STATISTICAL ANALYSIS PLAN

STUDY 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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PLAN PREPARED BY:	[redacted], M.Sc.

STATISTICAL ANALYSIS AMENDMENT PLAN APPROVAL

Date and Time(UTC)	Reason for Signing	Name
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STATISTICAL ANALYSIS PLAN VERSION HISTORY

This SAP was developed based on Roche SAP model document updated on 26 October 2020.

SAP Version	Approval Date	Based on Protocol (Version, Approval Date)
4	see electronic date stamp on title	Version 5, 13 May 2021
3	17 June 2021	Version 5, 13 May 2021
2	04 March 2021	Version 4, 24 February 2021
1	04 February 2021	Version 2, 10 December 2020

STATISTICAL ANALYSIS PLAN AMENDMENT RATIONALE

The key change in this SAP amendment (v4) is an update to how viral RNA negativity is defined. Samples that are below the limit of quantification (BLQ) are now to be considered as negative (BLQ was previously considered to be positive). This change affects the definition of the modified intent-to-treat infected (mITTi) population and some secondary virology endpoints. This change has been detailed in each of the relevant sections.

The rationale for this change is to align the definition of negativity with other external data (e.g., Fischer et al. 2021) to ensure our findings are comparable and interpretable. Previously, the definition of negativity was based only on the qualitative result determined using cycle threshold (Ct) values. Therefore, a particular viral load threshold was not used; to ensure we have a numeric viral load threshold to define negativity, the limit of quantification (120 copies/mL) is now also considered. Samples that are qualitatively "negative" or quantitatively "BLQ" are now defined to be negative.

Key changes to the SAP, along with the rationale for each change, are summarized below.

Section	Description of Change	Rationale for Change
4	The modified intent-to-treat infected (mITTi) population definition was updated to ensure that each patient has at least one reverse-transcription polymerase chain reaction (RT-PCR) test result above or equal to the limit of quantification (LOQ)	To align with external data
5.3.5	Additional subgroups of: days from symptom onset to treatment (≤ 3 days, > 3 days); and any underlying health conditions (yes, no) have been added for the primary endpoint	To allow analyses of additional subgroups that may be of interest and/or relevance
5.4.2.1	For the time to cessation of viral shedding and time to sustained non-detectable virus RNA endpoints, receiving a negative result or a result below the limit of quantification (BLQ) now meets the condition for cessation and non-detectability, respectively	To align with external data
5.4.2.1	Updated the censoring date and time for patients that do not meet the time-to-event virology endpoints to the final timepoint on Day 7	To ensure patients remain "at-risk" to assist with the interpretation of the Kaplan-Meier plots at Day 7
5.4.2.1	For the proportion of patients positive endpoint, the definition of positivity was amended to be any RT-PCR test result above or equal to the limit of quantification	To align with external data
5.4.2.3	Modified the censoring date and time for patients that do not meet the efficacy time-to-event endpoints to the final timepoint on Day 28	To align with our handling of other time-to-event endpoints

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

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Term	Description
AEs	adverse events
AESI	adverse events of special interest
ANCOVA	analysis of covariance
AUC	area under the curve
BID	twice a day
BLQ	limit of quantification
BMI	body mass index
C_{max}	maximum concentration
CoV	coronavirus
COVID-19	coronavirus disease 2019
CSR	Clinical Study Report
EC90	90% effective concentration
EUA	emergency use authorization
HCV	hepatitis C virus
IMC	Internal Monitoring Committee
IMP	investigational medicinal product
ITT	intent-to-treat
IxRS	interactive voice or web-based response system
LLOQ	lower limit of quantification
LOQ	limit of quantification
LSM	least squares mean
mITTi	modified intent-to-treat infected
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NLME	nonlinear mixed effects
PD	pharmacodynamics
PDs	protocol deviations
PGIS	Patient Global Impression of Severity
PK	pharmacokinetic
PTs	preferred terms
RT-PCR	reverse-transcription polymerase chain reaction
SARS	severe acute respiratory syndrome
SARS-CoV-1	severe acute respiratory syndrome coronavirus-1
SARS-CoV-2	SARS coronavirus-2
SE	standard error
SOC	System Organ Class
SpO_2	peripheral capillary oxygen saturation

1. INTRODUCTION

This Statistical Analysis Plan (SAP) provides details of the planned analyses and statistical methods used to evaluate the antiviral activity, safety, pharmacokinetics, and efficacy data for Study WV43042.

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 severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1) (90% effective concentration [EC_{90}] = 0.34 μM) and SARS-coronavirus-2 (CoV-2) (mean EC_{90} = 0.5 μM). AT-527 has been evaluated in two completed clinical studies in healthy volunteers and patients infected with hepatitis C virus (HCV). In the recently 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.

Despite the recent approval of vaccines for the prevention of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection and the recent emergency use authorization (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 intravenous infusion, making them unsuitable for use in the outpatient setting. There remains, therefore, an urgent unmet medical need to develop new therapies for COVID-19.

1.1 OBJECTIVES AND ENDPOINTS

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 SpO2 • 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	• 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 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.) • 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 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

Table 1 Objectives and Corresponding Endpoints (cont.)

Secondary Objectives	Corresponding Endpoints
• To evaluate the efficacy of AT-527 compared with placebo (contd..)	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) • 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

Table 1 Objectives and Corresponding Endpoints (cont.)

