will enroll approximately 40 patients in a 3:1 ratio to receive AT-527 or placebo, respectively. *Cohort E will enroll approximately to 224 patients in a 1:1 ratio to receive AT-527 or placebo, respectively.*

The sample size for Cohort A–D is based on the primary endpoint of change from baseline in SARS-CoV-2 virus RNA measured by RT-PCR at a single timepoint. A sample size of 27 patients per arm (active or placebo) in the modified intent-to-treat–infected population will result in at least 80% power to detect a mean reduction in change from baseline of between 0.7 and 1.1 log10 copies using a two-sample t-test for comparison of means, assuming a standard

deviation of between 1.2 and 1.85 and based on a 1-sided 10% alpha. The sample size has been adjusted to 30 patients per arm to account for an estimated 90% SARS-CoV-2 positive rate and is based on a single-cohort comparison of AT-527 versus placebo.

For Cohort E, a sample size of 100 patients per arm (active or placebo) in the mITTi population will result in at least 80% power to detect a mean reduction in change from baseline of 0.5 log10 copies using a two-sample t-test for comparison of means, assuming a standard deviation of 1.25 (deemed appropriate based on current data from WV43042) and based on a 1-sided 2.5% alpha. The sample size for Cohort E has been adjusted to 112 patients per arm to account for an estimated 90% SARS-CoV-2 positive rate. Based on emerging external data on direct acting antivirals in COVID-19 (Fischer et al 2021), a lower mean reduction in change from baseline of 0.5 log10 copies has been utilized in Cohort E. A lower alpha level has also been selected to reduce the likelihood of a false positive result and enable a more robust evaluation of the antiviral activity of AT-527 at the selected dose regimen. This sample size also allows for a better characterization of the secondary endpoints.

Interim Analyses

Planned Interim Analyses

An interim safety analysis is planned for Cohort A after the first 30 patients have completed assessments through Day 10.

Optional Interim Analysis

Given the hypothesis-generating nature of this study, the Sponsor may choose to conduct additional interim analyses (i.e., beyond what is specified in the protocol). 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, who will be unblinded at the treatment group level. Access to treatment assignment information will follow the Sponsor's standard procedures.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
ANCOVA	Analysis of Covariance
AUC	area under the curve
$AUC_{\tau,ss}$	area under the concentration–time curve over the dosing interval at steady state
BID	twice a day
BCRP	Breast Cancer Resistance Protein
$C_{12\ hr}$	concentration at 12 hours
CDC	Centers for Disease Control and Prevention
C_{max}	maximum concentration
COVID-19	coronavirus disease 2019
CoV	coronavirus
CYP	Cytochrome P450
EC	Ethics Committee
EC_{90}	90% effective concentration
eCRF	electronic Case Report Form
EDC	electronic data capture
EUA	emergency use authorization
FDA	Food and Drug Administration
HCV	hepatitis C virus
ICH	International Council for Harmonisation
IMC	Internal Monitoring Committee
IMP	investigational medicinal product
IND	Investigational New Drug (Application)
IRB	Institutional Review Board
IxRS	interactive voice or web-based response system
LLOQ	lower limit of quantification
LSM	least square means
mITTi	modified intent-to-treat infected
MN	mobile nursing
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NGS	next-generation sequencing
NLME	non-linear mixed effects
NOAEL	no-observed-adverse-effect level
OAT	Organic Anion Transporter
PBMC	peripheral blood mononuclear cell

Abbreviation	Definition
PD	pharmacodynamic
PGIS	Patient Global Impression of Severity
P-gp	P-glycoprotein
PK	pharmacokinetic
PRO	patient-reported outcome
QD	once a day
QTcF	QT interval corrected through use of Fridericia's formula
RT-PCR	reverse-transcription polymerase chain reaction
SAP	Statistical Analysis Plan
SARS	severe acute respiratory syndrome
SARS-CoV-2	SARS coronavirus-2
SpO_2	peripheral capillary oxygen saturation
ULN	upper limit of normal
WGS	whole genome sequencing

1. BACKGROUND

1.1 BACKGROUND ON COVID-19

Coronaviruses are positive-sense, single-stranded RNA viruses, named for the crown-like appearance of their spike glycoproteins on the virus envelope. They are a large family of viruses that can cause illness ranging from the common cold to more severe diseases such as Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS). An epidemic of cases with unexplained lower respiratory tract infections was first detected in Wuhan, the largest metropolitan area in China's Hubei province, and was reported to the WHO Country Office in China on 31 December 2019. The WHO subsequently declared a pandemic on 11 March 2020. This infectious disease, named coronavirus disease 2019 (COVID-19) by the WHO, is caused by a novel coronavirus strain SARS coronavirus-2 (SARS-CoV-2). As of *5 September 2021, more than 220 million confirmed cases of COVID-19 and over 4.5 million COVID-19–related deaths have been reported globally (WHO 2021)*.

Infection with SARS-CoV-2 may be asymptomatic, or it may cause a wide spectrum of illness, ranging from a mild upper respiratory tract infection to severe life-threatening sepsis and multiorgan failure (Wiersinga et al. 2020). Commonly reported symptoms include fever, cough, shortness of breath, loss of taste or smell, sore throat, fatigue, headaches, muscle aches, and gastrointestinal disturbance. Symptoms are typically thought to last 2–3 weeks, but it is increasingly recognized that many patients continue to experience symptoms for many weeks—the so-called "long COVID" (NIHR 2020). Around 10% of non-hospitalized mild COVID-19 patients report symptoms lasting more than 4 weeks and up to 87% of hospitalized patients continue to experience symptoms 2 months after the onset of their illness. COVID-19 affects people of all ages; however, people who are immunocompromised, elderly, or have certain underlying medical conditions (e.g., chronic heart, lung and kidney disease; diabetes, obesity, cancer) are at increased risk of poor outcomes (Carfi et al. 2020; CDC 2020; PHE 2020).

Prevention and control of SARS-CoV-2 infection have focused on public health measures to slow transmission, such as social distancing, the use of face masks, and national or regional lockdowns. *The first of several effective COVID-19 vaccines was authorized in December 2020. However, despite large multi-national vaccination campaigns, as of September 2021, only 41.1% of the global population (1.9% of the population in low-income countries) have received at least one dose of vaccine (Mathieu et al. 2020). Furthermore, the duration of protection afforded by these vaccines is currently unknown and there are increasing reports of breakthrough infections, including among fully vaccinated individuals, partly driven by the emergence of SARS CoV-2 variants (Hacisuleyman et al. 2021). Additionally, there remains a subset of the population who refuse to be vaccinated, who may not respond to the available vaccines, or who have a contraindication to vaccination.* Intravenous remdesivir, an inhibitor of RNA-dependent RNA polymerase, and dexamethasone have shown benefit in hospitalized patients with severe COVID-19. However, in patients with

moderate COVID-19, dexamethasone is not efficacious (and may be harmful) and the use of remdesivir is not approved for non-hospitalized patients (Gandhi et al. 2020). Neutralizing monoclonal antibody cocktail therapies such as bamlanivimab plus etesevimab, and casirivimab plus imdevimab, have been *approved or* granted emergency use authorization (EUA) *in some countries* for the treatment of mild to moderate COVID-19 in adults and pediatric patients (12 years and older weighing at least 40 kilograms) with positive results of direct SARS-CoV-2 viral testing and who are at high risk for progressing to severe COVID-19 and/or hospitalization. *However, the risk of emergence of SARS-CoV-2 variants with resistance to monoclonal antibodies threatens the efficacy of these therapies as seen recently with bamlanivimab plus etesevimab, which was shown to have reduced in vitro activity against the gamma and beta variants, leading the United States government to temporarily pause distribution of this antibody cocktail with the US FDA recommending that healthcare providers use alternative authorized monoclonal antibodies (PHE 2021).* Additionally, the risk of viral variants to which available vaccines may offer insufficient protection further underscores the need for treatment options.

Therefore, there remains a significant and urgent unmet medical need for an orally administered, direct-acting antiviral drug with broad utility for the treatment of COVID-19. This will remain the case despite the availability of vaccinations *and current treatments*. An antiviral therapeutic that could be administered to ambulatory, non-hospitalized patients has the potential to significantly *reduce the burden of disease and* reduce the deterioration of these patients, thereby preventing progression of disease or hospitalization, and will have a significant impact on the pandemic and public health systems globally.

1.2 BACKGROUND ON AT-527

AT-527 (RO7496998) is a phosphoramidate prodrug of a unique, 6-modified purine nucleotide prodrug discovered by Atea Pharmaceuticals Inc. In laboratory studies, AT-527 potently inhibits the RNA-dependent RNA polymerase of several single-stranded RNA viruses. AT-527 is converted to its active intracellular triphosphate form (AT-9010) through a series of intermediate metabolites. AT-527 has demonstrated sub-micromolar potency against a range of coronaviruses, including SARS-CoV-1 (90% effective concentration [EC_{90}] = 0.34 μM) and SARS-CoV-2 (mean EC_{90} $[SD] = 0.42\ [0.18]\ \mu M$). AT-527 has been evaluated in two completed clinical studies in healthy volunteers and patients infected with hepatitis C virus (HCV). In the completed Phase II study AT-01B-002 in HCV-infected patients, AT-527 was well-tolerated for durations up to 12 weeks and achieved a high rate of antiviral efficacy with no safety issues.

AT-527 is administered orally as tablets. In healthy volunteers and HCV-infected patients, AT-527 exhibited a *linear* pharmacokinetic and pharmacodynamic (PK/PD) profile associated with the observed antiviral efficacy in HCV-infected patients. This supportive human PK profile is likely to be observed with dosing of patients with COVID-

19 and is expected to be sufficient for achieving antiviral efficacy, based on the sub-micromolar EC_{90} for the active triphosphate of AT-527 against SARS-CoV-2 virus replication in laboratory studies.

The safety and efficacy of AT-527 in hospitalized patients aged ≥ 18 years with moderate COVID-19 who have risk factors for poor outcome is currently being evaluated in a Phase II study (AT-03A-001). The safety and pharmacokinetics of AT-527 is also being evaluated in healthy volunteers (AT-03A-002). Summaries from these studies are briefly outlined below.

AT-03A-002

The ongoing Phase I Study AT-03A-002 is a randomized (1:1), double-blind, placebo controlled study to assess the safety and PK of multiple doses of AT-527 in healthy subjects. In Cohort 1, 20 subjects received 550 mg AT-527 (as 1 × 550 mg tablet) or matching placebo BID for 5 days. In Cohort 2, 10 subjects received a single dose of 550 mg AT-527 (as 2 × 275 mg tablets) or matching placebo. In Cohort 3, 11 subjects received 825 mg AT-527 (as 3 × 275 mg tablets) or matching placebo BID for 10 days. Two additional cohorts are planned to further evaluate the PK and safety of AT-527 at different dose regimens.

Preliminary safety data from 41 subjects in the completed Cohorts 1–3 show no premature discontinuations due to AT-527-related AEs, no SAEs, and no patterns with regards to laboratory-, ECG-, or vital sign abnormalities.

AT-03A-001

This ongoing Phase II study is randomized (1:1), double blind, placebo-controlled study to evaluate the safety and efficacy of AT-527 in hospitalized patients with moderate COVID-19 and risk factors for poor outcomes. The study targets enrollment of 190 patients.

An interim analysis was performed after the first 70 patients completed assessments through Day 14. Interim virology results based on 62 patients who were evaluable for virology analysis indicated that AT-527 rapidly reduced viral load levels. At Day 2, a 0.7 log_{10} greater mean reduction from baseline viral load was observed in patients who received AT-527 compared to placebo. A sustained difference in viral load reduction was maintained through Day 8.

AT-527 was generally well tolerated. In this hospitalized study, there were no drug-related serious adverse events. Non-serious adverse events were equally distributed across treatment arms. Most were mild-to-moderate in severity and assessed as not related to the study drug by the investigator. No safety concerns or newly determined risks were identified.

Refer to the AT-527 Investigator's Brochure for details on nonclinical and clinical studies.

1.3 STUDY RATIONALE AND BENEFIT-RISK ASSESSMENT

Despite the recent approval of vaccines for the prevention of SARS-CoV-2 infection and the recent *approvals and* EUA for monoclonal antibody therapies for mild to moderate COVID-19, there remains a significant and urgent unmet medical need to develop new therapies for COVID-19. Logistical challenges to widespread vaccination globally are likely to remain in the near future and there will be populations who cannot be safely vaccinated or for whom vaccination may offer insufficient protection. Currently available treatments, remdesivir and dexamethasone, have demonstrated benefit only in hospitalized patients with COVID-19, and the recently authorized monoclonal antibody therapies for mild to moderate COVID-19 have to be administered by *the parenteral route and in a healthcare setting due to the risk of hypersensitivity reactions,* making them unsuitable for use in the outpatient setting. There remains, therefore, an urgent unmet medical need to develop new therapies for COVID-19.

Extensive nonclinical safety data, Phase I and Phase II clinical safety data in healthy volunteers, HCV-infected *and COVID-19* patients, the favorable human PK data, and the potent in vitro antiviral activity of AT-527 against SARS-CoV-2 replication support further clinical evaluation of AT-527 in patients with COVID-19. The main objective of the current study is to evaluate the antiviral activity and PK of AT-527 in patients with mild or moderate COVID-19 and to obtain early proof of concept to support further development of AT-527 in this patient population.

Nonclinical and clinical safety studies have not revealed any pattern of drug-attributable adverse effects or any consistent clinically significant laboratory abnormalities related to any body system. Repeat-dose toxicity studies of up to 13 weeks duration in rats and monkeys did not show target-organ toxicity up to the highest tested dose levels. In rats, the no-observed-adverse-effect level (NOAEL) was the highest tested dose of 1000 mg/kg/day in these studies. In monkeys, the NOAEL in a 7-day study was 1000 mg/kg/day, a dose that was reduced to 650 mg/kg/day on Day 8 of a 14-day study due to loose stools, emesis, inappetence, and weight loss. 650 mg/kg/day was the NOAEL and the highest tested dose in the subsequent 13-week monkey toxicity study. As detailed in Section 3.3.1, the highest AT-527 dose that may be evaluated in this study is 1100 mg, administered twice a day (BID) for 5 days. While this dose has not previously been tested in humans, the plasma exposures achieved in the repeat-dose toxicity studies either exceed or approximate the anticipated clinical exposures for the unchanged drug and different metabolites in one or both species, depending on the metabolite. The potential for reproductive toxicity was assessed in dose range–finding and pivotal GLP-compliant embryofetal development studies in rats and rabbits and in a GLP-compliant fertility study in the rat. These studies have not revealed a concern for reproductive toxicity and achieved exposures exceeded the anticipated clinical exposures (see Appendix 6). Thus, the nonclinical safety data are considered to be supportive of the intended clinical dose levels and regimens and consistent with international guidelines, ICH Topic M3 (R2) and associated question and answer

document (ICH 2008; ICH 2012). The potential for gastrointestinal effects in humans will be monitored through medical data review and safety review on an ongoing basis in this study.

Some nucleoside analogs have been associated with mitochondrial toxicity, resulting in damage to the liver, muscles, heart, nerve, pancreas and other organs. Nephrotoxicity has also been associated with the use of some nucleoside analogs (e.g., tenofovir), but this is not a broad class effect. The completed nonclinical assessments of AT-527 indicate no potential for mitochondrial toxicity. The active triphosphate of AT-527, AT-9010, did not inhibit the in vitro enzyme activities of human cellular DNA-dependent DNA polymerases (α, β, or γ) and was poorly incorporated by human mitochondrial RNA polymerase. Additionally, using cell-based assays, AT-527 had no effect on mitochondrial cell membrane integrity, mitochondrial-dependent ATP production, or mitochondrial biogenesis. To date, there have been no clinical signs of adverse effects suggesting mitochondrial toxicity for AT-527 in animal toxicology studies or in completed clinical studies.

AT-527 has not been associated with hepatotoxicity in nonclinical or clinical experience to date. *As of 28 May 2021, a total of 164 subjects have received unblinded AT-527 (82 healthy volunteers, 82 patients with HCV , including patients with cirrhosis) and 80 subjects have received blinded AT-527 (28 healthy volunteers, 52 patients with COVID-19) at various dose levels in clinical studies.* In HCV-infected patients, liver function test values tend to normalize with AT-527 treatment. No hepatotoxicity was observed in animal models (multiple species).

Patients in this study will be monitored closely for any adverse events through regular clinical assessments and safety laboratory monitoring. The inclusion and exclusion criteria have been designed to exclude patients who may be at risk of toxicity. Although it is reasonable to expect that a SARS-CoV-2 direct-acting antiviral with potent in vitro antiviral activity will result in clinical benefit in SARS-CoV-2 infected patients, AT-527 has not yet been proven to be efficacious in patients with COVID-19. Thus, it is possible that patients will not receive any benefit from participation in this study. However, the high unmet medical need for the treatment of COVID-19, the nonclinical and clinical data for AT-527, and the risk mitigation outlined for this study support the conduct of a placebo-controlled study to evaluate the antiviral activity of AT-527 in non-hospitalized adult patients with mild or moderate COVID-19.

2. OBJECTIVES AND ENDPOINTS

This study will evaluate the antiviral activity, safety, pharmacokinetics, and efficacy of AT-527 compared with placebo in non-hospitalized adult patients with mild or moderate COVID-19. Specific objectives and corresponding endpoints for the study are outlined in Table 1.

Table 1 Objectives and Corresponding Endpoints

Primary Objective	Corresponding Endpoint
• To evaluate the antiviral activity of AT-527 compared with placebo	• Change from baseline in amount of SARS-CoV-2 virus RNA as measured by RT-PCR at specified timepoints
Secondary Objectives	**Corresponding Endpoints**
• To evaluate the antiviral activity of AT-527 compared with placebo	• Time to cessation of SARS-CoV-2 viral shedding as measured by RT-PCR • Time to sustained non-detectable SARS-CoV-2 virus RNA • Proportion of patients positive for SARS-CoV-2 virus RNA by RT-PCR at specified timepoints • Area under the curve in the amount of SARS-CoV-2 virus RNA as measured by RT-PCR
• To evaluate the safety of AT-527 compared with placebo	• Incidence and severity of adverse events, with severity determined according to NCI CTCAE v5.0 • Change from baseline in targeted vital signs, including SpO_2 • Change from baseline in targeted clinical laboratory test results
• To characterize the PK profile of AT-511 (free base form of AT-527) and its major metabolites	• Plasma concentration of AT-511, AT-551, AT-229, and AT-273 (surrogate for the intracellular concentration of the active triphosphate metabolite AT-9010) at specified timepoints

NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events; RT-PCR = reverse-transcription polymerase chain reaction; PK=pharmacokinetic; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; SpO_2= peripheral capillary oxygen saturation

Table 1 Objectives and Corresponding Endpoints (cont.)

Secondary Objectives (cont.)	Corresponding Endpoints
• To evaluate the efficacy of AT-527 compared with placebo	• The time to alleviation or improvement of COVID-19 symptoms (Items 1–12 of the COVID-19 Symptom Diary) maintained for a duration of 21.5 hours, defined as follows: – For new symptoms: time from start of treatment to the alleviation of COVID-19 symptoms (i.e., a score of 0 [none] or 1 [mild] on the COVID-19 Symptom Diary) – For preexisting symptoms: time from start of treatment to when a patient's symptoms have been maintained or improved (Note: Improved requires at least a single category improvement from baseline on the Likert scale.) • The time to alleviation or improvement of COVID-19 symptoms (Items 1–12 of the COVID-19 symptom diary) maintained for a duration of 43 hours, defined as follows: – For new symptoms: time from start of treatment to the alleviation of COVID-19 symptoms (i.e., a score of 0 [none] or 1 [mild] on the COVID-19 Symptom Diary) – For preexisting symptoms: time from start of treatment to when a patient's symptoms have been maintained or improved (Note: Improved requires at least a single category improvement from baseline on the Likert scale.) • Time to alleviation of COVID-19 symptoms, defined as the length of time taken from start of treatment to the point at which the following criterion is met and maintained for at least 21.5 hours: Score of 0 or 1 on Items 1–12 of the COVID-19 Symptom Diary (without consideration for presence of pre-existing symptoms) • Time to alleviation of COVID-19 symptoms, defined as the length of time taken from start of treatment to the point at which the following criterion is met and maintained for at least 43 hours: Score of 0 or 1 on Items 1–12 of the COVID-19 Symptom Diary (without consideration for presence of pre-existing symptoms) • Duration of fever, defined as the time to return to an afebrile state (temperature ≤37.5°C) maintained for at least 21.5 hours • Frequency of COVID-19–related complications (death, hospitalization, radiologically confirmed pneumonia, acute respiratory failure, sepsis, coagulopathy, pericarditis, myocarditis, cardiac failure) • *Frequency of hospitalizations and death (all cause)* • Time to alleviation of an individual symptom, defined as the time taken from the start of treatment to the point at which the following criterion is met and maintained (for each individual symptom) for at least 21.5 hours: – Score of 0 or 1 for Items 1–12 of the COVID-19 Symptom Diary – Score of 0 for Items 13 and 14 of the COVID-19 Symptom Diary

COVID-19 = coronavirus disease 2019; NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events; PK = pharmacokinetic; SARS-CoV-2 = severe acute respiratory syndrome

Table 1 Objectives and Corresponding Endpoints (cont.)

Exploratory Objectives	Corresponding Endpoints
• To evaluate the antiviral activity of AT-527 compared with placebo	• Treatment-emergent amino acid substitutions in SARS-CoV-2 viral genes (*nsp12* and potentially other genes) • Anti–SARS-CoV-2 antibody status/titer at specified timepoints • Change from baseline in SARS-CoV-2 virus titer at specified timepoints • Time to cessation of SARS-CoV-2 viral shedding as measured by virus titer • Proportion of patients with positive SARS-CoV-2 virus titer at specified timepoints • Area under the curve of SARS-CoV-2 virus titer • Drug susceptibility in patients with evaluable virus at specified timepoints
• To evaluate the relationship between drug exposure and antiviral activity of AT-527	• Relationship between plasma concentration of AT-273 and anti-viral activity
• To evaluate patient-reported COVID-19 symptom severity	• Patient's global impression of severity of COVID-19 symptoms as assessed through the use of PGIS at Day 14 and Day 28
• To identify and/or evaluate biomarkers that are predictive of response to AT-527 (i.e., predictive biomarkers), are early surrogates of efficacy, are associated with progression to a more severe disease state (i.e., prognostic biomarkers), can provide evidence of AT-527 activity (i.e., pharmacodynamic biomarkers), or can increase the knowledge and understanding of disease biology and drug safety	• Relationship between biomarkers in blood, saliva, nasopharyngeal swab, and nasosorption samples (listed in Section 4.5.5) and efficacy, safety, PK, or other biomarker endpoints

COVID-19 = coronavirus disease 2019; PGIS = Patient Global Impression of Severity;
PK = pharmacokinetic SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2

3. STUDY DESIGN

3.1 DESCRIPTION OF THE STUDY

This is a Phase II, randomized, double-blind, placebo-controlled study to evaluate the antiviral activity, safety, pharmacokinetics, and efficacy of selected dose regimens of AT-527 compared with placebo in non-hospitalized adult patients with mild or moderate COVID-19. If all potential cohorts are fully enrolled, the study will enroll up to approximately *404 patients* globally.

Patients will be screened up to 72 hours prior to randomization. Patients who do not meet the criteria for participation in this study (screen failure) cannot be re-screened. The investigator will record reasons for screen failure in the screening log.

Eligible patients will be enrolled sequentially into one of up to five possible treatment cohorts (Cohorts A–E). Sixty patients will be enrolled into Cohort A; Cohorts B–*D* will enroll 40 patients each*, and Cohort E will enroll approximately 224 patients*.

Patients who withdraw from the study or who discontinue study treatment will not be replaced. However, patients who withdraw from the study after screening, but before receiving the first dose, will be replaced.

In Cohort A, patients will be randomized in a 1:1 ratio to receive either AT-527 550 mg (or placebo) BID on Days 1–5, with assessment visits on Days 3, 5, and 7. After 30 patients (50% enrollment) in Cohort A have completed assessments through Day 10, an interim review of safety data will be conducted to determine the AT-527 dose and regimen for Cohort B.

