February 14, 2023

To Whom It May Concern:

This is the protocol for the below mentioned study.

Study Title: A Phase 1-2, dose-escalating, 6-part study to evaluate the safety and pharmacokinetics and pharmacodynamics of single and multiple doses of AT-007 in healthy adult subjects and adult subjects with Classic Galactosemia (CG) or GALK Deficient Galactosemia

NCT: NCT04117711

Document Date of Protocol: 03 September 2021

Please contact me with any questions.

Respectfully,

Evan Bailey, MD
Exective Clinical Director

CURRENT STUDY PROTOCOL

A Phase 1-2, Dose-Escalating, 6-Part Study to Evaluate the Safety and Pharmacokinetics of Single and Multiple Doses of AT-007 in Healthy Adult Subjects and Adult Subjects with Classic Galactosemia (CG) or GALK-deficient Galactosemia

Protocol Number
AT-007-1001
Version 10.0
3 September 2021

IND Number: 139699

Applied Therapeutics Inc.
340 Madison Avenue | 19th Floor
New York, NY 10173
USA

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PROTOCOL APPROVAL PAGE

A Phase 1-2, Dose-Escalating, 6-Part Study to Evaluate the Safety and Pharmacokinetics of Single and Multiple Doses of AT-007 in Healthy Adult Subjects and Adult Subjects with Classic Galactosemia (CG) or GALK-deficient Galactosemia

Protocol Number: AT-007-1001

Sponsor: Applied Therapeutics Inc.

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PROTOCOL SYNOPSIS

Name of Sponsor/Company: Applied Therapeutics Inc.

Name of Active Ingredient: AT-007

Title of Study: A Phase 1-2, Dose-Escalating, 6-Part Study to Evaluate the Safety and Pharmacokinetics of Single and Multiple Doses of AT-007 in Healthy Adult Subjects and Adult Subjects with Classic Galactosemia (CG) or GALK-deficient Galactosemia

Study Center: The study will be conducted at up to 6 sites in the United States (US) and up to 3 sites in Europe.

Phase of Development: 1-2

Study Objectives:

Primary:

- To evaluate the safety of single and multiple ascending doses of orally administered AT-007 in healthy adult subjects and adult subjects with CG or GALK-deficient Galactosemia

Secondary:

- To evaluate the pharmacokinetic (PK) parameters of single and multiple doses of orally administered AT-007 in healthy adult subjects and adult subjects with CG or GALK-deficient Galactosemia
- To evaluate the effect of single and multiple doses of orally administered AT-007 on the level of galactitol, a biomarker of aldose reductase (AR) activity, in adult subjects with CG or GALK-deficient Galactosemia
- To evaluate the effect of single and multiple doses of orally administered AT-007 on the levels of galactose and its other metabolites in adult subjects with CG or GALK-deficient Galactosemia

Exploratory:

- To measure the levels of AT-007 in the cerebrospinal fluid (CSF) of healthy adult subjects

Study Design:

This study is a first-in-human, randomized, placebo-controlled, 6-Part, single ascending dose (SAD) and multiple ascending dose (MAD) study in healthy adult subjects and adult subjects with CG or GALK-deficient Galactosemia. The study is designed to assess the safety and PK of AT-007 in healthy subjects and subjects with CG or GALK-deficient Galactosemia as well as the effect of AT-007 on biomarkers of galactose metabolism (galactose, galactitol, and other galactose metabolites) in subjects with CG or GALK-deficient Galactosemia.

This study consists of 6 parts:

- Part A (SAD) in 40 healthy subjects: 5 double-blind cohorts of 8 subjects (6 active and 2 placebo) each
- Part B (MAD for 7 days) in 28 healthy subjects: 2 double-blind cohorts of 8 subjects (6 active and 2 placebo) each and 2 double-blind cohorts of 6 subjects (4 active and 2 placebo) each
- Part C (MAD for 7 days) in 12 healthy subjects: 3 cohorts of 4 subjects each (open-label AT-007)

- Part D (SAD followed by 5-day washout followed by MAD for 27 days in up to 18 subjects with CG: 3 cohorts of 6 subjects (4 active and 2 placebo) each (the clinical site staff and the subjects will be blinded to study treatment).
- Part E (SAD followed by 5-day washout followed by MAD for 27 days) in up to 18 subjects with GALK-deficient Galactosemia: 1 to 3 cohorts of 6 subjects (4 active and 2 placebo) each (the clinical site staff and the subjects will be blinded to study treatment)
- Part D Extension for 90 days in up to 28 subjects (up to 21 active and 7 placebo) with CG in up to 2 cohorts (the clinical site staff and the subjects will be blinded to study treatment)

Note: In Parts D and E, overall number of subjects per cohort may be increased up to 8 subjects to address any potential lost-to-follow-up among subjects treated with either placebo or AT-007.

For all cohorts in all parts (A, B, C, D, and E), the study consists of a Screening Period (28 days [Day -28 to Day -1] for Parts A, B, and C and 40 days [Day -40 to Day -1] for Parts D and E [and D Extension for de novo subjects who did not participate in Part D and subjects whose last visit in Part D was >60 days before Day 1 in the Part D Extension]), a period of study treatment and associated assessments, and an end of study (EOS) or end of extension (EOE) Visit (28 days after the last dose of study drug). Subjects will be randomized and receive the first dose of study drug on Day 1. At a minimum, subjects will remain at the clinical research unit (CRU) through 24 hours after the first dose. Subjects in Part A will have one in-clinic stay during the Treatment Period with the single dose taken in the CRU. Subjects in Parts B and C will have 1 in-clinic stay with all 7 doses taken in the CRU. Subjects in Parts D and E will have 3 in-clinic stays with most doses taken at home. Safety will be monitored and blood samples will be collected for PK in all parts of the study. Also, subjects in selected dose cohorts of Parts A (SAD) and D (SAD portion only) will have urine collected for PK. In addition, subjects in Part C will have one lumbar puncture for CSF collection. Also, subjects in Parts D and E will have blood samples and urine collected for biomarkers of galactose metabolism.

The 90-day Part D Extension (separate informed consent required) is open for subjects with CG who completed Part D, participated in but did not complete Part D for reasons other than study drug related treatment emergent adverse event (related TEAE), or did not participate in Part D. Subjects who previously completed Part D may participate in the 90-day Extension at any time after the EOS Visit (scheduled for 28 days after the last dose in Part D MAD). Subjects who participated in but did not complete Part D may participate in the Extension after either the EOS Visit (if they have this visit and it occurs beyond 14 days after the last dose) or a minimum 2-week wash-out period after the last dose received in Part D (if they do not have the EOS Visit or have it before 2 weeks after the last dose). Subjects with CG who have not previously participated in Part D may be prescreened (optional) and participate in the 90-day Extension after they undergo all the required screening procedures from Day -60 to Day -1. Subjects with last visit in Part D more than 60 days before Day 1 in Part D Extension will also need a screening visit although some screening procedures done before Part D do not need to be repeated. Subjects in the Part D Extension will be randomized in a 3:1 ratio to active drug at the daily dose of 20 mg/kg or placebo. Most doses during the Part D Extension will be taken at home, but there are 2 in-clinic stays. A second cohort may be initiated in the Part D Extension to evaluate a dose higher than 20 mg/kg/day. If so, subjects will be randomized in a 3:1 ratio to active drug or placebo and will take most doses at home, except for 2 in-clinic stays. Also, subjects in Part D Extension will have blood samples collected for both PK and biomarkers of galactose metabolism.

The starting dose in Part A was 0.5 mg/kg as a single dose. Subsequent doses in Part A and all doses in Parts B, C, D, and E are based on the results of previous cohorts and/or previous parts of the study. Part A of the study started first. Part A Cohort 5 (40 mg/kg single dose) was recently added to the protocol (version 4.0) and results showed the dose was safe and well tolerated. Part B started after all subjects in Cohorts A1 through A4 completed the study (minus the EOS Visit). Given that the results for 40 mg/kg in Cohort A5 were acceptable, Part B Cohort 4 (40 mg/kg) will start with this version of the protocol (version 5.0). The first cohort in Part C (C1) started after both safety and PK data from

Cohorts B1 and B2 were reviewed. Cohort C1 was conducted simultaneously with Cohort B3 and used the same dose as Cohort B2 (10 mg/kg). The second cohort in Part C (C2) started after safety and PK data from Cohort B3 were reviewed and used the same dose as Cohort B3 (20 mg/kg); safety and PK results for Cohort C1 were also considered before starting Cohort C2. The third cohort in Part C (C3) will start with this version of the protocol (version 5.0) and use the same dose as Cohort B4 (given the results from Cohort A5); safety and PK results for Cohort C2 were also considered before starting Cohort C3. The first cohort in Part D (D1) started after all subjects in Cohorts A1 through A4 completed the study including the EOS Visit and all subjects in Cohorts B1 and B2 completed the study (minus the EOS Visit). The dose for Cohort D1 (5 mg/kg) was not higher than the dose for Cohort B2 (10 mg/kg). The second cohort in Part D (D2) started after all subjects in Cohort B3 completed the study (minus the EOS Visit) and the dose level (20 mg/kg) was the same as that for Cohort B3. The third cohort in Part D (D3) will start after all subjects in Cohort B4 complete the study (minus the EOS Visit) and the dose level will not be higher than the 40 mg/kg dose used for Cohort B4. The starting dose for Part E (Cohort E1) will be the most clinically appropriate dose as determined by the evaluation of available safety, tolerability, and PD (reduction in galactitol level) in Part D and the dose will not be higher than the highest acceptable dose in Part D at the time of starting Part E (after all subjects complete Cohorts D1 and D2 but may be before, during, or after Cohort D3). The Safety Review Committee (SRC) reviewed/will review safety data before each dose escalation and before the start of Parts B, C, D, and E. Also, the Sponsor reviewed/will review all PK and galactitol data as they become available.

The dose for the first cohort of the Part D Extension (DE1) will be 20 mg/kg/day based on acceptable SAD and MAD safety and PK data from Cohort D2. After SRC review of all SAD and MAD data and Sponsor review of PK and galactitol data for Cohorts D1 through D3, a second cohort of the Part D Extension (DE2) may be initiated to evaluate a dose higher than 20 mg/kg/day if this dose was well tolerated in the SAD and MAD portions of the study in both healthy volunteers and subjects with CG.

The review of the PK data from Cohorts A1 through A4 and the first 2 cohorts of Part B confirmed that once daily (QD) dosing is adequate.

Number of Subjects: Up to 80 healthy subjects, between 18 and 46 subjects with CG depending on the number of Part D Extension subjects who are or are not the same subjects as those in Part D, and up to 18 subjects with GALK-deficient Galactosemia are planned to be enrolled. More subjects may be enrolled if the number of cohorts increases to study AT-007 doses >40 mg/kg. Also, more subjects may be enrolled if the overall number of subjects per cohort in Parts D and E are increased up to 8 subjects to address any potential lost-to-follow-up among subjects treated with either placebo or AT-007.

Diagnosis and Main Study Entry Criteria for Parts A, B, and C:

Healthy male or non-pregnant, non-lactating female adult between 18 and 65 years of age, inclusive

Diagnosis and Main Study Entry Criteria for Part D and Part D Extension:

Male and non-pregnant, non-lactating female adult between the ages of 18 and 65 years, inclusive, with a Classic Galactosemia (CG) diagnosis confirmed by evidence of absent or significantly decreased (<1%) galactose-1-phosphate uridylyltransferase (GALT) activity in red blood cells or by historical record of diagnosis of GALT deficiency (medical record or gene analysis report or written communication by health care professional), who have no other significant health problems unrelated to CG.

For the Part D Extension, subjects who previously participated in but did not complete Part D must not have discontinued Part D because of study drug related TEAE(s).

Diagnosis and Main Study Entry Criteria for Part E:

Male and non-pregnant, non-lactating female adult between the ages of 18 and 65 years, inclusive, with a GALK-deficient Galactosemia diagnosis documented by historical record of either absent or significantly decreased galactokinase (GALK) activity in red blood cells (medical record, enzyme activity report, genetic mutation report, or written communication by health care professional), who have no other significant health problems unrelated to GALK-deficient Galactosemia.

Investigational Product, Dosage and Mode of Administration:

AT-007 (capsules or liquid suspension; single and multiple oral doses): once daily [QD] under fasting conditions for up to 7 days in healthy subjects; up to 27 days in subjects with CG who tolerated a previous single dose (and up to 90 days during an Extension); and up to 27 days in subjects with GALK-deficient Galactosemia who tolerated a previous single dose. Doses between 0.5 and 40 mg/kg, oral, were administered in Part A of the study. Dose levels in subsequent parts of the study are based on the safety, PK, and galactitol (Parts D and E only) results of previous cohorts and/or previous parts of the study. Doses higher than 40 mg/kg may be evaluated if the human maximum observed concentration (C_{max}) and area under the curve (AUC) are substantially lower than the corresponding values calculated in nonclinical experiments and no dose limiting toxicity (DLT) is observed up to and including at the 40 mg/kg dose. If dose escalation above 40 mg/kg is feasible based on the above criteria, these higher doses may be investigated in additional SAD and MAD cohorts in healthy subjects and in subjects with Galactosemia if no AT-007 levels are detected in the CSF collected from the healthy subjects enrolled in Cohort C3. Doses will be rounded according to the available SKU as described in the Pharmacy Manual

Duration of Treatment: Subject participation will be a maximum of approximately 56, 63, 63, 100, and 100 days (from screening through EOS Visit) for Parts A, B, C, D, and E, respectively. For subjects in the Part D Extension who did not previously participated in Part D (SAD and/or MAD), study participation will be a maximum of approximately 178 days (screening through EOE Visit at 28 days after the last dose of study drug during the extension). For subjects in the Part D Extension who previous participated in Part D (SAD and MAD), study participation will be a maximum of approximately 218 days (SAD and MAD) (screening through EOE Visit), assuming no re-screening needed for Part D Extension and not counting the wash-out period between the end of Part D MAD and the initiation of Part D Extension.

Reference Therapy, Dosage and Mode of Administration: Matching placebo (capsules or liquid suspension) will be administered under the same conditions and on the same schedule as the investigational product in Parts A, B, D, E, and D Extension. Part C is open-label AT-007 (no placebo).

Concomitant Medications and Dietary Restrictions:

The following medications are prohibited within 5 half-lives prior to the first dose of study drug through the last dose of study drug: 1) sensitive substrates of Breast Cancer Resistance Protein (BCRP) (e.g., rosuvastatin, sulfasalazine) or potent inhibitors of BCRP (e.g., cyclosporine A); 2) sensitive substrates of Organic Anion Transporter (OAT)1 and OAT3 (e.g., famotidine, adefovir, furosemide, ganciclovir, cefaclor, ceftizoxime, pen-G); 3) sensitive substrates of cytochrome P450 3A4 (CYP3A4) (e.g. midazolam, triazolam, buspirone, alfentanil, dronedarone, eletriptan, conivaptan, lovastatin, simvastatin), or CYP2B6 (e.g. bupropion), or CYP2C19 (e.g. omeprazole), or CYP1A2 (e.g. alosetron, caffeine, duloxetine, melatonin, ramelteon, tasimelteon,tizanidine).

Drugs potentially associated with transaminase elevations, such as mirtazapine, are prohibited within 5 half-lives prior to the first dose of study drug through the last dose of study drug.

The following potentially nephrotoxic drugs are prohibited within < 5 half-lives prior to the first dose of study drug through the last dose of study drug: amitriptyline, aspirin, doxepin, lithium, amphotericin B, foscarnet, ganciclovir, pentamidine, rifampin, antiretrovirals (e.g. adefovir, tenofovir), calcineurin inhibitors (e.g. cyclosporine, tacrolimus), angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, clopidogrel, ticlopidine, pamidronate, zoledronate, statins, chemotherapeutics, contrast dye, loop diuretics, thiazides, triamterene, allopurinol, gold therapy, haloperidol, quinine, ranitidine.