Exploratory Objectives	Corresponding Endpoints
• 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 of the study protocol) and efficacy, safety, PK, or other biomarker endpoints

COVID-19=coronavirus disease 2019; NCI CTCAE=National Cancer Institute Common Terminology Criteria for Adverse Events; nsp12=non-structural protein 12; PGIS=Patient Global Impression of Severity; PK=pharmacokinetic; RT-PCR=reverse-transcription polymerase chain reaction; SARS-CoV-2=severe acute respiratory syndrome coronavirus-2; SpO_2=peripheral capillary oxygen saturation.

1.2 STUDY DESIGN

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 220 patients at approximately 52 sites 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–E will enroll 40 patients each. 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. However, they may not receive the same treatment as the patient they are replacing.

In Cohort A, patients will be randomized in a 1:1 ratio to receive either AT-527 550 mg tablets (or placebo) twice a day (BID) on Days 1–5, with assessment visits on Days 3, 5,

and 7. After 30 patients (50% enrolment) 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–E, 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–E 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 evaluating such lower doses may be initiated at any time following the Cohort A interim safety review and will not require IMC oversight.

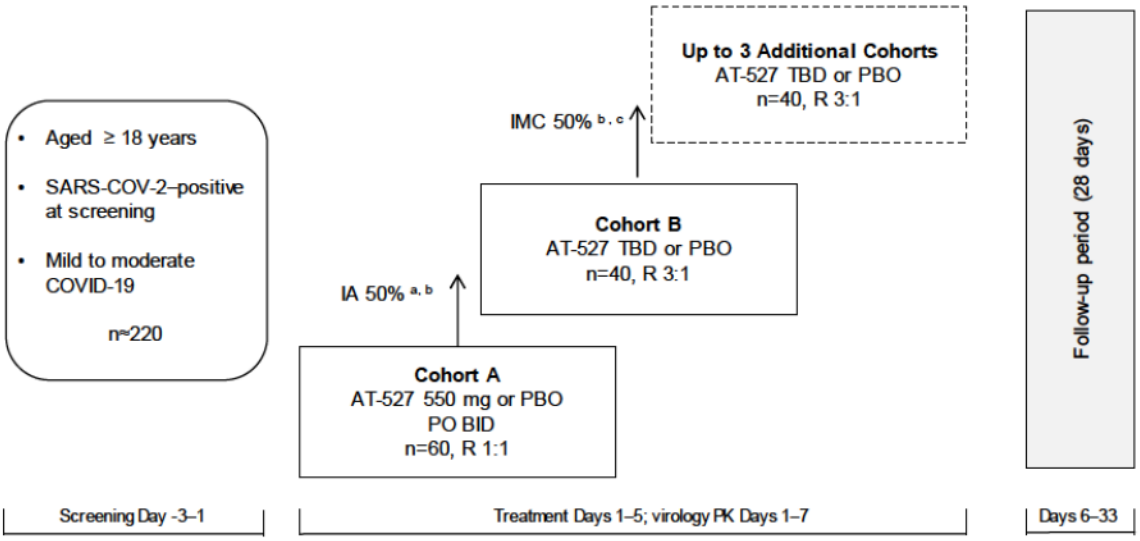
For all cohorts, visits may be conducted in clinic or by mobile nursing. Where local policies and local site logistics permit, patients may also be confined in clinic during Days 1 – 7. Where treatment occurs in the inpatient setting, the purpose of hospitalization must not be for severe COVID-19 requiring inpatient treatment. A safety follow-up telephone call will be conducted on Day 14 and 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 (AEs); AEs will be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v5.0.

During Cohorts B–E, an IMC will provide additional safety oversight for cohorts evaluating regimens with AT-527 doses higher than 550 mg BID (refer to Section 1.2.2 for details).

The study schema is shown in Figure 1.

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 will be randomized in a 1:1 ratio (AT-527: placebo). Subsequent cohorts 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] If a dose lower than 550 mg BID is evaluated in any of Cohorts B–E, no IMC input is required, and the cohort may be initiated at any time after the Cohort A interim safety analysis.

[c] For cohorts evaluating regimens with AT-527 doses higher than 550 mg BID, the IMC will review unblinded safety data through Day 10 for the first 20 patients in the cohort. Dose regimen decisions for the next cohort will be made by the Sponsor study team following IMC review.

1.2.1 Treatment Assignment and Blinding

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, patients will be randomly assigned to receive AT-527 or placebo in a 1:1 ratio. In subsequent cohorts, 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 group.

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 (independent of the project team) will have access to the unblinded data to allow for data transfer, unblinding, and summarizing of safety data for IMC review.

While PK samples must be collected from patients assigned to the placebo group 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 group 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 (SAE) 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 that are considered by the investigator or Sponsor to be related to an investigational medicinal product (IMP). 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.

1.2.2 Data Monitoring

During Cohorts B–E, 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 AEs, SAEs, deaths, and vital signs 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 SAE for which the causal relationship is judged by the investigator to have a reasonable possibility of relatedness to the study drug
- Greater than 20% of the enrolled patients in any cohort experience clinically significant Grade 3 or 4 AEs 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 550 mg BID will not require additional IMC oversight.

2. STATISTICAL HYPOTHESES

The primary virology analysis for this trial will evaluate the antiviral activity of AT-527 compared with placebo at Days 3, 5, and 7 to demonstrate superiority of AT-527 over placebo.