In Cohorts B–*D*, patients will be randomized in a 3:1 ratio to receive either AT-527 or placebo on Days 1–5, with assessment visits on Days 3, 5 and 7. Dose regimen decisions for Cohorts C–*D* will be made by the Sponsor study team following unblinded review of safety data by an Internal Monitoring Committee (IMC) after 20 patients (approximately 15 active and 5 placebo) enrolled in the previous cohort have completed assessments through Day 10. The maximum dose to be tested in the study will not exceed AT-527 1100 mg BID for 5 days. Depending on emerging data, the Sponsor may also evaluate regimens with doses of AT-527 lower than 550 mg BID. *Cohorts that evaluate regimens with AT-527 doses lower than a previously tested dose regimen will not require IMC oversight.*

In Cohort E, patients will be randomized in a 1:1 ratio to receive either AT-527 (or placebo) BID on Days 1–5, with assessment visits on Days 3, 5, 7 and 14. Cohort E may be initiated prior to the completion of all four prior cohorts, and will utilize a dose regimen selected from a previous cohort. As Cohort E dose regimen will have been previously tested, this cohort does not require an IMC.

For all cohorts, visits may be conducted in clinic or by mobile nursing. Where local policies and local site logistics permit, patients may also be confined in clinic during Days 1–7. Where treatment occurs in the inpatient setting, the purpose of hospitalization must not be for severe COVID-19 requiring inpatient treatment. An end of study safety follow-up telephone call on Day 33. The total study duration for each patient will be 33 days.

For all cohorts, a selection of different samples will be collected to assess SARS-CoV-2 viral status at various timepoints. To evaluate the PK properties of AT-527, PK samples will be collected at various timepoints. Patients will be closely monitored for COVID-19 signs and symptoms and adverse events; adverse events will be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v5.0.

During Cohorts B–D, an IMC will provide additional safety oversight for cohorts evaluating regimens with AT-527 doses higher than 550 mg BID. The IMC will include representatives from Clinical Science and Clinical Safety who are independent of the Sponsor study team, and a Biostatistician who does not have regular contact with the sites. In addition to the ongoing assessment of the incidence, nature, and severity of adverse events, serious adverse events, deaths, and vital sign and laboratory abnormalities performed by the Medical Monitor, the IMC will review safety data through Day 10 for the first 20 patients enrolled in those cohorts.

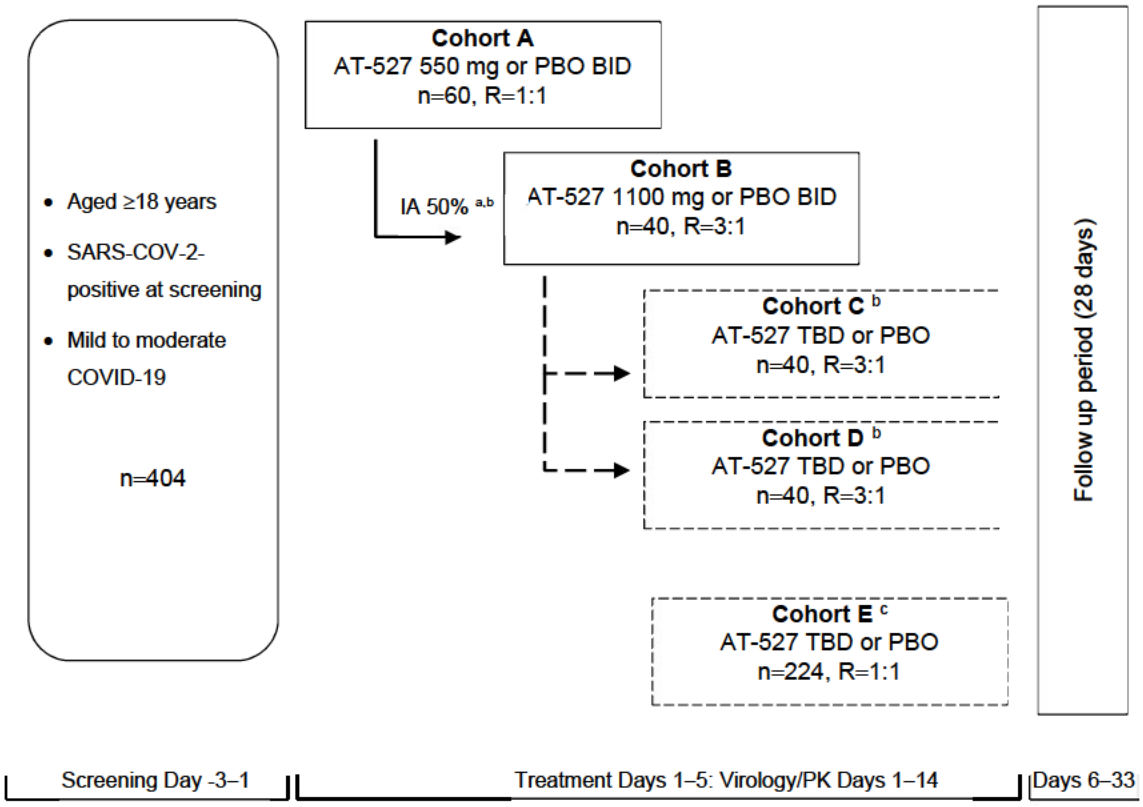
The IMC will pause further enrollment in the study to conduct a comprehensive safety assessment of all cumulative data if any of the following criteria are met.

- Death in any patient for which the cause of death is judged by the investigator to have a reasonable possibility of relatedness to the study drug
- Occurrence in any patient of a life-threatening serious adverse event for which the causal relationship is judged by the investigator to have a reasonable possibility of relatedness to the study drug
- $>$20% of the enrolled patients in any cohort experience clinically significant Grade 3 or 4 adverse events judged by the investigator to have a reasonable possibility of relatedness to the study drug

The IMC will make recommendations as to the further conduct of the study as outlined in the IMC Charter. Other specific operational details such as the IMC's composition, frequency and timing of meetings, and members' roles and responsibilities will be detailed in the IMC Charter. *Cohorts evaluating regimens with AT-527 doses lower than a previously tested dose regimen will not require additional IMC oversight..*

Figure 1 presents an overview of the study design. Schedule of activities are provided in Appendix 1, Appendix 2, and Appendix 3.

Figure 1 Study Schema



BID=twice a day; COVID-19=coronavirus disease 2019; IA=Interim Analysis; IMC=Internal Monitoring Committee; PBO=placebo; PO=orally; R=randomization; SARS-CoV-2=severe acute respiratory syndrome coronavirus-2.

Note: Patients in Cohort A *and Cohort E* will be randomized in a 1:1 ratio (AT-527:placebo). *Cohorts B–D* will be randomized in a 3:1 ratio (AT-527:placebo).

[a] Planned interim safety analysis after the first 30 patients enrolled have completed assessments through Day 10 will inform dose regimen decision for Cohort B.

[b] *Cohorts evaluating regimens with AT-527 doses lower than a previously tested dose regimen will not require additional IMC oversight.*

[c] *Cohort E will utilize a dose regimen selected from a previous cohort and may be initiated prior to the completion of all four prior cohorts.*

3.2 END OF STUDY AND LENGTH OF STUDY

The end of this study is defined as the date when the last patient, last visit (i.e., Day 33 safety follow-up telephone call), 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 33 days after the last patient has been enrolled.

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

The total length of the study, from screening of the first patient to the end of the study, is expected to be approximately *12 months*.

3.3 RATIONALE FOR STUDY DESIGN

3.3.1 Rationale for AT-527 Dose and Schedule

In healthy volunteers and HCV-infected patients, the pharmacokinetics of AT-511 (the free base form of AT-527), AT-551, AT-229, and AT-273 (a major nucleoside metabolite of AT-511 and a surrogate for the intracellular concentration of the active triphosphate metabolite AT-9010) were comparable. AT-511 metabolites exhibited dose-related plasma exposure in both populations. The PK parameters of AT-511 and its metabolites AT-551, AT-229, and AT-273 were also comparable between non-cirrhotic and cirrhotic HCV-infected patients. Similar disposition characteristics of AT-527 are assumed in non-hospitalized adult patients with mild or moderate COVID-19.

3.3.1.1 Rationale for AT-527 550 mg BID Dose Regimen

The starting dose regimen (Cohort A) for this study is 550 mg AT-527 BID for 5 days. This regimen is supported by the following data:

- Tissue distribution data for the active triphosphate metabolite AT-9010 in non-human primate and production rates of AT-9010 in human and cynomolgus monkey hepatocytes incubated with 10 μM AT-511 (see details in the AT-527 Investigator's Brochure, Section 4.2.2.2 and Table 6-1)
- Predicted PK profiles for AT-9010 in the lung at the proposed 550 mg BID regimen (see details in the AT-527 Investigator's Brochure, in Figure 6-1)

At steady state, the 550 mg BID regimen is predicted to deliver the following values:

- Mean trough AT-9010 (the active triphosphate metabolite) concentrations at 12 hours ($C_{12\,hr}$) in the lung at about 0.9 μM, *2.1-fold* higher than the mean EC_{90} of *0.42 μM* determined for the free base AT-511 in an in vitro antiviral activity assay in SARS-CoV-2–infected human airway epithelial cells (AT-527 Investigator's Brochure)
- Mean maximum lung AT-9010 concentrations at about 1.5 μM (time to maximum concentration at about 4 hours postdose), 3.6-fold higher than the mean EC_{90} of 0.42 μM.

The 550 mg BID regimen has been evaluated in the ongoing phase I Study AT-03A-002; 20 healthy subjects (blinded, 1:1 randomization active vs. placebo) were enrolled and have completed dosing and no safety concerns were identified. In the ongoing Phase II study AT-03A-001 *using the 550 mg BID regimen* in hospitalized patients with moderate COVID-19 and risk factors for poor outcomes (blinded, 1:1 randomization active vs. placebo), *an interim analysis after the first 70 patients* have not revealed any safety concerns. *Furthermore, a planned interim safety analysis was performed after 30 patients (50% enrollment) in Cohort A (550 mg BID regimen) of the current study had completed assessments through Day 10, and no safety concerns were identified.*

Data for AT-527 550 mg once a day (QD) combined with daclatasvir 60 mg QD in HCV-infected patients is available from the recently completed 12-week study (AT-01B-002). In that study, most patients received a cumulative total dose of AT-527 30800 mg. Safety and tolerability data from the study provide a safety margin of 5.6-fold for a 5-day (5500 mg cumulative dose) treatment regimen for COVID-19 patients.

The animal and human systemic exposure parameters summarized in Table 6-2 of the AT-527 Investigator's Brochure indicate that the steady-state exposures of AT-511 and its metabolites achieved at the NOAEL doses in the 13-week toxicology studies exceed or approximate unity for the AT-527 550 mg BID dose in the target population of this study.

These considerations support an expected favorable safety profile for the evaluation of the 550 mg BID dose regimen in the target population.

Depending on emerging data, subsequent cohorts may evaluate doses higher or lower than 550 mg BID.

3.3.1.2 Rationale for Higher AT-527 Dose Regimens

The available nonclinical and clinical evidence supports the proposed starting regimen of 550 mg BID. However, AT-527 dose regimens higher than the starting regimen (550 mg BID) may be selected to further explore the dose-exposure-response profile for AT-527 and delineate the relationship between plasma concentration of AT-273, a surrogate for the intracellular concentration of the active triphosphate metabolite AT-9010, and antiviral activity.

The maximum dose regimen tested will not exceed 1100 mg BID for five days. At steady state, this regimen is predicted to achieve the following values:

- Mean trough AT-9010 concentrations at 12 hours ($C_{12\ hr}$) in the lung at about 1.8 μM, $4.3\text{-}fold$ higher than the mean EC_{90} of 0.42 μM determined for AT-511 in the in vitro antiviral activity assay in SARS-CoV-2-infected human airway epithelial cells
- Mean maximum AT-9010 concentrations in the lung at about 3.0 μM, 7.1-fold higher than the mean EC_{90} of $0.42\ \mu M$

Exposure multiples for the maximum concentration (C_{max}) and the area under the concentration–time curve over the dosing interval at steady state ($AUC_{\tau,ss}$) of AT-511, AT-551, AT-229, and AT-273 in plasma at the highest planned AT-527 dose in this study (1100 mg BID) are presented in Table 2.

Plasma exposures achieved in the repeat-dose toxicity studies in the rat and the non-human primate either exceed or approximate the anticipated clinical exposures for the different metabolites in one or both species, depending on the metabolite, and are consistent with the International Council for Harmonisation (ICH) document on metabolite coverage including prodrug approaches (ICH 2008).

Table 2 Predicted Rat and Non-Human Primate Exposure Multiples for AT-511 (Free Base Form of AT-527) and Its Major Metabolites AT-551, AT-229 and AT-273 at a Human Dose of AT-527 1100 mg BID

	Monkey: Day 5 1000 mg/kg		Monkey: 13-wk 650 mg/kg		Rat: 13-wk 1000 mg/kg	
	Males	Females	Males	Females	Males	Females
C_{max}						
AT-511	1.6	1.2	2.0	1.0	0.01	0.04
AT-551	8.0	17.5	5.5	3.2	15.1	27.8
AT-229	2.1	2.0	1.0	0.8	2.0	4.3
AT-273	0.4	1.1	0.7	0.6	1.1	1.5
$AUC_{\tau,ss}$						
AT-511	2.9	1.0	1.3	1.5	0.01	0.02
AT-551	8.0	11.4	4.2	2.4	6.0	6.6
AT-229	1.6	0.9	0.6	0.7	1.7	2.8
AT-273	0.3	0.5	0.4	0.4	0.8	1.1

$AUC_{\tau,ss}$ = area under the concentration–time curve during the dosing interval at steady state; Cmax = maximum concentration.

3.3.2 Rationale for Patient Population

Despite the recent *approval* and EUA of monoclonal antibody therapies for patients with mild to moderate COVID-19, the utility of these treatments in this patient population is limited by the requirement for *parenteral administration within a healthcare setting*. An antiviral therapeutic that could be administered orally to ambulatory non-hospitalized patients with mild to moderate COVID-19 has the potential to significantly reduce their viral load and symptoms which may in turn reduce the risk of deterioration of these patients, preventing progression of disease or hospitalization, and will have a significant impact on the pandemic and public health systems globally. Moreover, together with vaccines, safe, effective oral antivirals are expected to form the mainstay of routine patient management for COVID-19 in the years to come. Given the acceptable safety profile so far in the ongoing Phase I and Phase II studies, this study is justified to confirm the antiviral activity, safety, and pharmacokinetics of AT-527 to inform further

development and selection of the appropriate dosing regimen of AT-527 in this patient population.

3.3.3 Rationale for Control Group

A placebo-controlled study design is essential for the evaluation of antiviral activity of AT-527 given the visit-by-visit reduction in SARS-CoV-2 viral load that is expected in the natural course of a generally self-limiting infection. The use of a placebo control will also allow unbiased evaluation of the safety and efficacy of AT-527. Although new monoclonal antibody therapies have recently been *approved or* authorized for use in this patient population, their use is currently limited and supportive or symptomatic treatment remains the mainstay of treatment for this patient population. Participants in this study will be allowed to use appropriate symptomatic treatment at the investigator's discretion as detailed in Section 4.4. Therefore, a placebo control is considered appropriate in this study.

3.3.4 Rationale for Biomarker Assessments

COVID-19 is a heterogeneous disease, and expression of certain biomarkers has been shown to vary among patients, especially those that are hospitalized (Arunachalam et al. 2020). Biomarker expression has also been shown to vary significantly among patients with mild or moderate disease. Therefore, all patients may not be equally likely to benefit from treatment. Predictive biomarker samples collected prior to dosing will be assessed in an effort to identify those patients with COVID-19–driven pathogenesis who are most likely to respond to AT-527. PD biomarkers will be assessed to demonstrate evidence of biologic activity of AT-527 in patients, to support selection of a recommended dose and dosing regimen, and to inform potential revisions to the PK sample collection schedule. As these biomarkers may also have prognostic value, their potential association with disease progression will also be explored.

3.3.5 Rationale for Non-Standard Clinical Outcome Assessments

At the onset of the COVID-19 pandemic, there was limited understanding of what constituted disease progression in COVID-19 and how the symptoms were manifested and impacted patients' lives. Given the heterogeneous nature of COVID-19–related symptoms in outpatients, key COVID-19–related symptoms should be assessed systematically to provide an accurate evaluation. Currently, there are no validated patient-reported outcome (PRO) tools for the assessment of COVID-19 symptoms. This study will utilize a PRO instrument based on the example U.S. Food and Drug Administration (FDA) COVID-19 PRO instrument that assesses 14 of the most common COVID-19 symptoms (FDA 2020). The symptom items used in this PRO are derived from information provided by the Centers for Disease Control and Prevention (CDC) as of 28 August 2020.

4. MATERIALS AND METHODS

4.1 PATIENTS

Up to approximately 404 non-hospitalized adult patients with mild or moderate COVID-19 will be enrolled in this study.

4.1.1 Inclusion Criteria

Patients must meet the following criteria for study entry:

- Signed Informed Consent Form
- Age ≥18 years at time of signing Informed Consent Form
- Willing and able to comply with the study protocol and all study procedures, in the opinion of the investigator
- Positive SARS-CoV-2 diagnostic test (RT-PCR or rapid antigen test) at screening
- Has symptoms consistent with mild or moderate COVID-19, as determined by the investigator, with onset $\leq$5 days prior to randomization
- For women of childbearing potential: agreement to remain abstinent (refrain from heterosexual intercourse) or use adequate contraception during the treatment period and for 30 days after the final dose of study drug.

  A woman is considered to be of childbearing potential if she is postmenarchal, has not reached a postmenopausal state ($\geq$12 continuous months of amenorrhea with no identified cause other than menopause), and is not permanently infertile due to surgery (i.e., removal of ovaries, fallopian tubes, and/or uterus) or another cause as determined by the investigator (e.g., Müllerian agenesis). The definition of childbearing potential may be adapted for alignment with local guidelines or regulations. Women of childbearing potential must be established on their chosen method of contraception at screening.

  The following are examples of adequate contraceptive methods: bilateral tubal ligation; male sterilization; hormonal contraceptives; hormone-releasing intrauterine devices; copper intrauterine devices; male or female condom with or without spermicide; and cap, diaphragm, or sponge with spermicide.

  The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not adequate methods of contraception.
  If required per local guidelines or regulations, locally recognized adequate methods of contraception and information about the reliability of abstinence will be described in the local Informed Consent Form.

4.1.2 Exclusion Criteria

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

- Clinical signs indicative of COVID-19 illness requiring hospitalization, defined as any of the following: shortness of breath at rest, respiratory rate ≥ 30, heart rate ≥ 125, peripheral capillary oxygen saturation (SpO_2) $\leq 93\%$ on room air
- Treatment with a therapeutic agent against SARS-CoV-2 including, but not limited to, other direct-acting antivirals, convalescent plasma, monoclonal antibodies against SARS-CoV-2, or intravenous immunoglobulin within 3 months or less than 5 drug-elimination half-lives (whichever is longer) prior to screening
- Requirement, in the opinion of the investigator, for any of the prohibited medications during the study
- Use of hydroxychloroquine or amiodarone within 3 months of screening
- Pregnant or breastfeeding, or intending to become pregnant during the study or within 30 days after the final dose of study drug. Women of childbearing potential must have a negative pregnancy test result at screening
- Abnormal laboratory test results at screening, defined as meeting any of the following sets of criteria:
  - ALT or AST $> 5 \times$ upper limit of normal (ULN)
  - Total bilirubin $> 1.5 \times$ ULN, unless the patient has known Gilbert disease
  - Creatinine clearance < 60 mL/min *(Cockcroft-Gault formula)*
  - Total WBC $< 2{,}500/mm^3$ or ANC $< 800/mm^3$
  - Platelet count $< 80 \times 10^9/L$
- Clinically significant abnormal ECG, as determined by the Investigator, at screening
- Planned procedure or surgery during the study
- Known allergy or hypersensitivity to study drug or drug product excipients
- Substance abuse, as determined by the investigator, within 12 months prior to screening
- Poor peripheral venous access
- Malabsorption syndrome or other condition that would interfere with enteral absorption
- Any clinically significant history of epistaxis within the last 3 months and/or history of being hospitalized due to epistaxis of any previous occasion
- History of anaphylaxis
- Any uncontrolled serious medical condition or other clinically significant abnormality in laboratory tests that, in the investigator's judgment, precludes the patient's safe participation in and completion of the study

4.2 METHOD OF TREATMENT ASSIGNMENT AND BLINDING

4.2.1 Treatment Assignment

This is a randomized, double-blind study. After initial written or electronic informed consent has been obtained, all screening procedures and assessments have been completed, and eligibility has been established for a patient, the study site will obtain the patient's identification number and treatment assignment from an interactive voice or web-based response system (IxRS).

In Cohort A *and Cohort E*, patients will be randomly assigned to receive AT-527 or placebo in a 1:1 ratio. In *Cohorts B–D,* patients will be randomized to receive AT-527 or placebo in a 3:1 ratio. A permuted-block randomization method will be utilized to ensure a balanced assignment to each treatment arm (see Section 3.1 for details).

4.2.2 Blinding

Study site personnel and patients will be blinded to treatment assignment during the study. Following interim analyses, the Sponsor study team and appropriate senior management personnel will be unblinded at a treatment group level, however the Sponsor and its agents will be blinded to individual treatment assignment, with the exception of individuals who require access to patient treatment assignments to fulfill their job roles during a clinical trial. These roles include the unblinding group responsible, clinical supply chain managers, sample handling staff, operational assay group personnel, IxRS service provider, and IMC members. Supporting biometrics staff will have access to the unblinded data to allow for data transfer, unblinding, and summarizing of *interim analyses and* IMC review.

While PK samples must be collected from patients assigned to the placebo arm to maintain the blinding of treatment assignment, PK assay results for these patients are generally not needed for the safe conduct or proper interpretation of the study data. Laboratories responsible for performing study drug PK assays will be unblinded to patient treatment assignments to identify appropriate samples for analysis. PK samples from patients assigned to the placebo arm will not be analyzed for study drug PK concentration except by request (e.g., to evaluate a possible error in dosing).

If unblinding is necessary for a medical emergency (e.g., in the case of a serious adverse event for which patient management might be affected by knowledge of treatment assignment), the investigator will be able to break the treatment code by contacting the IxRS. The treatment code should not be broken except in emergency situations. The investigator is not required to contact the Medical Monitor prior to breaking the treatment code. However, the investigator should contact the Medical Monitor to discuss emergency unblinding after the treatment code has been broken.

If the investigator wishes to know the identity of the study drug for any reason other than a medical emergency, he or she should contact the Medical Monitor directly. The

investigator should document and provide an explanation for any non-emergency unblinding. If the Medical Monitor agrees to patient unblinding, the investigator will be able to break the treatment code by contacting the IxRS.

As per health authority reporting requirements, the Sponsor's Drug Safety representative will break the treatment code for all serious, unexpected suspected adverse reactions (see Section 5.7) that are considered by the investigator or Sponsor to be related to an investigational medicinal product (IMP); defined in Section 4.3). The patient may continue to receive treatment, and the investigator, patient, and Sponsor personnel, with the exception of the Drug Safety representative and personnel who must have access to patient treatment assignments to fulfill their roles (as defined above), will remain blinded to treatment assignment.

4.3 STUDY TREATMENT AND OTHER TREATMENTS RELEVANT TO THE STUDY DESIGN

The investigational medicinal product for this study is RO7496998 (AT-527) or matching placebo.

4.3.1 Study Treatment Formulation and Packaging

4.3.1.1 AT-527 and Placebo

AT-527 and matching placebo will be supplied by the Sponsor as 275 mg or 550 mg tablets. For information on the AT-527 formulation, see the pharmacy manual or AT-527 Investigator's Brochure.

4.3.2 Study Treatment Dosage, Administration, and Compliance

The treatment regimens are summarized in Section 3.1.