In Parts D, E, and D Extension, subjects will be allowed to take concomitant medications for Galactosemia complications provided that these concomitant medications are reviewed/approved by the Investigator and that the subjects are on stable doses for at least a month prior to screening (or first dose of study drug if no screening visit) through the EOS or EOE Visit.

Study Endpoints:

Primary Endpoints

The primary endpoints of this study are overall safety and adverse events (AEs). Safety will be assessed by the following:

- AEs
- Clinical safety laboratory tests (hematology, chemistry, urinalysis)
- Physical examinations
- Vital signs
- Electrocardiograms (ECGs)

Secondary Endpoints

- PK parameters in healthy subjects, subjects with CG, and subjects with GALK-deficient Galactosemia
- Galactose, galactitol, and other galactose metabolites in the blood of subjects with CG and subjects with GALK-deficient Galactosemia
- Urine galactitol for subjects with CG and subjects with GALK-deficient Galactosemia

Exploratory Endpoints:

- AT-007 level in the CSF of healthy subjects (Part C only)

Statistical Methods:

Sample Size: The sample size was determined outside of statistical considerations. The number of subjects is typical for Phase 1 studies of this type and is adequate to meet study objectives.

Safety Analyses:

The evaluation of safety will be based on the occurrence of TEAEs; discontinuations from study drug and/or the study because of AEs; clinical safety laboratory test results, physical examination findings, vital sign evaluations, and 12-lead ECGs results. All safety endpoints will be summarized descriptively. No statistical inference will be applied to the safety endpoints. Descriptive statistics will be calculated for quantitative safety data and frequency counts will be compiled for qualitative safety data.

PK Analyses:

PK calculations will be performed using appropriate software, e.g., Phoenix™ WinNonlin® (Version 6.3 or higher, Pharsight Corporation) and/or SAS® (Version 9.4 or higher, SAS Institute Inc.).

PK parameters will be calculated using noncompartmental methods, using an appropriate model. Concentrations below the lower limit of quantitation (BLQ) will be treated as zero from the time of the first quantifiable concentration; embedded and terminal BLQ concentrations will be treated as "missing." Actual sample times, if available, will be used in the PK analysis.

AT-007 concentration-time data and PK parameters will be summarized by study part and cohort (dose level) using descriptive statistics (n, mean, standard deviation [SD], median, minimum, maximum, percent coefficient of variation [CV%]).

Dose proportionality, the proportionality of a change in systemic exposure with a change in dose, may be assessed using a power model or other appropriate methods.

Details of the PK analyses, including any additional exploratory analyses, will be provided in the Statistical Analysis Plan (SAP).

PK samples will be stored for possible future analysis of AT-007 metabolites.

Biomarker Analyses:

For Parts D, E, and D Extension, blood and/or urine levels of galactose and/or its metabolites (including galactitol) will be summarized using descriptive statistics. Details of these analyses will be provided in the SAP.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Definition or Explanation
AE	Adverse event
ADP	Adenosine diphosphate
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AR	Aldose reductase
ARI	Aldose reductase inhibitor
AST	Aspartate transaminase
ATP	Adenosine triphosphate
AUC	Area under the curve
BCRP	Breast cancer resistance protein
BMD	Bone marrow density
BMI	Body mass index
BLQ	Below the limit of quantitation
BSA	Body surface area
BUN	Blood urea nitrogen
CFR	Code of Federal Regulations (in US)
CG	Classic Galactosemia
CI	Confidence interval
CLIA	Clinical Laboratory Improvement Amendments
C_{max}	Maximum observed concentration
CNS	Central nervous system
CRF	Case report form
CRISPR-Cas9	Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9
CRO	Contract research organization
CRU	Clinical research unit
CSF	Cerebrospinal fluid
CV	Coefficient of variation
CYP	Cytochrome P450
DBP	Diastolic blood pressure
DLT	Dose-limiting toxicity
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
EOS	End-of-study

Abbreviation	Definition or Explanation
FDA	Food and Drug Administration (of the US)
FSH	Follicle-stimulating hormone
Gal	Galactose
gal-NAc	N-acetylgalactosamine
GALE	Uridine diphosphate galactose 4'-epimerase
GALK	Galactokinase
GalNet	International Galactosemia Network
GALT	Galactose-1-phosphate uridyltransferase
GCP	Good Clinical Practice
Glc	Glucose
glc-1P	Glucose-1-phosphate
glc-NAc	N-acetylglucosamine
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HED	Human equivalent dose
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
ICF	Informed Consent Form
ICH	International Council for Harmonisation
I/E	Inclusion/exclusion
IEC	Independent Ethics Committee
IPD	Important protocol deviation
IQ	Intelligence quotient
IRB	Institutional Review Board
IUD	Intrauterine device
IVRS/IWRS	Interactive Voice/Web Randomization System
LAR	Legally authorized representative
LDH	Lactate dehydrogenase
LV	Left ventricular
MAD	Multiple ascending dose
Max	Maximum
MDRD	Modification of Diet in Renal Disease study
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum

Abbreviation	Definition or Explanation
MRI	Magnetic resonance imaging
MRSD	Maximum recommended starting dose
MTD	Maximum tolerated dose
NASH	Non-alcoholic steatohepatitis
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
OAT	Organic anion transporter
OATP	Organic anion transporting polypeptide
OMIM	Online Mendelian Inheritance in Man
OTC	Over-the-counter
PK	Pharmacokinetic(s)
POI	Primary ovarian insufficiency
PT	Preferred Term
QD	Once daily
RBC	Red blood cell
RDW	Red cell distribution width
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SBP	Systolic blood pressure
SD	Standard deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
SRC	Safety Review Committee
$T_{1/2}$	Half-life
TBD	To be determined
TEAE	Treatment-emergent adverse event
T_{max}	Time of maximum observed concentration
UDP	Uridine diphosphate
US	United States

Note: Additional PK-related abbreviations can be found in Section 7.2.

1. INTRODUCTION

AT-007 is an aldose reductase inhibitor (ARI) that is being developed by Applied Therapeutics Inc. (the Sponsor) for the treatment of Classic Galactosemia (CG) and other severe enzyme deficiencies resulting in Galactosemia, such as GALK Deficiency.

There is no previous human experience with AT-007. This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and all applicable regulatory requirements.

1.1. Background

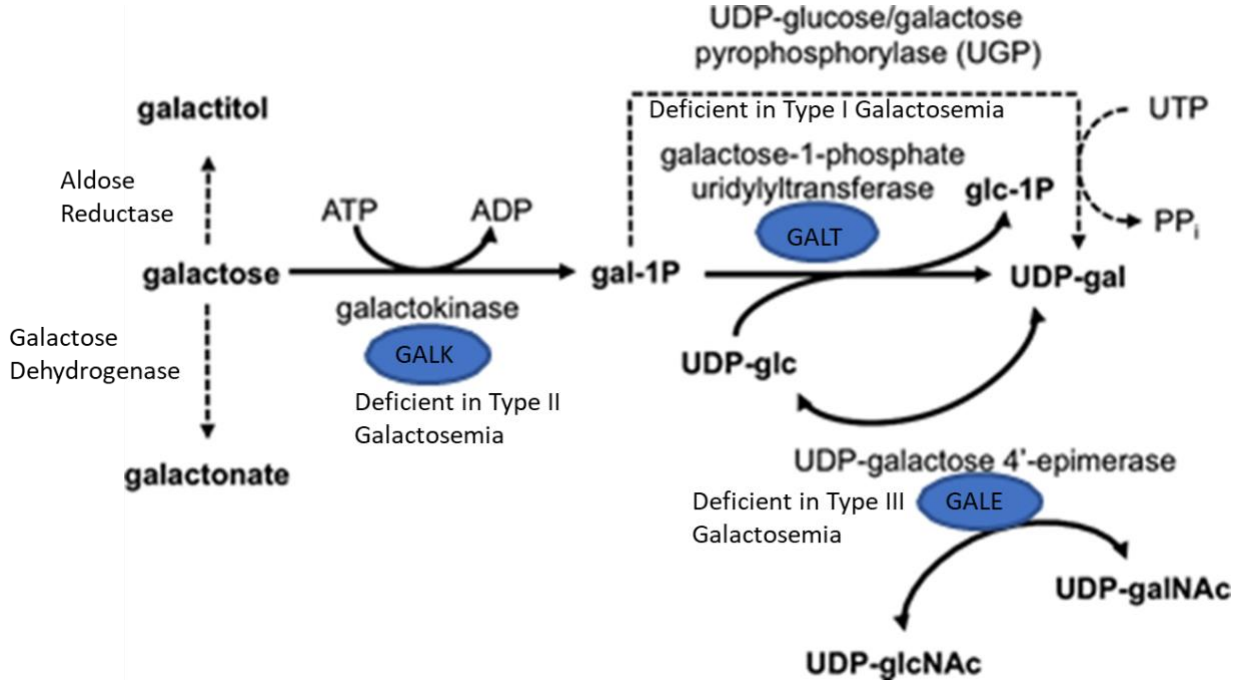
Galactosemia is a potentially life-threatening condition caused by genetic defects to enzymes involved in the metabolism of galactose. The clinical picture evolves in two phases. The first acute phase occurs after birth when the dietary intake of breast milk and formulas containing lactose produces a galactose toxicity syndrome manifested by hyperbilirubinemia, failure to thrive, vomiting, cataract formation, blood coagulation defects, and renal tubule dysfunction. The second phase, which results in long term complications of disease, occurs despite dietary restriction of galactose, with later development of speech abnormalities, mental retardation, neurological ataxias, cataract, and ovarian failure. This has been shown to be due to endogenous production of galactose within the body and cant be prevented by dietary restriction.

1.1.1. Galactosemia Types, Treatment, Prevalence, and Symptoms

Galactosemia is an inborn metabolic disease caused by aberrant metabolism of the sugar galactose, a metabolite of lactose (Berry 2000; Augustin 2010). Galactose, the C-4 epimer of glucose, cannot be metabolized directly by the glycolytic pathway. Thus, in organisms ranging from bacteria to humans, galactose is first converted to UDP-glucose via the sequential actions of three enzymes of the evolutionarily conserved Leloir pathway: galactokinase (GALK), galactose-1-phosphate uridyltransferase (GALT) and UDP-galactose 4'-epimerase (GALE) (Figure 1). Mutations in the genes coding for these enzymes give rise to different types of Galactosemia, which in the developed world are usually detected by routine screening of newborn infants.

AT-007 is in clinical development for the treatment of CG, a subtype of Type I Galactosemia further described in Section 1.1.2 and Section 1.1.3 and other severe enzyme deficiencies, such as GALK deficiency.

Figure 1: The Leloir Pathway and Alternative Route of Galactose Metabolism



Source: Modified from Daenzer et al, 2016.

1.1.2. GALT Deficiency (Includes Classic Galactosemia)

GALT deficiency can be classified into three categories based on its residual activity: Classic Galactosemia (CG), Clinical Variant Galactosemia, and Duarte Galactosemia. Classic Galactosemia patients exhibit severe GALT deficiency with absent or barely detectable erythrocyte and hepatic activity, whereas Clinical Variant Galactosemia is associated with residual GALT activity in the RBC and/or liver. Duarte Galactosemia is associated with 15% to 35% GALT activity. The residual or low GALT activity displayed by Clinical Variant and Duarte Galactosemia patients is sufficient to metabolize endogenously produced galactose, and in some cases minimal external lactose/galactose intake through the diet can be tolerated. Thus, Clinical Variant and Duarte Galactosemia patients often do not display the long-term complications seen in CG patients, as long as a stringent diet is adhered to. In contrast, patients with severe GALT deficiency (CG) display severe long-term complications, despite dietary restriction, and require additional therapeutic intervention to prevent these outcomes.

Type I Galactosemia (GALT deficiency) is the best studied form of the disease. Classic Galactosemia (CG) is characterized by severe GALT deficiency/absent or barely detectable GALT activity; Clinical Variant Galactosemia is associated with residual GALT activity in the RBC and/or liver; and biochemical variant Galactosemia is associated with 15% to 35% GALT activity (e.g., Duarte Galactosemia) (Fridovich-Keil et al, 2014). CG occurs with a frequency of 150,000 live births in the United States and in other multiethnic populations (Pyhtila et al, 2014). In the US, this translates to an incidence of approximately 80 infants born with CG every year.

Individuals with CG have elevated levels of galactose-1-phosphate (gal-1P), galactitol and galactonate in tissues, blood and urine. Galactitol and galactonate are products of alternative metabolism of excess galactose which accumulates when the Leloir pathway is blocked

(Figure 1). Erythrocyte gal-1P levels have been reported to be 20 to 30 times higher than normal, and erythrocyte galactitol levels up to 8 times higher than normal (Yager et al, 2003). Galactitol is not detectable in the plasma of healthy controls but is present in the plasma of treated individuals with CG at 10 to 12 µmol/L on average, and urinary galactitol can be ≥10-fold higher than in normal subjects (Palmieri et al, 1999). Galactitol levels in the brain have also been shown to be elevated in CG patients via magnetic resonance imaging (MRI).

CG typically presents in the neonatal period, with symptoms of poor feeding and poor growth, jaundice, liver enlargement and dysfunction, lethargy, hypotonia, renal tubular disease, cataracts and *E. coli* sepsis (Berry 2000). In the newborn period, CG is life-threatening but early implementation of a galactose-restricted diet, within the first 10 to 14 days of life, typically resolves the acute neonatal complications, and infants appear normal until 1 to 2 years of age, when long-term complications begin to emerge (Berry 1997, Demirbas 2018).

Despite early diagnosis/implementation of a galactose-restricted diet, the majority of patients with CG still develop long-term complications. Such chronic complications include CNS-related abnormalities (below average IQ [72%], tremor [46%], ataxia [15%], dysarthria [24%], speech and language deficiency [88%], depression [39%], anxiety [67%]); low bone density (24%); presenile cataracts (>21%); growth delay; and, in almost all females, primary ovarian insufficiency (POI) (Berry et al, 2018; Weisbren et al, 2012). It is unclear whether the CNS impairment is progressive throughout life, as there is a lack of well-controlled longitudinal assessments; however, a negative correlation has been demonstrated between age and cognition, suggesting the disease may be progressive through adulthood.

CNS dysfunction seen in Galactosemia patients appears to be due to broad structural and functional abnormalities, which may be the result of diffuse substrate or metabolite deposition within the brain. Brain imaging studies have shown cerebral and cerebellar atrophy as well as white matter abnormalities, including increased neurite dispersion and lower neurite density. Grey matter abnormalities have also been detected using voxel based morphometry. Functional MRI has shown altered neural activity and connectivity within several regions of the brain.

1.1.3. GALK Deficiency

Type II Galactosemia (GALK Deficiency or GALKD) displays wide variation in incidence due to three distinct founder mutations, in the Romani population (common in Eastern Europe, Ireland and the UK), Costa Rican population, and Japanese population. General incidence in Europe is approximately 1:50,000, but incidence in Germany was recently reported to be 1:40,000 due to an increased population of those of Romani descent. Incidence in the US population has not been well studied, but it is estimated to be 1:100,000. Patients with GALK deficiency cannot convert galactose into gal-1P and thus do not have elevated levels of this metabolite of galactose. Instead, they exhibit elevated levels of galactitol and galactonate. The typical presentation is neonatal cataracts (Hennermann et al, 2011), although cases of pseudotumor cerebri in the neonatal period have been also described, believed to be due to increased intracranial pressure caused by elevated galactitol levels in the brain (Bosch et al, 2002).

Until recently, long-term complications, specifically CNS complications (microcephaly, low intelligence, and speech and motor difficulties) such as those seen in GALT deficiency were not considered features of GALKD. However, a detailed analysis of a GALKD cohort of the Bosnian

population that had immigrated to Germany revealed high rates of intellectual deficits, microcephaly, and failure to thrive, even in those patients with good compliance on a galactose-restricted diet. Rates of these CNS complications were similar to those seen in GALT deficiency. Because GALKD patients display elevated galactitol levels, but not gal-1P (which does not form in GALKD patients), this study supported the strong likelihood that galactitol, not gal-1P, may be responsible for CNS complications seen in Galactosemia patients (Hennermann, 2011).