The following null and alternative hypothesis will be tested at a one-sided 0.10 significance level (equivalent to a two-sided 0.20 significance level):

$$H_0: CFB_{AT-527} \leq CFB_{Placebo} \qquad versus \qquad H_a: CFB_{AT-527} > CFB_{Placebo},$$

for which the CFB_{AT-527} and $CFB_{Placebo}$ refer to the decrease from baseline in amount of SARS-CoV-2 virus RNA as measured by reverse-transcription polymerase chain reaction (RT-PCR) for AT-527 and placebo at specified timepoints, respectively.

No alpha adjustments for multiple comparisons are planned.

3. SAMPLE SIZE DETERMINATION

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. Each additional cohort will enroll approximately 40 patients in a 3:1 ratio to receive AT-527 or placebo, respectively.

The sample size 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 treatment group (active or placebo) in the modified intent-to-treat infected (mITTi; defined in Table 2) 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 treatment group to account for an estimated 90% SARS-CoV-2 positive rate and is based on a single-cohort comparison of AT-527 versus placebo.

4. ANALYSIS SETS

Disposition summaries will be based on the intent-to-treat (ITT) population. All randomized patients will be included in the ITT population, and data will be summarized according to the randomized treatment assignment.

All primary and secondary virology and efficacy outcomes will be analyzed in the 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. Patients who received any dose of AT-527 will be summarized in the AT-527 group.

Pharmacokinetic analyses will be performed on the PK analysis population, which will consist of all patients in the ITT population who had at least one post-dose drug concentration measurement at a scheduled visit timepoint.

The analysis populations are defined in Table 2.

Table 2 Analysis Sets

Population	Definition
ITT	All randomized patients, whether or not the patient received the assigned treatment.
Safety-evaluable	All patients who received at least one dose of study treatment.
mITTi	All patients randomized in the study that received any amount of study drug and had at least one positive central SARS-CoV-2 RT-PCR test result above or equal to the LOQ (by nasopharyngeal swab) during the study, with patients grouped according to the treatment assignment at randomization
PK population	All patients in the ITT population who had at least one post-dose drug concentration measurement at a scheduled visit timepoint

ITT=intent-to-treat; mITTi=modified intent-to-treat infected; LOQ=limit of quantification; PK=pharmacokinetic; RT-PCR=reverse-transcription polymerase chain reaction; SARS-CoV-2= severe acute respiratory syndrome coronavirus-2.

5. STATISTICAL ANALYSES

5.1 GENERAL CONSIDERATION

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

All primary and secondary virology endpoints will be produced using the nasopharyngeal swab samples. Additional descriptive analyses for saliva and serum samples will be performed on an exploratory basis for the primary and secondary virology endpoints, as required. A summary of the sampling differences between cohorts is provided in Table 3. Refer to Appendix 1 and Appendix 2 of the study protocol for further details.

No alpha adjustments for multiple comparisons are planned.

For all cohorts, the primary virology analysis will be performed as part of a Day 10 Review (accompanied with key safety review if deemed necessary, see Section 5.7.1) 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. 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 all cohorts.

Table 3 Differences in Virology Sampling Between Cohorts

Assessment	Cohort A	Cohorts B – E
Nasopharyngeal swab sample for COVID-19 virology	Once a day on Days 1, 3, 5, and 7	Once a day on Days 1, 3, 5, and 7
Saliva sample for COVID-19 virology	NA	Once a day on Days 1, 3, 5, and 7
Serum sample for anti-SARS-CoV-2 antibodies and viral RNA	Once a day on Days 1 and 7	Once a day on Days 1, 3, 5, and 7

COVID-19 = coronavirus disease 2019; NA = not applicable; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2.

For virology endpoints, the time windows for each assessment are given in Table 4. The time windows will be used for all virology endpoints except the time-to-event endpoints.

Table 4 Virology Assessment Time Windows

Scheduled Timepoint [a, b, d]	Acceptable Time Window
Baseline (Day 1 pre-dose)	Day 1 pre-dose[c]
Day 3	Day 2 – Day 3
Day 5	Day 4 – Day 5
Day 7	Day 6 – Day 8

[a] 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.

[b] 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.

[c] If no pre-dose sample is available, a sample taken up to 2 hours post-dose can be used as baseline.

[d] No unscheduled visit or early termination serum sample to be taken for Cohort A.

For vital signs, including oxygen saturation, the time windows for each assessment are given in Table 5.

Table 5 Vital Signs (Including Oxygen Saturation) Time Windows

Scheduled Timepoint [a, b, d]	Acceptable Time Window
Baseline (Day 1 pre-dose)	Day 1 pre-dose[c]
Day 3	Day 2 – Day 3
Day 5	Day 4 – Day 5
Day 7	Day 6 – Day 8

[a] 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.

[b] 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.

[c] Baseline is defined as the last assessment prior to treatment.

[d] These time windows do not apply to temperature. Temperature is collected as part of the COVID-19 Symptom Diary (twice a day on Days 1–14; once a day on Days 15–28).

For lab parameters (hematology, chemistry), the time windows for each assessment are given in Table 6.

Table 6 Laboratory (Hematology, Chemistry) Time Windows

Scheduled Timepoint [a]	Acceptable Time Window
Baseline (Day 1 pre-dose)	Day 1 pre-dose[b]
Day 3	Day 2 – Day 4
Day 7	Day 5 – Day 9

[a] 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.