Patients in Cohort A will self-administer AT-527 (or placebo) tablets at a dose of 550 mg orally BID on Days 1–5. AT-527 (or placebo) tablets should be taken approximately 12 hours apart. If the first dose is taken after 4 p.m. (16:00 hours) on Day 1, the next dose will be taken the morning of Day 2. For these patients, the tenth dose will be taken on the morning of Day 6. If the first dose is taken prior to 4 p.m. (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 8 hours between doses). For these patients, the tenth dose will be taken in the evening of Day 5. AT-527 (or placebo) may be taken with or without a meal. AT-527 (or placebo) tablets should be swallowed whole with a glass of water and should not be chewed, cut, or crushed. If a dose of AT-527 (or placebo) is missed (i.e., not taken within 12 hours after the scheduled dosing time), the patient should resume dosing with the next scheduled dose. If a patient vomits after taking a dose, a replacement tablet should not be taken, dosing should resume with the next scheduled dose. To assess patient compliance with self-administration of AT-527 (or placebo), patients will be required to record the time and date they took each dose in a medication diary; missed doses will also be recorded. Patients will be instructed to bring their bottles of AT-527 (or placebo) and their medication diaries to each study visit.

If a BID regimen is selected for Cohorts B–E, patients will follow the dose administration guidance described above for Cohort A. For other dose regimens, refer to the pharmacy manual for detailed instructions on drug preparation and administration.

Details on treatment administration (e.g., dose and timing) should be noted on the Study Drug Administration electronic Case Report Form (eCRF). Cases of overdose or medication error, along with any associated adverse events, should be reported as described in Section 5.3.5.11.

Guidelines for dosage modification and treatment interruption or discontinuation for patients who experience adverse events are provided in Section 5.1.2.

4.3.3 Investigational Medicinal Product Handling and Accountability

All IMPs required for completion of this study will be provided by the Sponsor. The study site (i.e., investigator or other authorized personnel [e.g., pharmacist or nurse]) is responsible for maintaining records of IMP delivery to the site, IMP inventory at the site, IMP use by each patient, and disposition or return of unused IMP, thus enabling reconciliation of all IMP received, and for ensuring that patients are provided with doses specified by the protocol.

The study site should follow all instructions included with each shipment of IMP. The study site will acknowledge receipt of IMPs supplied by the Sponsor, using the IxRS to confirm the shipment condition and content. Any damaged shipments will be replaced. The investigator or designee must confirm that appropriate temperature conditions have been maintained during transit, either by time monitoring (shipment arrival date and time) or temperature monitoring, for all IMPs received and that any discrepancies have been reported and resolved before use of the IMPs. All IMPs must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions, with access limited to the investigator and authorized staff.

Only patients enrolled in the study may receive IMPs, and only authorized staff may supply or administer IMPs.

IMPs will either be disposed of at the study site according to the study site's institutional standard operating procedure or be returned to the Sponsor with the appropriate documentation. The site's method of destroying Sponsor-supplied IMPs must be agreed to by the Sponsor. The site must obtain written authorization from the Sponsor before any Sponsor-supplied IMP is destroyed, and IMP destruction must be documented on the appropriate form.

Accurate records of all IMPs received at, dispensed from, returned to, and disposed of by the study site should be recorded on the drug accountability log.

Refer to the pharmacy manual and/or the AT-527 Investigator's Brochure for information on IMP handling, including preparation and storage, and accountability.

4.3.4 Continued Access to AT-527

Currently, the Sponsor does not have any plans to provide IMP (RO7496998 [AT-527]) or any other study treatments to patients who have completed the study. The Sponsor may evaluate whether to continue providing AT-527 in accordance with the Roche Global Policy on Continued Access to Investigational Medicinal Product, available at the following website:

http://www.roche.com/policy_continued_access_to_investigational_medicines.pdf

4.4 CONCOMITANT THERAPY

Concomitant therapy consists of any medication (e.g., prescription drugs, over-the-counter drugs, vaccines, herbal or homeopathic remedies, nutritional supplements) used by a patient in addition to protocol-mandated treatment from 7 days prior to initiation of study drug (12 months for COVID-19 vaccines) to the end of study telephone call (Day 33). All such medications should be reported to the investigator and recorded on the Concomitant Medications eCRF.

4.4.1 Permitted Therapy

Patients are permitted to use the following therapies during the study:

- Hormonal contraceptives (see Section 4.1.1)
- Hormone-replacement therapy
- Paracetamol (acetaminophen)
- Non-steroidal anti-inflammatory drugs
- COVID-19 vaccines are permitted from Day 15 onwards (see Section 4.5.2 for documentation requirements pertaining to vaccine type and date of dosing).
  - Patients must have a 40-day window since their last dose of vaccination (second dose if applicable) prior to enrollment.

4.4.2 Cautionary Therapy

4.4.2.1 *Herbal Therapies*

Concomitant use of herbal therapies is not recommended because their pharmacokinetics, safety profiles, and potential drug–drug interactions are generally unknown.

4.4.2.2 *Effects of Co-administered Therapies on AT-511 and Metabolites*

P-glycoprotein (P-gp) inhibitors

- *Concomitant use of P-gp inhibitors is permitted but dosing of study drug and the P-gp inhibitor must be staggered by 2 hours (with study drug dosed first).*

Common examples of P-gp inhibitors include carvedilol, imidazoles, macrolides, ritonavir and verapamil.

For a list of P-gp inhibitors, refer to https://go.drugbank.com/categories/DBCAT002667

Breast Cancer Resistance Protein (BCRP) inducers

- *BCRP inducers may affect plasma levels of AT-229. However, AT-229 is considered an inactive metabolite, and changes in AT-229 PK are not expected to affect the antiviral activity of AT-527. Consider substituting or stopping drugs that are major BCRP inducers during study treatment and resume 12 hours after the final dose of study drug. In the event that the BCRP inducers cannot be safely discontinued or substituted, they should be used with caution. Common examples of BCRP inducers include venlafaxine.*

  For a list of BCRP inducers, refer to https://go.drugbank.com/categories/DBCAT003986.

Organic Anion Transporter 1 and 3 (OAT1 and OAT3) inhibitors

- *OAT1 and OAT3 inhibitors may affect plasma levels of AT-273. However, AT-273 is considered an inactive metabolite, and changes in AT-273 PK are not expected to affect the antiviral activity of AT-527. Consider stopping drugs that are major OAT1/3 inhibitors during study treatment and resume 12 hours after the final dose of study drug. In the event that the OAT1/3 inhibitors cannot be safely discontinued or substituted, they should be used with caution. Common examples of OAT1/3 inhibitors include furosemide, losartan and cimetidine.*

  For a list of OAT1 and OAT3 inhibitors, refer to https://go.drugbank.com/categories/DBCAT004041 and https://go.drugbank.com/categories/DBCAT003946, respectively.

4.4.2.3 *Effects of AT-511 on Co-administered Therapies*

P-glycoprotein (P-gp) sensitive substrates

- *P-gp inhibition: In the event that sensitive substrates of P-gp with a NTI cannot be safely discontinued or substituted, they should be dosed 2 hours after study drug administration, by which time absorption of AT-511 is considered essentially complete. Common examples of P-gp sensitive substrates with NTI include digoxin and tamoxifen.*

  For a list of sensitive P-gp substrates with NTI refer to https://go.drugbank.com/categories/DBCAT004027.

4.4.3 Prohibited Therapy

Use of the following concomitant therapies is prohibited from Day 1 until study completion or early termination, except as otherwise indicated, as described below:

- Investigational therapy (other than protocol-mandated study treatment) is prohibited within 90 days prior to initiation of study treatment and during study treatment
- Sofosbuvir, for patients with active HCV

- Abacavir, for patients with HIV
- Hydroxychloroquine or amiodarone within 3 months prior to screening and during the study

4.5 STUDY ASSESSMENTS

The schedules of activities to be performed during the study are provided in Appendix 1–Appendix 3. All activities should be performed and documented for each patient.

Patients will be monitored for safety and tolerability throughout the study.

At applicable sites, informed consent may be obtained and certain study assessments may be performed by a mobile nursing (MN) professional at the patient's home to improve access and convenience for patients participating in the study. If participating sites cannot provide MN services, the Sponsor will select a healthcare organization that will be responsible for providing MN services (the MN vendor). The site or MN vendor is responsible for ensuring that all MN professionals are licensed, qualified, and in good standing, as per applicable regulations, and that appropriate background checks have been performed. If the investigator at a participating site determines that MN services are appropriate for a patient and the patient gives written or electronic informed consent to participate in MN visits, the MN network will communicate with the patient and the patient's site. MN visits will be scheduled on specified visit days, to allow for the relevant assessments to be performed by the MN professional. The schedule of activities will specify the assessments that can be performed by an MN professional.

4.5.1 Informed Consent Forms and Screening Log

Written or electronic informed consent for participation in the study must be obtained before performing any study-related procedures (including screening evaluations). Informed Consent Forms for enrolled patients and for patients who are not subsequently enrolled will be maintained at the study site.

All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before enrollment. The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

4.5.2 Medical History, Baseline Conditions, Concomitant Medication, and Demographic Data

Medical history, including clinically significant diseases, surgeries, cancer history (including prior cancer therapies and procedures), reproductive status, smoking history, and use of alcohol and drugs of abuse, will be recorded at baseline. In addition, all medications (e.g., prescription drugs, over-the-counter drugs, vaccines, herbal or

homeopathic remedies, nutritional supplements) used by the patient within 7 days prior to initiation of study treatment (12 months for COVID-19 vaccines) will be recorded. At the time of each study visit and at the follow-up telephone call (Day 33), an interval medical history should be obtained and any changes in medications and allergies should be recorded.

Demographic data will include age, sex, and self-reported race/ethnicity.

COVID-19 vaccines should be reported by the brand name or company manufacturer, if available (examples: Pfizer COVID-19 vaccine, Moderna COVID-19 vaccine) and date administered if known. If not available, report as COVID-19 vaccine.

4.5.3 Physical Examinations

A complete physical examination, performed at screening and other specified visits, should include an evaluation of the head, eyes, ears, nose, and throat, and the cardiovascular, dermatologic, musculoskeletal, respiratory, gastrointestinal, genitourinary, and neurologic systems. Any abnormality identified at baseline should be recorded on the General Medical History and Baseline Conditions eCRF.

Limited, symptom-directed physical examinations should be performed as clinically indicated. Changes from baseline abnormalities should be recorded in patient notes. New or worsened clinically significant abnormalities should be recorded as adverse events on the Adverse Event eCRF.

4.5.4 Vital Signs

Vital signs will include measurements of respiratory rate, pulse rate, and systolic and diastolic blood pressure and SpO_2 while the patient is in a supine or seated position, and temperature. Record abnormalities observed at baseline on the General Medical History and Baseline Conditions eCRF. At subsequent visits, record new or worsened clinically significant abnormalities on the Adverse Event eCRF.

4.5.5 Laboratory, Biomarker, and Other Biological Samples

At screening only, samples for the following laboratory tests will be sent to the study site's local laboratory for analysis:

- Pregnancy test *(urine or serum)*

  All women of childbearing potential will have a pregnancy test at screening
- SARS-CoV-2 diagnostic test (RT-PCR or rapid antigen test) at screening: nasopharyngeal swab
- Hematology: WBC count, RBC count, hemoglobin, hematocrit, platelet count, and differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes, other cells)
- Chemistry panel (serum or plasma): bicarbonate or total carbon dioxide (if considered standard of care for the region), sodium, potassium, chloride, glucose,

BUN or urea, creatinine, *creatinine clearance (Cockcroft-Gault formula),* total protein, albumin, phosphate, calcium, magnesium, total and direct bilirubin, ALP, ALT, AST, triglycerides, cholesterol, amylase and lipase

On all other study days, samples for the following laboratory tests will be sent to designated central laboratories for analysis, unless otherwise noted:

- Hematology: WBC count, RBC count, hemoglobin, hematocrit, platelet count, and differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes, other cells)
- Chemistry panel (serum or plasma): bicarbonate or total carbon dioxide (if considered standard of care for the region), sodium, potassium, chloride, glucose, BUN or urea, creatinine, *creatinine clearance (Cockcroft-Gault formula),* total protein, albumin, phosphate, calcium, magnesium, total and direct bilirubin, ALP, ALT, AST, urate, CK (isoenzyme analysis if CK is elevated), cardiac troponins, BNP, triglycerides, cholesterol, amylase, LDH, PCT, and lipase
- Coagulation: INR, aPTT, PT, D-dimer, and fibrinogen
- Plasma samples for PK analysis and exploratory PK research
- Serum samples for PD analysis and exploratory biomarker research
- Serum samples for SARS-COV-2 antibody titer and serum viral load
- Nasopharyngeal swab, saliva, and blood samples for SARS-COV-2 virology tests (viral load and exploratory analysis, including sequencing)
- Nasopharyngeal swab sample for respiratory pathogen co-infections panel (multiplex assay)
- Nasosorption™ samples for exploratory research on biomarkers
- Saliva samples for exploratory research on biomarker (viral) assay development
- Blood PAXgene RNA for RNA sequencing or quantitative polymerase chain reaction
- Cryopreserved peripheral blood mononuclear cells (PBMCs) for high dimensional cytometry analysis
- Plasma obtained during PBMC processing will be retained for further exploratory research

Exploratory biomarker research and virologic testing may include, but will not be limited to, analysis of inflammatory mediators and/or cytokines, immune cells, serum viral load, SARS-COV-2 antibody titer, and virus genotypic analysis. Research may also involve extraction of DNA, cell-free DNA, or RNA; analysis of mutations, single nucleotide polymorphisms, and other genomic variants; and genomic profiling through use of next-generation sequencing of a comprehensive panel of genes. Genomic research will be aimed at exploring inherited characteristics.

For sampling procedures, storage conditions, and shipment instructions, see the laboratory manual.

Biological samples will be destroyed no later than the time of completion of the final Clinical Study Report, with the following exceptions:

- Plasma samples collected for PK analysis may be needed for additional PK assay development and validation; therefore, these samples will be destroyed no later than 5 years after the final Clinical Study Report has been completed.
- Blood (serum, PMBCs, plasma [from PBMC processing]), blood PAXgene RNA, nasopharyngeal swabs , nasosorption and saliva samples collected for biomarker research and biomarker (viral) assay development will be destroyed no later than 15 years after the final Clinical Study Report has been completed.

When a patient withdraws from the study, samples collected prior to the date of withdrawal may still be analyzed, unless the patient specifically requests that the samples be destroyed or local laws require destruction of the samples. However, if samples have been tested prior to withdrawal, results from those tests will remain as part of the overall research data.

Data arising from sample analysis, including data on genomic variants, will be subject to the confidentiality standards described in Section 8.4.

Given the complexity and exploratory nature of exploratory biomarker analyses, data derived from these analyses will generally not be provided to study investigators or patients unless required by law. The aggregate results of any conducted research will be available in accordance with the effective Sponsor policy on study data publication.

4.5.6 Electrocardiograms

Single ECG recordings will be obtained at screening and specified timepoints, as outlined in the schedule of activities (see Appendix 1–Appendix 3), and may be obtained thereafter at unscheduled timepoints as at the investigator's discretion.

All ECG recordings must be performed using a standard high-quality, high-fidelity digital electrocardiograph machine equipped with computer-based interval measurements. Lead placement should be as consistent as possible. ECG recordings must be performed after the patient has been resting for at least 10 minutes. All ECGs are to be obtained prior to other procedures scheduled at that same time (e.g., vital sign measurements, blood draws) and should not be obtained within 3 hours after any meal. Circumstances that may induce changes in heart rate, including environmental distractions (e.g., television, radio, conversation) should be avoided during the pre-ECG resting period and during ECG recording.

For safety monitoring purposes, the investigator or delegate must review, sign, and date all ECG tracings. Paper copies of ECG tracings will be kept as part of the patient's permanent study file at the site.

4.5.7 Clinical Outcome Assessments

PRO instruments will be completed to assess the clinical efficacy of AT-527 compared with placebo. In addition, PRO instruments will enable the capture of each patient's direct experience with AT-527.

PRO data will be collected through use of the following instruments: COVID-19 Symptom Diary and Patient Global Impression of Severity (PGIS).

4.5.7.1 Data Collection Methods for Clinical Outcome Assessments

PRO instruments will be self-administered at specified timepoints during the study (see the schedules of activities in Appendix 1–Appendix 3). At the clinic, instruments will be administered before the patient receives any information on disease status, prior to the performance of non-PRO assessments, and prior to the administration of study treatment unless otherwise specified.

PRO instruments, translated into the local language as appropriate, will be provided by the Sponsor in pre-printed booklets to enable the instrument to be administered. The booklets will be labeled to capture timepoint of administration.

Patients should be given the following instructions for completing PRO instruments at home or in the clinic:

- Patients should complete the instruments in a quiet area with minimal distractions and disruptions.
- Patients should answer questions to the best of their ability; there are no right or wrong answers.
- Patients should not obtain advice or help from others (e.g., family members or friends) when completing the instruments.

4.5.7.2 Description of Clinical Outcome Assessment Instruments

COVID-19 Symptom Diary

The COVID-19 Symptom Diary (see Appendix 5) is adapted from a general influenza-like illness symptom patient diary. The diary will be used to characterize key symptoms recognized as attributable to COVID-19 illness per FDA guidance (FDA 2020) as well as to document the treatment benefit of AT-527. The COVID-19 Symptom Diary is composed of 14 individual symptom item questions, each with a 2- or 4-point Likert response option.

As outlined in the schedules of activities (Appendix 1–Appendix 3), the COVID-19 Symptom Diary will be administered on paper and completed in its entirety by the patient twice a day (morning and evening) on Days 1–14 and once a day in the evening on Days 15–28. Patients will be provided with separate versions of the diary for the Days 1–14 assessments (morning vs. evening) and Days 15–28 assessment (evening).

Patient Global Impression of Severity

The PGIS (see Appendix 5) is a single item assessment of a patient's impression of severity of his or her COVID-19 symptoms over the course of the study and is administered in conjunction with the COVID-19 Symptom Diary. Change in COVID-19 symptoms is rated on a 4-point Likert scale from "none" (0) to "severe" (3).

Additional COVID-19 Related Assessment

Patients will measure their temperature twice a day on Days 1–14 and once a day on Days 15–28. Temperature will be recorded as an additional entry in the COVID-19 Symptom Diary.

4.5.8 Optional Blood Samples for Whole Genome Sequencing (Patients at Participating Sites)

At participating sites, optional blood samples will be collected from consenting patients for DNA extraction to enable whole genome sequencing (WGS) to identify variants that are predictive of response to study drug, are associated with progression to a more severe disease state, are associated with susceptibility to developing adverse events, can lead to improved adverse event monitoring or investigation, or can increase the knowledge and understanding of disease biology and drug safety. Research will be aimed at exploring inherited characteristics. The samples may be sent to one or more laboratories for analysis.

Collection and submission of blood samples for WGS is contingent upon the review and approval of the exploratory research by each site's Institutional Review Board/ Ethics Committee (IRB/EC) and, if applicable, an appropriate regulatory body. If a site has not been granted approval for WGS, this section of the protocol (Section 4.5.8) will not be applicable at that site.

The Informed Consent Form will contain a separate section that addresses optional blood samples for WGS. A separate, specific signature will be required to document a patient's agreement to provide optional blood samples. The investigator should document whether or not the patient has given consent to participate and (if applicable) the date(s) of consent, by completing the Optional Whole Genome Sequencing Informed Consent/Withdrawal eCRF.

Genomics is increasingly informing researchers' understanding of disease pathobiology. WGS provides a comprehensive characterization of the genome and, along with clinical data collected in this study, may increase the opportunity for developing new therapeutic approaches or new methods for monitoring efficacy and safety or predicting which patients are more likely to respond to a drug or develop adverse events. Data will be analyzed in the context of this study but will also be explored in aggregate with data from other studies. The availability of a larger dataset will assist in identification and characterization of important biomarkers and pathways to support future drug development.

For sampling procedures, storage conditions, and shipment instructions, see the laboratory manual.

Blood samples collected for WGS are to be stored until they are no longer needed or until they are exhausted. However, the storage period will be in accordance with the IRB/EC–approved Informed Consent Form and applicable laws (e.g., health authority requirements).

Refer to Section 4.5.5 for details on use of samples after patient withdrawal, confidentiality standards for data, and availability of data from biomarker analyses.

4.6 TREATMENT, PATIENT, STUDY, AND SITE DISCONTINUATION

4.6.1 Study Treatment Discontinuation

Patients must permanently discontinue study treatment if they experience any of the following:

- Any medical condition that the investigator or Sponsor determines may jeopardize the patient's safety if he or she continues to receive study treatment
- Investigator or Sponsor determination that treatment discontinuation is in the best interest of the patient
- Pregnancy
- Any event that meets stopping criteria defined in Section 3.1

The primary reason for study treatment discontinuation should be documented on the appropriate eCRF. Patients who discontinue study treatment prematurely will not be replaced.

Patients who discontinue study drug prematurely will complete assessments as indicated in the schedules of activities (see Appendix 1–Appendix 3).

Refer to the schedules of activities (see Appendix 1–Appendix 3) for details on follow-up assessments to be performed for patients who permanently discontinue study treatment. If a patient requests to be withdrawn from treatment or follow-up assessments, this request must be documented in the source documents and signed by the investigator.

4.6.2 Patient Discontinuation from the Study

Patients who discontinue from study participation will have an early termination visit 7 (±2) days after their final dose of study drug. Patients who discontinue from the study after Day 14 will not be required to complete an early termination visit.

Patients have the right to voluntarily withdraw from the study at any time for any reason. In addition, the investigator has the right to withdraw a patient from the study at any time.

Reasons for patient discontinuation from the study may include, but are not limited to, the following:

- Patient withdrawal of consent
- Study termination or site closure
- Adverse event
- Loss to follow-up
- Patient non-compliance, defined as failure to comply with protocol requirements as determined by the investigator or Sponsor

Every effort should be made to obtain a reason for patient discontinuation from the study. The primary reason for discontinuation from the study should be documented on the appropriate eCRF. If a patient requests to be withdrawn from the study, this request must be documented in the source documents and signed by the investigator. Patients who withdraw from the study will not be replaced.

4.6.3 Study Discontinuation

The Sponsor has the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of adverse events in this or other studies indicates a potential health hazard to patients
- Patient enrollment is unsatisfactory

The Sponsor will notify the investigator if the Sponsor decides to discontinue the study.

4.6.4 Site Discontinuation

The Sponsor has the right to close a site at any time. Reasons for closing a site may include, but are not limited to, the following:

- Excessively slow recruitment
- Poor protocol adherence
- Inaccurate or incomplete data recording
- Non-compliance with the ICH guideline for Good Clinical Practice
- No study activity (i.e., all patients have completed the study and all obligations have been fulfilled)

5. ASSESSMENT OF SAFETY

5.1 SAFETY PLAN

AT-527 is not approved, and clinical development is ongoing. The safety plan for patients in this study is based on clinical experience with AT-527 completed and ongoing studies. The anticipated important safety risks for AT-527 are outlined below. Please

refer to the AT-527 Investigator's Brochure for a complete summary of safety information.

Several measures will be taken to ensure the safety of patients participating in this study. Eligibility criteria have been designed to exclude patients at higher risk for toxicities. Throughout the duration of the study, patients will undergo safety monitoring including assessment of the nature, frequency, and severity of adverse events.

5.1.1 Risks Associated with AT-527

AT-527 has been evaluated in two completed clinical studies (AT-01B-001 and AT-01B-002) in healthy volunteers and HCV-infected patients. In the first study, 88 subjects were dosed with blinded study drug (AT-527 or placebo), with 72 of the 88 subjects receiving active study drug. AT-527 was well-tolerated in both healthy volunteers and HCV-infected patients as either single or multiple doses (seven daily doses) up to 600 mg of AT-527 (equivalent to 553 mg AT-511 free base). In the second study, 10 HCV-infected patients received AT-527 550 mg in combination with 60 mg daclatasvir QD for 8-12 weeks. There were no serious adverse events, dose-limiting toxicities, or premature discontinuations.