1.1.4. Enzyme Defects and Mechanisms of Disease

It was long hypothesized that accumulation of gal-1P was responsible for the CNS complications and ovarian insufficiency observed in patients with GALT deficiency (Type I Galactosemia) because patients with GALK deficiency (Type II Galactosemia), who do not produce or accumulate gal-1P, were thought to be free of such complications. However, a study of GALK deficiency in a cohort of a Bosnian population that had immigrated to Germany (Hennermann, 2011), revealed high rates of CNS dysfunction and developmental deficiencies similar to those seen in GALT-deficient patients. GALK-deficient patients did not have elevated gal-1P, either before or after implementation of a galactose-restricted diet, but did demonstrate highly elevated levels of galactitol. On a galactose-restricted diet, GALKD patients' galactose levels largely normalized, but galactitol levels remained elevated by about 10-fold compared with healthy control values. This study was the first prospective study of GALK-deficient patients identified via newborn screening. Before this study, these patients had been found primarily in countries where newborn screening and careful evaluations of Galactosemia outcomes were not performed (e.g., Bosnia and Bulgaria). This new information provided by the German GALKD patient cohort suggested that galactitol (not gal-1P) is the metabolite responsible for CNS complications in patients with Galactosemia.

Several other clinical findings support the role of galactitol (either in addition to gal-1P or instead of gal-1P) in long-term complications in patients with GALT and GALK deficiencies. For example, little correlation has been found in Galactosemia patients between the levels of gal-1P and clinical manifestations (Pyhtila et al, 2014). In addition, galactitol accumulation in the brain (detected by in vivo brain MRI with spectroscopy) has been reported to cause cerebral edema (Martineli et al, 2016), encephalopathy (Berry et al, 2001), and delay of psychomotor development with bilateral ocular cataracts (Otaduy et al, 2006). A restricted lactose-free diet induced almost complete resolution of the MRI white matter signal intensity abnormalities and disappearance of the galactitol peaks (Otaduy et al, 2006).

Experimental results detailed below provide additional evidence against gal-1p (or gal-1p alone) and/or point toward galactitol as a critical factor in the pathogenesis of the delayed and/or long-term complications of Galactosemia such as CNS disturbances and possibly ovarian insufficiency later in life:

- AR inhibition was shown to prevent galactose-induced ovarian dysfunction in the Sprague-Dawley rat (Meyer et al, 1992).
- A model of CG in *Drosophila melanogaster*, whose genome contains sequences related to all three Leloir pathway genes, mimics aspects of both the acute (survival of larvae) and long-term (climbing ability of adult males and fecundity of females) outcomes of CG (Daenzer et al, 2016). Like human patients, and unlike mice, GALT-deficient *Drosophila* larvae exhibit galactose-dependent lethality, which can

be rescued by the initiation of a galactose-restricted diet early in development or by the expression of a human GALT transgene. GALT-deficient flies that are raised and maintained exclusively on a galactose-restricted diet still demonstrate a clear deficiency in the normal negative geotaxic response seen in controls. As with the acute galactose-dependent phenotype, this delayed, galactose-independent muscular or neuromuscular deficit is rescued by the expression of a human GALT transgene. In this model, as expected, loss of GALK lowered or eliminated gal-1P accumulation in GALT-null animals. However, there was no concomitant rescue of larval survival or adult climbing or fecundity phenotypes. These findings strongly contradict the long-standing hypothesis that gal-1P accumulation underlies the pathophysiology of acute and long-term outcomes in GALT-null *Drosophila* and suggests that other metabolite(s) of galactose are responsible for the phenotype.

- Another model of CG, the GALT-null Sprague-Dawley rat model was created by using CRISPR-Cas9 gene editing technology [J. Fridovich-Keil, PhD, personal communication]. The GALT-null animals are homozygous for M3, a small frameshift insertion designated as rGALTm3. All M3/M3 neonates demonstrate complete absence of GALT activity in all tissues tested with apparently normal levels of the other Leloir enzymes, GALK and GALE. Like human patients with CG, following exposure to galactose, M3/M3 rats demonstrate abnormal accumulation of galactose, galactitol, galactonate, and gal-1P. All homozygotes also demonstrate conspicuous bilateral cataracts that are not seen in heterozygotes or wild- type animals. In contrast to human patients with CG, the GALT-null rats do not exhibit the lethal neonatal symptoms following exposure to mothers' milk (possibly because rat breast milk contains 10% of the lactose content of human breast milk), but they do demonstrate, in addition to cataracts, mild growth restriction both before and after weaning and a variety of CNS deficits as adults. These deficits can be revealed by tests including the rotarod (tests balance and coordination), Morris Water Maze (tests memory and cognitive flexibility), and Novel Object Recognition tests.

1.1.5. Clinical Management of Galactosemia

Currently, there are no therapies available for treatment of Galactosemia or prevention of the long-term complications associated with severe deficiencies in GALT or GALK. Standard of care is immediate implementation of a life-long galactose-restricted diet. Although the diet can reverse the acute clinical picture in the newborn and in many cases prevent fatalities in newborns if initiated early enough, it does not prevent the appearance of long-term complications in patients with severe enzyme deficiencies.

Guidelines recommend that children with severe GALT or GALK deficiencies be screened for speech and language delay, hearing and cognitive disorders at ages 7 to 12 months, and at 2, 3, and 5 years. Interventions include intensive therapy by a speech expert, psychologist, developmental pediatrician, etc. Also recommended are regular neurological and psychosocial evaluations, bone density and ophthalmologic assessments, a comprehensive dietary evaluation including monitoring of calcium intake, vitamins D and K supplementation if necessary, hormonal status evaluation and hormone replacement therapy consideration, as well as a regular exercise and assessment of skeletal problems. Periodic assessment of galactose metabolites in erythrocytes can help clinicians confirm dietary compliance (Welling et al, 2017).

Despite dietary restriction, patients with severe enzyme deficiencies still present with significant long-term complications of disease. Most adults with Galactosemia have an IQ under 85 and live at home with their parents or in a care facility. Because there are not many patients with Galactosemia over the age of 40 years old (because most died at birth prior to the initiation of newborn screening), the long-term prognosis of adults with Galactosemia is unclear. Complications of disease that present in adulthood, such as tremor and seizures, have not been studied prospectively in adults, nor has progression of cognitive impairment and IQ.

1.1.6. Galactosemia Prevalence

Prior to the initiation of newborn screening, which began in the US in the 1960s and was mandatory in all 50 States in 2004, nearly all infants with CG died at birth due to acute exposure to lactose. The current living population of CG patients in the US is estimated to be approximately 2,800 individuals. This is based on cumulative newborn screening results identifying 2,500 patients in the US from initiation of screening through 2014, and a yearly birth rate of approximately 80 patients per year in the US (providing an additional estimated 240 births in 2014 through 2018). The GALK-deficient Galactosemia population is largely undetermined in the US, but is estimated to be significantly lower than the CG population, based on an extremely low incidence in the US ethnic population.

1.2. Development of Aldose Reductase Inhibitors (ARIs)

If accumulation of galactitol is responsible for the long-term complications of Galactosemia, then it stands to reason that blocking AR from converting galactose to galactitol, in combination with a galactose-restricted diet, may be able to prevent/reduce these Galactosemia complications.

Applied Therapeutics Inc. (the Sponsor) is currently developing two structurally similar ARIs: one ARI is designated as AT-001 and another one is designated as AT-007. AT-001 is not CNS penetrant, while AT-007 penetrates the CNS when administered orally.

Using a genetic rat model of Galactosemia ("GALT null"), the Sponsor has successfully recapitulated critical features of Type I Galactosemia seen in humans. The accumulation of galactitol in various parenchyma and the onset of cataracts in this animal model can serve as biochemical and clinical markers of disease and disease progression.

In a pilot study in GALT-null neonatal rats, AT-001 (or placebo) was administered orally, suspended in the liquid formula, starting on the day after they were born and continuing for 9 to 18 days. Treatment with AT-001 decreased galactitol levels in the liver and brain and reduced or prevented the development of cataracts.

The Sponsor decided to develop AT-007 for the treatment of CG because it is both CNS penetrant and retinally penetrant. In the same "GALT null" rat model, treatment with AT-007 was able to normalize biochemical markers of Galactosemia and prevented the onset of cataracts, but importantly also prevented and CNS dysfunction.

This protocol (Study AT-007-1001) is the first-in-human study for AT-007.

1.3. Choice of Study Population

Study AT-007-1001 is being conducted in healthy adult subjects (Parts A [single ascending dose or SAD] and B and C [multiple ascending dose or MAD]); adult subjects with CG (Part D [SAD,

MAD] and Part D Extension); and adult subjects with GALK-deficient Galactosemia (Part E [SAD and MAD]).

1.4. Dose Rationale

The initial first-in-human dose for Part A (SAD) was 0.5 mg/kg. This did not exceed the maximum recommended starting dose (MRSD) using a safety factor of 10.

The MRSD is based on the 13-week rat and dog Good Laboratory Practice (GLP) studies evaluating doses of 50, 200, and 1000 mg/kg/day in rats and 50, 200, and 400 mg/kg/day in dogs.

AT-007 was administered to Beagle dogs once daily (QD) for at least 91 consecutive days via oral gavage. The following parameters and endpoints were evaluated in this study: clinical signs, body weights, body weight gains, food consumption, ophthalmology, electrocardiography, clinical pathology parameters (hematology, coagulation, serum chemistry, and urinalysis), toxicokinetic parameters, gross necropsy findings, organ weights, and histopathologic examinations.

There were no test article-related changes for ophthalmology, electrocardiography, clinical pathology, or organ weight data throughout the study.

Administration of AT-007 by once daily oral gavage was well tolerated in dogs at levels of 50, 200, and 400 mg/kg/day. The early termination (Day 85) in 1 male dog from the 400 mg/kg/day was associated with hematopoietic changes that were not observed in any other male or female dog at any dose level; as such, this observation was considered sporadic and a relationship with the test-article could not be established.

Therefore, the no-observed-adverse-effect level (NOAEL) was considered to be 400 mg/kg/day. This dosage corresponded to mean $AUC_{0\text{-}24}$ values of 1,130,000 and 1,160,000 h*ng/mL on Day 91 for males and females, respectively, and C_{max} values of AT-007 of 145,000 and 155,000 ng/mL on Day 91 for males and females, respectively

In the 13-week rat toxicology study, doses of 50, 200, and 1000 mg/kg/day were administered QD for 91 consecutive days via oral gavage. The following parameters and endpoints were evaluated in this study: clinical signs, body weights, body weight gains, food consumption, ophthalmology, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), toxicokinetic parameters, gross necropsy findings, organ weights, and histopathologic examinations.

There were no control or test article-related clinical observations or significant effects on body weights, food consumption, ophthalmology, clinical pathology, or mortality findings throughout the study. All gross necropsy and histopathological findings for the main and recovery periods resulted in no evidence of test article-related effects. Therefore, the NOAEL in the rat was considered to be 1000 mg/kg/day. This dosage corresponded to mean $AUC_{0\text{-}24}$ values of 2,040,000 and 2,360,000 h*ng/mL on Day 90 for males and females, respectively, and C_{max} values of AT-007 of 182,000 and 306,000 ng/mL on Day 90 for males and females, respectively.

Based on the NOAEL of 1000 mg/kg in rats and the NOAEL of 400 mg/kg in dogs, the human equivalent doses (HEDs) were calculated to be the following: 160 mg/kg/day from the rat and 216 mg/kg/day from the dog. Because the NOAEL of 400 mg/kg/day in dogs is the lower of the two, the HED of 216 mg/kg/day was chosen for the derivation of the MRSD. Therefore, using

the default safety factor of 10, the MRSD for AT-007 is 21.6 mg/kg/day. After consideration of other factors related to known pharmacology and another significant clinical program in the same mechanistic class, the starting dose for AT-007 in Part A was 0.5 mg/kg/day. This equates to a safety margin of 432 (216/0.5).

Dose levels in Part A escalated from 0.5 mg/kg to 5 mg/kg, 10 mg/kg, 20 mg/kg, and 40 mg/kg in the 5 cohorts (Cohorts A1 through A5) with no dose-limiting toxicity (DLT) identified in any cohort.

The first 3 cohorts in Part B (Cohorts B1, B2, and B3) escalated from 5 mg/kg to 10 mg/kg to 20 mg/kg following acceptable results for the same doses in Part A. Cohort B4 will start with this version of the protocol (version 5.0) and use a dose of 40 mg/kg based on that dose being acceptable in Cohort A5. The study drug exposure results were carefully considered to ensure that the plasma concentrations measured in Part A were in the range to detect changes in AR activity and galactitol levels in subjects with CG.

The doses in Part C are based on those in Part B. Cohort C1 was conducted with the same dose as Cohort B2 (10 mg/kg). Cohort C2 used the dose as Cohort B3 (20 mg/kg). Cohort C3 will use the same dose as Cohort B4 (40 mg/kg).

The initial dose in Part D (5 mg/kg) was based on data from Part A (Cohorts A1 through A4) and Part B (first 2 cohorts).

The starting dose in Part E will be the most clinically appropriate dose as determined by the evaluation of safety, tolerability, and PD (reduction in galactitol level) in Part D. The dose will not be higher than the highest acceptable dose in Part D at the time of starting Part E (after all subjects complete Cohorts D1 and D2 but may be before, during, or after Cohort D3).

The dose for the first cohort of the Part D Extension (Cohort DE1) will be 20 mg/kg/day based on acceptable SAD and MAD safety and PK data from Cohort D2.

AT-007 dose levels in Part A ranged from 0.5 and 40 mg/kg/day. Dose levels in subsequent parts of the study are based on the safety and PK results (and galactitol results in Parts D, E, and D Extension only) of previous cohorts and/or previous parts of the study. Doses higher than 40 mg/kg may be evaluated if the human C_{max} and AUC are substantially lower than the corresponding values calculated in nonclinical experiments and no DLT is observed up to and including at the 40 mg/kg dose. If dose escalation above 40 mg/kg is feasible based on the above criteria, these higher doses may be investigated in additional SAD and MAD cohorts in healthy subjects and in subjects with Galactosemia if no AT-007 levels are detected in the cerebrospinal fluid (CSF) collected from the healthy subjects enrolled in Cohort C3.

Although it is anticipated that daily doses do not need to be higher than 40 mg/kg, there is a chance that the PK data observed during the study will show otherwise. If this happens, then additional subjects may be enrolled.

2. OBJECTIVES AND ENDPOINTS

The primary objective of this multicenter, first-in-human study is the following:

- To evaluate the safety of single and multiple ascending doses of orally administered AT-007 in healthy adult subjects and adult subjects with CG or GALK-deficient Galactosemia

The secondary objectives of this study are the following:

- To evaluate the pharmacokinetic (PK) parameters of single and multiple doses of orally administered AT-007 in healthy adult subjects and adult subjects with CG or GALK-deficient Galactosemia
- To evaluate the effect of single and multiple doses of orally administered AT-007 on the level of galactitol, a biomarker of AR activity, in adult subjects with CG or GALK-deficient Galactosemia
- To evaluate the effect of single and multiple doses of orally administered AT-007 on the levels of galactose and its other metabolites in adult subjects with CG or GALK-deficient Galactosemia

The exploratory objectives are the following:

- To measure the levels of AT-007 in the cerebrospinal fluid (CSF) of healthy adult subjects

The primary endpoints of this study are overall safety and adverse events (AEs). Safety will be assessed by the following:

- AEs
- Clinical safety laboratory tests (hematology, chemistry, urinalysis)
- Physical examinations
- Vital signs
- Electrocardiograms (ECGs)

The secondary endpoints are the following:

- PK parameters (Section 7.2) in healthy subjects, subjects with CG, and subjects with GALK-deficient Galactosemia
- Galactose, galactitol, and other galactose metabolites in the blood of subjects with CG and subjects with GALK-deficient Galactosemia
- Urine galactitol for subjects with CG and subjects with GALK-deficient Galactosemia

The exploratory endpoints are the following:

- AT-007 level in the CSF of healthy subjects (Part C only)

PK samples will be stored for possible future analysis of AT-007 metabolites.