[b] Baseline is defined as the last assessment prior to treatment.

Unscheduled visit and early termination visit data will also be mapped according to the acceptable time windows per Table 4, Table 5, and Table 6. Where multiple measurements within a visit window are available for a patient, the value obtained closest to the target timepoint (center of the acceptable time window) will be used. If two measurements collected with the same time deviation exist before and after the target timepoint, the measurement obtained before the target timepoint will be adopted for analysis. However, preference will always be given to measurements collected on the day specified in the scheduled timepoint. The assessment timepoint having no

measurements within the corresponding acceptable time window will be considered as missing and will not be included in the analysis for the timepoint in question.

5.2 PARTICIPANT DISPOSITION

Disposition summaries will be based on the ITT population.

The number of patients who enroll, discontinue, or complete the study will be summarized by treatment group and pooled placebo group. Summaries may also be produced for each individual placebo group. Reasons for premature study withdrawal will be listed and summarized by treatment group. Major protocol deviations (PDs) will be listed and evaluated for their potential effects on the interpretation of study results.

Patients will not be excluded from the analysis populations based on major PDs unless they do not otherwise meet the definitions for each population. Sensitivity analyses evaluating the impact of major PDs may be conducted if a large number of major PD's are observed during the study.

5.3 PRIMARY ENDPOINT ANALYSIS

5.3.1 Definition of 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.

The primary analysis will use the nasopharyngeal swab data. For all Cohorts, the primary analysis will be compared at Days 3, 5, and 7 and will be performed as part of the Day 10 Review (Section 5.7.1).

All cohorts will use a pooled placebo group including all patients up to the cohort being analyzed. At the end of the study, sensitivity analysis will be performed for each cohort with a combined placebo group from all cohorts (Section 5.3.3).

The primary analysis for each cohort will be tested at a one-sided 10% alpha (equivalent to a two-sided 20% alpha).

5.3.2 Main Analytical Approach for Primary Virology Endpoint

The primary virology endpoint analyses will be performed on the mITTi population using the nasopharyngeal swab data.

For each cohort, the change from baseline in the amount of SARS-CoV-2 virus RNA will be compared between the AT-527 group and the pooled placebo group (meaning all placebo patients up to and including the cohort being analyzed will be used to form the placebo comparator group) at Days 3, 5, and 7 using analysis of covariance (ANCOVA) with baseline viral load as a covariate.

The least squares mean (LSM) change and its standard error (SE) of means in each group, the difference between two groups in LSM change, SE, and 80% CI, and p-values will be presented. The LSM change will be presented graphically over time.

Additionally, descriptive summaries for the change from baseline will also be presented by treatment group. The change from baseline and the amount of SARS-CoV-2 virus RNA will also be presented graphically over time.

Baseline is defined as the Day 1 (pre-dose) nasopharyngeal swab (Table 4).

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

The qualitative result of the SARS-CoV-2 RT-PCR test is determined using cycle threshold (Ct) values. If the qualitative result is positive, then an associated quantitative result is also provided. The quantitative result is either numerical or below the limit of quantification (BLQ). The limit of quantification (LOQ) for the virus RNA is 120 copies/ml. If the virus RNA sample has a positive qualitative result, but a quantitative result BLQ, virus RNA will be imputed as LOQ – 1 (119 copies/mL). Negative qualitative samples will be imputed as LOQ/2 (60 copies/ml) before being transformed to the log10 scale. Samples reported as positive, but not quantifiable, will contribute to qualitative summaries, but will be treated as missing (not imputed) for quantitative summaries.

Missing data will not be imputed. Any missing data will not be included in the analyses.

5.3.3 Sensitivity Analyses for Primary Virology Endpoint

A sensitivity analysis may be performed, whereby only data obtained at the nominal timepoint is used. This means that the time windows in Table 4 would not be applicable; only samples collected on the scheduled timepoints (Days 3, 5, and 7) would be included in the analysis.

At the end of the study, sensitivity analyses will also be performed for each cohort with a combined placebo group from all cohorts.

5.3.4 Supplementary Analyses for Primary Virology Endpoint

To supplement the main analysis, an exploratory time-weighted average change from baseline may also be performed. This will be calculated as the AUC of the change from baseline viral load divided by its total time interval (time of last observation minus time of first observation).

5.3.5 Subgroup Analyses for Primary Virology Endpoint

The change from baseline in the amount of SARS-CoV-2 virus RNA may be compared between the treatment groups and pooled placebo group for the following subgroups:

- Baseline viral load (for example: high, low; based on median baseline viral load)
- Baseline anti-SARS-CoV-2 antibody (spike protein) status (positive, negative)
- Time from symptom onset to treatment ($\leq$ 3 days, $>$ 3 days)
- Any underlying health conditions (yes, no)

Subgroup analyses will be analyzed descriptively at each timepoint and may be plotted over time.

5.4 SECONDARY ENDPOINTS ANALYSES

All secondary endpoints will be analyzed descriptively and summary statistics will be presented. Descriptive comparisons will be made for each AT-527 group and a pooled placebo group containing patients from all cohorts. For time to event endpoints, a Kaplan-Meier plot and the median time to response will be presented along with the 80% CI. For proportion, frequency and incidence endpoints, the number, proportion and difference in proportions along with 80% CIs will be presented. For area under the curve (AUC) endpoints, summary statistics will be presented.