In the COVID-19 development program, AT-527 is being evaluated in one Phase I study (AT-03A-002) in healthy volunteers and two Phase II studies in COVID-19 patients (AT-03A-001 and WV43042). In the ongoing study AT-03A-002, the completed Cohorts 1 and 3 investigated AT-527 doses at 550 mg (as 1 × 550 mg tablet) or placebo BID for 5 days and 825 mg (as 3 × 275 mg) or placebo BID for 10 days, respectively. The completed Cohort 2 investigated a single dose of 550 mg (as 2 × 275 mg tablets). Preliminary safety data from 41 subjects in the completed Cohorts 1–3 show no premature discontinuations due to AT-527-related AEs, no SAEs, and no patterns with regards to laboratory-, ECG-, or vital sign abnormalities. In the ongoing study AT-03A-001, AT-527 is being evaluated at a dose of 550 mg BID in hospitalized patients with moderate COVID-19 and risk factors for poor outcomes. Preliminary results from an interim analysis performed after the first 70 patients show that AT-527 was generally well tolerated. There were no drug-related SAEs. Non-serious AEs were equally distributed across treatment arms. Most were mild-to-moderate in severity and assessed as not related to the study drug. No safety concerns or newly determined risks were identified. (see further detail in Section 1.2 and 1.3).

In the current study (WV43042), a planned interim safety analysis after the first 30 patients enrolled in Cohort A (dosed at 550 mg BID for 5 days) had completed assessments through Day 10, identified no safety concerns. All AEs were Grade 1 or 2 in severity and no SAEs were reported.

Some nucleoside analogs have been associated with mitochondrial toxicity, resulting in damage to liver, muscles, heart, nerve, pancreas, and other organs. Nephrotoxicity has also been associated with the use of some nucleoside analogs (e.g., tenofovir) but is not a broad class effect. The completed nonclinical assessments suggested no potential for mitochondrial toxicity with AT-527. To date, there have been no clinical signs of adverse events suggesting mitochondrial toxicity for AT-527 dosing.

To date, there have been no appreciable patterns of treatment-related or dose-related clinical adverse events or laboratory abnormalities from nonclinical or clinical studies.

5.1.2 Management of Patients Who Experience Adverse Events

5.1.2.1 Dose Modifications

There will be no dose modifications for study drug in this study.

5.1.2.2 Treatment Interruption

Treatment interruptions will not be permitted for study drug in this study.

5.1.2.3 Management of Increases in QT Interval

Study drug should be discontinued in patients who develop any of the following, unless there is a clear alternative cause for the changes:

- Sustained (at least two ECG measurements $>$30 minutes apart) QTcF that is $>$500 ms and $>$60 ms longer than the baseline value
- An episode of torsades de pointes or a new ECG finding of clinical concern

Management of patients with sustained QTcF prolongation should include close monitoring, with ECGs repeated at least hourly until two successive ECGs show resolution of the findings, correction of any electrolyte abnormalities, and possible discontinuation of other concomitant medications that are known to prolong the QT interval. Consultation with a cardiologist or electrophysiologist is recommended, to help in the management of such patients.

5.2 SAFETY PARAMETERS AND DEFINITIONS

Safety assessments will consist of monitoring and recording adverse events, including serious adverse events and adverse events of special interest, performing protocol-specified safety laboratory assessments, measuring protocol-specified vital signs, and conducting other protocol-specified tests that are deemed critical to the safety evaluation of the study.

Certain types of events require immediate reporting to the Sponsor, as outlined in Section 5.4.

5.2.1 Adverse Events

According to the ICH guideline for Good Clinical Practice, an adverse event is any untoward medical occurrence in a clinical investigation subject administered a

pharmaceutical product, regardless of causal attribution. An adverse event can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition) (see Sections 5.3.5.9 and 5.3.5.10 for more information)
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline
- Any deterioration in a laboratory value or other clinical test (e.g., ECG, X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study drug
- Adverse events that are related to a protocol-mandated intervention, including those that occur prior to assignment of study treatment (e.g., screening invasive procedures such as biopsies)

5.2.2 Serious Adverse Events (Immediately Reportable to the Sponsor)

A serious adverse event is any adverse event that meets any of the following criteria:

- Is fatal (i.e., the adverse event actually causes or leads to death)
- Is life-threatening (i.e., the adverse event, in the view of the investigator, places the patient at immediate risk of death)

  This does not include any adverse event that, had it occurred in a more severe form or was allowed to continue, might have caused death.
- Requires or prolongs inpatient hospitalization (see Section 5.3.5.11)
- Results in persistent or significant disability/incapacity (i.e., the adverse event results in substantial disruption of the patient's ability to conduct normal life functions)
- Is a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to study drug
- Is a significant medical event in the investigator's judgment (e.g., may jeopardize the patient or may require medical/surgical intervention to prevent one of the outcomes listed above)

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an adverse event (e.g., rated as mild, moderate, or severe, or according to NCI CTCAE; see Section 5.3.3); the event itself may be of relatively minor medical significance (such as severe headache without any further findings).

Severity and seriousness need to be independently assessed for each adverse event recorded on the eCRF.

Serious adverse events are required to be reported by the investigator to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2 for reporting instructions).

5.2.3 Adverse Events of Special Interest (Immediately Reportable to the Sponsor)

Adverse events of special interest are required to be reported by the investigator to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2 for reporting instructions). Adverse events of special interest for this study are as follows:

- Cases of potential drug-induced liver injury that include an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's Law (see Section 5.3.5.6)
- Suspected transmission of an infectious agent by the study drug, as defined below

  Any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term applies only when a contamination of the study drug is suspected.

5.3 METHODS AND TIMING FOR CAPTURING AND ASSESSING SAFETY PARAMETERS

The investigator is responsible for ensuring that all adverse events (see Section 5.2.1 for definition) are recorded on the Adverse Event eCRF and reported to the Sponsor in accordance with instructions provided in this section and in Sections 5.4–5.6.

For each adverse event recorded on the Adverse Event eCRF, the investigator will make an assessment of seriousness (see Section 5.2.2 for seriousness criteria), severity (see Section 5.3.3), and causality (see Section 5.3.4).

5.3.1 Adverse Event Reporting Period

Investigators will seek information on adverse events at each patient contact. All adverse events, whether reported by the patient or noted by study personnel, will be recorded in the patient's medical record and on the Adverse Event eCRF.

After informed consent has been obtained but prior to initiation of study drug, only serious adverse events caused by a protocol-mandated intervention (e.g., invasive procedures such as biopsies, discontinuation of medications) should be reported (see Section 5.4.2 for instructions for reporting serious adverse events).

After initiation of study drug, all adverse events will be reported until 28 days after the final dose of study drug.

Instructions for reporting adverse events that occur after the adverse event reporting period are provided in Section 5.6.

5.3.2 Eliciting Adverse Event Information

A consistent methodology of non-directive questioning should be adopted for eliciting adverse event information at all patient evaluation timepoints. Examples of non-directive questions include the following:

"How have you felt since your last clinic visit?"

"Have you had any new or changed health problems since you were last here?"

5.3.3 Assessment of Severity of Adverse Events

The adverse event severity grading scale for the NCI CTCAE (v5.0) will be used for assessing adverse event severity. Table 3 will be used for assessing severity for adverse events that are not specifically listed in the NCI CTCAE.

Table 3 Adverse Event Severity Grading Scale for Events Not Specifically Listed in NCI CTCAE

Grade	Severity
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; or intervention not indicated
2	Moderate; minimal, local, or non-invasive intervention indicated; or limiting age-appropriate instrumental activities of daily living [a]
3	Severe or medically significant, but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; or limiting self-care activities of daily living [b, c]
4	Life-threatening consequences or urgent intervention indicated [d]
5	Death related to adverse event [d]

NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events.

Note: Based on the most recent version of NCI CTCAE (v5.0), which can be found at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

[a] Instrumental activities of daily living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

[b] Examples of self-care activities of daily living include bathing, dressing and undressing, feeding oneself, using the toilet, and taking medications, as performed by patients who are not bedridden.

[c] If an event is assessed as a "significant medical event," it must be reported as a serious adverse event (see Section 5.4.2 for reporting instructions), per the definition of serious adverse event in Section 5.2.2.

[d] Grade 4 and 5 events must be reported as serious adverse events (see Section 5.4.2 for reporting instructions), per the definition of serious adverse event in Section 5.2.2.

5.3.4 Assessment of Causality of Adverse Events

Investigators should use their knowledge of the patient, the circumstances surrounding the event, and an evaluation of any potential alternative causes to determine whether an adverse event is considered to be related to the study drug, indicating "yes" or "no" accordingly. The following guidance should be taken into consideration (see also Table 4):

- Temporal relationship of event onset to the initiation of study drug
- Course of the event, with special consideration of the effects of dose reduction, discontinuation of study drug, or reintroduction of study drug (as applicable)
- Known association of the event with the study drug or with similar treatments
- Known association of the event with the disease under study
- Presence of risk factors in the patient or use of concomitant medications known to increase the occurrence of the event
- Presence of non-treatment-related factors that are known to be associated with the occurrence of the event

Table 4 Causal Attribution Guidance

Is the adverse event suspected to be caused by the study drug on the basis of facts, evidence, science-based rationales, and clinical judgment?	
YES	There is a plausible temporal relationship between the onset of the adverse event and administration of the study drug, and the adverse event cannot be readily explained by the patient's clinical state, intercurrent illness, or concomitant therapies; and/or the adverse event follows a known pattern of response to the study drug; and/or the adverse event abates or resolves upon discontinuation of the study drug or dose reduction and, if applicable, reappears upon re-challenge.
NO	An adverse event will be considered related, unless it fulfills the criteria specified below. Evidence exists that the adverse event has an etiology other than the study drug (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the adverse event has no plausible temporal relationship to administration of the study drug (e.g., cancer diagnosed 2 days after first dose of study drug).

5.3.5 Procedures for Recording Adverse Events

Investigators should use correct medical terminology/concepts when recording adverse events on the Adverse Event eCRF. Avoid colloquialisms and abbreviations.

Only one adverse event term should be recorded in the event field on the Adverse Event eCRF.

5.3.5.1 Diagnosis versus Signs and Symptoms

A diagnosis (if known) should be recorded on the Adverse Event eCRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than

jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded on the Adverse Event eCRF. If a diagnosis is subsequently established, all previously reported adverse events based on signs and symptoms should be nullified and replaced by one adverse event report based on the single diagnosis, with a starting date that corresponds to the starting date of the first symptom of the eventual diagnosis.

5.3.5.2 Adverse Events that Are Secondary to Other Events

In general, adverse events that are secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause, with the exception of severe or serious secondary events. A medically significant secondary adverse event that is separated in time from the initiating event should be recorded as an independent event on the Adverse Event eCRF. For example:

- If vomiting results in mild dehydration with no additional treatment in a healthy adult, only vomiting should be reported on the eCRF.
- If vomiting results in severe dehydration, both events should be reported separately on the eCRF.
- If a severe gastrointestinal hemorrhage leads to renal failure, both events should be reported separately on the eCRF.
- If dizziness leads to a fall and consequent fracture, all three events should be reported separately on the eCRF.
- If neutropenia is accompanied by an infection, both events should be reported separately on the eCRF.

All adverse events should be recorded separately on the Adverse Event eCRF if it is unclear as to whether the events are associated.

5.3.5.3 Persistent or Recurrent Adverse Events

A persistent adverse event is one that extends continuously, without resolution, between patient evaluation timepoints. Such events should only be recorded once on the Adverse Event eCRF. The initial severity (intensity or grade) of the event will be recorded at the time the event is first reported. If a persistent adverse event becomes more severe, the most extreme severity should also be recorded on the Adverse Event eCRF. If the event becomes serious, it should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning that the event became serious; see Section 5.4.2 for reporting instructions). The Adverse Event eCRF should be updated by changing the event from "non-serious" to "serious," providing the date that the event became serious, and completing all data fields related to serious adverse events.

A recurrent adverse event is one that resolves between patient evaluation timepoints and subsequently recurs. Each recurrence of an adverse event should be recorded as a separate event on the Adverse Event eCRF.

5.3.5.4 Abnormal Laboratory Values

Not every laboratory abnormality qualifies as an adverse event. A laboratory test result must be reported as an adverse event if it meets any of the following criteria:

- Is accompanied by clinical symptoms
- Results in a change in study treatment (e.g., dosage modification, treatment interruption, or treatment discontinuation)
- Results in a medical intervention (e.g., potassium supplementation for hypokalemia) or a change in concomitant therapy
- Is clinically significant in the investigator's judgment

It is the investigator's responsibility to review all laboratory findings. Medical and scientific judgment should be exercised in deciding whether an isolated laboratory abnormality should be classified as an adverse event.

If a clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., ALP and bilirubin 5 × ULN associated with cholestasis), only the diagnosis (i.e., cholestasis) should be recorded on the Adverse Event eCRF.

If a clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded on the Adverse Event eCRF, along with a descriptor indicating whether the test result is above or below the normal range (e.g., "elevated potassium," as opposed to "abnormal potassium"). If the laboratory abnormality can be characterized by a precise clinical term per standard definitions, the clinical term should be recorded as the adverse event. For example, an elevated serum potassium level of 7.0 mEq/L should be recorded as "hyperkalemia."

Observations of the same clinically significant laboratory abnormality from visit to visit should only be recorded once on the Adverse Event eCRF (see Section 5.3.5.3 for details on recording persistent adverse events).

5.3.5.5 Abnormal Vital Sign Values

Not every vital sign abnormality qualifies as an adverse event. A vital sign result must be reported as an adverse event if it meets any of the following criteria:

- Is accompanied by clinical symptoms
- Results in a change in study treatment (e.g., dosage modification, treatment interruption, or treatment discontinuation)
- Results in a medical intervention or a change in concomitant therapy
- Is clinically significant in the investigator's judgment

It is the investigator's responsibility to review all vital sign findings. Medical and scientific judgment should be exercised in deciding whether an isolated vital sign abnormality should be classified as an adverse event.

If a clinically significant vital sign abnormality is a sign of a disease or syndrome (e.g., high blood pressure), only the diagnosis (e.g., hypertension) should be recorded on the Adverse Event eCRF.

Observations of the same clinically significant vital sign abnormality from visit to visit should only be recorded once on the Adverse Event eCRF (see Section 5.3.5.3 for details on recording persistent adverse events).

5.3.5.6 Abnormal Liver Function Tests

The finding of an elevated ALT or AST ($>3\times$ baseline value) in combination with either an elevated total bilirubin ($>2\times$ ULN) or clinical jaundice in the absence of cholestasis or other causes of hyperbilirubinemia is considered to be an indicator of severe liver injury (as defined by Hy's Law). Therefore, investigators must report as an adverse event the occurrence of either of the following:

- Treatment-emergent ALT or AST $>3\times$ baseline value in combination with total bilirubin $>2\times$ ULN (of which $\geq 35\%$ is direct bilirubin)
- Treatment-emergent ALT or AST $>3\times$ baseline value in combination with clinical jaundice

The most appropriate diagnosis or (if a diagnosis cannot be established) the abnormal laboratory values should be recorded on the Adverse Event eCRF (see Section 5.3.5.4) and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event), either as a serious adverse event or an adverse event of special interest (see Section 5.4.2).

5.3.5.7 Deaths

All deaths that occur during the protocol-specified adverse event reporting period (see Section 5.3.1), regardless of relationship to study drug, must be recorded on the Adverse Event eCRF and immediately reported to the Sponsor (see Section 5.4.2). This includes death attributed to progression of COVID-19.

Death should be considered an outcome and not a distinct event. The event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the Adverse Event eCRF. Generally, only one such event should be reported. If the cause of death is unknown and cannot be ascertained at the time of reporting, **"unexplained death"** should be recorded on the Adverse Event eCRF. If the cause of death later becomes available (e.g., after autopsy), "unexplained death" should be replaced by the established cause of death. The term **"sudden death"** should not be used unless combined with the presumed cause of death (e.g., "sudden cardiac death").

If the death is attributed solely to progression of COVID-19, “coronavirus disease-2019 progression" should be recorded on the Adverse Event eCRF.

Deaths that occur after the adverse event reporting period should be reported as described in Section 5.6.

5.3.5.8 Preexisting Medical Conditions

A preexisting medical condition is one that is present at the screening visit for this study. Such conditions should be recorded on the General Medical History and Baseline Conditions eCRF.

A preexisting medical condition should be recorded as an adverse event only if the frequency, severity, or character of the condition worsens during the study. When recording such events on the Adverse Event eCRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

5.3.5.9 Lack of Efficacy or Worsening of COVID-19

Medical occurrences or symptoms of deterioration that are anticipated as part of COVID-19, such as fluctuations in symptoms, should not be recorded as adverse events. However, deterioration that is judged by the investigator to have unexpectedly worsened in severity or frequency or changed in nature at any time during the study should be recorded as an adverse event. When recording an unanticipated worsening of COVID-19 on the Adverse Event eCRF, it is important to convey the concept that the condition has changed by including applicable descriptors (e.g., "accelerated worsening of coronavirus disease 2019").

5.3.5.10 Hospitalization or Prolonged Hospitalization

Any adverse event that results in hospitalization (i.e., inpatient admission to a hospital) or prolonged hospitalization should be documented and reported as a serious adverse event (per the definition of serious adverse event in Section 5.2.2), except as outlined below.

An event that leads to hospitalization under the following circumstances should not be reported as an adverse event or a serious adverse event:

- Hospitalization for a preexisting condition, provided that all of the following criteria are met:

  The hospitalization was planned prior to the study or was scheduled during the study when elective surgery became necessary because of the expected normal progression of the disease

  The patient has not experienced an adverse event

An event that leads to hospitalization under the following circumstances is not considered to be a serious adverse event, but should be reported as an adverse event instead:

- Hospitalization that was necessary because of patient requirement for outpatient care outside of normal outpatient clinic operating hours

5.3.5.11 Cases of Overdose, Medication Error, Drug Abuse, or Drug Misuse

Overdose (accidental or intentional), medication error, drug abuse, and drug misuse (hereafter collectively referred to as "special situations"), are defined as follows:

- Accidental overdose: accidental administration of a drug in a quantity that is higher than the assigned dose
- Intentional overdose: intentional administration of a drug in a quantity that is higher than the assigned dose
- Medication error: accidental deviation in the administration of a drug

  In some cases, a medication error may be intercepted prior to administration of the drug.
- Drug abuse: intentional excessive use of a drug that may lead to addiction or dependence, physical harm, and/or psychological harm
- Drug misuse: intentional deviation in the administration of a drug that does not qualify as drug abuse

  In cases where drug is to be self-administered by the patient, drug misuse could involve the drug being administered to someone other than the patient.

Special situations are not in themselves adverse events, but may result in adverse events. Each adverse event associated with a special situation should be recorded separately on the Adverse Event eCRF. If the associated adverse event fulfills seriousness criteria or qualifies as an adverse event of special interest, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2). For AT-527 (or matching placebo), adverse events associated with special situations should be recorded as described below for each situation:

- Accidental overdose: Enter the adverse event term. Check the "Accidental overdose" and "Medication error" boxes.
- Intentional overdose: Enter the adverse event term. Check the "Intentional overdose" box. If drug abuse is suspected, check the "Drug abuse" box. If drug abuse is not suspected, check the "Drug misuse" box.
- Medication error that does not qualify as an overdose: Enter the adverse event term. Check the "Medication error" box.
- Medication error that qualifies as an overdose: Enter the adverse event term. Check the "Accidental overdose" and "Medication error" boxes.

- Drug abuse that does not qualify as an overdose: Enter the adverse event term. Check the "Drug abuse" box.
- Drug abuse that qualifies as an overdose: Enter the adverse event term. Check the "Intentional overdose" and "Drug abuse" boxes.
- Drug misuse that does not qualify as an overdose: Enter the adverse event term. Check the "Drug misuse" box.
- Drug misuse that qualifies as an overdose: Enter the adverse event term. Check the "Intentional overdose" and "Drug misuse" boxes.

In addition, all special situations associated with AT-527 (or matching placebo), regardless of whether they result in an adverse event, should be recorded on the Adverse Event eCRF as described below:

- Accidental overdose: Enter the drug name and "accidental overdose" as the event term. Check the "Accidental overdose" and "Medication error" boxes.
- Intentional overdose: Enter the drug name and "intentional overdose" as the event term. Check the "Intentional overdose" box. If drug abuse is suspected, check the "Drug abuse" box. If drug abuse is not suspected, check the "Drug misuse" box.
- Medication error that does not qualify as an overdose: Enter the name of the drug administered and a description of the error (e.g., wrong dose administered, wrong dosing schedule, incorrect route of administration, wrong drug, expired drug administered) as the event term. Check the "Medication error" box.
- Medication error that qualifies as an overdose: Enter the drug name and "accidental overdose" as the event term. Check the "Accidental overdose" and "Medication error" boxes. Enter a description of the error in the additional case details.
- Intercepted medication error: Enter the drug name and "intercepted medication error" as the event term. Check the "Medication error" box. Enter a description of the error in the additional case details.
- Drug abuse that does not qualify as an overdose: Enter the drug name and "drug abuse" as the event term. Check the "Drug abuse" box.
- Drug abuse that qualifies as an overdose: Enter the drug name and "intentional overdose" as the event term. Check the "Intentional overdose" and "Drug abuse" boxes.
- Drug misuse that does not qualify as an overdose: Enter the drug name and "drug misuse" as the event term. Check the "Drug misuse" box.
- Drug misuse that qualifies as an overdose: Enter the drug name and "intentional overdose" as the event term. Check the "Intentional overdose" and "Drug misuse" boxes.
- Drug administered to someone other than the patient: Enter the drug name and "patient supplied drug to third party" as the event term. Check the "Drug misuse" box.

As an example, an accidental overdose that resulted in a headache would require two entries on the Adverse Event eCRF, one entry to report the accidental overdose and one entry to report the headache. The "Accidental overdose" and "Medication error" boxes would need to be checked for both entries.

5.3.5.12 Patient-Reported Outcome Data

Adverse event reports will not be derived from PRO data by the Sponsor. Sites are not expected to review the PRO data for adverse events.

5.4 IMMEDIATE REPORTING REQUIREMENTS FROM INVESTIGATOR TO SPONSOR

Certain events require immediate reporting to allow the Sponsor to take appropriate measures to address potential new risks in a clinical trial. The investigator must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours after the investigator learns of the event. The following is a list of events that the investigator must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study drug:

- Serious adverse events (defined in Section 5.2.2; see Section 5.4.2 for details on reporting requirements)
- Adverse events of special interest (defined in Section 5.2.3; see Section 5.4.2 for details on reporting requirements)
- Pregnancies (see Section 5.4.3 for details on reporting requirements)

For serious adverse events and adverse events of special interest, the investigator must report new significant follow-up information to the Sponsor immediately (i.e., no more than 24 hours after becoming aware of the information). New significant information includes the following:

- New signs or symptoms or a change in the diagnosis
- Significant new diagnostic test results
- Change in causality based on new information
- Change in the event's outcome, including recovery
- Additional narrative information on the clinical course of the event

Investigators must also comply with local requirements for reporting serious adverse events to the local health authority and IRB/EC.

5.4.1 Medical Monitors and Emergency Medical Contacts

Contact Information for All Sites

Medical Monitor/Emergency Medical Contact: [redacted], M.D.

Telephone No.: [redacted]

To ensure the safety of study patients, an Emergency Medical Call Center will be available 24 hours per day, 7 days per week, in case the above-listed contacts cannot be reached. The Emergency Medical Call Center will connect the investigator with an Emergency Medical Contact, provide medical translation service if necessary, and track all calls. Contact information, including toll-free numbers for the Emergency Medical Call Center, will be distributed to investigators.

5.4.2 Reporting Requirements for Serious Adverse Events and Adverse Events of Special Interest

5.4.2.1 Events That Occur prior to Study Drug Initiation

After informed consent has been obtained but prior to initiation of study drug, only serious adverse events caused by a protocol-mandated intervention should be reported. The paper Clinical Trial Serious Adverse Event/Adverse Event of Special Interest Reporting Form provided to investigators should be completed and submitted to the Sponsor or its designee immediately (i.e., no more than 24 hours after learning of the event), either by faxing or by scanning and emailing the form using the fax number or email address provided to investigators.