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design

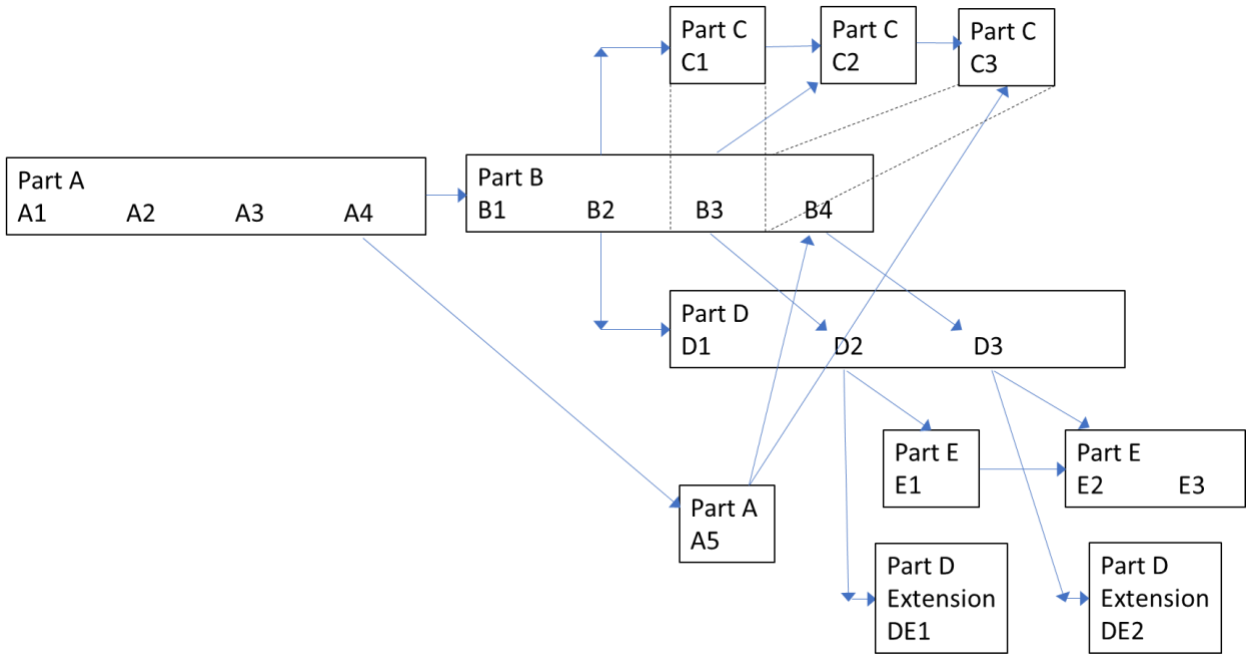
This is a first-in-human, randomized, placebo-controlled, 6-Part, SAD and MAD study in healthy adult subjects, adult subjects with CG, and adult subjects with GALK-deficient Galactosemia. The study will assess the safety and PK of AT-007 in these subjects as well as the effect of AT-007 on biomarkers of galactose metabolism (galactose and galactose metabolites) in subjects with galactosemia. The study is being conducted in multiple centers in the US and Europe. This study consists of 6 parts (Table 1) with the timing of the parts as shown in Figure 2.

Table 1: Study Design

Study Part	Study Population	Design	Planned Number of Cohorts	Subjects Per Cohort	Total # of Subjects
Part A	Healthy Adult Subjects	SAD	5	8	40
Part B	Healthy Adult Subjects	MAD (QD for 7 days)	4	6 in 2 cohorts & 8 in 2 cohorts	28
Part C	Healthy Adult Subjects	MAD (QD for 7 days)	3	4	12
Part D	Adult Subjects with CG	SAD, 5-day washout, MAD (QD for 27 days)	3	6	18
Part E	Adult Subjects with GALK-deficient Galactosemia	SAD, 5-day washout, MAD (QD for 27 days)	1 to 3	6	18
Part D Extension	Adult Subjects with CG	MAD (QD for 90 Days)	1 to 2	8 to 28	28

CG = Classic Galactosemia; EOS = end of study; MAD = multiple ascending dose; QD = once daily (dosing); SAD = single ascending dose; TEAE = treatment emergent adverse event.
The total number of subjects column reflects the maximum number of subjects if all cohorts are conducted with the planned number of subjects and no subject participates in more than 1 cohort and/or part. However, in Parts D and E, subjects are allowed to participate in multiple cohorts (assuming no safety or tolerability issues in the prior cohort) on any treatment assignment with a ≥ 5-day washout period between the last dose of study drug in one cohort and the first dose of study drug in the next cohort. Also, Part D subjects can participate in Part D Extension at any time after the Part D EOS Visit (Part D completers and those who discontinued for any reason other than study drug related TEAE and had an EOS Visit more than 14 days after the last dose) or any time beyond 14 days after the last dose in Part D (Part D subjects who discontinued Part D for any reason other than study drug related TEAE and did not have an EOS Visit more than 14 days after the last dose).
Parts A and B are double-blind. Part C is open-label. In Parts D, E, and D Extension, the clinical site staff (except for the unblinded pharmacist) and the subjects are blinded to study treatment but the Sponsor is not blinded.
The number of cohorts and total number of subjects may increase if doses > 40 mg/kg are tested in additional cohorts (see Section 1.4). Also, in Parts D and E, the overall number of subjects per cohort may be increased up to 8 subjects to address any potential lost-to-follow-up among subjects treated with either placebo or AT-007. Up to 28 total subjects in up to 2 cohorts are planned for Part D Extension.

Figure 2: Study Schematic



Within each Part, the Cohorts are sequential. Solid lines with arrows indicate that data from the part or cohort at the non-arrow end are required before the part or cohort at the arrow end can start. Dotted lines indicate cohorts (C1 and B3; C3 and B4) that will be conducted simultaneously. Parts D, E, and D Extension are not dependent on Part C results, and no temporal relationship between Parts D, E, and D Extension and Part C is implied. Also, with the exception of Cohorts B4, C3, D3, E2, E3, and DE2 being directly or indirectly dependent on Cohort A5 results, no temporal relationship between Cohort A5 and any other part/cohort is implied. Cohort DE2 (start and dose level) are not dependent on results from Cohort DE1. Figure is not to scale.

Parts A, B, and C are being conducted in a single clinical research unit (CRU) in the US. Subjects within each cohort in Part A were enrolled and dosed at approximately the same time (within 2 to 3 days for the first cohort and on the same day for the subsequent cohorts). Subjects within each cohort in Part B will be enrolled and dosed on the same days. The first cohort of Part C (C1) was conducted simultaneously with the third cohort of Part B (B3) and received the same dose (10 mg/kg) as the second cohort of Part B (B2). The second cohort of Part C (C2) started after all subjects in the third cohort of Part B (B3) completed the study (minus the End-of-Study [EOS] Visit) and received the same dose (20 mg/kg) as Cohort B3; safety and PK results for Cohort C1 were also considered before starting Cohort C2. The third cohort in Part C (C3) will be conducted simultaneously with the fourth cohort of Part B (B4) and receive the same dose (40 mg/kg); safety and PK results for Cohort C2 were also considered before starting Cohort C3. For Cohorts B3 and C1, subjects were enrolled and dosed on the same day but in different parts of the CRU to separate double-blind (B3) from open-label (C1) dosing. All subjects in Cohort C2 were enrolled and dosed on the same day. All subjects in Cohorts B4 and C3 will be enrolled and dosed on the same day but in different parts of the CRU to separate double-blind (B4) from open-label (C3) dosing.

Parts D, E, and D Extension are being conducted in multiple CRUs. It is not anticipated that subjects within each cohort will be enrolled/dosed at approximately the same time.

Initially, 4 double-blind dosing cohorts were planned in the SAD portion of the study in healthy subjects (Part A); subsequently, a fifth cohort (A5) was added to Part A. Within each cohort of 8 subjects, 6 subjects were randomly assigned to AT-007 and 2 subjects were randomly assigned to placebo. For the first cohort, the first 2 subjects were sentinel subjects with 1 subject randomly assigned to AT-007 and 1 subject randomly assigned to placebo. These 2 sentinel subjects were observed for at least 24 hours before any of the additional subjects (6 subjects: 5 randomly assigned to AT-007 and 1 randomly assigned to placebo) in the first cohort were dosed. The dose levels of AT-007 for Cohorts A1, A2, A3, A4, and A5 were 0.5, 5, 10, 20, and 40 mg/kg.

Before the study proceeded to Part B (MAD in healthy subjects), safety data from Cohorts A1 through A4 in Part A (SAD in healthy subjects) were evaluated by the Safety Review Committee (SRC) (Section 3.4) and all PK data from Cohorts A1 through A4 were evaluated by the Sponsor. As of this protocol version, Cohorts A1 through A5 (0.5, 5, 10, 20, and 40 mg/kg) have completed the study with acceptable results.

Four double-blind dosing cohorts are planned in the MAD portion of the study in healthy subjects (Part B). Within each cohort of 6 or 8 subjects, 4 or 6 subjects will be randomly assigned to AT-007 and the remaining 2 subjects will be randomly assigned to placebo. Subjects will receive study drug (AT-007 or placebo) QD for 7 consecutive days. The starting dose level of AT-007 for the first cohort (B1) was 5 mg/kg based on the results of Part A. Subsequent cohorts (B2 and B3) were given 10 and 20 mg/kg based on results from the previous cohorts in both Parts A and B. Cohort B4 is planned with a dose level of 40 mg/kg based on data from Cohort A5.

The review of the PK data from Cohorts A1 through A4 and the first 2 cohorts of Part B confirmed that once daily (QD) dosing is adequate.

Part C consists of 3 open-label MAD dosing cohorts (same dose levels as Cohorts B2, B3, and B4) in healthy subjects. Cohort C1 was conducted at the same time as Cohort B3 using the same dose (10 mg/kg) as Cohort B2. Cohort C2 was conducted after all subjects in Cohort B3 completed the study (minus the EOS Visit) and used the same dose (20 mg/kg) as Cohort B3; data from Cohort C1 were also considered before starting Cohort C2. Cohort C3 will run concurrently with Cohort B4 and use the same dose (40 mg/kg); data from Cohort C2 were also considered before starting Cohort C3. Within each Part C cohort, all 4 subjects will be treated with AT-007. Subjects will have a lumbar puncture on Day 7 to collect CSF for the measurement of AT-007 with the exact timing of the lumbar puncture based on PK data from Part B. Otherwise, the subjects in Part C have the same schedule as the subjects in Part B (minus randomization).

Before the study proceeded to Part D (SAD and MAD in subjects with CG), safety data from Cohorts A1 through A4 in Part A and the first 2 cohorts in Part B were evaluated by the SRC and all PK data from these cohorts were evaluated by the Sponsor.

Three dosing cohorts are planned for subjects with CG (Part D). The same subjects will receive both the single dose and the multiple doses. Within each cohort of 6 subjects, 4 subjects will be randomly assigned to AT-007 and 2 subjects will be randomly assigned to placebo. Following a single dose of study drug (AT-007 or placebo), there will be a 5-day washout. Subjects who tolerate the single-dose will receive 1 daily dose of the same study drug (AT-007 or placebo) for 27 consecutive days. The decision of whether each individual subject can start multiple dosing

will be made by the Investigator in consultation with the Sponsor. **No subject in Part D can start multiple dosing without explicit agreement from the Sponsor.** Subjects will complete the 27 days of treatment and the associated PK, PD, and safety assessments through the 2 days after the MAD portion and then have an EOS Visit at 28 days after the last dose.

The starting dose for Part D (i.e., for Cohort D1) was based on results from Part A (Cohorts A1 through A4) and Cohorts B1 and B2 and at 5 mg/kg was lower than the dose for Cohort B2 (10 mg/kg). The dose for Cohort D2 (20 mg/kg) was based on results from these cohorts plus Cohorts B3 and D1; results for Cohort C1 were also considered. The dose for Cohort D3 will be based on results from all cohorts in Parts A and B and Cohorts D1 and D2 and will not be higher than the dose for Cohort B4.

Before the study proceeds to Part E (SAD and MAD in subjects with GALK-deficient Galactosemia), safety data from all completed cohorts in Parts A, B, and D of the study will be evaluated by the SRC and all PK data from these cohorts will be evaluated by the Sponsor. The starting dose for Part E will not be higher than the highest acceptable dose in Part D at the time of starting Part E (after all subjects complete Cohorts D1 and D2 but may be before, during, or after Cohort D3). The dose for subsequent cohorts (if any) in Part E will be based on results from the previous cohort(s) in Part E.

One to 3 dosing cohort are planned for subjects with GALK-deficient Galactosemia (Part E). The same subjects will receive both the single dose and the multiple doses. Within each cohort of 6 subjects, 4 subjects will be randomly assigned to AT-007 and 2 subjects will be randomly assigned to placebo. Following a single dose of study drug (AT-007 or placebo), there will be a 5-day washout. Subjects who tolerate the single-dose will receive 1 daily dose of the same study drug (AT-007 or placebo) for 27 consecutive days. The decision of whether each individual subject can start multiple dosing will be made by the Investigator in consultation with the Sponsor. **No subject in Part E can start multiple dosing without explicit agreement from the Sponsor.** Subjects will complete the 27 days of treatment and the associated PK, PD, and safety assessments through the 2 days after the MAD portion and then have an EOS Visit at 28 days after the last dose.

Notes for Parts D and E: The overall number of subjects per cohort may be increased up to 8 subjects to address any potential lost-to-follow-up among subjects treated with either placebo or AT-007. Subjects treated with study drug (placebo or AT-007) in a cohort may be randomized to treatment in another cohort (separate consent required) within the same Part (assuming no safety or tolerability issues in the prior cohort) as long as there is a washout period of ≥5 days between the last dose in a cohort and the first dose in the next cohort.

The dose for the first cohort of the Part D Extension (DE1) will be 20 mg/kg/day based on acceptable SAD and MAD safety and PK data from Cohort D2. After SRC review of all SAD and MAD data and Sponsor review of PK and galactitol data for Cohorts D1 through D3, a second cohort of the Part D Extension (DE2) may be initiated to evaluate a dose >20 mg/kg/day if this dose was well tolerated in the SAD and MAD portions of the study in both healthy volunteers and subjects with CG.

In Part D Extension, within each cohort of up to 28 subjects, subjects will be randomly assigned in a 3:1 to AT-007 and placebo. Subjects will receive 1 daily dose of the same study drug (AT-007 or placebo) for 90 consecutive days. Subjects will complete the 90 days of treatment

and the associated PK, PD, and safety assessments through the day after the last dose and then have an EOE Visit at 28 days after the last dose. The EOE Visit will not be required for subjects who transition to a separate Open-Label Extenstion Study. For these subjects the study completion will be the completion of the Treatment Period at Day 91.

For all its parts (A, B, C, D, E, and D Extension), the study consists of a Screening Period (28 days [Day -28 to Day -1] in Parts A, B, and C; 40 days [Day -40 to Day -1] in Parts D and E; and Day -60 to Day -1 in Part D Extension for de novo subjects who did not participate in Part D and subjects whose last visit in Part D was >60 days before Day 1 in the Part D Extension]), a period of study treatment and associated assessments, and an EOS Visit (28 days after the last dose of study drug) (for Part D Extension, the visit at 28 days after the last dose is called the end of extension [EOE] Visit). Part D Extension de novo subjects may also participate in optional prescreening. Subjects will be randomized and receive the first dose of study drug on Day 1. At a minimum, subjects will remain at the clinical research unit (CRU) through 24 hours after the first dose. Subjects in Part A will have 1 in-clinic stay during the Treatment Period with the single dose taken in the CRU. Subjects in Parts B and C will have 1 in-clinic stay with all 7 doses taken in the CRU. Subjects in Parts D and E will have 3 in-clinic stays with most doses taken at home. Subjects in Part D Extension will have 2 in-clinic stays and monthly visits (home health or in-clinic) with most doses taken at home. Subjects who withdraw or are withdrawn from the study after taking study drug will not be replaced.