5.4.1 Potential Further Statistical Analyses

If required, in addition to the descriptive analyses, formal statistical analysis may also be presented for secondary endpoints.

Time to event secondary virology and efficacy endpoints may be analyzed using the generalized Wilcoxon test or log-rank test (as appropriate), which may be stratified for appropriate covariates. Secondary proportion, frequency and incidence endpoints for virology and efficacy may be analyzed using the Fisher's Exact test. AUC and duration secondary endpoints may be analyzed using the Mann–Whitney U test, where AUC is calculated using the trapezoidal method.

5.4.2 Secondary Endpoints

5.4.2.1 Secondary Endpoints (Virology)

All secondary virology analyses will be performed on the mITTi population.

All virology analyses will be performed using the nasopharyngeal swab data. Descriptive analyses of saliva and serum samples will be performed on an exploratory basis for the primary and secondary virology endpoints, as required.

Time to event virology secondary endpoints

For time to event virology secondary endpoints, time will be calculated in hours.

- **Time to cessation of SARS-CoV-2 viral shedding as measured by RT-PCR**
  - Time to cessation of viral shedding by RT-PCR, in hours, is defined as the time between the initiation of any study treatment and first time when a negative or BLQ test result by RT-PCR is obtained. Patients who do not receive a negative or BLQ test result by RT-PCR by the last observation timepoint will be treated as censored at the final timepoint on Day 7.
- **Time to sustained non-detectable SARS-CoV-2 virus RNA**
  - Time to sustained non-detectable SARS-CoV-2 virus RNA, in hours, is defined as the time between the initiation of any study treatment and first time when a negative or BLQ test result by RT-PCR is obtained after which no positive test above or equal to the LOQ was reported. Patients who do not meet this endpoint by the last observation timepoint will be treated as censored at the final timepoint on Day 7.

These time-to-event endpoints will be summarized using descriptive statistics by treatment, as well as Kaplan-Meier plots. The median time to response from the Kaplan-Meier plots will be presented along with the 80% CI. If required, a hazard ratio may be produced adjusting for baseline viral load using a Cox proportional hazards model.

For all cohorts, the virology time-to-event endpoints will be described for the AT-527 group and the pooled placebo group through Day 7 using the mITTi population.

For virology time to event endpoints, deaths will be right censored at Day 7. Consequently, for these endpoints, participants censored on the final day reflect two different states, death or failure to meet the improvement outcome criterion. Therefore, it is important to understand the outcome in the context of the number and timing of deaths by treatment group.

Intercurrent events are those that occur after treatment initiation and either preclude observation of the variable or affect its interpretation. Intercurrent events and missing data will be accounted for through censoring rules, as described in Table 7 below.

Table 7 Virology Time to Event Censoring

Event	Censor	Date and Time
Death prior to meeting endpoint	Yes	Final timepoint on Day 7
Discontinuation or lost to follow-up up to Day 7 for any reason prior to meeting endpoint	Yes	Last observed virology assessment
Do not meet the endpoint	Yes	Final timepoint on Day 7

Proportion virology secondary endpoint

- **Proportion of patients positive for SARS-CoV-2 virus RNA by RT-PCR at specified timepoints**
  - Proportion of patients positive for SARS-CoV-2 virus RNA by RT-PCR will be presented by treatment at each visit. It is defined as the percentage of patients with a positive virus RNA by RT-PCR test result above or equal to the LOQ.

For virology proportion endpoints in all cohorts, the proportion of patients positive for SARS-CoV-2 virus RNA by RT-PCR will be compared between the AT-527 group and the combined placebo group. For all cohorts, the number, proportion and difference in proportions along with 80% CIs will be presented by treatment group for all timepoints.

Missing data will be treated as missing and will not be included in the analysis. However, if a patient dies, the non-responder rule will apply and the patient will be imputed as positive for all timepoints post-death.

Area under the curve (AUC) virology secondary endpoint

- **Area under the curve in the amount of SARS-CoV-2 virus RNA as measured by RT-PCR**
  - AUC in virus RNA (RT-PCR) will be presented by treatment group and is defined as AUC of the amount of virus RNA (RT-PCR). AUC will be calculated using the trapezoidal method. AUC of change from time 0 (t_0) to time K (t_k) is given by the formula:

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

where t_k (hours) represents the date of the k^{th} virus RNA (RT-PCR) assessment ($k = 0, \ldots, K$) and y_k represents the $\log_{10}$ value of the k^{th} virus RNA (RT-PCR) assessment.

For the virology AUC endpoint, the AUC of the amount of virus RNA (RT-PCR) will be compared between the AT-527 group and a combined placebo group. For all cohorts, summary statistics will be presented to Day 7 using the full combined placebo group.

Missing data will be treated as such and not included in the analysis.

If the virus RNA sample has a positive qualitative result, but a quantitative result below the BLQ, virus RNA will be imputed as LOQ – 1 (119 copies/mL). Negative qualitative samples will be imputed as LOQ/2 (60 copies/ml) before being transformed to the log10 scale.

5.4.2.2 Secondary Endpoints (Safety)

The safety objective for this study is to evaluate the safety of AT-527 compared with placebo.

The safety population will be used for all safety analyses. Patients will be analyzed according to the treatment they actually received.