5.4.2.2 Events That Occur after Study Drug Initiation

After initiation of study drug, serious adverse events and adverse events of special interest will be reported until 28 days after the final dose of study drug. Investigators should record all case details that can be gathered immediately (i.e., within 24 hours after learning of the event) on the Adverse Event eCRF and submit the report via the electronic data capture (EDC) system. A report will be generated and sent to Roche Safety Risk Management by the EDC system.

In the event that the EDC system is unavailable, the paper Clinical Trial Serious Adverse Event/Adverse Event of Special Interest Reporting Form provided to investigators should be completed and submitted to the Sponsor or its designee immediately (i.e., no more than 24 hours after learning of the event), either by faxing or by scanning and emailing the form using the fax number or email address provided to investigators. Once the EDC system is available, all information will need to be entered and submitted via the EDC system.

Instructions for reporting serious adverse events that occur > 28 days after the final dose of study treatment are provided in Section 5.6.

5.4.3 Reporting Requirements for Pregnancies

5.4.3.1 Pregnancies in Female Patients

Female patients of childbearing potential will be instructed through the Informed Consent Form to immediately inform the investigator if they become pregnant during the study or within 30 days after the final dose of study drug. A paper Clinical Trial Pregnancy Reporting Form should be completed and submitted to the Sponsor or its designee immediately (i.e., no more than 24 hours after learning of the pregnancy), either by

faxing or by scanning and emailing the form using the fax number or email address provided to investigators. Pregnancy should not be recorded on the Adverse Event eCRF. The investigator should discontinue study drug and counsel the patient, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Any serious adverse events associated with the pregnancy (e.g., an event in the fetus, an event in the mother during or after the pregnancy, or a congenital anomaly/birth defect in the child) should be reported on the Adverse Event eCRF. In addition, the investigator will submit a Clinical Trial Pregnancy Reporting Form when updated information on the course and outcome of the pregnancy becomes available.

5.4.3.2 Abortions

A spontaneous abortion should be classified as a serious adverse event (as the Sponsor considers abortions to be medically significant), recorded on the Adverse Event eCRF, and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2).

If a therapeutic or elective abortion was performed because of an underlying maternal or embryofetal toxicity, the toxicity should be classified as a serious adverse event, recorded on the Adverse Event eCRF, and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2). A therapeutic or elective abortion performed for reasons other than an underlying maternal or embryofetal toxicity is not considered an adverse event.

All abortions should be reported as pregnancy outcomes on the paper Clinical Trial Pregnancy Reporting Form.

5.4.3.3 Congenital Anomalies/Birth Defects

Any congenital anomaly/birth defect in a child born to a female patient exposed to study drug should be classified as a serious adverse event, recorded on the Adverse Event eCRF, and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2).

5.5 FOLLOW-UP OF PATIENTS AFTER ADVERSE EVENTS

5.5.1 Investigator Follow-Up

The investigator should follow each adverse event until the event has resolved to baseline grade or better, the event is assessed as stable by the investigator, the patient is lost to follow-up, or the patient withdraws consent. Every effort should be made to follow all serious adverse events considered to be related to study treatment or trial-related procedures until a final outcome can be reported.

During the adverse event reporting period (defined in Section 5.3.1), resolution of adverse events (with dates) should be documented on the Adverse Event eCRF and in the patient's medical record to facilitate source data verification.

All pregnancies reported during the study should be followed until pregnancy outcome.

5.5.2 Sponsor Follow-Up

For serious adverse events, adverse events of special interest, and pregnancies, the Sponsor or a designee may follow up by telephone, fax, email, and/or a monitoring visit to obtain additional case details and outcome information (e.g., from hospital discharge summaries, consultant reports, autopsy reports) in order to perform an independent medical assessment of the reported case.

5.6 ADVERSE EVENTS THAT OCCUR AFTER THE ADVERSE EVENT REPORTING PERIOD

The Sponsor should be notified if the investigator becomes aware of any serious adverse event that occurs after the end of the adverse event reporting period (defined as 28 days after the final dose of study drug), if the event is believed to be related to prior exposure to study drug. These events should be reported through use of the Adverse Event eCRF. However, if the EDC system is not available, the investigator should report these events directly to the Sponsor or its designee, either by faxing or by scanning and emailing the paper Clinical Trial Serious Adverse Event/Adverse Event of Special Interest Reporting Form using the fax number or email address provided to investigators.

5.7 EXPEDITED REPORTING TO HEALTH AUTHORITIES, INVESTIGATORS, INSTITUTIONAL REVIEW BOARDS, AND ETHICS COMMITTEES

The Sponsor will promptly evaluate all serious adverse events and adverse events of special interest against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators, IRBs, ECs, and applicable health authorities based on applicable legislation.

To determine reporting requirements for single adverse event cases, the Sponsor will assess the expectedness of these events through use of the reference safety information in the document listed below:

Drug	Document
AT-527	AT-527 Investigator's Brochure

The Sponsor will compare the severity of each event and the cumulative event frequency reported for the study with the severity and frequency reported in the applicable reference document.

Reporting requirements will also be based on the investigator's assessment of causality and seriousness, with allowance for upgrading by the Sponsor as needed.

An aggregate report of any clinically relevant imbalances that do not favor the test product will be submitted to health authorities.

6. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

All primary and secondary virology and efficacy outcomes will be analyzed in the modified intent-to-treat infected (mITTi) population. The mITTi population is defined as all patients randomized in the study who received any amount of study drug and had at least one positive SARS-CoV-2 RT-PCR test result *above or equal to the limit of quantification (LOQ)* during the study, with patients grouped according to the treatment assignment at randomization.

Safety analyses will be performed on the safety evaluable population, which consists of all patients who receive any amount of study drug. In all safety analyses, patients will be grouped according to the treatment that the patients actually received rather than the treatment assigned at randomization.

For *Cohorts A–D*, the primary virology analysis will be performed as part of a Day 10 review (accompanied with key safety review if deemed necessary, further details will be included in the SAP) which is to be completed after all patients enrolled in the current cohort have completed assessments through Day 10. Other endpoints will be analyzed at the completion of each full cohort or at the completion of the study.

For Cohorts A–D, all analyses at the Day 10 review or at the completion of each cohort will compare each AT-527 dose group and a combined placebo group, including all placebo patients enrolled across cohorts up to the cohort being analyzed, while analyses performed at the completion of the study will compare each AT-527 dose group and a combined placebo group from *Cohorts A–D.*

For Cohort E, the primary virology analysis will be performed as part of a Day 14 review (accompanied with key safety review if deemed necessary, further details will be included in the SAP) which is to be completed after all patients in Cohort E have completed assessments through Day 14. Other endpoints will be analyzed at the completion of the cohort.

For Cohort E, all analyses will compare the AT-527 dose group and the placebo group from Cohort E only. Data from cohorts with the same dose may be pooled to further evaluate the primary endpoint as part of a sensitivity analysis. Full details of all planned tables, listings, graphs, and statistical methods will be provided in a Statistical Analysis Plan (SAP), which will be finalized prior to the end of the study.

6.1 DETERMINATION OF SAMPLE SIZE

The primary objective of this study is to evaluate the antiviral activity of AT-527 compared with placebo. Approximately 60 patients will be randomized in a 1:1 ratio in Cohort A to receive AT-527 or placebo. Cohorts B–*D* will enroll approximately 40 patients in a 3:1 ratio to receive AT-527 or placebo, respectively. *Cohort E will enroll approximately 224 patients in a 1:1 ratio to receive AT-527 or placebo.*

The sample size *for Cohort A–D* is based on the primary endpoint of change from baseline in SARS-CoV-2 virus RNA measured by RT-PCR at a single timepoint. A sample size of 27 patients per arm (active or placebo) in the mITTi population will result in at least 80% power to detect a mean reduction in change from baseline of between 0.7 and 1.1 log10 copies using a two-sample t-test for comparison of means, assuming a standard deviation of between 1.2 and 1.85 (Regeneron 2020) and based on a 1-sided 10% alpha. The sample size has been adjusted to 30 patients per arm to account for an estimated 90% SARS-CoV-2 positive rate and is based on a single-cohort comparison of AT-527 versus placebo.

For Cohort E, a sample size of 100 patients per arm (active or placebo) in the mITTi population will result in at least 80% power to detect a mean reduction in change from baseline of 0.5 log10 copies using a two-sample t-test for comparison of means, assuming a standard deviation of 1.25 (deemed appropriate based on current data from WV43042) and based on a 1-sided 2.5% alpha. The sample size for Cohort E has been adjusted to 112 patients per arm to account for an estimated 90% SARS-CoV-2 positive rate. Based on emerging external data on direct acting antivirals in COVID-19 (Fischer et al 2021), a lower mean reduction in change from baseline of 0.5 log10 copies has been utilized in Cohort E. A lower alpha level has also been selected to reduce the likelihood of a false positive result and enable a more robust evaluation of the antiviral activity of AT-527 at the selected dose regimen. This sample size also allows for a better characterization of the secondary endpoints.

6.2 SUMMARIES OF CONDUCT OF STUDY

The number of patients who are randomized, enroll, discontinue, or complete the study will be summarized. Reasons for premature study discontinuation will be listed and summarized.

Eligibility criteria and other major protocol deviations will be listed and summarized by treatment group.

6.3 SUMMARIES OF DEMOGRAPHIC AND BASELINE CHARACTERISTICS

Demographic and baseline characteristics (including, but not limited to, age, sex, race, onset of symptoms, baseline symptom severity, presence of underlying health conditions, pre-existing symptoms) will be summarized using means, standard

deviations, medians, and ranges for continuous variables and proportions for categorical variables, as appropriate. Summaries will be presented by treatment group and will be presented for the mITTi population and may, in addition, be presented for the safety population.

Medical history data, including surgery and procedures, and baseline conditions, will be summarized descriptively by treatment group using the safety population. Previous and concomitant treatments will be summarized descriptively by treatment group. Exposure to study drug will be summarized, including number of doses. A listing of patients by treatment group, detailing dosing of study drug will be prepared.

6.4 VIROLOGY AND EFFICACY ANALYSES

All virology and efficacy analyses will be performed on the mITTi population.

Sensitivity analyses to evaluate the robustness of results to the primary analysis methods (e.g., handling of dropouts) may be conducted and will be described in the SAP.

No alpha adjustments for multiple comparisons are planned.

6.4.1 Primary Virology Endpoint

The primary objective of this study is to evaluate the antiviral activity of AT-527 compared with placebo on the basis of the change from baseline in amount of SARS-CoV-2 virus RNA as measured by RT-PCR and based on the log-10 scale at specified timepoints.

In Cohorts A-D, the change from baseline in the amount of SARS-CoV-2 virus RNA will be compared between each AT-527 group and a combined placebo group at Days 3, 5, and 7 using Analysis of Covariance (ANCOVA) with baseline virus RNA as a covariate. The treatment group least squares mean (LSM) changes, difference in LSM changes, 80% CI and p-value will be presented for each comparison. Additionally, descriptive summaries for the change from baseline will also be presented by treatment group.

In Cohort E, the change from baseline in the amount of SARS-CoV-2 virus RNA will be compared between the AT-527 group and a placebo group containing only patients from Cohort E (unpooled) at Days 3, 5, 7, and 14 using Analysis of Covariance (ANCOVA) with baseline virus RNA as a covariate. The treatment group least squares mean (LSM) changes, difference in LSM changes, 95% CI and p-value will be presented for each comparison. Additionally, descriptive summaries for the change from baseline will also be presented by treatment group.

If the data are not normally distributed, a non-parametric alternative method will be used (e.g., Mann-Whitney U test) to compare distributions.

Further details of the primary endpoint analysis will be included in the SAP, including details of any missing data handling rules for the primary endpoint.

6.4.2 Secondary Virology and Efficacy Endpoints

The following virology and efficacy secondary endpoints will be compared between each AT-527 dose group and the pooled placebo group *for Cohorts A–D and an unpooled placebo group for Cohort E*:

6.4.2.1 Secondary Virology Endpoints

- Time to cessation of SARS-CoV-2 viral shedding as measured by RT-PCR
- Time to sustained non-detectable SARS-CoV-2 virus RNA
- Proportion of patients positive for SARS-CoV-2 virus RNA by RT-PCR at specified timepoints
- Area under the curve in the amount of SARS-CoV-2 virus RNA as measured by RT-PCR

6.4.2.2 Secondary Efficacy Endpoints

- Time to alleviation or improvement of COVID-19 symptoms (Items 1–12 of the COVID-19 Symptom Diary) maintained for a duration of 21.5 hours (24 hours minus 10%, to allow some flexibility in the timing of assessments), defined as follows:
  - For new symptoms: time from start of treatment to the alleviation of COVID-19 symptoms (i.e., a score of 0 [none] or 1 [mild] on the COVID-19 Symptom Diary)
  - For pre-existing symptoms: time from start of treatment to when a patient's symptoms have been maintained or improved (note improved requires at least a single category improvement from baseline on the Likert scale)

The efficacy endpoint definition includes both alleviation of new COVID-19 symptoms and maintenance/improvement of pre-existing COVID-19 symptoms to allow for the possibility that some patients may have underlying health conditions with symptoms similar to those observed with COVID-19 (e.g., cough in a patient with chronic obstructive pulmonary disease). At screening, patients will be assessed with a 14-item COVID-19 symptom severity assessment to identify pre-existing symptoms (within the prior 30 days), and assess if they worsened due to COVID-19. Symptoms that are not pre-existing are considered to be new symptoms and need to achieve sustained alleviation (score of 0 or 1) to meet the endpoint.

If a symptom is pre-existing, the patient is asked if the severity at screening is worse than what was experienced within the last 30 days prior to COVID-19:

- If the severity is not worse, the symptom must be maintained (no worsening) or improved to meet the endpoint.
- If the severity is worse, then a sustained improvement of at least one grade must be achieved to meet the endpoint. For example, a symptom classed as

severe at screening must improve to moderate, mild, or none, and a symptom classed as moderate at screening must improve to mild or none.

- The time to alleviation or improvement of COVID-19 symptoms (Items 1–12 of the COVID-19 Symptom Diary) maintained for a duration of 43 hours, defined as follows:
  - For new symptoms: time from start of treatment to the alleviation of COVID-19 symptoms (i.e., a score of 0 [none] or 1 [mild] on the COVID-19 Symptom Diary)
  - For preexisting symptoms: time from start of treatment to when a patient's symptoms have been maintained or improved (Note: Improved requires at least a single category improvement from baseline on the Likert scale.)
- Time to alleviation of COVID-19 symptoms, defined as the length of time taken from start of treatment to the point at which the following criterion is met and maintained for at least 21.5 hours: Score of 0 or 1 on Items 1–12 of the COVID-19 Symptom Diary (without consideration for presence of pre-existing symptoms)
- Time to alleviation of COVID-19 symptoms, defined as the length of time taken from start of treatment to the point at which the following criterion is met and maintained for at least 43 hours: Score of 0 or 1 on Items 1–12 of the COVID-19 Symptom Diary (without consideration for presence of pre-existing symptoms)
- Duration of fever, defined as the time to return to an afebrile state (temperature $\leq$37.5°C) maintained for at least 21.5 hours
- Frequency of COVID-19–related complications (death, hospitalization, radiologically confirmed pneumonia , acute respiratory failure, sepsis, coagulopathy, pericarditis, myocarditis, cardiac failure)
- *Frequency of hospitalizations and death (all cause)*
- Time to alleviation of an individual symptom, defined as the time taken from the start of treatment to the point at which the following criterion is met and maintained (for each individual symptom) for at least 21.5 hours:
  - Score of 0 or 1 for Items 1–12 of the COVID-19 Symptom Diary
  - Score of 0 for Items 13 and 14 of the COVID-19 Symptom Diary

All secondary endpoints will be analyzed descriptively and summary statistics will be presented. For time to event endpoints, a Kaplan-Meier plot and the median time to response will be presented along with CIs. For proportion, frequency and incidence endpoints, the number, proportion and difference in proportions along with CIs will be presented. For area under the curve (AUC) endpoints, summary statistics will be presented. Any exploratory statistical testing will be specified in the SAP as appropriate.

Rules for missing data will be specified in the SAP.

6.4.3 Exploratory Virology Endpoints

The following virology exploratory endpoints will be compared between each AT-527 dose group and the pooled placebo group *for Cohorts A–D and an unpooled placebo group for Cohort E*:

- Treatment-emergent amino acid substitutions in SARS-CoV-2 viral genes (*nsp12* and potentially other genes)
- Anti-SARS-CoV-2 antibody status/titer at specified timepoints
- Change from baseline in SARS-CoV-2 virus titer at specified timepoints
- Time to cessation of SARS-CoV-2 viral shedding as measured by virus titer
- Proportion of patients with positive SARS-CoV-2 virus titer at specified timepoints
- AUC of SARS-CoV-2 virus titer
- Drug susceptibility in patients with evaluable virus at specified timepoints

All endpoints will be summarized using descriptive statistics as specified for the secondary endpoints. Any exploratory statistical testing will be specified in the SAP.

6.4.4 Exploratory Patient Global Impression of Severity Endpoint

The exploratory endpoint to evaluate patients' self-assessment of COVID-19 symptom severity as assessed by PGIS at Days 14 and 28 will be summarized using descriptive statistics as specified for the secondary endpoints.

6.5 SAFETY ANALYSES

The safety analysis population will consist of all randomized patients who received any amount of study drug, with patients grouped according to treatment received.

Safety analyses will be presented by each AT-527 dose and the combined placebo group *for Cohorts A–D and an unpooled placebo group for Cohort E*.

Safety will be assessed through summaries of exposure to study treatment, adverse events, changes in laboratory test results, and changes in vital signs and ECGs.

Study treatment exposure (such as treatment duration and total dose received) will be summarized with descriptive statistics.

All verbatim adverse event terms will be mapped to Medical Dictionary for Regulatory Activities thesaurus terms, and adverse event severity will be graded according to NCI CTCAE v5.0. All adverse events, serious adverse events, adverse events leading to death, adverse events of special interest, and adverse events leading to study treatment discontinuation that occur on or after the first dose of study treatment will be summarized by mapped term, appropriate thesaurus level, and severity grade. For events of varying severity, the highest grade will be used in the summaries. Deaths and cause of death will be summarized.

Relevant laboratory and vital sign (pulse rate, respiratory rate, blood pressure, SpO_2, and temperature), data will be displayed by time, with grades identified where appropriate. Additionally, a shift table of selected laboratory tests will be used to summarize the baseline and maximum postbaseline severity grade. Changes in vital signs and ECGs will be summarized.

6.6 PHARMACOKINETIC ANALYSES

The PK analysis population will consist of patients with sufficient data to enable estimation of key parameters (including, but not limited to C_{max}, and minimum concentration), with patients grouped according to treatment received. Due to sparse sampling, non-compartmental analysis will not be performed.

Samples taken for pharmacokinetic analysis will be processed to plasma and concentrations of AT-511 (free base form of AT-527) and its major metabolites AT-551, AT-229, and AT-273, will be quantified by a validated liquid chromatography-tandem mass spectrometry method.

Descriptive summary statistics for PK concentration data will be provided by cohort and visit/sampling time point. Concentrations below the lower limit of quantification (LLOQ) will be reported as zero. Summary statistics will include mean (arithmetic and geometric), SD, CV (arithmetic and geometric), median, minimum, and maximum. Concentrations below LLOQ will be treated as zero in summary statistics and for PK parameter calculations.

Nonlinear mixed effects (NLME) modeling may be used to analyze the dose-concentration-time data of AT-511 and AT-273 in plasma. Population and individual PK parameters (e.g., CL/F and V_{ss}/F) will be estimated and the influence of various covariates (such as age, gender and body weight) on these parameters will be investigated. The data collected during this study may be pooled with data collected in other clinical studies (previous Phase I and/or Phase II study in healthy volunteers and HCV-infected patients) as appropriate to build the pharmacokinetic model. Secondary PK parameters such as AUC and C_{max} may be derived from the individual post-hoc predictions. Results of the NLME PK analysis will be reported in a standalone document separate from the Clinical Study Report.

The exposure-response profile of AT-511 and its major metabolites will be explored (e.g., relationship between plasma concentration of AT-273 and antiviral activity) and will be reported in a standalone document separate from the Clinical Study Report.

Additional PK analyses may be conducted as appropriate. The PK data and parameters derived from these analyses may be used for exploratory graphical analyses of the PD data and parameters.

6.7 BIOMARKER ANALYSES

Although no formal statistical analysis of exploratory biomarkers will be performed, data may be analyzed in the context of this study and in aggregate with data from other studies.

6.8 INTERIM ANALYSES

6.8.1 Planned Interim Analyses

An interim safety analysis is planned for Cohort A after the first 30 patients have completed assessments through Day 10.

6.8.2 Optional Interim Analysis

Given the hypothesis-generating nature of this study, the Sponsor may choose to conduct additional interim analyses (i.e., beyond what is specified in Section 6.8.1). 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, who will be unblinded at the treatment group level. Access to treatment assignment information will follow the Sponsor's standard procedures.

7. DATA COLLECTION AND MANAGEMENT

7.1 DATA QUALITY ASSURANCE

The Sponsor will be responsible for data management of this study, including quality checking of the data. Data entered manually will be collected via EDC through use of eCRFs. Sites will be responsible for data entry into the EDC system. In the event of discrepant data, the Sponsor will request data clarification from the sites, which the sites will resolve electronically in the EDC system.

The Sponsor will produce an EDC Study Specification document that describes the quality checking to be performed on the data. Central laboratory data and IxRS data will be sent directly to the Sponsor, using the Sponsor's standard procedures to handle and process the electronic transfer of these data.

eCRFs and correction documentation will be maintained in the EDC system's audit trail. System backups for data stored by the Sponsor and records retention for the study data will be consistent with the Sponsor's standard procedures.

PRO data will be collected on paper questionnaires. The data from the questionnaires will be entered into the EDC system by site staff.

7.2 ELECTRONIC CASE REPORT FORMS

eCRFs are to be completed through use of a Sponsor-designated EDC system. Sites will receive training and have access to a manual for appropriate eCRF completion. eCRFs will be submitted electronically to the Sponsor and should be handled in accordance with instructions from the Sponsor.

All eCRFs should be completed by designated, trained site staff. eCRFs should be reviewed and electronically signed and dated by the investigator or a designee.

At the end of the study, the investigator will receive patient data for his or her site in a readable format that must be kept with the study records. Acknowledgement of receipt of the data is required.

7.3 SOURCE DATA DOCUMENTATION

Study monitors will perform ongoing source data verification and review to confirm that critical protocol data (i.e., source data) entered into the eCRFs by authorized site personnel are accurate, complete, and verifiable from source documents.

Source documents (paper or electronic) are those in which patient data are recorded and documented for the first time. They include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, patient-reported outcomes, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, microfiche, photographic negatives, microfilm or magnetic media, X-rays, patient files, and records kept at pharmacies, laboratories, and medico-technical departments involved in a clinical trial.

Before study initiation, the types of source documents that are to be generated will be clearly defined in the Trial Monitoring Plan. This includes any protocol data to be entered directly into the eCRFs (i.e., no prior written or electronic record of the data) and considered source data.

Source documents that are required to verify the validity and completeness of data entered into the eCRFs must not be obliterated or destroyed and must be retained per the policy for retention of records described in Section 7.5.

To facilitate source data verification and review, the investigators and institutions must provide the Sponsor direct access to applicable source documents and reports for trial-related monitoring, Sponsor audits, and IRB/EC review. The study site must also allow inspection by applicable health authorities.

7.4 USE OF COMPUTERIZED SYSTEMS

When clinical observations are entered directly into a study site's computerized medical record system (i.e., in lieu of original hardcopy records), the electronic record can serve as the source document if the system has been validated in accordance with health authority requirements pertaining to computerized systems used in clinical research. An acceptable computerized data collection system allows preservation of the original entry of data. If original data are modified, the system should maintain a viewable audit trail that shows the original data as well as the reason for the change, name of the person making the change, and date of the change.