The Schedules of Assessments for Parts A, B, C, D, E, and D Extension are provided in Appendix A (Part A), Appendix B (Part B), Appendix C (Part C), Appendix D (Part D), Appendix E (Part E), and Appendix F (Part D Extension), respectively.

At selected time points during all parts of the study, all subjects will have the following:

- Assessment of AEs
- Blood and urine samples collected for routine safety laboratory assessments
- Physical examinations, assessment of vital signs, and ECGs
- Pregnancy tests (all females at screening and females of childbearing potential subsequently)
- Blood samples collected for PK

For subjects enrolled in selected cohorts in Parts A and D (Cohorts A4, A5, and D3), urine samples will be collected at selected time points after the single dose for the assessment of AT-007.

Subjects in Part C will have a lumbar puncture on Day 7 after the last dose to collect CSF for measurement of AT-007. The timing of the lumbar puncture relative to the last dose will be based on the PK data from completed cohorts in Part B. The intent is for the lumbar puncture to be taken close to the time of the maximum observed concentration (T_{max}) of AT-007 after the last dose.

During Part D, at selected time points, subjects will have blood samples taken for the assessment of GALT activity in red blood cells, GALT gene analysis, and aldose reductase activity; these assessments will also be done for Part D Extension subjects who did not participate in Part D but will not be repeated for those who did participate in Part D. Also, subjects in both Part D and

Part D Extension will have blood samples taken for the assessment of galactose and its metabolites, and subjects in Part D will also have urine collected for the assessment of galactitol.

During Part E, at selected time points, subjects will have blood samples taken for the assessment of GALK activity in red blood cells, GALK gene analysis, aldose reductase activity, as well as galactose and its metabolites and urine collected for the assessment of galactitol.

Within each multi-cohort part (A, B, C, and D [and E, if applicable]) of the study, in the subsequent cohorts after the first cohort, the dose may be increased, decreased, or repeated. The SRC will review safety (including AE, laboratory, physical examination, vital signs, and ECG data) through Days 3, 8, 8, 12, and 12 during Parts A, B, C, D, and E, respectively, for each cohort and provide a recommendation for the next cohort as needed. If there is a second cohort in Part D Extension, the dose will be higher than 20 mg/kg and depend on the results for that dose in healthy subjects and subjects with CG in Part D.

3.2. Rationale for Overall Study Design

The overall study design is commonly used for Phase 1 safety and PK studies. Incorporation of sentinel subjects in any cohort, especially the first cohort, is designed to limit the number of subjects exposed to a potentially unsafe drug or unsafe dose. The durations of the in-clinic stays are based on safety and practical considerations to ensure proper assessment of potential AEs and collection of biological samples and various examinations/measurements required by the study. An EOS or EOE Visit is conducted at 28 days after the last dose to assess the outcome of AEs (if any) after study drug treatment and to ensure proper reporting of AEs with onset during the post-treatment period. Use of an SRC to assess safety and PK data before dose escalation and before transitioning from single doses (Part A) to multiple doses (Parts B and C) is standard practice in such studies. Having safety and PK data in healthy subjects before dosing subjects with the proposed indication(s)) (Parts D, E, and D Extension) is also standard practice. Having longer duration doses not exceed doses that have been studied for shorter durations is standard practice as well. Having the Investigator (with explicit agreement from the Sponsor) rather than the SRC decide if safety for the sentinel subjects in Cohort A1 is acceptable and if a subject in Part D or E will start multiple dosing with study drug after the single dose are practical consideration because the decisions needs to be made within 24 hours (Cohort A1) and within the 5-day washout period (individual subjects in Parts D and E). Galactose and its metabolites are only measured in subjects with CG or GALK-deficient Galactosemia (Parts D, E, and D Extension) because AR (the enzyme inhibited by AT-007) converts galactose to galactitol only in the presence of extremely high levels of galactose (found in subjects with CG or GALK-deficient Galactosemia but not healthy subjects).

3.3. Study Drug Administration Schedules

Parts A, B, C, and D of the study will start more or less sequentially followed by starting Parts E and D Extension more or less concurrently. However, conduct of all study parts will overlap (Figure 2 and Section 3.1).

Within each multi-cohort part, the cohorts (dose levels) will be dosed sequentially. The SRC will review available safety data through a pre-specified timeframe (Day 3 for Part A, Day 8 for Parts B and C, and Day 12 for Part D [and E, if applicable]) and provide recommendations for the next cohort. If there is a second cohort in Part D Extension, the dose will be higher than

20 mg/kg and depend on the results for that dose in healthy subjects and subjects with CG in Part D. Additional cohorts will be enrolled if it is deemed appropriate after safety assessment by the SRC and confirmation by the Sponsor. A decision to repeat a dose level or to study another higher or lower dose level may be made. The Investigator(s) and Institutional Review Boards (IRBs) / Independent Ethics Committees (IECs) will be notified of these decisions.

The SRC will also review data for the last cohort for each study part except that there will be no next cohort within the same part of the study.

AT-007 dose levels in Part A were between 0.5 and 40 mg/kg. Dose levels in subsequent parts of the study are based on the safety and PK results (and galactitol results in Parts D, E, and E Extension only) of previous cohorts and/or previous parts of the study. Doses higher than 40 mg/kg may be evaluated if the human C_{max} and AUC are substantially lower than the corresponding values calculated in nonclinical experiments and no dose limiting toxicity is observed up to and including at the 40 mg/kg dose. If dose escalation above 40 mg/kg is feasible based on the above criteria, these higher doses may be investigated in additional SAD and MAD cohorts in healthy subjects and in subjects with CG or GALK-deficient Galactosemia if no AT-007 levels are detected in the CSF collected from the healthy subjects enrolled in Cohort C3.

All doses of study drug (AT-007 or placebo) should be taken in the morning under fasting conditions (after at least a 10-hour overnight fast that is broken 2 hours after dosing [mandatory for doses with PK sampling time points and recommended for all other doses]). This will be the case regardless of whether the study drug dose is taken at the CRU or at home.

3.3.1. Part A: Double-blind Single Ascending Dose (SAD) in Healthy Subjects

Four dose levels were initially planned for the SAD group and a fifth dose level was recently added and completed (Table 2). Subjects were enrolled in 5 sequential cohorts of 8 subjects each, for a total of 40 subjects. The initial dose level was 0.5 mg/kg based on nonclinical study results (Section 1.4). The first cohort included a sentinel group (first 2 subjects with 1 per treatment) dosed at the same time. Dose administration for the remainder of the first cohort occurred only at least 24 hours after the 2 sentinel subjects received study drug (AT-007 or placebo) and were contingent upon acceptable safety results through 24 hours for the sentinel subjects per Investigator in consultation with the Sponsor and with explicit agreement from the Sponsor. Subjects were housed at the CRU for observation for at least 48 hours after dosing. Subjects returned for the EOS Visit at 28 days after the dose. Progression to and the dose level for the next cohort were dependent upon safety data through Day 3 from the previous cohort. The Schedule of Assessments for Part A is provided in Appendix A. Maximum duration of study participation (Screening through EOS Visit) for an individual subject was approximately 56 days.

Table 2: SAD Dose Cohorts in Healthy Subjects (Part A)

Cohort	Dose	
A1 (sentinel group)	Single dose of AT-007 oral capsule, dose 0.5 mg/kg (n=1)	placebo (n=1)
A1 (remainder of cohort)	Single dose of AT-007 oral capsule, dose 0.5 mg/kg (n=5)	placebo (n=1)
A2	Single dose of AT-007 oral capsule, dose 5 mg/kg (n=6)	placebo (n=2)
A3	Single dose of AT-007 oral capsule, dose 10 mg/kg (n=6)	placebo (n=2)
A4	Single dose of AT-007 oral capsule, dose 20 mg/kg (n=6)	placebo (n=2)
A5	Single dose of AT-007 oral capsule, dose 40 mg/kg (n=6)	placebo (n=2)

n = number of subjects; SAD = single ascending dose.
Note: The number of cohorts and total number of subjects may increase if doses above 40 mg/kg are tested in additional cohorts (see Section 1.4). This table has been updated to reflect the dose levels actually tested in completed Cohorts A1 through A5. At this time, no additional cohorts are planned in Part A.

3.3.2. Part B: Double-blind Multiple Ascending Dose (MAD) in Healthy Subjects

Four dose levels are planned for the MAD group in Part B (Table 3). Subjects will be enrolled in 4 sequential cohorts of 6 or 8 subjects each, for a total of 28 subjects. The initial dose level for Part B (5 mg/kg) was based on the results from Part A Cohorts A1 through A4. Subjects will receive study drug (AT-007 or placebo) QD for 7 consecutive days. Subjects will be housed at the CRU for observation from Day -1 (day before the first dose) through at least 48 hours after the last dose. Subjects will return for the EOS Visit at 28 days after the last dose. Progression to and the dose level for the next cohort will be dependent upon safety data through Day 8 from the previous cohort as well as results from Part A. The Schedule of Assessments for Part B is provided in Appendix B. Maximum duration of study participation (Screening through EOS Visit) for an individual subject will be approximately 63 days.

Table 3: MAD Dose Cohorts in Healthy Subjects (Part B)

Cohort	Dose	
B1	AT-007 oral capsule QD for 7 consecutive days, dose 5 mg/kg (n=6)	placebo (n=2)
B2	AT-007 oral capsule QD for 7 consecutive days, dose 10 mg/kg (n=4)	placebo (n=2)
B3	AT-007 oral capsule QD for 7 consecutive days, dose 20 mg/kg (n=6)	placebo (n=2)
B4	AT-007 oral capsule QD for 7 consecutive days, dose 40 mg/kg (n=4)	placebo (n=2)

n = number of subjects; MAD = multiple ascending dose; QD = once daily.
Note: The number of cohorts and total number of subjects may increase if doses above 40 mg/kg are tested in additional cohorts (see Section 1.4). The numbers of subjects in the cohorts are intended to ensure at least 6 subjects on AT-007 at each well-tolerated dose level when Cohort C subjects at the same dose level are considered. This table has been updated to reflect the dose levels actually tested in completed Cohorts B1, B2, and B3 and the planned dose in Cohort B4.

3.3.3. Part C: Open-label Multiple Ascending Dose (MAD) in Healthy Subjects

The first cohort of Part C (i.e., Cohort C1) was conducted concurrently with Cohort B3 but at different locations with the same CRU, and the second cohort of Part C (Cohort C2) was conducted after Cohort B3. Cohort C1 used the same dose (10 mg/kg) as Cohort B2; Cohort C2

used the same dose (20 mg/kg) as Cohort B3; and Cohort C3 will use the same dose as and run concurrently with Cohort B4 (40 mg/kg) (Table 4). Results for prior Part C cohorts will be considered before starting the next Part C cohort. Each of the cohorts in Part C will consist of only 4 subjects and all subjects will be assigned to open-label AT-007. The subjects will follow the same schedule as the subjects in Part B except that subjects in Part C will have a lumbar puncture at approximately T_{max} after the last dose (Day 7 dose) for the collection of CSF (Appendix C).

Table 4: MAD Dose Cohorts in Healthy Subjects with CSF Collection (Part C)

Cohort	Dose
C1	AT-007 oral capsule QD for 7 consecutive days, dose 10 mg/kg (n=4)
C2	AT-007 oral capsule QD for 7 consecutive days, dose 20 mg/kg (n=4)
C3	AT-007 oral capsule QD for 7 consecutive days, dose 40 mg/kg (n=4)

n = number of subjects; MAD = multiple ascending dose; QD = once daily.
This table has been updated to reflect the dose levels actually tested in completed Cohorts C1 and C2 and the planned dose in Cohort C3.

3.3.4. Part D: Single Ascending Dose (SAD) and Multiple Ascending Dose (MAD) in Subjects with Classic Galactosemia (CG)

Three dose levels are planned for the subjects with CG (Table 5). Subjects will be enrolled in 3 sequential cohorts of 6 subjects each for a total of up to 18 subjects. The initial dose level for Part D was based on the results from Cohorts A1 through A4 and Cohorts B1 and B2. Subjects will receive study drug (AT-007 or placebo) on Day 1 and remain at the CRU for at least 48 hours after the first dose. Subjects will then have a 5-day washout period (Day 1 postdose through Day 6 predose). Then, subjects who tolerated the single dose will take the same study drug QD for 27 consecutive days (Day 6 through Day 32, inclusive) for the multiple dosing portion of Part D. **(Note: For each individual subject, the Investigator and the Sponsor will decide whether the subject can start the multiple dosing portion of Part D. The Investigator must consult the Sponsor. Starting the multiple dosing portion requires explicit agreement from the Sponsor.)** In the 27-day multiple dosing portion, subjects will take most of their doses at home (fasting conditions [Section 5.3] recommended). Subjects will return to the site for brief in-clinic stays after the first week of multiple dosing and at the end of multiple dosing as indicated in the Schedule of Assessments for Part D (Appendix D). Subjects will return for the EOS Visit at 28 days after the last dose.

Progression to and the dose level for the next cohort will be dependent upon safety data through Day 12 and at least single dose PK and galactitol results from the previous cohort as well as additional Part B (and possibly Part C) results.

The Schedule of Assessments for Part D is provided in Appendix D. Maximum duration of study participation (Screening through EOS Visit) for an individual subject will be approximately 100 days.

Table 5: Dose Cohorts in Subjects with Classic Galactosemia (Part D)

Cohort	Dose	
D1	AT-007 oral capsule on Day 1, dose 5 mg/kg (SAD portion), followed by a 5-day-washout, and AT-007 oral capsule QD for 27 days (MAD portion), dose 5 mg/kg (n=4)	placebo (n=2)
D2	AT-007 oral capsule on Day 1, dose 20 mg/kg (SAD portion), followed by a 5-day-washout, and AT-007 oral capsule QD for 27 days (MAD portion), dose 20 mg/kg (n=4)	placebo (n=2)
D3	AT-007 oral capsule on Day 1, dose TBD mg/kg (SAD portion), followed by a 5-day-washout, and AT-007 oral capsule QD for 27 days (MAD portion), dose TBD mg/kg (n=4)	placebo (n=2)

n = number of subjects; MAD = multiple ascending dose; QD = once daily; SAD = single ascending dose; TBD = to be determined.

Note: The number of cohorts and total number of subjects may increase if doses above 40 mg/kg are tested in additional cohorts (see Section 1.4). The overall number of subjects per cohort may be increased up to 8 subjects to address any potential lost-to-follow-up among subjects treated with either placebo or AT-007. Subjects treated with study drug (placebo or AT-007) in a cohort may be randomized to treatment in another cohort (separate consent required) within the same Part (assuming no safety or tolerability issues in the prior cohort) as long as there is a washout period ≥5 days between the last dose in a cohort and the first dose in the next cohort. This table has been updated to reflect the dose levels actually tested in completed Cohorts D1 and D2. The dose level for Cohort D3 is TBD as of the writing of protocol version 5.0.

3.3.5. Part E: Single Ascending Dose (SAD) and Multiple Ascending Dose (MAD) in Subjects with GALK-deficient Galactosemia

The starting dose level for subjects with GALK-deficient Galactosemia will be the most clinically appropriate dose as determined by the evaluation of safety, tolerability, and PD (reduction in galactitol level) in Part D cohorts completed at the time of starting Part E (Table 6). If needed, additional dose levels may be tested in additional cohorts. If applicable, dose levels for subsequent cohorts in Part E will be based on safety data through Day 12 and at least single dose PK and galactitol results from the previous cohort(s) in Part E and possibly additional cohorts in Part D.