Safety analyses will be presented by each AT-527 dose and a combined placebo group containing patients from all cohorts. Safety will be assessed through summaries of exposure to study treatment, AEs, changes in laboratory test results, and changes in vital signs and ECGs. If a low number of events is observed, a listing may be produced in place of a summary table. A listing of pregnancies will also be produced.

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

- **Incidence and severity of AEs, with severity determined according to NCI CTCAE v5.0**

All verbatim AE terms will be mapped to Medical Dictionary for Regulatory Activities (MedDRA) thesaurus terms and will be classified by System Organ Class (SOC) and preferred term. AE severity will be graded according to NCI CTCAE v5.0. For AEs of varying severity, the highest grade will be used in the summaries. Deaths and cause of death will be listed.

Only treatment-emergent AEs will be summarized. Treatment-emergent AEs are defined as those AEs with observed or imputed onset date on or after the start date of trial treatment. Only where the most extreme intensity is greater than the initial intensity (or if most extreme intensity is not missing and initial intensity is missing) will events with an onset date prior to the start of study treatment be considered treatment-emergent.

An AE with a completely missing start date will be assumed to be treatment-emergent unless the AE has a complete non-imputed end date that is prior to study Day 1. AEs will be coded and tabulated by SOC and/or Preferred Terms (PTs). In tabulations, PTs

and their associated SOC will be presented in order of descending frequency summed across the treatment groups.

The following will be summarized for each AT-527 group and a pooled placebo group, and listings produced where required:

- AEs
- SAEs
- AEs related to study treatment
- AEs leading to death
- AEs leading to withdrawal of study drug
- AEs leading to withdrawal from study
- AEs occurring in ≥5% of patients in at least one treatment group
- AEs occurring in ≥10% of patients in at least one treatment group
- AEs of NCI CTCAE Grade 3 or higher
- AEs by most extreme NCI CTCAE grade
- AEs and SAEs by outcome
- AEs and SAEs by time of onset according to the following categories:
  - Week 1 (Days 1-7)
  - Week 2 (Days 8-14)
  - Days ≥ 15

All adverse events of special interest (AESI) will be presented by treatment group. AESI are as follows for this study:

- Cases of potential drug-induced liver injury that include an elevated alanine transaminase (ALT) or aspartate aminotransferase (AST) in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's Law (as detailed in protocol)
- 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.

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

- **Change from baseline in targeted vital signs, including peripheral capillary oxygen saturation (SpO_2)**

For each of the vital signs (pulse rate, respiratory rate, blood pressure, peripheral capillary oxygen saturation [SpO_2], and temperature), summary statistics on absolute value and the change from baseline will be presented over time. For all cohorts, this will be presented by treatment group at all timepoints using a combined placebo group containing patients from all cohorts.

Additionally, for all cohorts, a graphical representation of means over time of temperature (daily to Day 28) will be presented. Summaries of other temperature related secondary efficacy endpoint are described in Section 5.4.2.3.

Baseline is defined as the last assessment prior to treatment.

- **Change from baseline in targeted clinical laboratory test results**

For each of the hematological and biochemical test parameters, summary statistics of observed and change from baseline values at each timepoint will be presented by treatment group for each scheduled timepoint. Baseline is defined as the last assessment prior to treatment.

Additionally, shift tables of selected laboratory tests will be used to summarize the baseline and maximum post-baseline severity grade. NCI CTCAE v5.0 grading will be used with an indication of the direction of the abnormality (High, Low). A summary of the number and percentage of patients with abnormal laboratory outcomes will be produced by treatment group and pooled placebo group for each parameter for each scheduled timepoint.

The number and proportion of patients who meet the pre-specified criteria shown in Table 8 will be presented by treatment group during the study.

Table 8 Pre-specified Treatment-Emergent Abnormal Laboratory Criteria

AST (U/L) or ALT (U/L) and Total bilirubin (mg/dL)
AST (U/L) or ALT (U/L) > 3 × Baseline
AST (U/L) or ALT (U/L) > 5 × Baseline
AST (U/L) or ALT (U/L) > 3 × Baseline and Total bilirubin (mg/dL) > 2 × ULN

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

5.4.2.3 Secondary Endpoints (Efficacy)

Time to event and duration efficacy secondary endpoints include:

- **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**
  - Time to alleviation or improvement is defined as the length of time (hours) taken from start of treatment to the point at which all of the following three criteria are met and maintained for a concurrent duration of at least 21.5 hours:
    - Alleviation of all new COVID-19 symptoms, defined as a score of 0 [none] or 1 [mild] of the COVID-19 Symptom Diary
    - Improvement of all pre-existing symptoms that had worsened due to COVID-19, defined as at least a single category improvement from baseline on the Likert scale. For example, a symptom classed as severe at baseline must improve to moderate, mild, or none, and a symptom classed as moderate at baseline must improve to mild or none.
    - Maintain/improvement of all pre-existing symptoms that had not worsened due to COVID-19, defined as either remaining the same score or at least a single category improvement from baseline on the Likert scale.

- **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**
  - Time to alleviation or improvement is defined as the length of time (hours) taken from start of treatment to the point at which all of the following three criteria are met and maintained for a concurrent duration of at least 43 hours:
    - Alleviation of all new COVID-19 symptoms, defined as a score of 0 [none] or 1 [mild] of the COVID-19 Symptom Diary
    - Improvement of all pre-existing symptoms that had worsened due to COVID-19, defined as at least a single category improvement from baseline on the Likert scale. For example, a symptom classed as severe at baseline must improve to moderate, mild, or none, and a symptom classed as moderate at baseline must improve to mild or none.
    - Maintain/improvement of all pre-existing symptoms that had not worsened due to COVID-19, defined as either remaining the same score or 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, as described above.