7.5 RETENTION OF RECORDS

Records and documents pertaining to the conduct of this study and the distribution of IMP, including eCRFs, electronic or paper PRO data (if applicable), Informed Consent Forms, laboratory test results, and medication inventory records, and images, must be retained by the Principal Investigator for 15 years after completion or discontinuation of the study or for the length of time required by relevant national or local health authorities, whichever is longer. After that period of time, the documents may be destroyed, subject to local regulations.

No records may be disposed of without the written approval of the Sponsor. Written notification should be provided to the Sponsor prior to transferring any records to another party or moving them to another location.

The Sponsor will retain study data for 25 years after the final study results have been reported or for the length of time required by relevant national or local health authorities, whichever is longer.

8. ETHICAL CONSIDERATIONS

8.1 COMPLIANCE WITH LAWS AND REGULATIONS

This study will be conducted in full conformance with the ICH E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki, or the applicable laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study will comply with the requirements of the ICH E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting). Studies conducted in the United States or under a U.S. Investigational New Drug (IND) Application will comply with U.S. FDA regulations and applicable local, state, and federal laws. Studies conducted in the European Union or European Economic Area will comply with the E.U. Clinical Trial Directive (2001/20/EC) and applicable local, regional, and national laws.

8.2 INFORMED CONSENT

The Sponsor's sample Informed Consent Form (and ancillary sample Informed Consent Forms such as a Mobile Nursing Informed Consent Form, if applicable) will be provided to each site. If applicable, it will be provided in a certified translation of the local language. The Sponsor or its designee must review and approve any proposed deviations from the Sponsor's sample Informed Consent Forms or any alternate consent forms proposed by the site (collectively, the "Consent Forms") before IRB/EC submission. The final IRB/EC–approved Consent Forms must be provided to the Sponsor for health authority submission purposes according to local requirements.

If applicable, the Informed Consent Form will contain separate sections for any optional procedures. The investigator or authorized designee will explain to each patient the objectives, methods, and potential risks associated with each optional procedure. Patients will be told that they are free to refuse to participate and may withdraw their consent at any time for any reason. A separate, specific signature will be required to document a patient's agreement to participate in optional procedures. Patients who decline to participate will not provide a separate signature.

The Consent Forms must be signed and dated by the patient or the patient's legally authorized representative before his or her participation in the study. The case history or clinical records for each patient shall document the informed consent process and that written or electronic informed consent was obtained prior to participation in the study.

The Consent Forms should be revised whenever there are changes to study procedures or when new information becomes available that may affect the willingness of the patient to participate. The final revised IRB/EC–approved Consent Forms must be provided to the Sponsor for health authority submission purposes.

If the Consent Forms are revised (through an amendment or an addendum) to communicate information that might affect a patient's willingness to continue in the study, the patient or a legally authorized representative must re-consent by signing the most current version of the Consent Forms or the addendum, in accordance with applicable laws and IRB/EC policy. For any updated or revised Consent Forms, the case history or clinical records for each patient shall document the informed consent process and that written or electronic informed consent was obtained using the updated/revised Consent Forms for continued participation in the study.

A copy of each signed Consent Form must be provided to the patient or the patient's legally authorized representative. All signed and dated Consent Forms must remain in each patient's study file or in the site file and must be available for verification by study monitors at any time.

8.3 INSTITUTIONAL REVIEW BOARD OR ETHICS COMMITTEE

This protocol, the Informed Consent Forms, any information to be given to the patient, and relevant supporting information must be submitted to the IRB/EC by the Principal Investigator and reviewed and approved by the IRB/EC before the study is initiated. In addition, any patient recruitment materials must be approved by the IRB/EC.

The Principal Investigator is responsible for providing written summaries of the status of the study to the IRB/EC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC. Investigators are also responsible for promptly informing the IRB/EC of any protocol amendments (see Section 9.7).

In addition to the requirements for reporting all adverse events to the Sponsor, investigators must comply with requirements for reporting serious adverse events to the local health authority and IRB/EC. Investigators may receive written IND safety reports or other safety-related communications from the Sponsor. Investigators are responsible for ensuring that such reports are reviewed and processed in accordance with health authority requirements and the policies and procedures established by their IRB/EC, and archived in the site's study file.

8.4 CONFIDENTIALITY

The Sponsor maintains confidentiality standards by coding each patient enrolled in the study through assignment of a unique patient identification number. This means that patient names are not included in data sets that are transmitted to any Sponsor location.

Patient medical information obtained by this study is confidential and may be disclosed to third parties only as permitted by the Informed Consent Form (or separate authorization for use and disclosure of personal health information) signed by the patient, unless permitted or required by law.

Medical information may be given to a patient's personal physician or other appropriate medical personnel responsible for the patient's welfare, for treatment purposes.

Given the complexity and exploratory nature of exploratory biomarker analyses, data derived from these analyses will generally not be provided to study investigators or patients unless required by law. The aggregate results of any conducted research will be available in accordance with the effective Sponsor policy on study data publication (see Section 9.6).

Data generated by this study must be available for inspection upon request by representatives of national and local health authorities, Sponsor monitors, representatives, and collaborators, and the IRB/EC for each study site, as appropriate.

Study data may be submitted to government or other health research databases or shared with researchers, government agencies, companies, or other groups that are not participating in this study. These data may be combined with or linked to other data and used for research purposes, to advance science and public health, or for analysis, development, and commercialization of products to treat and diagnose disease. In addition, redacted Clinical Study Reports and other summary reports will be provided upon request (see Section 9.6).

8.5 FINANCIAL DISCLOSURE

Investigators will provide the Sponsor with sufficient, accurate financial information in accordance with local regulations to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate health authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study (see definition of end of study in Section 3.2).

9. STUDY DOCUMENTATION, MONITORING, AND ADMINISTRATION

9.1 STUDY DOCUMENTATION

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented, including, but not limited to, the protocol, protocol amendments, Informed Consent Forms, and documentation of IRB/EC and governmental approval. In addition, at the end of the study, the investigator will receive the patient data, including an audit trail containing a complete record of all changes to data.

9.2 PROTOCOL DEVIATIONS

The investigator should document and explain any protocol deviations. The investigator should promptly report any deviations that might have an impact on patient safety and data integrity to the Sponsor and to the IRB/EC in accordance with established IRB/EC policies and procedures. The Sponsor will review all protocol deviations and assess whether any represent a serious breach of Good Clinical Practice guidelines and require reporting to health authorities. As per the Sponsor's standard operating procedures, prospective requests to deviate from the protocol, including requests to waive protocol eligibility criteria, are not allowed.

9.3 MANAGEMENT OF STUDY QUALITY

The Sponsor has implemented a system to manage the quality of the study, focusing on processes and data that are essential to ensuring patient safety and data integrity. Prior to study initiation, the Sponsor identified potential risks associated with critical trial processes and data and implemented plans for evaluating and controlling these risks. Risk evaluation and control included the selection of risk-based parameters

(e.g., adverse event rate, protocol deviation rate) and the establishment of quality tolerance limits for these parameters prior to study initiation. Detection of deviations from quality tolerance limits will trigger an evaluation to determine if action is needed. Details on the establishment and monitoring of quality tolerance limits are provided in a Quality Tolerance Limit Management Plan.

9.4 SITE INSPECTIONS

Site visits will be conducted by the Sponsor or an authorized representative for inspection of study data, patients' medical records, and eCRFs. The investigator will permit national and local health authorities; Sponsor monitors, representatives, and collaborators; and the IRBs/ECs to inspect facilities and records relevant to this study.

9.5 ADMINISTRATIVE STRUCTURE

This trial will be sponsored and managed by F. Hoffmann-La Roche Ltd (outside the United States) and Atea Pharmaceuticals, Inc. (in the United States). F. Hoffmann-La Roche Ltd will provide clinical operations management and data management, and both, F. Hoffmann-La Roche Ltd and Atea Pharmaceuticals, Inc. will provide medical monitoring.

Up to approximately 220 patients will be enrolled across approximately 52 sites globally. Enrollment will occur through an IxRS.

Central facilities will be used for certain study assessments throughout the study (e.g., specified laboratory tests, biomarker and PK analyses), as specified in Section 4.5. Accredited local laboratories will be used for assessing eligibility at screening and routine monitoring if required; local laboratory ranges will be collected.

An IMC will be employed to monitor and evaluate patient safety as specified in Section 3.1.

9.6 DISSEMINATION OF DATA AND PROTECTION OF TRADE SECRETS

Regardless of the outcome of a trial, the Sponsor is dedicated to openly providing information on the trial to healthcare professionals and to the public, at scientific congresses, in clinical trial registries, and in peer-reviewed journals. The Sponsor will comply with all requirements for publication of study results. Study data may be shared with others who are not participating in this study (see Section 8.4 for details), and redacted Clinical Study Reports and other summary reports will be made available upon request. For more information, refer to the Roche Global Policy on Sharing of Clinical Study Information at the following website:

www.roche.com/roche_global_policy_on_sharing_of_clinical_study_information.pdf

The results of this study may be published or presented at scientific congresses. For all clinical trials in patients involving an IMP for which a marketing authorization application has been filed or approved in any country, the Sponsor aims to submit a journal manuscript reporting primary clinical trial results within 6 months after the availability of the respective Clinical Study Report. In addition, for all clinical trials in patients involving an IMP for which a marketing authorization application has been filed or approved in any country, the Sponsor aims to publish results from analyses of additional endpoints and exploratory data that are clinically meaningful and statistically sound.

The investigator must agree to submit all manuscripts or abstracts to the Sponsor prior to submission for publication or presentation. This allows the Sponsor to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the investigator.

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

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements. Any formal publication of the study in which contribution of Sponsor personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate Sponsor personnel.

Any inventions and resulting patents, improvements, and/or know-how originating from the use of data from this study will become and remain the exclusive and unburdened property of the Sponsor, except where agreed otherwise.

9.7 PROTOCOL AMENDMENTS

Any protocol amendments will be prepared by the Sponsor. Protocol amendments will be submitted to the IRB/EC and to regulatory authorities in accordance with local regulatory requirements.

Approval must be obtained from the IRB/EC and regulatory authorities (as locally required) before implementation of any changes, except for changes necessary to eliminate an immediate hazard to patients or changes that involve logistical or administrative aspects only (e.g., change in Medical Monitor or contact information).

10. REFERENCES

Arunachalam PS, Wimmers F, Mok CKP, et al. Systems biological assessment of immunity to mild versus severe COVID-19 infection in humans. Science 2020;369:1210–20.

Carfi A, Bernabei R, Landi F, et al. Persistent symptoms in patients after acute COVID-19. JAMA 2020;324:603–5.

[CDC] Centers for Disease Control and Prevention. People with certain medical conditions [resource on the internet]. 2020 [updated 16 October 2020; cited 1 November 2020]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html.

[FDA] U.S. Food and Drug Administration. Assessing COVID-19–related symptoms in outpatient adult and adolescent subjects in clinical trials of drugs and biological products for COVID-19 prevention or treatment: guidance for industry [resource on the Internet]. 2020 [published September 2020; cited 1 November 2020]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/assessing-covid-19-related-symptoms-outpatient-adult-and-adolescent-subjects-clinical-trials-drugs.

Fischer W, Eron JJ, Holman W, et al. Molnupiravir, an Oral Antiviral Treatment for COVID-19. medRxiv [Preprint]. 2021 June 17.

Gandhi RT, Lynch JB, Del Rio C. Mild to moderate Covid-19. N Engl J Med 2020;383:1757–66.

[ICH] International Council for Harmonisation Topic M3 (R2) Non-clinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals [resource on the Internet]. 2008 [published July 2008; cited 9 November 2020]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-m-3-r2-non-clinical-safety-studies-conduct-human-clinical-trials-marketing-authorization_en.pdf.

[ICH] International Council for Harmonisation guideline M3 (R2)—questions and answers [resource on the Internet]. 2012 [published May 2012; cited 9 November 2020]. Available from: https://www.ema.europa.eu/en/documents/other/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en.pdf.

Mathieu E, Ritchie H, Ortiz-Ospina E, et al. A global database of COVID-19 vaccinations.[resource on the Internet] Nat Hum Behav (2021). Accessed 10 September 2021. Available from: https://ourworldindata.org/coronavirus.

[NIHR] National Institute for Health Research. Living with Covid19 [resource on the Internet]. 2020 [published 15 October 2020; cited 1 November 2020]. Available from: https://evidence.nihr.ac.uk/themedreview/living-with-covid19/#Ref.

[PHE] Public Health England. Guidance: COVID-19: long-term health effects [resource on the Internet]. 2020 [published 7 September 2020; cited 1 November 2020]. Available from: https://www.gov.uk/government/publications/covid-19-long-term-health-effects/covid-19-long-term-health-effects.

[PHE] Public Health England. Pause in the distribution of bamlanivimab/etesevimab [resource on the Internet]. 2021 [published 25 June 2021; cited 10 September 2020]. Available from: https://www.phe.gov/emergency/events/COVID19/investigation-MCM/Bamlanivimab-etesevimab/Pages/bamlanivimab-etesevimab-distribution-pause.aspx.

[Regeneron] Regeneron Pharmaceuticals, Inc. REGN-COV2 antibody cocktail program update: September 29, 2020. Accessed: 16 November 2020. Available from: https://investor.regeneron.com/static-files/a596a85e-e72d-4529-8eb5-d52d87a99070).

[WHO] World Health Organization. Weekly operational update on COVID-19—*07 September 2021* [resource on the Internet]. *2021* [published *07 September 2021; cited: 10 September 2021*]. Available from: *https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---7-september-2021*.

Wiersinga WJ, Rhodes A, Cheng AC, et al. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. JAMA 2020;324:782–93.

Appendix 1
Schedule of Activities for Cohort A

	Screening [a]	Treatment					Monitoring					UV [b]	ET [c]	Follow-Up
Day	–3 to 1	1	2	3	4	5	6	7	8–13	14 [d]	15–28			33 [d] (±3)
Informed consent	x [e, f]													
Demographic data [f]	x													
Medical history and baseline conditions [f]	x													
Pre-existing symptoms assessment	x													
COVID-19 Symptom Diary [g]		x	x	x	x	x	x	x	x [g]	x [g]	x [g, h]			
Vital signs, including oxygen saturation [f, i]	x	x		x		x		x				x	x	
Weight [f]	x													
Height [f]	x													
Complete physical examination [f, j]	x							x						
Limited physical examination [f, k,]		As clinically indicated										x		
Single ECG	x					x						x		
Hematology [f, l]	x	x		x				x				x		
Chemistry [f, m]	x	x		x				x				x		
Pregnancy test [f, n]	x													

Appendix 1: Schedule of Activities for Cohort A

	Screening [a]	Treatment					Monitoring					UV [b]	ET [c]	Follow-Up
Day	–3 to 1	1	2	3	4	5	6	7	8–13	14 [d]	15–28			33 [d] (±3)
Nasopharyngeal swab sample for COVID-19 virology [f, o]	x	x [p]		x		x		x				x	x	
Nasosorption™ sample for biomarkers [f]		x		x		x		x						
Serum sample for biomarkers, anti-SARS-COV-2 antibodies, and viral RNA [f]		x [p]						x						
Randomization [q]	x													
AT-527 (or placebo) administration		x [r]	x	x	x	x								
Telephone call										x				x
Concomitant medications [f, s]	x	x										x	x	x
Adverse events [f, t]	x	x										x	x	x

Appendix 1: Schedule of Activities for Cohort A

COVID-19 = coronavirus disease 2019; ET = early termination; PK = pharmacokinetic; RT-PCR = reverse-transcription polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; PRO = patient-reported outcome; UV = unscheduled visit.

Notes: On treatment days (Days 1–5), all assessments should be performed prior to dosing, unless otherwise specified.

[a] Screening assessments may be performed up to 72 hours prior to Day 1 or on Day 1.

[b] An unscheduled visit represents a visit that is not specified by the protocol but is determined to be necessary by the investigator or Sponsor (e.g., for evaluation of an adverse event). Assessments (including PK sample collection) should be performed as clinically indicated. For unscheduled visits occurring after Day 7, PK sample collection is not required.

[c] Patients who discontinue study drug prematurely will continue to complete assessments as indicated until the end of the study. Patients who discontinue from study participation will have an early termination visit 7 (±2) days after their final dose of study drug. Patients who discontinue from the study after Day 14 will not be required to complete an early termination visit.

[d] Required follow-up information will be collected via telephone call at Day 14 (± 2 days) and Day 33 (±3 days).

[e] Informed consent must be documented before any study-specific screening procedure is performed.

[f] For patients at participating sites who have provided written or electronic informed consent to participate in mobile nursing visits, this assessment or procedure may be performed by a trained nursing professional at the patient's home.

[g] COVID-19 Symptom Diary includes questions regarding symptoms (Items 1–14) and the Patient Global Impression of Severity. On assessment visit days, questionnaires will be self-administered before the patient receives any information on disease status, prior to the performance of non-PRO assessments, and prior to the administration of study treatment. On Day 1, the first diary questionnaire should be completed prior to the first dose. On Days 1–14, the questionnaire (including recording of temperature) should be completed twice a day (morning and evening) at the same time each day.

[h] On Days 15–28, the questionnaire (including recording of temperature) should be completed once a day in the evening at the same time each day.

[i] Includes oxygen saturation (pulse oximetry), respiratory rate, pulse rate, and systolic and diastolic blood pressure while the patient is in a supine or seated position, and temperature.

[j] Includes evaluation of the head, eyes, ears, nose, and throat, and the cardiovascular, dermatologic, musculoskeletal, respiratory, gastrointestinal, genitourinary, and neurologic systems.

[k] Perform a limited, symptom-directed examination as clinically indicated.

[l] Hematology includes WBC count, RBC count, hemoglobin, hematocrit, platelet count, and differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes, other cells).

[m] Chemistry panel (serum or plasma) at screening includes bicarbonate or total carbon dioxide (if considered standard of care for the region), sodium, potassium, chloride, glucose, BUN or urea, creatinine, *creatinine clearance (Cockcroft-Gault formula),* total protein, albumin, phosphate, calcium, magnesium, total and direct bilirubin, ALP, ALT, AST, triglycerides, cholesterol, amylase and lipase. On all other study days,

chemistry panel (serum or plasma) includes bicarbonate or total carbon dioxide (if considered standard of care for the region), sodium, potassium, chloride, glucose, BUN or urea, creatinine, total protein, albumin, phosphate, calcium, magnesium, total and direct bilirubin, ALP, ALT, AST, urate, CK (isoenzyme analysis if CK elevated), cardiac troponins, BNP, triglycerides, cholesterol, amylase, LDH, PCT, and lipase.

[n] All women of childbearing potential will have a pregnancy test *(urine or serum)* at screening.

[o] Nasopharyngeal swab samples should be collected at screening and once per day on Days 1, 3, 5, and 7. Swab samples should be collected from both nostrils at all timepoints. Screening samples will be used for point-of-care RT-PCR or rapid antigen testing to determine eligibility.

[p] Sample must be collected prior to dosing.

[q] Randomization will be performed as soon as possible after screening and within 72 hours.

[r] First dose on Day 1 must be administered as soon as possible after randomization and within 24 hours. AT-527 (or placebo), should be taken approximately 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 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 8 hours between doses). For these patients, the 10th dose will be taken in the evening of Day 5.

[s] Medication (e.g., prescription drugs, over-the-counter drugs, vaccines, herbal or homeopathic remedies, nutritional supplements) used by a patient in addition to protocol-mandated treatment from 7 days prior to initiation of study drug until the end of study follow-up telephone call on Day 33.

[t] After informed consent has been obtained but prior to initiation of study drug, only serious adverse events caused by a protocol-mandated intervention should be reported. After initiation of study drug, all adverse events will be reported until the end of study follow-up telephone call on Day 33.

Appendix 2
Schedule of Activities for Cohorts B–*D*

	Screening [a]	Treatment					Monitoring					UV [b]	ET [c]	Follow-up
Day	–3 to 1	1	2	3	4	5	6	7	8–13	14 [d]	15–28			33 [d] (±3)
Informed consent	x [e, f]													
Demographic data [f]	x													
Medical history and baseline conditions [f]	x													
Pre-existing symptoms assessment	x													
COVID-19 Symptom Diary [g]		x	x	x	x	x	x	x	x	x	x [h]			
Vital signs, including oxygen saturation [f, i]	x	x		x		x		x				x	x	
Weight [f]	x													
Height [f]	x													
Complete physical examination [f, j]	x							x						
Limited physical examination [f, k]		As clinically indicated										x		
Single ECG	x					x						x		
Hematology [f, l]	x	x		x				x				x		
Chemistry [f, m]	x	x		x				x				x		
Coagulation (INR, aPTT, PT, D-dimer, and fibrinogen)		x		x				x						
Pregnancy test [f, n]	x													
Nasopharyngeal swab sample for COVID-19 virology [f, o]	x	x [p]		x		x		x				x	x	

Appendix 2: Schedule of Activities for Cohorts B–D

	Screening [a]	Treatment					Monitoring					UV [b]	ET [c]	Follow-up
Day	–3 to 1	1	2	3	4	5	6	7	8–13	14 [d]	15–28			33 [d] (±3)
Nasosorption™ sample for biomarkers [f]		x		x		x		x						
Saliva sample for COVID-19 virology [f]		x [p]		x		x		x				x	x	
Serum sample for biomarkers, anti-SARS-COV-2 antibodies, and viral RNA [f]		x [p]		x		x		x				x	x	
Blood sample for cryopreserved PBMCs [f, q]		x				x		x						
Blood sample for RNA analysis [f, r]		x				x		x					x	
Blood sample for WGS (optional) [f, s]		x												
Randomization [t]	x													
AT-527 (or placebo) administration [u]		x	x	x	x	x								
Telephone Call										x				x
Concomitant medications [f, v]	x	x										x	x	x
Adverse events [f, w]	x	x										x	x	x

COVID-19 = coronavirus disease 2019; ET = early termination; PBMC = peripheral blood mononuclear cell; PK = pharmacokinetic; PRO = patient-reported outcome; RT-PCR = reverse-transcription polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; UV = unscheduled visit; WGS = whole genome sequencing.

Notes: On treatment days (Days 1–5), all assessments should be performed prior to dosing, unless otherwise specified.

[a] Screening assessments may be performed up to 72 hours prior to Day 1 or on Day 1.

[b] An unscheduled visit represents a visit that is not specified by the protocol but is determined to be necessary by the investigator or Sponsor (e.g., for evaluation of an adverse event). Assessments (including PK sample collection) should be performed as clinically indicated. For unscheduled visits occurring after Day 7, PK sample collection is not required.

[c] Patients who discontinue study drug prematurely will continue to complete assessments as indicated until the end of the study. Patients who discontinue from study participation will have an early termination visit 7 (± 2) days after their final dose of study drug. Patients who discontinue from the study after Day 14 will not be required to complete an early termination visit.

[d] Required follow-up information will be collected via telephone call at Day 14 (± 2 days) and Day 33 (±3 days).

[e] Informed consent must be documented before any study-specific screening procedure is performed.

[f] For patients at participating sites who have provided written or electronic informed consent to participate in mobile nursing visits, this assessment or procedure may be performed by a trained nursing professional at the patient's home.

[g] COVID-19 Symptom Diary includes questions regarding symptoms (Items 1–14) and the Patient Global Impression of Severity. Questionnaires will be self-administered before the patient receives any information on disease status, prior to the performance of non-PRO assessments, and prior to the administration of study treatment. On Day 1, the first diary questionnaire should be completed prior to the first dose. On Days 1–14, the questionnaire (including recording of temperature) should be administered twice a day (morning and evening) at the same time each day.

[h] On Days 15–28, the questionnaire (including recording of temperature) should be completed once a day in the evening at the same time each day.

[i] Includes oxygen saturation (pulse oximetry), respiratory rate, pulse rate, and systolic and diastolic blood pressure while the patient is in a supine or seated position, and temperature.