Subjects will receive study drug (AT-007 or placebo) on Day 1 and remain at the CRU for at least 48 hours after the first dose. Subjects will then have a 5-day washout period (Day 1 postdose through Day 6 predose). Then, subjects who tolerated the single dose will take the same study drug QD for 27 consecutive days (Day 6 through Day 32, inclusive) for the multiple dosing portion of Part E. **(Note: For each individual subject, the Investigator and the Sponsor will decide whether the subject can start the multiple dosing portion of Part E. The Investigator must consult the Sponsor. Starting the multiple dosing portion requires explicit agreement from the Sponsor.)** In the multiple dosing portion, subjects will take most of their doses at home (fasting conditions [Section 5.3] recommended). Subjects will return to the site for brief in-clinic stays after the first week of multiple dosing and at the end of multiple dosing as indicated in the Schedule of Assessments for Part E (Appendix E). Subjects will return for the EOS Visit at 28 days after the last dose.

The Schedule of Assessments for Part E is provided in Appendix E. Maximum duration of study participation (Screening through EOS Visit) for an individual subject will be approximately 100 days.

Table 6: Dose Cohort in Subjects with GALK-deficient Galactosemia (Part E)

Cohort	Dose	
E1	AT-007 oral capsule on Day 1, dose TBD mg/kg (SAD portion), followed by a 5-day-washout, and AT-007 oral capsule QD for 27 days (MAD portion), dose TBD mg/kg (n=4)	placebo (n=2)

n = number of subjects; MAD = multiple ascending dose; QD = once daily; SAD = single ascending dose; TBD = to be determined.

Note: There may be up to 2 additional cohorts if the decision is made to test more than 1 dose level. Also, the number of cohorts and total number of subjects may increase if doses above 40 mg/kg are tested in additional cohorts (see Section 1.4). The overall number of subjects per cohort may be increased up to 8 subjects to address any potential lost-to-follow-up among subjects treated with either placebo or AT-007. Subjects treated with study drug (placebo or AT-007) in a cohort may be randomized to treatment in another cohort (separate consent required) within the same Part (assuming no safety or tolerability issues in the prior cohort) as long as there is a washout period ≥5 days between the last dose in a cohort and the first dose in the next cohort.

3.3.6. Part D Extension: 90-Day Dosing in Subjects with Classic Galactosemia

The Part D Extension is open to the following subjects:

- Subjects who completed Part D may start in the extension at any time after the EOS Visit in Part D.
- Subjects who participated in but discontinued Part D for reasons other than study drug related TEAE (and completed an EOS Visit at any time >14 days after the last dose) may start in the extension at any time after the EOS Visit in Part D.
- Subjects who participated in but discontinued Part D for reasons other than study drug related TEAE (and who either did not have an EOS Visit or had an EOS Visit before 14 days after the last dose) may start in the extension at any time beyond 14 days after the last dose in Part D.
- De novo subjects who did not participate in Part D may participate in the 90-day Extension after they undergo all the required screening procedures from Day -60 to Day -1.

The dose for the first cohort of the Part D Extension (DE1) will be 20 mg/kg/day based on acceptable SAD and MAD safety and PK data from Cohort D2. After SRC review of all SAD and MAD data and Sponsor review of PK and galactitol data for Cohorts D1 through D3, a second cohort of the Part D Extension (DE2) may be initiated to evaluate a dose >20 mg/kg/day if this dose was well tolerated in the SAD and MAD portions of the study in both healthy volunteers and subjects with CG.

A screening visit is required for de novo subjects and subjects whose last visit in Part D was >60 days before Day 1 in the Part D Extension. The de novo subjects may also participate in optional prescreening.

Within each cohort of up to 28 subjects, subjects will be randomly assigned in a 3:1 to AT-007 and placebo. Subjects will receive study drug (AT-007 or placebo) on Day 1 and remain at the CRU for at least 24 hours after the first dose. Subjects will receive 1 daily dose of the same study drug (AT-007 or placebo) for a total of 90 consecutive days. In the extension, subjects will take most of their doses at home (fasting conditions [Section 5.3] recommended). Subjects will have

1 in-clinic stay associated with the first dose, a second in-clinic stay associated with the last dose, and home health or in-clinic visits at Months 1 and 2 as indicated in the Schedule of Assessments for Part D Extension (Appendix F). At any time during Part D Extension, an interview with the patient and caregiver (when appropriate) will be performed to assess the impact of disease. (detailed processes described in a separate Interview Guide). Subjects will return for the EOE Visit at 28 days after the last dose. The EOE Visit will not be required for subjects who transition to a separate Open-Label Extenstion Study. For these subjects the EOE will be Day 91.

The Schedule of Assessments for Part D Extension is provided in Appendix F. Maximum duration of study participation (Screening through EOE Visit) for an individual subject will be approximately 178 days for a de novo subject and approximately 218 days for a subject who was screened for Part D, completed Part D, and completed Part D Extension without requiring a screening visit for the Part D Extension and without counting any washout period between Part D and Part D Extension.

Table 7: 90-Day Dose Cohort in Subjects with Classic Galactosemia (Part D Extension)

Cohort	Dose	
DE1	AT-007 oral capsule or liquid suspension on Day 1, dose 20 mg/kg (SAD portion), followed by AT-007 oral capsule or liquid suspension QD for a total of 90 days, dose 20 mg/kg (n=up to 21)	placebo (n=up to 7)

n = number of subjects; MAD = multiple ascending dose; QD = once daily; SAD = single ascending dose; TBD = to be determined.

Note: There may be up to 1 additional cohort if the decision is made to test more than 1 dose level. Regardless of whether there is 1 or 2 cohorts, the maximum number of subjects is 28. However, the number of cohorts and total number of subjects may increase if doses above 40 mg/kg are tested in additional cohorts (see Section 1.4). The overall number of subjects per cohort may be increased to address any potential lost-to-follow-up among subjects treated with either placebo or AT-007.

3.4. Safety Review Committee (SRC)

An SRC consisting of the Principal Investigator(s) or designee (e.g., a medically qualified sub-investigator), the pharmacokineticist, and Sponsor (including external consultants) will meet and review the safety data from each cohort in each part of the study. Study progression to the next cohort (within each part of the study) will occur only after review of the safety data through Day 3 in Part A, Day 8 in Parts B and C, and Day 12 in Parts D and E.

Safety data to be assessed prior to each dose escalation decision within a part of the study include but are not limited to the following:

- AEs (including dose-limiting toxicities [DLTs], see Section 3.5)
- Clinical safety laboratory tests (hematology, chemistry, coagulation, urinalysis)
- Physical examinations
- Vital signs
- ECGs

In addition, PK data for AT-007 will be considered during all parts of the study. PK data will be evaluated by the Sponsor rather than the SRC. Safety and PK data from Cohorts A1 through A4 in Part A were evaluated by the SRC (safety) and Sponsor (PK) before Part B (MAD) was allowed to start. Safety and PK data from Cohorts A1 through A4 in Part A and Cohorts B1 and B2 were evaluated before Cohort C1 (MAD with lumbar puncture) or Cohort D1 (subjects with CG) were allowed to start. Safety and PK data from these cohorts plus Cohort D2 (and possibly Cohorts A5, B4, and D3, depending on timing) will be evaluated before Cohort E1 (subjects with GALK-deficient Galactosemia) will be allowed to start. (Notes: Cohort A5 [40 mg/kg] was added as part of the amendment resulting in protocol version 4.0 and completed before the writing of protocol version 5.0. Addition of Cohort A5 resulted in the addition of Cohort C3 [as part of the same protocol amendment that added Cohort A5] and will result in Cohorts B4 and D3 [both not yet used as of the writing of protocol version 5.0] being conducted.) Safety and PK data from Cohort D2 were evaluated before deciding the dose (20 mg/kg) for Cohort DE1.

The review of the PK data from Cohorts A1 through A4 and the first 2 cohorts of Part B confirmed that once daily (QD) dosing is adequate.

Safety and PK data from Cohorts A1 through A4 and Cohorts B1, B2, and B3 were evaluated before Cohort C2 (MAD with lumbar puncture) was allowed to start.

Safety and PK data from Cohorts A1 through A5, Cohorts B1 through B3, and Cohorts C1 and C2 were evaluated before deciding to start Cohorts B4 and C3 (MAD with lumbar puncture) simultaneously at the same dose (40 mg/kg).

Safety and PK data from Cohorts A1 through A4 and Cohorts B1 and B2 were evaluated before Part D (SAD and MAD in subjects with CG) was allowed to start. In addition to safety data through Day 12 and at least single dose PK and galactitol results from the previous cohort(s) within Part D and safety and PK data from Cohort B3 were evaluated before Cohort D2 was allowed to start. Safety and PK data from Cohort B4 will be evaluated before Cohort D3 will be allowed to start.

Safety data through Day 12 and at least single dose PK and galactitol results from all completed cohorts within Part D (at least Cohorts D1 and D2 [possibly Cohort D3, depending on timing]) will be evaluated before Cohort E1 will be allowed to start. The starting dose for Part E will not be higher than the highest acceptable dose in Part D at the time that Part E starts. In case of multiple cohorts in Part E, safety data through Day 12 and at least single dose PK and galactitol results from previous cohort(s) within Part E will be evaluated before subsequent cohorts in Part E will be allowed to start.

Safety and PK data from Cohort D2 were evaluated before deciding the dose (20 mg/kg) for Cohort DE1 (Part D Extension). In case of a second cohort in the Part D Extension, safety data through Day 12 and at least single dose PK and galactitol results from all completed cohorts within Part D (including Cohort D3) and safety and PK data for Cohorts A5 and B4 will be evaluated before deciding upon a dose.

At each dose escalation decision, the SRC will recommend proceeding or not proceeding to the next cohort as well as the dose level for AT-007. The AT-007 dose level may be increased, decreased, or repeated from one cohort to the next.

Parts A, B, and C will be conducted at a single CRU. The Principal Investigator (or designee) at that study center will be part of the SRC during Parts A, B, and C.

Parts D, E, and D Extension will be conducted at multiple centers. The Principal Investigators (or designees) will be part of the SRC during Parts D, E, and D Extension.

At minimum, the SRC will meet and review data at the following times:

- Review of safety data through Day 3 for the following cohorts to determine whether to proceed with the next cohort and at what dose level:
  - Cohort A1
  - Cohort A2
  - Cohort A3
  - Cohort A4 (At this SRC meeting, a starting dose level for Part B was recommended and subsequently confirmed after Sponsor review of all PK data from Cohorts A1 through A4.)
  - Cohort A5 (At this SRC meeting, a dose level for both Cohorts B4 and C3 was recommended and subsequently confirmed after Sponsor review of all PK data from Cohorts A1 through A5.)
- Review of safety data through Day 8 for the following cohorts to determine whether to proceed with the next cohort and at what dose level:
  - Cohort B1
  - Cohort B2 (At this SRC meeting, starting dose level[s] for Part C and Part D were recommended and subsequently confirmed after Sponsor review of all PK data from Cohorts A1 through A4 and Cohorts B1 and B2.)
  - Cohort B3/C1 (At this SRC meeting, a dose level for Cohorts C2 and D2 was recommended and subsequently confirmed after Sponsor review of all PK data from Cohorts A1 through A4 and Cohorts B1 through B3 and Cohort C1 as well as single dose PK and galactitol data from Cohort D1 and SRC review of safety data from Cohort D1.)
  - Cohort C2
  - Cohort B4/C3 (At this SRC meeting, a dose level for Cohort D3 will be recommended and require confirmation [or change] after Sponsor review of all PK data from all of Parts A and B as well as single dose PK and galactitol data from Cohorts D1 and D2. Note: Because Cohorts A5, B4, and C3 are new or newly opened, SRC review of safety from Cohorts D1 and D2 was already done by the time Cohort A5 was added to the protocol.)
- Review of safety data through Day 12 (Study Day 12, not the 12th day of multiple dosing) for the following cohorts to confirm whether to proceed with the next cohort and at what dose level:
  - Cohort D1

- Cohort D2

- Review of safety data through Day 12 (Study Day 12, not the 12th day of multiple dosing) for Cohort D3 (At this SRC meeting, a dose level for Cohort E1 may be recommended if Cohort E1 has not already started with a dose level not higher than that for Cohort D2. A dose level may also be recommended for Cohort DE2. Any recommended dose level requires confirmation [or change] after Sponsor review of all PK data for Parts A, B, C, and D as well as single dose galactitol data from Cohorts D1 through D3.)
- Review safety data through Day 12 (Study Day 12, not the 12th day of multiple dosing) for Cohort E1 to confirm whether to proceed with Cohort E2 and at what dose level (if needed) (Any recommended dose level for Cohort E2 requires confirmation [or change] after Sponsor review of single dose PK and galactitol data from Cohort E1.)
- Review safety data through Day 12 (Study Day 12, not the 12th day of multiple dosing) for Cohort E2 to confirm whether to proceed with Cohort E3 and at what dose level (if needed) (Any recommended dose level for Cohort E3 requires confirmation [or change] after Sponsor review of single dose PK and galactitol data from Cohort E2.)
- Review of data for the last Part E cohort to finish assessing safety of AT-007 treatment of subjects with GALK-deficient Galactosemia
- Review of data for Cohort DE1 (and Cohort DE2 if applicable) to finish assessing safety of AT-007 treatment of subjects with CG

3.5. Dose Escalation/De-escalation

For Parts A, B, and C of the study (healthy subjects), safety and drug exposure will determine escalation or de-escalation. If the initial dose tested in Part A had not been well tolerated, the Sponsor may have choosen to de-escalate until a well-tolerated dose was identified. A careful analysis of available safety, laboratory evaluation, and drug PK data in healthy subjects was done to determine the starting dose for Part D in subjects with CG. A careful analysis of available safety, laboratory evaluation, and drug PK data in subjects with CG will determine the starting dose for Part E in subjects with GALK-deficient Galactosemia. The starting dose in Part E will not be higher than the highest acceptable dose used in Part D at the time of starting Part E. The starting dose in Part D Extension will be 20 mg/kg based on acceptable SAD and MAD safety and PK data from Cohort D2.

For Part D of the study (subjects with CG), safety, drug exposure, and biomarker analysis of galactitol will determine escalation or de-escalation. If the drug exposure in subjects with CG is similar to that seen in healthy subjects in Part A, the same or similar approach to dose escalation may be utilized. If there is any concern of altered drug exposure or drug clearance based on PK analysis after the single dose in the initial cohort of Part D, then alternative dose escalation to lower levels may be selected until the relationship between dose and exposure in subjects with CG is determined. Dose escalation may be stopped if an effect on galactitol (reduction in galactitol) is achieved, but it becomes apparent that higher drug exposure is not increasing reduction of galactitol or providing any additional benefit. If any tested dose is not well tolerated

while markedly reducing galactitol levels, lower dose levels may be tested until no/minimal reduction in galactitol levels is detected.

In case of multiple cohorts in Parts E, the dose escalation process will be the same as for Part D.

A second cohort of the Part D Extension (DE2) may be initiated to evaluate a dose higher than 20 mg/kg/day if this dose was well tolerated in the SAD and MAD portions of the study in both healthy volunteers and subjects with CG.

The SRC will make recommendations to the Sponsor regarding dose escalation or de-escalation for each cohort based on the results from the previous cohort as described in Section 3.4. The Sponsor will act upon this recommendation and inform the Investigator(s) and the IRB(s)/IEC(s).

The SRC will pay particular attention to DLTs defined as any AT-007-related AE or clinically relevant finding on laboratory tests, ECGs, vital signs, or physical examinations that, in the opinion of the Investigator and/or the SRC, precludes administering the dose to any subject and/or continuing to administer daily doses to the subject who experienced the event. Specific individual subject-level dose stopping criteria for hepatic and renal toxicity are described in Appendix G and Appendix H. For DLTs, as well as fatal serious adverse events (SAEs) and life-threatening SAEs, breaking the blind will be considered and initiated (as needed) per SRC discussion and agreement.

Dose-escalation will be stopped and de-escalation initiated if:

- ≥2 subjects on AT-007 (B and C cohorts of the same dose level considered together) experience a DLT at any time through 24 hours after the last dose of study treatment
- Any subject on AT-007 has a fatal SAE or life-threatening SAE requiring urgent intervention at any time through 24 hours of the last dose of study treatment

The maximum tolerated dose (MTD) will be the dose level below the dose level at which one or more of the events listed above occurs.