- **Time to alleviation of COVID-19 signs and symptoms maintained for a duration of 21.5 hours**
  - Time to alleviation of COVID-19 symptoms is defined as the length of time (hours) 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, regardless of if the symptom is pre-existing or new.
- **Time to alleviation of COVID-19 signs and symptoms maintained for a duration of 43 hours**
  - Time to alleviation of COVID-19 symptoms is defined as the length of time (hours) 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, regardless of if the symptom is pre-existing or new.
- **Duration of fever**
  - Duration of fever is defined as the time from start of treatment to return to an afebrile state (temperature ≤ 37.5°C) maintained for at least 21.5 hours.
- **Time to alleviation of an individual symptom**
  - Time to alleviation of an individual symptom is defined as the length of time (hours) 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

For each of these endpoints, the time at which the endpoint is met is the time of the start of the period when the criterion is met.

These endpoints will be summarized using descriptive statistics by treatment group, as well as Kaplan-Meier plots where appropriate. The median time to response will be presented along with the 80% CI. For all cohorts, the efficacy time to event endpoints will be described through Day 28 between the AT-527 group and the combined placebo group from all available cohorts using the mITTi population.

A summary of the symptom severity for each symptom in the COVID-19 symptom diary will be provided at baseline, Day 14 and Day 28 for each treatment group and pooled placebo group. Day 14 (morning) assessments are to be used for the Day 14 summary. If the morning sample is missing, the evening sample will be used.

For efficacy time to event endpoints, deaths will be right censored at Day 28. Consequently, for these endpoints, participants censored on Day 28 reflect two different states, death and failure to meet the improvement outcome criterion. Therefore, it is

important to understand the outcome in the context of the number and timing of deaths by treatment group.

Intercurrent events are those that occur after treatment initiation and either preclude observation of the variable or affect its interpretation. Intercurrent events and missing data will be accounted for through censoring rules, as described in Table 9 below.

Table 9 Efficacy Time to Event Censoring

Event	Censor	Date and Time
Death prior to meeting endpoint	Yes	Final timepoint on Day 28
Discontinuation or lost to follow-up up to Day 28 for any reason prior to meeting endpoint	Yes	Last observed COVID-19 symptom diary assessment
Do not meet the endpoint	Yes	Final timepoint on Day 28

Frequency efficacy endpoints include:

- **Frequency of COVID-19–related complications**
  - COVID-19-related complications include: death, hospitalization, radiologically confirmed pneumonia, acute respiratory failure, sepsis, coagulopathy, pericarditis, myocarditis, and cardiac failure.

For frequency efficacy endpoints, the number, proportion and difference in proportions between the AT-527 group and the pooled placebo group from all available cohorts, along with 80% CIs will be presented by treatment group.

5.4.2.4 Secondary Endpoint (Pharmacokinetics)

- **Plasma concentration of AT-511, AT-551, AT-229, and AT-273 at specified timepoints**

The PK analysis population will consist of patients with sufficient data to enable estimation of key parameters (including, but not limited to, maximum concentration [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 PK 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.

For AT-511, AT-551, AT-229, and AT-273 plasma concentration-time profiles will be plotted

- By cohort,

  - Overlaying arithmetic mean and individual values (log concentration scale)
  - Overlaying arithmetic mean (+ SD) and individual values (linear concentration scale)
  - Overlaying individual plots (linear and log concentration scale)
- Group by cohort
  - Overlaying arithmetic mean (log concentration scale)
  - Overlaying arithmetic mean (+ SD) (linear concentration scale)

Individual PK concentration data will be listed by cohort.

Descriptive summary statistics tables for PK concentration data will be provided by cohort and visit/sampling timepoint. Summary statistics will include mean (arithmetic and geometric), SD, CV (arithmetic and geometric), median, minimum, and maximum. Concentrations below the lower limit of quantification (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 (CSR).

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 pharmacodynamic (PD) data and parameters.

5.4.2.5 Sensitivity Analyses for Secondary Endpoints

Due to practical constraints, patients may receive first study treatment a day post randomization. Sensitivity analyses may be performed on the following time-to-event virology and efficacy secondary endpoints, defined as the time from randomization to meeting the endpoint:

- Time to cessation of SARS-CoV-2 viral shedding as measured by RT-PCR
- Time to sustained non-detectable SARS-CoV-2 virus RNA
- Time to alleviation or improvement of COVID-19 symptoms (Items 1–12 of the COVID-19 symptom diary)
- Time to alleviation of COVID-19 signs and symptoms

Sensitivity analyses may also be performed for the following time-to-event virology secondary endpoints where results reported as BLQ are treated as positive (instead of negative):

- Time to cessation of SARS-CoV-2 viral shedding as measured by RT-PCR
- Time to sustained non-detectable SARS-CoV-2 virus RNA

5.5 EXPLORATORY ENDPOINTS ANALYSIS

Exploratory Virology Endpoint Analyses

The following virology exploratory endpoints will be compared between each AT-527 dose group and the pooled placebo group:

- 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

Polymorphic and treatment-emergent substitutions in nsp12 and potentially other genes will be reported descriptively, and the percentage of patients with those viral mutations will be calculated.