[j] Includes evaluation of the head, eyes, ears, nose, and throat, and the cardiovascular, dermatologic, musculoskeletal, respiratory, gastrointestinal, genitourinary, and neurologic systems.

[k] Perform a limited, symptom-directed examination as clinically indicated.

[l] Hematology includes WBC count, RBC count, hemoglobin, hematocrit, platelet count, and differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes, other cells).

[m] Chemistry panel (serum or plasma) at screening includes bicarbonate or total carbon dioxide (if considered standard of care for the region), sodium, potassium, chloride, glucose, BUN or urea, creatinine, *creatine clearance (Cockcroft-Gault formula),* total protein, albumin, phosphate, calcium, magnesium, total and direct bilirubin, ALP, ALT, AST, triglycerides, cholesterol, amylase and lipase. On all other study days, chemistry panel (serum or plasma) includes bicarbonate or total carbon dioxide (if considered standard of care for the region), sodium, potassium, chloride, glucose, BUN or urea, creatinine, total protein, albumin, phosphate, calcium, magnesium, total and direct bilirubin, ALP, ALT, AST, urate, CK (isoenzyme analysis if CK elevated), cardiac troponins, BNP, triglycerides, cholesterol, amylase, LDH, PCT, and lipase.

[n] All women of childbearing potential will have a pregnancy test *(urine or serum)* at screening.

[o] Nasopharyngeal swab samples should be collected at screening and once per day on Days 1, 3, 5, and 7. Swab samples should be collected from both nostrils at all timepoints. Screening samples will be used for point-of-care RT-PCR or rapid antigen testing to determine eligibility.

[p] Sample must be collected prior to dosing.

[q] Samples for PBMC may not be collected at some timepoints if sample stability requirements for processing will not be met. Please refer to the laboratory manual for more detail. A plasma sample will be generated from the blood sample obtained following PBMC processing and will be stored for biomarker research. No additional blood draw is needed.

[r] Collection of blood sample for RNA analysis using PAXgene tubes.

[s] Not applicable for a site that has not been granted approval for WGS. If the sample is not collected on Day 1 for any reason, it may be collected at any later timepoint.

[t] Randomization will be performed as soon as possible after screening and within 72 hours.

[u] First dose on Day 1 must be administered as soon as possible after randomization and within 24 hours. For BID regimens, AT-527 (or placebo), should be taken approximately 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 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 8 hours between doses). For these patients, the 10th dose will be taken in the evening of Day 5. For other dose regimens see Pharmacy manual for details of dosing instruction.

[v] Medication (e.g., prescription drugs, over-the-counter drugs, vaccines, herbal or homeopathic remedies, nutritional supplements) used by a patient in addition to protocol-mandated treatment from 7 days (12 months for COVID-19 vaccines) prior to initiation of study drug until the end of study follow-up telephone call on Day 33.

[w] After informed consent has been obtained but prior to initiation of study drug, only serious adverse events caused by a protocol-mandated intervention should be reported. After initiation of study drug, all adverse events will be reported until the end of study follow-up telephone call on Day 33.

Appendix 3
Schedule of Activities for Cohorts E

	Screening [a]	Treatment					Monitoring					UV [b]	ET [c]	Follow-up
Day	–3 to 1	1	2	3	4	5	6	7	8–13	14 [d]	15–28			33 [d] (±3)
Informed consent	x [e, f]													
Demographic data [f]	x													
Medical history and baseline conditions [f]	x													
Pre-existing symptoms assessment	x													
COVID-19 Symptom Diary [g]		x	x	x	x	x	x	x	x	x	x [h]			
Vital signs, including oxygen saturation [f, i]	x	x		x		x		x		x		x	x	
Weight [f]	x													
Height [f]	x													
Complete physical examination [f, j]	x									x				
Limited physical examination [f, k]		As clinically indicated										x		
Single ECG	x					x						x		
Hematology [f, l]	x	x		x				x				x		
Chemistry [f, m]	x	x		x				x				x		
Coagulation (INR, aPTT, PT, D-dimer, and fibrinogen)		x		x				x						
Pregnancy test [f, n]	x													
Nasopharyngeal swab sample for COVID-19 virology [f, o]	x	x [p]		x		x		x		x		x	x	

Appendix 3: Schedule of Activities for Cohort E

	Screening [a]	Treatment					Monitoring					UV [b]	ET [c]	Follow-up
Day	−3 to 1	1	2	3	4	5	6	7	8–13	14 [d]	15–28			33 [d] (±3)
Nasosorption™ sample for biomarkers [f]		x		x		x		x		x				
Saliva sample for COVID-19 virology [f]		x [p]		x		x		x		x		x	x	
Serum sample for biomarkers, anti-SARS-COV-2 antibodies, and viral RNA [f]		x [p]						x		x		x	x	
Blood sample for cryopreserved PBMCs [f, q]		x						x						
Blood sample for RNA analysis [f, r]		x						x						
Blood sample for WGS (optional) [f, s]		x												
Randomization [t]	x													
AT-527 (or placebo) administration [u]		x	x	x	x	x								
Telephone Call														x
Concomitant medications [f, v]	x	x										x	x	x
Adverse events [f, w]	x	x										x	x	x

COVID-19 = coronavirus disease 2019; ET = early termination; PBMC = peripheral blood mononuclear cell; PK = pharmacokinetic; PRO = patient-reported outcome; RT-PCR = reverse-transcription polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; UV = unscheduled visit; WGS = whole genome sequencing.

Notes: On treatment days (Days 1–5), all assessments should be performed prior to dosing, unless otherwise specified.

[a] Screening assessments may be performed up to 72 hours prior to Day 1 or on Day 1.

[b] An unscheduled visit represents a visit that is not specified by the protocol but is determined to be necessary by the investigator or Sponsor (e.g., for evaluation of an adverse event). Assessments (including PK sample collection) should be performed as clinically indicated. For unscheduled visits occurring after Day 7, PK sample collection is not required.

[c] Patients who discontinue study drug prematurely will continue to complete assessments as indicated until the end of the study. Patients who discontinue from study participation will have an early termination visit 7 (± 2) days after their final dose of study drug. Patients who discontinue from the study after Day 14 will not be required to complete an early termination visit.

[d] Required follow-up information will be collected at Day 14 (± 2 days). and Day 33 (±3 days). Patients in Cohort E are required to have an in-person visit for nasopharyngeal swab collection.

[e] Informed consent must be documented before any study-specific screening procedure is performed.

[f] For patients at participating sites who have provided written or electronic informed consent to participate in mobile nursing visits, this assessment or procedure may be performed by a trained nursing professional at the patient's home.

[g] COVID-19 Symptom Diary includes questions regarding symptoms (Items 1–14) and the Patient Global Impression of Severity. Questionnaires will be self-administered before the patient receives any information on disease status, prior to the performance of non-PRO assessments, and prior to the administration of study treatment. On Day 1, the first diary questionnaire should be completed prior to the first dose. On Days 1–14, the questionnaire (including recording of temperature) should be administered twice a day (morning and evening) at the same time each day.

[h] On Days 15–28, the questionnaire (including recording of temperature) should be completed once a day in the evening at the same time each day.

[i] Includes oxygen saturation (pulse oximetry), respiratory rate, pulse rate, and systolic and diastolic blood pressure while the patient is in a supine or seated position, and temperature.

[j] Includes evaluation of the head, eyes, ears, nose, and throat, and the cardiovascular, dermatologic, musculoskeletal, respiratory, gastrointestinal, genitourinary, and neurologic systems.

[k] Perform a limited, symptom-directed examination as clinically indicated.

[l] Hematology includes WBC count, RBC count, hemoglobin, hematocrit, platelet count, and differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes, other cells).

[m] Chemistry panel (serum or plasma) at screening includes bicarbonate or total carbon dioxide (if considered standard of care for the region), sodium, potassium, chloride, glucose, BUN or urea, creatinine, creatinine clearance (Cockcroft-Gault formula), total protein, albumin, phosphate, calcium, magnesium, total and direct bilirubin, ALP, ALT, AST, triglycerides, cholesterol, amylase and lipase. On all other study days, chemistry panel (serum or plasma) includes bicarbonate or total carbon dioxide (if considered standard of care for the region), sodium, potassium, chloride, glucose, BUN or urea, creatinine, total protein, albumin, phosphate, calcium, magnesium, total and direct bilirubin, ALP, ALT, AST, urate, CK (isoenzyme analysis if CK elevated), cardiac troponins, BNP, triglycerides, cholesterol, amylase, LDH, PCT, and lipase.

[n] All women of childbearing potential will have a pregnancy test (urine or serum) at screening.

[o] Nasopharyngeal swab samples should be collected at screening and once per day on Days 1, 3, 5, and 7. Swab samples should be collected from both nostrils at all timepoints. Screening samples will be used for point-of-care RT-PCR or rapid antigen testing to determine eligibility.

[p] Sample must be collected prior to dosing.

[q] Samples for PBMC may not be collected at some timepoints if sample stability requirements for processing will not be met. Please refer to the laboratory manual for more detail. A plasma sample will be generated from the blood sample obtained following PBMC processing and will be stored for biomarker research. No additional blood draw is needed.

[r] Collection of blood sample for RNA analysis using PAXgene tubes.

[s] Not applicable for a site that has not been granted approval for WGS. If the sample is not collected on Day 1 for any reason, it may be collected at any later timepoint.

[t] Randomization will be performed as soon as possible after screening and within 72 hours.

[u] First dose on Day 1 must be administered as soon as possible after randomization and within 24 hours. For BID regimens, AT-527 (or placebo), should be taken approximately 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 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 8 hours between doses). For these patients, the 10th dose will be taken in the evening of Day 5. For other dose regimens see Pharmacy manual for details of dosing instruction.

[v] Medication (e.g., prescription drugs, over-the-counter drugs, vaccines, herbal or homeopathic remedies, nutritional supplements) used by a patient in addition to protocol-mandated treatment from 7 days (12 months for COVID-19 vaccines) prior to initiation of study drug until the end of study follow-up telephone call on Day 33.

[w] After informed consent has been obtained but prior to initiation of study drug, only serious adverse events caused by a protocol-mandated intervention should be reported. After initiation of study drug, all adverse events will be reported until the end of study follow-up telephone call on Day 33.

Appendix 4
Schedule of Pharmacokinetic Samples

<table>
<tr><td></td><td>Cohort A</td><td colspan="3">Cohorts B–E</td></tr>
<tr><td></td><td>BID Regimen</td><td>BID Regimen</td><td>TID Regimen</td><td>QD Regimen</td></tr>
<tr><td rowspan="2">Day 1</td><td>Predose [a, c]</td><td>Predose [a, c]</td><td>Predose [a, c]</td><td>Predose [a, c]</td></tr>
<tr><td>1 and 3 hours postdose [b, c]</td><td>1 and 4 hours postdose [b, c]</td><td>1 hour postdose [b, c]
Predose [with reference to second dose of the day] [a, c]</td><td>1 and 4 hours postdose [b, c]</td></tr>
<tr><td>Day 3</td><td>Predose [a, c]</td><td>Predose [a, c]</td><td>Predose [a, c]</td><td>Predose [a, c]</td></tr>
<tr><td rowspan="2">Day 5</td><td rowspan="2">Predose [a, c] and 3 hours postdose [b, c]</td><td>Predose [a, c]</td><td>Predose [a, c]</td><td>Predose [a, c]</td></tr>
<tr><td>1 and 4 hours postdose [b, c]</td><td>1 hour postdose [b, c]
Predose [with reference to second dose of the day] [a, c]</td><td>1 and 4 hours postdose [b, c]</td></tr>
<tr><td></td><td></td><td colspan="3"></td></tr>
<tr><td>Day 7</td><td>48 (±1) hours [c, e]</td><td>Anytime [c, d]</td><td>Anytime [c, d]</td><td>Anytime [c, d]</td></tr>
<tr><td></td><td></td><td colspan="3"></td></tr>
<tr><td>Early termination</td><td>—</td><td colspan="3">Sample to be collected if early termination occurs on or before Day 7</td></tr>
</table>

BID = twice a day; QD = once a day; TID = three times a day.

Note: Unless otherwise specified (TID regimen), all timepoints are in relation to the first dose administered on a given day.

[a] Predose sampling window: within 30 minutes prior to dose administration.

[b] Postdose sampling window: ±30 minutes from scheduled sampling time.

[c] For patients at participating sites who have provided written or electronic informed consent to participate in mobile nursing visits, this assessment or procedure may be performed by a trained nursing professional at the patient's home.

[d] The exact sampling time will be captured in relation to the time of last (10^{th}) dose.

[e] In Cohort A, the 48-hour PK sampling on Day 7 is with reference to the first dose on Day 5.

Appendix 5
COVID-19 Symptom Diary
and Patient Global Impression of Severity

<u>Do not reproduce or distribute</u>. The Sponsor will provide sites with all instruments to be completed in this study.

Note that the following is a sample. Sites will be provided with separate versions of the diary for Days 1–14 (morning vs. evening, including recording of temperature) and Days 15–28 (evening, including recording of temperature).

Today's date: _____ Time completing: _____

<u>COVID-19 Evening Symptom Diary</u>

This diary will keep track of your COVID-19 symptoms and temperature during the study. Please complete this diary before you go to bed each evening. For each symptom, please tick the box that best describes your experience.

Please rate the severity of each symptom at its worst since you got up this morning.

	None	Mild	Moderate	Severe
1. Nasal congestion or runny nose				
2. Sore throat				
3. Cough				
4. Shortness of breath (difficulty breathing)				
5. Aches and pains				
6. Fatigue (tiredness)				
7. Headache				
8. Chills/Sweats				
9. Feeling hot or feverish				
10. Nausea (wanting to throw up)				
11. Vomiting (thrown up)				
12. Diarrhea (mostly or completely liquid bowel movements)				

	Same as usual / no change	Not as good as usual / no sense
13. Rate your sense of **smell** since you got up this morning.		
14. Rate your sense of **taste** since you got up this morning.		

Patient Global Impression of Severity

Please rate your overall COVID-19 symptoms at their worst since you got up this morning.

☐ None

☐ Mild

☐ Moderate

☐ Severe

Appendix 6
Update on AT-527 Reproductive Toxicity, In Vitro, *and Clinical* Drug–Drug Interaction Data

New data from reproductive toxicity studies have become available since the release of the AT-527 Investigator's Brochure Version 7. The new data are summarized below. Overall, these new data did not provide new safety concerns and support further development including the planned Phase 3 clinical trial.

In vitro *drug-drug interaction (DDI)* flags for AT-527 with potential clinical relevance *as well as preliminary data from clinical DDI studies* were taken into account in the concomitant therapy section.

REPRODUCTIVE TOXICITY

The potential for reproductive toxicity was assessed in dose range-finding and pivotal Good Laboratory Practice (GLP)-compliant embryofetal development studies in rats and rabbits and in a GLP-compliant fertility study in the rat.

EMBRYOFETAL DEVELOPMENT

Definitive GLP-compliant embryofetal development (EFD) studies did not reveal evidence of teratogenicity or embryofetal toxicity up to the highest maternal dose level tested of 1000 mg/kg/day in rats and 100 mg/kg/day in rabbits.

Administration of AT-527 by oral gavage to female rats at dose levels of 250, 500, and 1000 mg/kg/day once daily from gestation day (GD) 7 to GD17 in an initial dose range-finding study and in a subsequent definitive study induced no maternal or developmental toxicity at any dose level.

In a dose range finding EFD study in pregnant rabbits, the two highest dose groups of 500 and 250 mg/kg/day were terminated prematurely due to severe maternal toxicity. The low dose level of 125 mg/kg/day was tolerated, but induced maternal toxicity characterized by apparent reductions of food consumption, minimal body weight loss and slightly reduced gravid uterine weight. There were no external fetal abnormalities at 125 mg/kg/day and resorption incidence was not noticeably increased, but fetal weight was reduced by 10%, which was secondary to maternal toxicity.

In a definitive GLP-compliant EFD study in pregnant rabbits, AT-527 was administered by once daily oral gavage to time-mated female New Zealand White rabbits at 25, 50, or 100 mg/kg/day from GD 7 through GD 19. The highest dose level of 100 mg/kg/day was above the maximum maternal tolerated dose, characterized by marked reductions in food consumption, body weight loss in the majority of rabbits and 3 associated abortions. Reductions in maternal food consumption and body weight gain were also noted at both lower dose levels of 50 and 25 mg/kg/day. Maternal administration of AT-527 did not affect embryofetal viability or fetal body weights at any dose level. There were no

AT-527-related fetal abnormalities at any dose level. Therefore, the developmental no-adverse-effect level (NOAEL) for AT-527 was 100 mg/kg/day.

FERTILITY AND EARLY EMBRYONIC DEVELOPMENT (FEED)

In a GLP-compliant FEED study, AT-527 was given to male and female rats by daily oral gavage at dose levels of 250, 500 and 1000 mg/kg/day. Males were treated for 4 weeks and females for 2 weeks before pairing. For females dosing stopped on GD6 and they were submitted to C-section on GD13. Males were dosed for at least 7 weeks prior to necropsy. Administration of AT-527 reduced body weights and body weight gains in males at 1000 mg/kg/day and caused increased incidence of abnormal breathing sounds at 500 and 1000 mg/kg/day. There were no AT-527-related effects on mating, fertility, or reproductive organ weights in males or females, estrous cycling and ovarian and uterine parameters in females, or male reproductive assessments (sperm motility or concentration) in males. Therefore, the NOAEL for AT-527 for general toxicity, reproductive performance, and early embryonic development was 1000 mg/kg/day, the highest dose tested.

Exposures for AT-511 (Free Base Form of AT-527) and Its Major Metabolites AT-551, AT-229 and AT-273 at No-Adverse Effect Level Doses from Pivotal Reproductive Toxicity Studies

	Pivotal EFD rat 1000 mg/kg/day		Pivotal EFD rabbit 100 mg/kg/day	
	GD17 AUC_{tlast} (hr*ng/mL)	Multiple to human for 550 mg bid/ 1100 mg bid	GD19 AUC_{tlast} (hr*ng/mL)	Multiple to human for 550 mg bid/ 1100 mg bid
AT-511	145	0.02/0.01	4080	0.5/0.3
AT-551	135000	26/13	33800	7/3
AT-229	160000	10/5	70100	4/2
AT-273	28600	5/2	14300	2/1

AUC = area under the concentration–time curve; BID = twice a day; EFD = embryofetal development; GD = gestation day.

Anticipated human exposures at 550 mg bid are an AUC of 7503 hr*ng/mL for AT-511, 5134 hr*ng/mL for AT-551, 16154 hr*ng/mL for AT-229 and 6136 hr*ng/mL for AT-273.

Anticipated human exposures at 1100 mg bid are an AUC of 15006 hr*ng/mL for AT-511, 10268 hr*ng/mL for AT-551, 32308 hr*ng/mL for AT-229 and 12631 hr*ng/mL for AT-273.

All studies were performed in the United States of America with bioanalysis and toxicokinetic evaluations performed in Canada, both countries members of the Organisation for Economic Co-operation and Development (OECD) Mutual Acceptance

of Data (MAD) program. All definitive reproductive toxicity studies were performed in compliance with OECD GLP Regulations.

IN VITRO DRUG-DRUG INTERACTION

The parent compound AT-511 and metabolites AT-551, AT-229 and AT-273 were characterized in vitro to determine the potential of CYP450 and transporter related victim and perpetrator drug-drug interaction (DDI). In vitro DDI flags with potential clinical relevance were taken into account in the concomitant therapy section.

AT-511 as the parent drug is a substrate of P-gp, and likely not a substrate of BCRP. The breakdown of AT-511 is not CYP450 dependent, and several catabolism/metabolism processes have been postulated and are presented in the most recent version of the Investigator Brochure [v7].

The metabolites AT-551 and AT-273 are not substrates of P-gp or BCRP, metabolite AT-229 is a substrate of BCRP but not P-gp. AT-229 is not a substrate of a renal transporter (Organic Anion Transporter 1 [OAT1] and 3 [OAT3], Organic Cation Transporter 2 [OCT2], Multidrug and Toxin Extrusion protein 1 [MATE1] and 2-K [MATE2-K]) while AT-273 is a substrate of OAT1 and OAT3 but not OCT2, MATE1, MATE2-K.

The in vitro potential of the parent drug AT-511 and the metabolites (AT-551, AT-229 and AT-273) for perpetrator DDI against drug transporters (P-gp, BCRP, Organic Anion Transporting Polypeptide 1B1 [OATP1B1] and 1B3 [OATP1B3], OAT1, OAT3, OCT2, MATE1 and MATE2-K) and CYP450's (1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A) has been characterized.

AT-511 is an in vitro inhibitor of P-gp, BCRP and OATP1B1 with IC50 values below the estimated gut concentration (550 mg dose/intake water 250 mL) and for which a DDI risk may be anticipated.

The in vitro reversible/time-dependent inhibition and induction potential of the parent drug AT-511 on CYP3A4 is likely indicated, however, the impact of the perpetrator potential on net enzyme DDI effects in liver and intestine remains uncertain. The direct inhibition IC50 values of AT-511 on CYP2C8 and CYP2C19 in vitro are much higher than the unbound systemic exposure in the historical clinical studies, and no additional CYP related in DDI risks were identified.

For metabolites AT-551, AT-229 and AT-273, no or weak perpetrator DDI risk for transporters and CYPs is anticipated based on in vitro data and the static perpetrator DDI risk assessment results.

CLINICAL DRUG-DRUG INTERACTION STUDIES

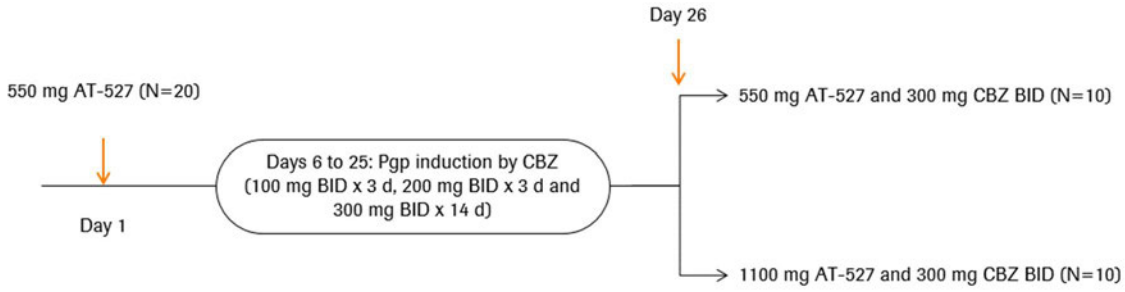
Preliminary data are available from 3 clinical studies, namely AT-03A-004, AT-03A-005 and AT-03A-009, and are summarized below.

Study AT-03A-004: A Phase 1 Study Assessing the Effect of Carbamazepine, a P-Glycoprotein Inducer, on the Pharmacokinetics of AT-527 in Healthy Adult Subjects.

Design elements

This is a single center, open-label DDI study with 3 Periods. In Periods 1 and 2, healthy subjects (n=20) undergo sequential treatments. Subjects who complete Periods 1 and 2 are subsequently be randomized to one of two treatments in Period 3.

The below figure presents an overview of the study design.