Dose escalation within each part of the study will continue until the MTD is identified per the rules described earlier in this section or the SRC and/or Sponsor decide to stop dose escalation. Dose de-escalation, if initiated within any part of the study, will continue until the MTD is identified or the SRC and/or Sponsor decide to stop.

If the events outlined in the above MTD definition do not occur at any dose level in a study part, then the MTD will be the highest dose level in that study part.

If the events outlined in the above MTD definition occur at any given dose level, the Sponsor may decide to investigate a dose higher that the previously well-tolerated dose but lower than the dose at which one of the events outlined in the above MTD definition occurred.

3.6. Study Termination

The study will be terminated if the MTD is associated with insufficient drug exposure.

The study may be discontinued for any clinically significant changes, or pattern of changes (for example, clinically significant laboratory abnormalities), that are judged by the Investigator and/or Sponsor to be related to the study drug and represent a major tolerability concern.

The Principal Investigator reserves the right to terminate the study in the interest of subject safety and welfare. The Sponsor reserves the right to discontinue the study due to administrative reasons at any time.

4. SELECTION OF SUBJECTS

4.1. Healthy Subjects in Study Parts A, B, and C

Subjects must meet all of the inclusion criteria and none of the exclusion criteria to be eligible for the study.

***Criteria that are marked with an asterisk and in bold font also apply to subjects with CG and subjects with GALK-deficient Galactosemia.**

4.1.1. Inclusion Criteria

1. Healthy male or non-pregnant, non-lactating female subject between 18 and 65 years of age, inclusive.
2. ***Females must be of non-childbearing potential (defined as surgically sterile [i.e., had a bilateral tubal ligation, hysterectomy, or bilateral oophorectomy ≥6 months prior to the first dose of study drug] or postmenopausal for ≥1 year [confirmatory follicle-stimulating hormone or FSH test results required] prior to the first dose of study drug) or agree to use an acceptable form of birth control from screening until 30 days after study completion.**
3. ***Males must be unable to procreate (defined as surgically sterile [i.e., had a vasectomy ≥6 months prior to screening]) or must agree to use an acceptable form of birth control from screening through 30 days after study completion.**
4. No significant disease as determined by the Investigator during screening.
5. ***No clinically significant abnormal laboratory value as determined by the Investigator during screening.**
6. No significant abnormality on 12-lead ECG as determined by the Investigator during screening.
7. ***Subject's vital signs (measured after 5 minutes rest in a seated position) at screening must be within the following ranges: systolic blood pressure (SBP) <140 mmHg; diastolic blood pressure (DBP) <90 mmHg; and heart rate between 50 and 100 bpm, inclusive.**
8. No regular medical treatment outside of female birth control methods (females of childbearing potential only).
9. ***Able to communicate effectively with study personnel.**
10. ***Willing and able to be confined to the CRU as required by the protocol.**
11. Willing and able to give written informed consent after being informed of and understanding the nature of the study.

4.1.2. Exclusion Criteria

1. History or presence of clinically significant hematopoietic, renal (estimated glomerular filtration rate [eGFR] < 90 mL/min/1.73 m^2), hepatic, endocrine, metabolic, pulmonary, neurological, psychiatric, cardiovascular, immunological, dermatological, or gastrointestinal

diseases; conditions capable of altering the absorption, metabolism, or elimination of drugs; or conditions that constitute a risk factor when taking the study drug and/or impact the conduct or results of the study.

2. ***Body Mass Index (BMI) >35 kg/m^2.**
3. ***Underweight or BMI < 17.5 kg/m^2.**
4. ***Positive test for hepatitis B surface antigen, hepatitis C antibody, or human immunodeficiency virus (HIV) at screening or previous treatment for hepatitis B, hepatitis C, or HIV infection.**
5. Evidence of significant active neuropsychiatric disease.
6. Has smoked or used tobacco- or nicotine-containing products ≤6 months prior to the first dose of study drug.
7. ***Pregnant, lactating, or not using/not willing to use appropriate means of contraception.**
8. ***Any prior history of substance abuse (including alcohol) or treatment for such.**
9. Positive urine screen for drugs of abuse (amphetamines, barbiturates, benzodiazepines, cocaine, cannabinoids, opiates) or cotinine.
10. ***History of significant drug allergy or drug hypersensitivity.**
11. ***Investigators, site personnel directly affiliated with this study, and their immediate families (defined as a spouse, parent, child, or sibling, whether biological or legally adopted).**
12. ***Any other condition that, in the opinion of the Investigator, precludes the subject from following and completing the protocol.**
13. A clinically significant abnormal finding on the physical exam, medical history, ECG, or clinical laboratory results at screening.
14. A significantly abnormal diet (per Investigator judgment) during the 4 weeks preceding the first dose of study drug.
15. ***Participation in another clinical trial of a different investigational product (randomized subjects only) within 5 half-lives prior to the first dose of study drug.**
16. Use of any over-the-counter (OTC) medication (including nutritional or dietary supplements, herbal preparations, or vitamins) ≤7 days prior to the first dose of study drug through the last dose of study drug without evaluation and approval by the Investigator.
17. Use of any prescription medication, except that allowed per protocol (Section 4.6), from 14 days prior to the first dose of study drug through the last dose of study drug without evaluation and approval by the Investigator.
18. ***Treatment with any sensitive substrates of Breast Cancer Resistance Protein (BCRP) (e.g., rosuvastatin, sulfasalazine) or potent inhibitors of BCRP (e.g., cyclosporine A) ≤5 half-lives days prior to the first dose of study drug through the last dose of study drug.**

19. ***Treatment with sensitive substrates of Organic Anion Transporter (OAT)1 and OAT3 (e.g., famotidine, adefovir, furosemide, ganciclovir, cefaclor, ceftizoxime, pen-G) ≤5 half-lives prior to the first dose of study drug through the last dose of study drug.**
20. ***Treatment with sensitive substrates of cytochrome P450 3A4 (CYP3A4) (e.g. midazolam, triazolam, buspirone, alfentanil, dronedarone, eletriptan, conivaptan, lovastatin, simvastatin), or CYP2B6 (e.g. bupropion), or CYP2C19 (e.g. omeprazole), or CYP1A2 (e.g. alosetron, caffeine, duloxetine, melatonin, ramelteon, tasimelteon,tizanidine) ≤5 half-lives prior to the first dose of study drug through the last dose of study drug**
21. ***Consumption of beverages or foods that contain alcohol, high levels of sorbitol, grapefruit, poppy seeds, broccoli, Brussels sprouts, pomegranate, star fruit, char-grilled meat, or caffeine/xanthine from 48 hours prior to the first dose of study drug through the last dose of study drug. Subjects will be instructed not to consume any of the above products; however, allowance for an isolated single incidental consumption may be evaluated and approved by the Investigator based on the potential for interaction with the study drug.**
22. ***Treatment with medications potentially associated with transaminase elevations, such as mirtazapine ≤5 half-lives prior to the first dose of study drug through the last dose of study drug**.

4.2. Subjects with Classic Galactosemia (CG) in Study Part D and Part D Extension

Subjects must meet all of the inclusion criteria and none of the exclusion criteria to be eligible for the study.

***Criteria in Section 4.1 that are marked with an asterisk and in bold font also apply to subjects with CG (Part D and Part D Extension).**

4.2.1. Inclusion Criteria

1. Male or non-pregnant, non-lactating female subject between the ages of 18 and 65 years, inclusive, with a CG diagnosis confirmed by evidence of absent or significantly decreased (<1%) GALT activity in red blood cells or historical record of diagnosis of GALT deficiency (medical record or gene analysis report or written communication by health care professional), and who have no other significant health problems.
2. Subject may be on concomitant medications and dietary supplements; however, they must be on stable doses for at least 1 month prior to screening and throughout the study.
3. Subject may have long-term complications of Galactosemia, including mild neurological deficits such as ataxia, tremor, dysmetria and dystonia, mild cognitive impairment, mild language difficulty, and primary ovarian insufficiency, that, in the opinion of the Investigator, do not interfere with the subject's ability to participate in the study.
4. Galactose-restricted diet that eliminates sources of galactose from dairy products but permits minimal dietary intake of galactose from non-dairy products for ≥3 months prior to screening and throughout the study.

5. Screening 12-lead ECG with normal sinus rhythm without pathological Q wave or significant ST/T wave changes.
6. Willing and able to consent voluntarily to participate in this study by providing written informed consent prior to the start of any study-specific procedures, with participation and consent of caregiver/LAR as well.

4.2.2. Exclusion Criteria

1. History or presence of clinically significant hematopoietic, hepatic, endocrine, metabolic (other than CG), pulmonary, neurological (other than CG-related), psychiatric, immunological, dermatological, or gastrointestinal diseases; conditions capable of altering the absorption, metabolism, or elimination of drugs; or conditions that constitute a risk factor when taking the study drug and/or impact the conduct or results of the study.
2. Evidence of diagnosis of clinical variant Galactosemia.
3. Complications of CG resulting in disability that, in the opinion of the Investigator, may prevent the subject from completing all study requirements (e.g., severe neurological deficits, severe cognitive impairment, or severe language difficulty).
4. Impaired renal function or eGFR < 90 mL/min/1.73 m^2, or urine protein-to-creatinine ratio (UPCR) ≥ 200 mg/g, or urine albumin-to- creatinine ratio (UACR) (≥ 30 mg/g.Note: eGFR and UPCR/UACR are an estimation of renal function, and the ultimate decision of whether a patient has normal renal function (and can be included in the study) is at the discretion of the Investigator, assuming there are no safety concerns. Also, because eGFR, UPCR and UACR can vary day to day based on outside factors, patients can be re-screened for eGFR, UPCR and UACR multiple times to understand the renal function of the patient.
5. History or presence of significant cardiovascular disorders including myocardial infarction, stroke, uncontrolled hypertension (sitting blood pressure ≥140/90 mmHg), left ventricular (LV) hypertrophy, atrial fibrillation, or valvular heart disease.
6. Abnormal findings on the screening 12-lead ECG, such as ST/T wave changes of ischemia, pathological Q wave changes, or any rhythm other than normal sinus rhythm.
7. Evidence of significant active hematological disease and/or cumulative blood donation of 1 unit (500 mL) or more including blood drawn during clinical studies in the last 3 months.
8. Unwilling to comply with a galactose-restricted diet.
9. Positive urine screen for drugs of abuse (amphetamines, barbiturates, benzodiazepines, cocaine, cannabinoids, opiates) unless clinically indicated.
10. Use of any OTC medication (including nutritional or dietary supplements, herbal preparations, or vitamins) ≤7 days prior to the first dose of study drug through the last dose of study drug without evaluation and approval by the Investigator. Use of medications to treat Galactosemia complications will be permitted by approval of the Investigator.
11. Use of any prescription medication, except that allowed per protocol (Section 4.6), from 14 days prior to the first dose of study drug through the last dose of study drug without evaluation and approval by the Investigator. Use of medications to treat Galactosemia complications will be permitted by approval of the Investigator.

12. For Part D Extension only, discontinuation due to study drug related TEAE in Part D.
13. Use of the following following potentially nephrotoxic drugs within 5 half-lives prior to the first dose of study drug through the last dose of study drug: amitriptyline, aspirin, doxepin, lithium, amphotericin B, foscarnet, ganciclovir, pentamidine, rifampin, antiretrovirals (e.g. adefovir, tenofovir), calcineurin inhibitors (e.g. cyclosporine, tacrolimus), angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, clopidogrel, ticlopidine, pamidronate, zoledronate, statins, chemotherapeutics, contrast dye, loop diuretics, thiazides, triamterene, allopurinol, gold therapy, haloperidol, quinine, ranitidine.

4.3. Subjects with GALK-deficient Galactosemia in Study Part E

Subjects must meet all of the inclusion criteria and none of the exclusion criteria to be eligible for the study.

***Criteria in Section 4.1 that are marked with an asterisk and in bold font also apply to subjects with GALK-deficient Galactosemia (Part E).**

4.3.1. Inclusion Criteria

Same as for Part D except for Inclusion Criterion 1:

1. Male and non-pregnant, non-lactating female adult between the ages of 18 and 65 years, inclusive, with a GALK-deficient Galactosemia diagnosis documented by historical record of either absent or significantly decreased GALK activity in red blod cells (medical record, enzyme activity report, genetic mutation report or written communication by health care professional) and who have no other significant health problems.

4.3.2. Exclusion Criteria

Same as for Part D except that “GALK-deficient Galactosemia” replaces “CG” in Exclusion Criteria 1 and 3; and the exclusion criterion related to discontinuation from Part D does not apply.

4.4. Subject Participation in Multiple Cohorts

Subjects in Parts D and E may participate in multiple cohorts within the same study Part (assuming no safety or tolerability issues in the prior cohort) regardless of whether they are treated with AT-007 or placebo in any cohort. However, there must be a washout period ≥5 days between the last dose of study drug in a cohort and the first dose of study drug in the next cohort. Subject who participates in multiple cohorts must consent to each cohort separately.

Subjects in Part D may participate in Part D Extension (separate consent required) regardless of whether they completed Part D or not as long as Part D discontinuation was not due to a study drug related TEAE. The timing from the end of Part D to the start of Part D Extension is addressed in Section 3.3.6. A screening visit is required for subjects whose last visit in Part D was >60 days before Day 1 in the Part D Extension. However, some screening procedures done before Part D do not need to be repeated.

4.5. Subject Discontinuation Criteria

For an individual subject, study completion is defined as completing the Treatment Period and the EOS or EOE Visit with the exception for subjects that after Part D Extension transition to the separate Open-Label Extenstion Study. These subjects will not perform the EOE Visit, hence they will complete Part D Extension at the Day 91 Visit..

Subjects will be free to discontinue the study at any time for any reason. Subjects may be discontinued from the study by the Investigator, if necessary, to protect the subjects' health and safety or the integrity of the study data.

Reasons for discontinuation include, but are not limited to, the following:

- Adverse event (specify)
- Pregnancy
- Withdrawal by subject (specify)
- Noncompliance or protocol deviation/violation (specify)
- Lost to follow-up (specify)
- Other (specify)

Subjects who discontinue after taking the first dose of study drug will not be replaced. Subjects who discontinue before taking study drug will be replaced.

In the event of a premature study discontinuation, subjects will undergo the procedures for the EOS Visit (or EOE Visit if in the Part D Extension) described in Section 6.9. The reason for discontinuation must be recorded in the case report form (CRF).

4.6. Medication and Activity Restrictions

Subjects must not take any OTC medication (including nutritional or dietary supplements, herbal preparations, or vitamins) ≤7 days prior to the first dose of study drug until the EOS (or EOE) Visit without evaluation and approval by the Investigator. For subjects with CG or GALK-deficient Galactosemia, use of medications to treat Galactosemia complications will be permitted by approval of the Investigator.

Subjects must not take any prescription medication, except that allowed per protocol in this section, from 14 days prior to the first dose of study drug until the EOS (or EOE) Visit without evaluation and approval by the Investigator. For subjects with CG or GALK-deficient Galactosemia, use of medications to treat Galactosemia complications will be permitted by approval of the Investigator.

In addition, use or consumption of the following are not permitted:

- Tobacco- or nicotine-containing products within 6 months prior to the first dose of study drug (including negative cotinine test at screening) through the EOS Visit (subjects in Parts A, B, and C only)
- Drugs of abuse (amphetamines, barbiturates, benzodiazepines, cocaine, cannabinoids, opiates) from a negative drug test at screening through the EOS (or EOE) Visit

(Exception: Subjects with CG or GALK-deficient Galactosemia may take such drugs or drugs that produce positive drug screening results if clinically indicated and on a stable dose for ≥1 month prior to screening and throughout the study. Drugs of abuse are defined by the local regulations where a patient lives [i.e., a positive result for cannabinoids will not be disqualifying if this drug is legal/permitted in the patient's hometown].)