Exploratory endpoints defined by virus titer will be summarized using descriptive statistics similar as specified for the secondary endpoints defined by SARS-CoV-2 virus RNA.

Drug susceptibility will be reported as EC_{50} values, and respective fold-change values (ratio of EC_{50} sample / EC_{50} reference) will be calculated from those. A summary of drug susceptibility with summary statistics will be produced at baseline (and other timepoints, if required), together with an individual patient listing of drug susceptibility.

Exploratory Pharmacokinetic Endpoint Analyses

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 CSR.

Respiratory Coinfections (Biofire)

All baseline (nasopharyngeal) samples will be tested for respiratory pathogens using the qualitative PCR-based FDA cleared BioFire FilmArray® Respiratory Panel 2 assay. This

assay detects 19 respiratory viral or bacterial pathogens. Frequency and proportions for each co-infection will be presented.

Exploratory Patient Global Impression of Severity Endpoint

The exploratory endpoint to evaluate patients' self-assessment of COVID-19 symptom severity as assessed by Patient Global Impression of Severity (PGIS) at Days 14 and 28 will be summarized using mean scores on a 4-point Likert scale: "none" (0) to "severe (3).

5.6 OTHER SAFETY ANALYSES

5.6.1 Extent of Exposure

Study treatment exposure (such as treatment duration and total dose received) will be summarized with descriptive statistics. A listing of patients by treatment group, detailing dosing of study drug will be prepared. A pooled placebo group containing patients from all cohorts will be used.

The duration of treatment exposure will be summarized with descriptive statistics by treatment group for the safety population. The duration of treatment exposure [days] is defined as the dosing period during which a patient takes medication as follows:

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

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

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

Here, frequency represents the number of doses.

5.6.2 ECGs

A summary of patients with clinically significant ECG abnormalities will be produced. A listing will also be produced.

5.7 OTHER ANALYSES

5.7.1 Day 10 Review

The Day 10 analysis will include the primary virology analysis (nasopharyngeal swab) for each cohort, together with any sensitivity, supplementary, and subgroup (if data are available) analysis as described in Section 5.3. For Cohorts B-E, if the data are available in a timely manner, the change from baseline using saliva samples will also be summarized descriptively and plotted over time.

For all cohorts, if the data are available in a timely manner, the change from baseline in SARS-CoV-2 virus titer (Section 5.5) will also be described.

Demographic (including, but not limited to, age, sex and BMI) will be summarized as described in Section 5.7.3. A summary will be presented by treatment group (including a pooled placebo group up to the cohort being analyzed) and will be presented for the mITTi population and may, in addition, be presented for the safety population.

In addition, summaries of the placebo groups from each individual cohort up to the cohort being analyzed may be presented.

If deemed necessary, the following key safety data will be provided for the current AT-527 group and a pooled placebo group up to the cohort being analyzed:

- All AEs
- Serious AEs
- AEs leading to death
- Laboratory abnormalities

In order to avoid the risk of unblinding at patient level by the analysis of individual safety data, safety representatives independent to the study will be delegated to review any safety listings.

5.7.2 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 using a pooled placebo group. The pooled placebo group may also be split by cohort, if required.

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

5.7.3 Summaries of Demographics and Baseline Characteristics

Demographic and baseline characteristics (including, but not limited to, age, sex, BMI, self-reported race/ethnicity, onset of symptoms, baseline symptom severity, presence of underlying conditions, and pre-existing symptoms) will be summarized using means, SDs, medians, and ranges for continuous variables and proportions for categorical variables, as appropriate. A summary will be presented by treatment group (including a pooled placebo group) and will be presented for the mITTi population and may, in addition, be presented for the safety population.

In addition, summaries of the placebo groups from each individual cohort will be presented to allow for a high-level comparison of the patients to be pooled.

Medical history data, including surgery and procedures, and baseline conditions, will be listed using the safety population. Previous and concomitant treatments will be summarized descriptively by treatment group. Listings will also be provided.

In addition, there will be a summary and listing of all treatments with the indication given as 'COVID-19'.

5.8 INTERIM ANALYSES

5.8.1 Planned Interim Analyses

An interim safety analysis is planned for Cohort A after the first 30 patients have completed assessments through Day 10 to allow determination of the AT-527 dose and regimen for Cohort B. No other interim analyses are planned.

The following key safety data will be provided for the AT-527 group and placebo group:

- All AEs
- Serious AEs
- AEs leading to death
- Laboratory abnormalities

In order to avoid the risk of unblinding at patient level by the analysis of individual safety data, safety representatives independent to the study will be delegated to review the safety listings.

5.8.2 Optional Interim Analyses

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's 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.

6. SUPPORTING DOCUMENTATION

Appendix 1 Changes to Protocol-Planned Analyses

This section is not applicable, since there are no changes to protocol-planned analyses.

Appendix 2 COVID-19 Symptom Diary and Patient Global Impression of Severity

Do not reproduce or distribute. 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: _____

COVID-19 Evening Symptom Diary

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)				

Appendix 2 COVID-19 Symptom Diary and Patient Global Impression of Severity (cont.)

	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

7. REFERENCES

Fischer WA, Eron JJ, Holman W, et al. Molnupiravir, an Oral Antiviral Treatment for COVID-19. medRxiv; 2021. doi: https://doi.org/10.1101/2021.06.17.21258639.