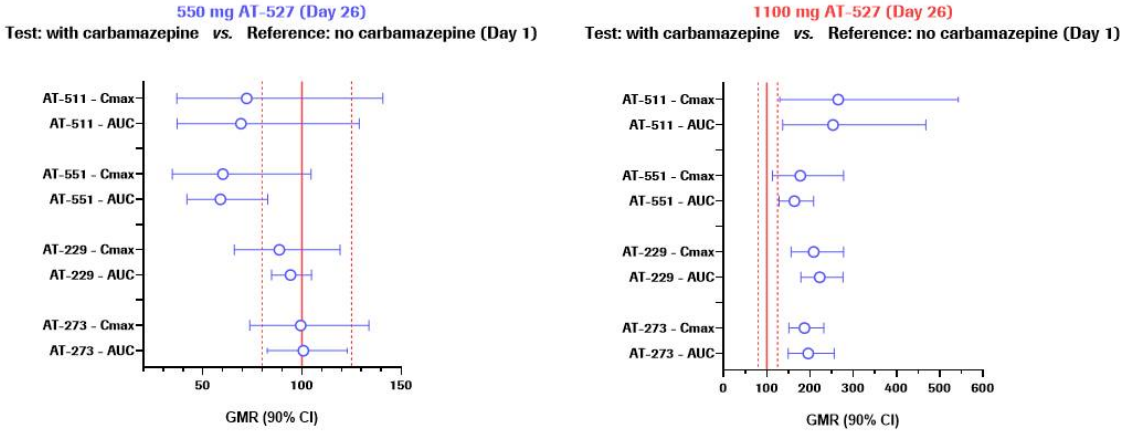
Period 1 (AT-527) (n=20). Day 1: AT-527 550 mg (1x550 mg tablet) in the morning. Days 2 to 5: No treatment for 4 days.
BID: twice a day, CBZ: Carbamazepine, PgP: P-Glycoprotein.

Period 2 (dose titration with carbamazepine) (n=20). Days 6 to 8 (3 days): carbamazepine 100 mg BID (1×100 mg tablet BID, in the morning and 12 hours later). Days 9 to 11 (3 days): carbamazepine 200 mg BID (2×100 mg tablets BID, in the morning and 12 hours later). Days 12 to 25 (14 days): carbamazepine 300 mg BID (3×100 mg tablets BID, in the morning and 12 hours later).

Period 3 (AT-527 + carbamazepine). Day 26: AT-527 550 mg (1×550 mg tablet, in the morning) + carbamazepine 300 mg BID (3×100 mg tablets BID, in the morning and 12 hours later) (n=10), or Day 26: AT-527 1100 mg (2×550 mg tablets, in the morning) + carbamazepine 300 mg BID (3×100 mg tablets BID, in the morning and 12 hours later) (n=10).

Preliminary results

The below figure presents the changes in pharmacokinetic parameters (C_{max} and AUC) for AT-511 and metabolites in the presence of the co-administered drug.



Legend to the figure. AUC: area under the plasma concentration-time curve, CI: confidence interval, C_{max}: maximum plasma concentration, GMR: geometric mean ratio.

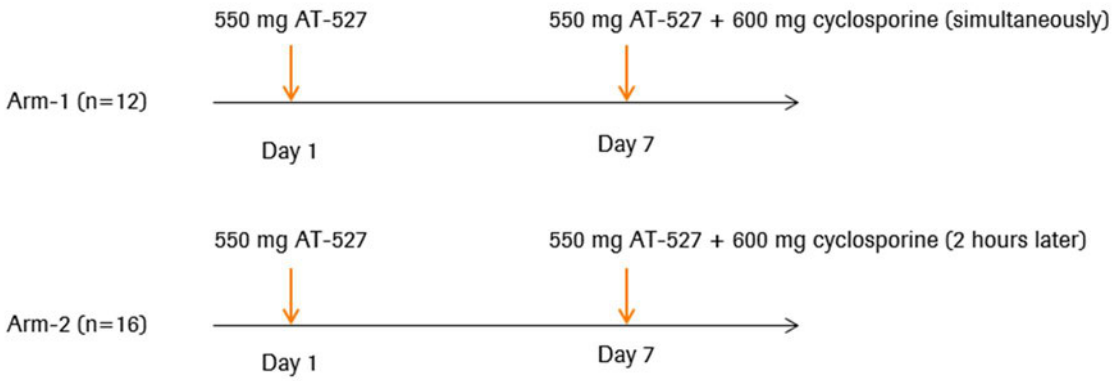
On Day 26, when 550 mg AT-527 was co-administered with carbamazepine, the exposure of AT-511 and AT-551 was reduced by about 30% and 40%, respectively. The exposure of AT-229 and AT-273 was not affected. This indicates that AT-527 can be administered with a strong P-gp inducer without dose adjustment.

On Day 26, when 1100 mg AT-527 was co-administered with carbamazepine, the exposure of AT-511 and metabolites essentially doubled. A single dose of 1100 mg AT-527 can mitigate the DDI risk associated with Pgp induction and can be administered with a strong Pgp inducer without dose adjustment.

Study AT-03A-005: A Phase 1, Randomized, Open-Label Study to Evaluate the Effect of Cyclosporine on the Plasma Pharmacokinetics of AT-527 in Healthy Adult Male Subjects.

Design elements

This is a single center, open-label, 2-arm study. The below figure presents an overview of the study design.



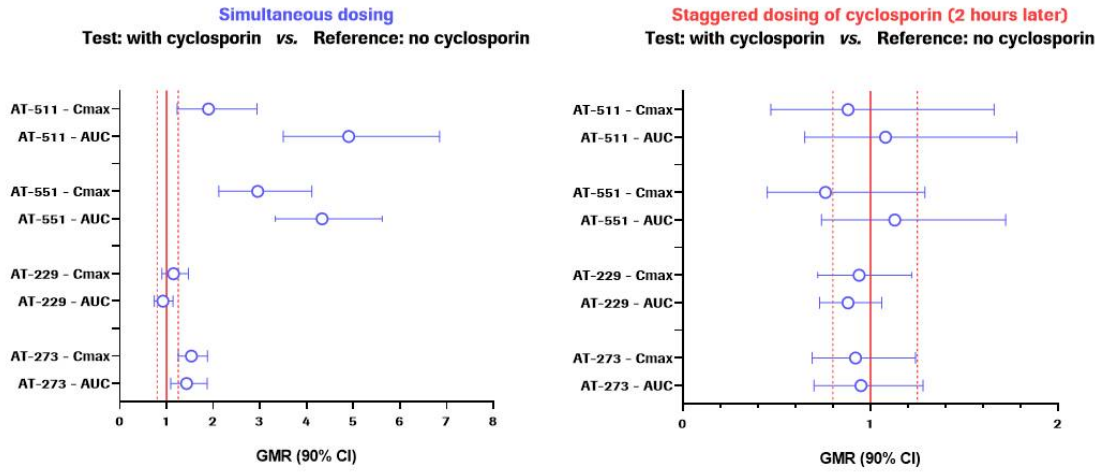
Arm-1 (n=12): Simultaneous dosing. Day 1: A single 550 mg dose of AT-527 (1×550 mg tablet) is administered to subjects. Day 7: A single 550 mg dose of AT-527 (1x550 mg tablet) and a single 600 mg dose of cyclosporine (6×100 mg capsules) is co-administered to subjects.

Arm-2 (n= 12): Staggered dosing. Day 1: A single 550 mg dose of AT-527 (1×550 mg tablet) is administered to subjects. Day 7: A single 550 mg dose of AT-527 (1×550 mg tablet) is administered to subjects. Then, a single 600 mg dose of cyclosporine (6×100 mg capsules) is administered approximately 2 hours later.

Preliminary results

The below figure presents the changes in pharmacokinetic parameters (C_{max} and AUC) for AT-511 and metabolites in the presence of the co-administered drug (left:

simultaneous dosing of cyclosporine [Arm-1], right: staggered dosing of cyclosporine [Arm-2]).



Legend to the figure. AUC: area under the plasma concentration-time curve, CI: confidence interval, Cmax: maximum plasma concentration, GMR: geometric mean ratio.

In Arm-1, the C_{max} of AT-511 and AT-551 doubled and tripled, respectively. The AUC of AT-511 and AT-551 increased by 5-fold and 4-fold, respectively. The exposure of AT-229 was not affected, and that of AT-273 increased by 50%.

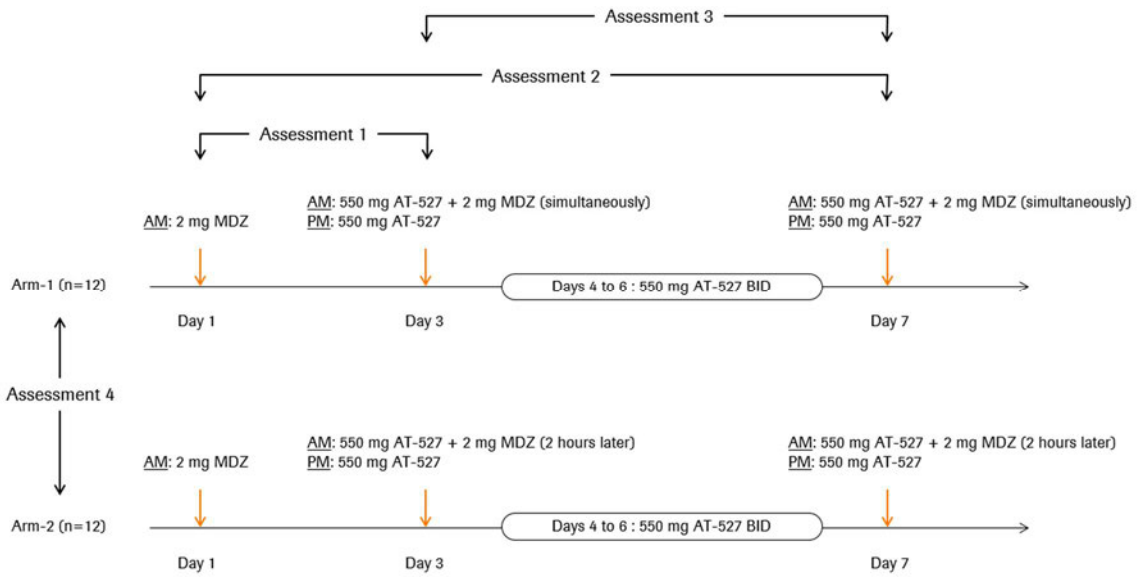
In Arm-2, a single dose of cyclosporine administered 2 hours after AT-527 did not affect the exposure of AT-511 and metabolites.

These data indicate that the substantial DDI risk associated with Pgp inhibition can be effectively mitigated by staggered dosing of the inhibitor.

Study AT-03A-009: A Phase 1 Open-Label Study to Evaluate the Interaction Potential between AT-527 and Midazolam in Healthy Adult Subjects

Design elements

This is a single center, open-label, 2-arm study. The below figure presents an overview of the study design.



MDZ: midazolam

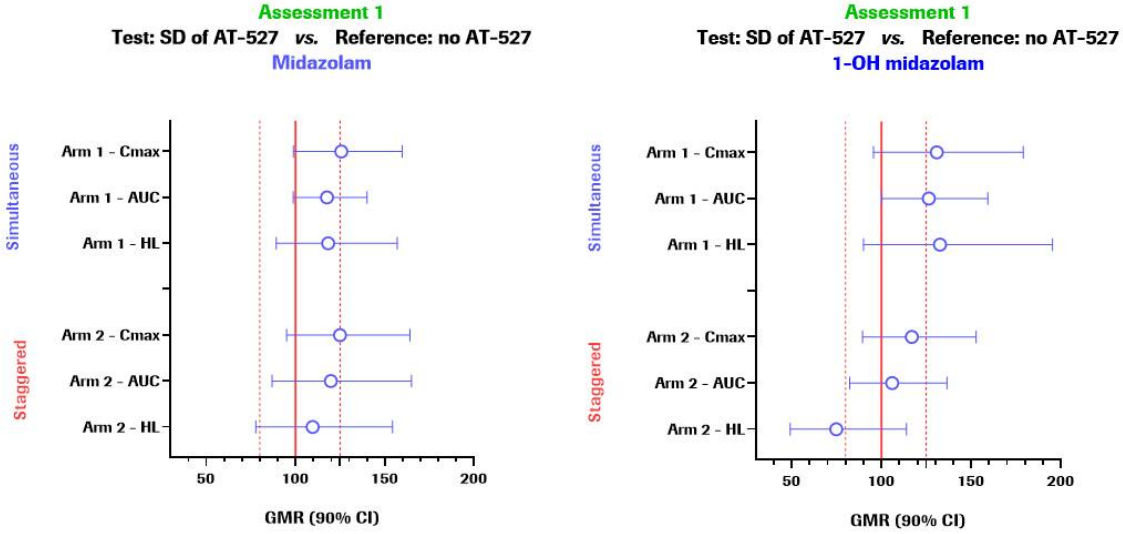
Arm-1 (n=12). Day 1: 2 mg midazolam (1 mL) is administered in the morning. Day 3: 550 mg AT-527 and 2 mg midazolam (1 mL) are co-administered (within 1 minute apart) together in the morning and a second 550 mg AT-527 dose is administered in the evening, approximately 12 hours after the morning dose. Days 4 to 6: 550 mg AT-527 is administered to subjects twice daily (BID), once in the morning and once in the evening, approximately 12 hours apart. Day 7: 550 mg AT-527 and 2 mg midazolam (1 mL) are co-administered (within 1 minute apart) in the morning and a second 550 mg AT-527 dose is administered in the evening, approximately 12 hours after the morning dose.

Arm-2 (n=12). Day 1: 2 mg midazolam (1 mL) is administered in the morning. Day 3: 550 mg AT-527 is administered in the morning then a 2 mg midazolam dose (1 mL) is administered 2 hours (±5 minutes) later (at approximately the same time when midazolam was administered in Day 1). A second 550 mg AT-527 dose is administered in the evening, approximately 12 hours after the AT-527 morning dose. Days 4 to 6: 550 mg AT-527 is administered to subjects BID, in the morning and in the evening, approximately 12 hours apart. Day 7: 550 mg AT-527 is administered in the morning

then a 2 mg midazolam dose (1 mL) is administered 2 hours (±5 minutes) later (at approximately the same time when midazolam was administered in Day 1). A second 550 mg AT-527 dose is administered in the evening, approximately 12 hours after the AT-527 morning dose.

Preliminary results

- *Assessment 1 (Day 3 is compared to Day 1). The below figure presents the changes in pharmacokinetic parameters (C_{max}, AUC, and half-life) for midazolam (left) and 1-hydroxy-midazolam (right) in the presence of AT-527 (single dose of 550 mg). In each forest plot, the changes in pharmacokinetic parameters are presented after the simultaneous (Arm-1; top) and staggered (Arm-2; bottom) administration of midazolam.*

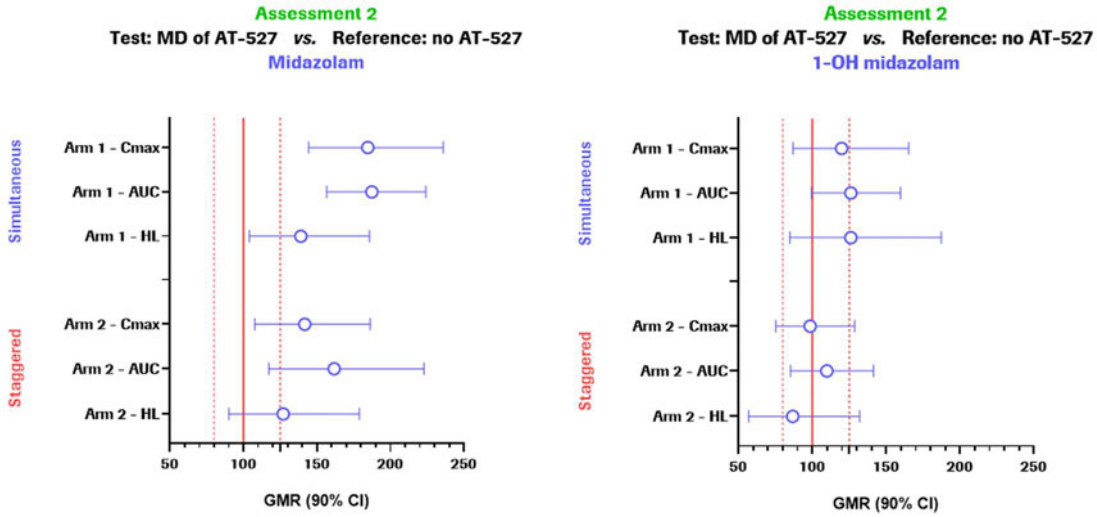


Legend to the figure. AUC: area under the plasma concentration-time curve, CI: confidence interval, Cmax: maximum plasma concentration, HL: half-life, SD: single dose, 1-OH midazolam: 1-hydroxy midazolam, GMR: geometric mean ratio.

A single dose of AT-527 increased the exposure of midazolam by 20–25% after simultaneous or staggered dosing. The exposure of 1-hydroxy midazolam was slightly increased by 25–30% after simultaneous dosing, and minimally increased by 5–15% after staggered dosing.

- *Assessment 2 (Day 7 is compared to Day 1). The below figure presents the changes in pharmacokinetic parameters (C_{max}, AUC, and half-life) for midazolam (left) and 1-hydroxy-midazolam (right) in the presence of AT-527 (multiple doses of 550 mg). In each forest plot, the changes in pharmacokinetic parameters are presented after*

the simultaneous (Arm-1; top) and staggered (Arm-2; bottom) administration of midazolam.

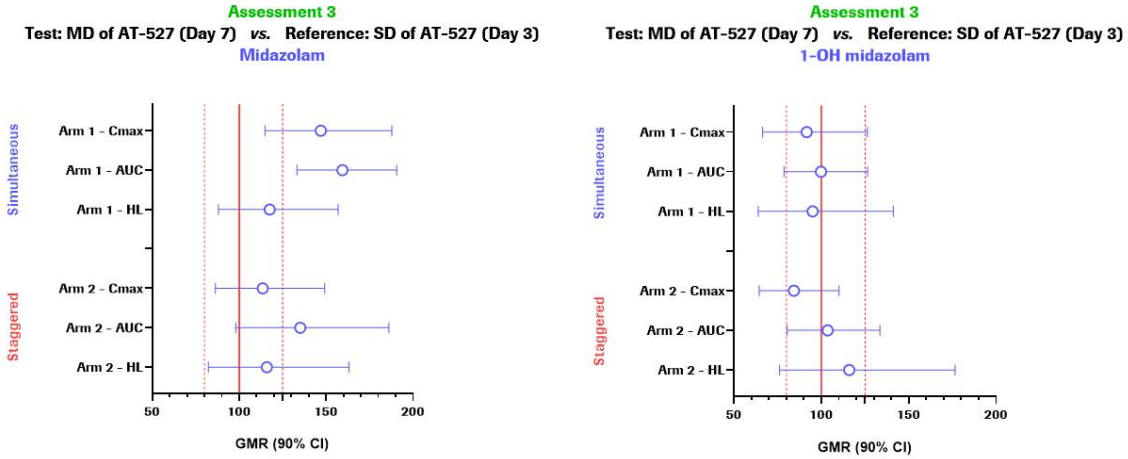


Legend to the figure. AUC: area under the plasma concentration-time curve, CI: confidence interval, Cmax: maximum plasma concentration, HL: half-life, SD: single dose, 1-OH midazolam: 1-hydroxy midazolam, GMR: geometric mean ratio.

Multiple doses of AT-527 increased the plasma exposure of midazolam by 90% (simultaneous dosing) and by 60% (staggered dosing), and had a slight to minimal effect on 1-hydroxy midazolam (an increase of 20–25% after simultaneous dosing, and an increase of 0–10% after staggered dosing).

- *Assessment 3 (Day 7 is compared to Day 3). The below figure presents the changes in pharmacokinetic parameters (C_{max}, AUC, and half-life) for midazolam (left) and 1-hydroxy-midazolam (right) in the presence of AT-527 (single dose of AT-527 on Day 3, and multiple dose of AT-527 on Day 7). In each forest plot, the changes in*

pharmacokinetic parameters are presented after the simultaneous (Arm-1; top) and staggered (Arm-2; bottom) administration of midazolam.

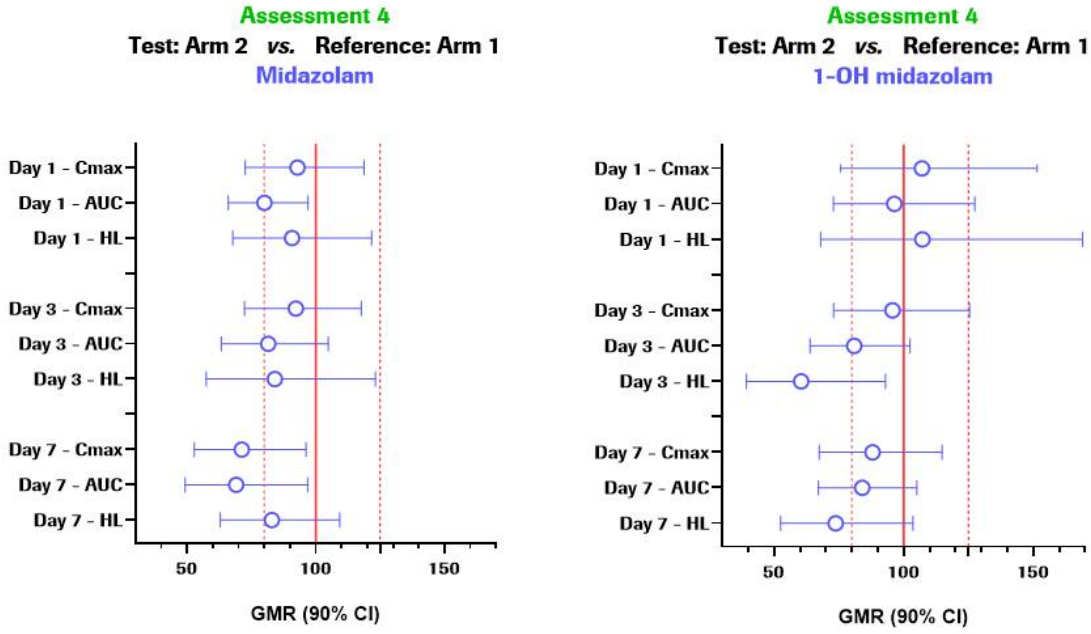


Legend to the figure. AUC: area under the plasma concentration-time curve, CI: confidence interval, Cmax: maximum plasma concentration, HL: half-life, SD: single dose, 1-OH midazolam: 1-hydroxy midazolam, GMR: geometric mean ratio.

After multiple doses of AT-527, the net effect of time dependent inhibition and induction (Day 7) over direct competitive inhibition (Day 3) on midazolam exposure was increased 45–60% (simultaneous dosing) and increased 15–35% (staggered dosing), and almost absent (0–5% increase) on 1-hydroxy midazolam.

- *Assessment 4 (Arm-2 [staggered administration of AT-527] is compared to Arm-1 [simultaneous administration of AT-527]). The below figure presents the changes in pharmacokinetic parameters (C_{max}, AUC, and half-life) for midazolam (left) and*

1-hydroxy-midazolam (right) in the absence (Day 1) or in the presence of AT-527 (single dose of AT-527 on Day 3, and multiple dose of AT-527 on Day 7).



Legend to the figure. AUC: area under the plasma concentration-time curve, CI: confidence interval, Cmax: maximum plasma concentration, HL: half-life, SD: single dose, 1-OH midazolam: 1-hydroxy midazolam, GMR: geometric mean ratio.

Day 1 (midazolam alone): the exposure of midazolam was slightly lower in Arm 2 (vs. Arm 1) by 10-20%, and was not changed for 1-hydroxy midazolam.

Day 3 (single dose of AT-527; competitive inhibition): 5–20% less effect of staggered dosing of AT-527 on the exposure of midazolam and 1-hydroxy midazolam.

Day 7 (multiple dose of AT-527; competitive plus time dependent inhibition and induction): 15–30% less effect of staggered dosing of AT-527 on the exposure of midazolam and 1-hydroxy midazolam.

Data interpretation

A single dose (simultaneous or staggered) of AT-527 increased the exposure of midazolam by 20–25%. AT-527 (AT-511) is a weak competitive inhibitor of CYP3A4.

Multiple doses of AT-527 increased the exposure of midazolam by 90 and 60% after simultaneous and staggered (30% less) dosing, respectively. This is consistent with time-dependent inhibition of CYP3A4.

Staggered dosing after single of multiple doses of AT-527 had limited effect on the PK parameters of midazolam.

The induction potential of AT-511, while confronted by a more pronounced time-dependent inhibition effect, is weak and unlikely to affect (reduce) the efficacy of hormonal contraceptives that are substrates of CYP3A4.

CYP3A4 substrates can be co-administered with AT-527 without dose adjustment. Staggered dosing of AT-527 is not warranted.