- Treatment with sensitive substrates of BCRP (e.g., rosuvastatin, sulfasalazine) or potent inhibitors of BCRP (e.g., cyclosporine A) within 5 half-lives prior to the first dose of study drug through the last dose of study drug
- Treatment with sensitive substrates of OAT1 and OAT3 (e.g., famotidine, adefovir, furosemide, ganciclovir, cefaclor, ceftizoxime, pen-G) within 5 half-lives prior to the first dose of study drug through the last dose of study drug
- Treatment with sensitive substrates of cytochrome P450 3A4 (CYP3A4) (e.g. midazolam, triazolam, buspirone, alfentanil, dronedarone, eletriptan, conivaptan, lovastatin, simvastatin), or CYP2B6 (e.g. bupropion), or CYP2C19 (e.g. omeprazole), or CYP1A2 (e.g. alosetron, caffeine, duloxetine, melatonin, ramelteon, tasimelteon,tizanidine) within 5 half-lives prior to the first dose of study drug through the last dose of study drug
- Drugs potentially associated with transaminase elevations, such as mirtazapine within 5 half-lives prior to the first dose of study drug through the last dose of study drug.
- Beverages or foods that contain alcohol, high levels of sorbitol, grapefruit, poppy seeds, broccoli, Brussels sprouts, pomegranate, star fruit, char-grilled meat, or caffeine/xanthine from 48 hours prior to the first dose of study drug through the visit before the EOS (or EOE) Visit (allowance for an isolated single incidental consumption may be evaluated and approved by the Investigator based on the potential for interaction with the study drug)
- The following following potentially nephrotoxic drugs within 5 half-lives prior to the first dose of study drug through the last dose of study drug: amitriptyline, aspirin, doxepin, lithium, amphotericin B, foscarnet, ganciclovir, pentamidine, rifampin, antiretrovirals (e.g. adefovir, tenofovir), calcineurin inhibitors (e.g. cyclosporine, tacrolimus), angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, clopidogrel, ticlopidine, pamidronate, zoledronate, statins, chemotherapeutics, contrast dye, loop diuretics, thiazides, triamterene, allopurinol, gold therapy, haloperidol, quinine, ranitidine

If the potential subject is currently taking any of the prohibited medications, a review with the investigator with consultation with the sponsor is encouraged. This is due to variability in potential drug-drug interactions and interpretation of what medications are sensitive substrates or moderate or severe inhibitors or inducers versus AT-007. As such, after investigation an exception to an above prohibited medication may be allowed.

A summary of potential drug-drug interactions (DDI) based on in vitro studies is provided in Appendix A.

Permitted concomitant medications in this study are the following:

- Female hormonal contraceptives or hormone replacement therapy (Parts A, B, C, D, E, and D Extension)
- Concomitant medications and dietary supplements with stable doses maintained for ≥1 month prior to screening and through the last dose of study drug (Parts A, B, C, D, E, and D Extension)
- Background medications for the management of subjects with history of seizures (Parts D, E, and D Extension) as long as they are not sensitive substrates of BCRP, OAT1, OAT3, CYP3A4, CYP2B6, CYP2C19, or CYP1A2 or potent inhibitors of BCRP. Patients who switched to an allowed anti-epiletic medication will need to be stable on treatment for at least 30 days prior to the first dose of study drug.

Subjects must not donate blood or plasma from 3 months prior to the first dose of study drug through the EOS (or EOE) Visit. It is recommended that blood/plasma donations not be made for at least 30 days after the EOS (or EOE) Visit.

Subject must not engage in strenuous exercise from 48 hours prior to the first dose of study drug through the EOS (or EOE) Visit.

Subjects must stay in the CRU during the time periods designated in the Schedules of Assessments (Appendix A, Appendix B, Appendix C, Appendix D, Appendix E, and Appendix F).

4.7. Contraception Requirements

Female subjects must be one of the following:

- Non-childbearing potential

  OR

- Childbearing potential and agree to use an acceptable form of birth control from screening until 30 days after the EOS (or EOE) Visit

Women of non-childbearing potential are defined as follows:

- Surgically sterile (i.e., had a bilateral tubal ligation, hysterectomy, or bilateral oophorectomy ≥6 months prior to the first dose of study drug)

  OR

- Postmenopausal for at least 1 year prior to the first dose of study drug.

Subjects claiming postmenopausal status will have an FSH test performed at screening to confirm postmenopausal status.

Females of childbearing potential must agree to use an acceptable form of birth control from the time point specified in the list below (or screening if no time point specified) until 30 days after the EOS (or EOE) Visit. The following are acceptable birth control methods for this study:

- Vasectomized partner (at least 6 months prior to dosing);
- Non-surgical permanent sterilization (e.g., Essure® procedure) at least 3 months prior to dosing;
- Double barrier method (e.g., diaphragm plus condom);
- Intrauterine device (IUD);
- Abstinence (and must agree to use a double barrier method if they become sexually active during the study);
- Implanted or intrauterine hormonal contraceptives in use for ≥6 consecutive months prior to dosing;
- Oral, patch, or injected contraceptives, or vaginal hormonal device (i.e., NuvaRing®) in use for ≥3 consecutive months prior to dosing.

Female subjects must agree to provide any requested information about the pregnancy should contraception fail and a pregnancy occurs within 90 days after the last dose of study drug.

A male subject with sexual partners who could become pregnant must meet the following criteria:

- Participant is unable to procreate, defined as surgically sterile (i.e., had a vasectomy ≥6 months prior to screening)

  OR

- Participant is able to procreate and agrees to use an acceptable contraceptive method from screening until 30 days after the EOS (or EOE) Visit. An acceptable method of contraception includes one of the following:
  - Abstinence from heterosexual intercourse
  - Condom with spermicide or condom with intra-vaginally applied spermicide

Male subjects must agree to provide any requested information about partner pregnancy should contraception fail and a pregnancy occurs within 90 days after the subject's last dose of study drug. If a partner pregnancy occurs, consent from the pregnant partner will also be obtained before any information about the pregnancy is requested or collected.

5. STUDY TREATMENTS

Subjects will be randomly assigned (Section 5.2) to either AT-007 or matching placebo in Parts A, B, D, E, and D Extension. The number of subjects to be treated with AT-007 and the number of subjects to be treated with placebo in each cohort are described in Section 3.3.

Subjects will be assigned to open-label AT-007 in Part C.

5.1. Study Drug Supplies

AT-007 drug substance is manufactured according to current Good Manufacturing Practice (GMP) by Worldwide Clinical Trials, Early Phase Services, located in San Antonio, TX. The chemical name for AT-007 is 2-(4-oxo-3-((5-(trifluoromethyl)benzo[d]thiazol-2-yl)methyl)-3,4-dihydrothieno[3,4-d]pyridazin-1-yl)acetic acid. Applied Therapeutics Inc. will supply sufficient quantities of the study drug formulation to allow completion of this study.

AT-007 and matching placebo will be shipped to the appropriate study sites pursuant to site standard operating procedures (SOPs). Upon receipt of the study drug products, the supplies will be inventoried and stored in an environmentally controlled and secure, limited access area. The lot numbers of the drugs along with the expiration dates (where available) will be recorded and copies of the Certificate of Analysis (where available) will be maintained on file. Records will be maintained of the receipt and dispensation of the drugs supplied.

Details regarding study drug shiptment, preparation, and dispensation are in a separate pharmacy manual. Dosing will be weight based. AT-007 (or matching placebo) will be taken orally, QD, under fasted conditions. Doses will be rounded according to the available stock keeping unit (SKU) as described in the Pharmacy Manual.

Retention samples of investigational AT-007 will not be required. At the conclusion of the study, any unused study drug will be returned to the Sponsor or destroyed by the study center pursuant to written authorization by the Sponsor and applicable regional/local regulations.

5.2. Study Drug Assignment and Blinding

Up to 116 subjects are planned to be dosed in this study:

- Part A: 5 cohorts of 8 healthy subjects each
- Part B: 2 cohorts of 6 healthy subjects each and 2 cohorts of 8 healthy subjects each
- Part C: 3 cohorts of 4 healthy subjects each
- Part D: 3 cohorts of 6 subjects with CG each
- Part E: 1 to 3 cohorts of 6 subjects with GALK-deficient Galactosemia each
- Part D Extension: 1 to 2 cohorts with a combined total of 28 subjects with CG

Note: The number of cohorts and total number of subjects may increase if doses above 40 mg/kg are tested in additional cohorts (see Section 1.4). In Parts D and E, the overall number of subjects per cohort may be increased up to 8 subjects to address any potential lost-to-follow-up among subjects treated with either placebo or AT-007. In Parts D and E, subjects treated with study drug (placebo or AT-007) in a cohort may be randomized to treatment in another cohort (separate consent required) within the same Part (assuming no safety or tolerability issues in the prior

cohort) as long as there is a washout period ≥5 days between the last dose in a cohort and the first dose in the next cohort. Both de novo and Part D subjects with CG may participate in Part D Extension (separate consent required) (see Section 3.3.6 for details regarding when a Part D subject can start study drug in the Part D Extension).

In Parts A, B, D, E, and D Extension, subjects will be randomized to receive either active drug (AT-007) or placebo (see Section 3.3 for the number of subjects to be randomized to each treatment within each cohort). Each subject will receive an assigned treatment based on the randomization schedule prepared by the study statistician.

Parts A and B will be double-blinded (the Sponsor/CRO, clinical site staff [except for unblinded pharmacist for Parts A and B], and the subjects will not know which subjects are treated with AT-007 or placebo).

In Part C, all subjects will be assigned to receive active drug (AT-007). Part C will be open-label.

In Parts D, E, and D Extension, the clinical site staff (except for the unblinded pharmacist) and the subjects will be blinded to study treatment. The Sponsor/CRO will not be blinded to study treatment because the relationship between exposure in Parts D, E, and D Extension (subjects with galactosemia) vs. exposure in healthy subjects will need to be evaluated on an ongoing basis to ensure that AT-007 blood levels remain within the limits achieved in Part B. This process will minimize the number of subjects with galactosemia potentially exposed to plasma concentrations of AT-007 not previously tested and shown to be safe in healthy subjects.

5.3. Study Drug Administration

For Parts A, B, and C, subjects will take the study drug (oral capsules of AT-007 or placebo) in the CRU under the supervision of study personnel. Study staff will conduct a hand and mouth check immediately after dose to ensure that the medication has been appropriately swallowed.

For Parts D and E, study drug was oral capsules of AT-007 or placebo. For Part D Extension, both oral capsules and liquid suspensions will be used. Most study drug doses in Parts D, E, and D Extension will be taken on an out-patient basis (i.e., at home). Each subject will be provided with a diary card to record the dates/times for all doses taken at home.

Study drug dose is in mg/kg. For each subject, body weight at screening will be used to determine the amount (mg) of study drug in each dose.

All doses of study drug should be taken under fasting conditions. Subjects should take all doses of study drug in the morning (with water) after overnight fasts of at least 10 hours. Subjects may end their fast at any time ≥2 hours after taking the study drug. These conditions are mandatory for doses associated with PK sampling and recommended for all other doses regardless of whether taken at home or in the clinic.

Subjects may drink water ad libitum.

5.4. Treatment Compliance

Treatment compliance is assured during Parts A, B, and C and for in-clinic doses during Parts D, E, and D Extension because subjects will take the study drug in the CRU under supervision by the study center staff.

During Parts D, E, and D Extension study drug will be dispensed and collected periodically because subjects will be taking most doses at home. Treatment compliance will be determined by counting the capsules (or otherwise measuring study drug dispensed to Part D Extension subjects taking the liquid suspension) dispensed and collected at each of these visits and by reviewing subjects' study drug diary cards.

5.5. Fasting/Meals/Beverages

During the stays in the CRU, subjects' meals and beverages will adhere to the study restrictions (Section 4.6). In addition, subjects will be fasted overnight for at least 10 hours before study drug administration on the mornings of study drug dosing days and the fast will be broken at ≥2 hours after the dose.

During the out-patient periods of the study, it is recommended that subjects take the study drug under the same fasting conditions. Subjects/caregivers will be instructed to have the subject fast overnight for at least 10 hours before study drug administration on the mornings of study drug dosing days and break the fast at ≥2 hours after the dose. Subjects/caregivers will also be instructed to adhere to the study restrictions (Section 4.6) and report any lapses.

Throughout the study, healthy subjects will maintain their normal/usual diet.

Throughout the study, subjects with CG or GALK-deficient Galactosemia will maintain their normal/usual galactose-restricted diet that eliminates sources of galactose from dairy products but permits minimal dietary intake of galactose from non-dairy products.

Subjects will be encouraged to drink water ad libitum.

6. STUDY PROCEDURES BY VISIT

The Schedules of Assessments for Parts A, B, C, D, E, and D Extension of the study are provided in Appendix A, Appendix B, Appendix C, Appendix D, Appendix E, and Appendix F, respectively.

The Screening Visit and EOS (or EOE) Visit can be done at any time of day, but the subject should arrive in a fasted state after fasting for at least 10 hours.

For the Check-in Visits for Parts A, B, C, and D Extension, the subject should arrive at the CRU around 11 am. For the Check-in Visits for Parts D and E, the subject should arrive at the CRU in the morning in a fasted state after fasting for at least 10 hours. The subject should not take study drug before arriving at the CRU because study drug will be administered at the visit as needed.

For the arrival times and conditions of all other visits, please refer to the Schedules of Assessments for Parts A, B, C, D, E, and D Extension (Appendix A, Appendix B, Appendix C, Appendix D, Appendix E, and Appendix F, respectively). If nothing is stated explicitly regarding arrival time, whether the subject should be in a fasted state, and/or whether the subject should have taken study drug or not, then assume that the subject can arrive at any time, fasted vs fed does not matter, and/or the subject should have taken study drug at home (if during a dosing period).

For each visit, the visit procedures are listed in the approximate order in which they should be performed. However, the order of some procedures may be changed for practical reasons (when the subject arrives relative to the planned time of dosing and when the subject is able to produce a urine sample) as long as the procedures are done within the required timeframe and under the required conditions (fasting vs non-fasting and/or before vs after the dose). For example, fasting blood and urine samples for clinical safety laboratories are usually written as occurring after the dose because of the importance of the timing of the dose; however, the fasting safety laboratory samples may be taken at any time before the subject breaks the fast after the dose.

Acceptable windows for visit procedures are provided in the footnotes in the Schedules of Assessments. In case of overlapping time points, meeting the nominal time point for study drug dosing and collecting blood samples for PK and/or biomarkers take priority over meeting the nominal time point ECGs and vital signs which take priority over meeting the nominal time point for physical examinations and collecting samples for clinical safety laboratory tests.

NOTE: If extenuating circumstances (i.e., COVID-19 pandemic) make clinic visits inadvisable/impractical/unwanted for subjects, study visits will be done as home visits by a visiting nurse with some equipment/supplies sent to the subjects' home for the duration of their study participation. The details regarding these home health visits are described in a separate document entitled "Work Instructions for Conduct of Home Health Protocol Visit(s) during COVID-19 Pandemic".

6.1. Prescreening Period (De novo Part D Extension Subjects Only)

Prescreening can be done at any time during a home health visit (in-clinic visit also permitted). The purpose of prescreening is to identify subjects who may be interested in and qualify for the study. A prescreening ICF must be completed before any study-specific prescreening procedure is conducted.

Prescreening is optional. Some subjects may do the prescreening activities as part of screening.

Prescreening activities may include the following:

- Collect blood sample for assessment of GALT activity in RBCs and GALT gene analysis
- Collect urine sample (spot collection) for analysis of galactitol
- Collect fasting blood sample(s) for clinical laboratory testing (hematology, coagulation, and chemistry) and serology testing (screening for HIV, hepatitis B, and hepatitis C)
- Measure body weight
- Measure height
- Obtainment of medical history only for documented diagnosis of Galactosemia, if available

6.2. Screening Period (Days -28 through -1 for Parts A, B, and C and Days -40 through -1 for Parts D and E, and Days -60 through -1 for Part D Extension)

The screening visit must occur within 28 days before the first dose of study drug for Parts A, B, and C; within 40 days before the first dose of study drug for Parts D andE; and within 60 days before the first dose of study drug for Part D Extension.

The Part D Extension screening visit is only applicable to the following subjects:

- De novo subjects
- Subjects whose last visit in Part D was >60 days before Day 1 in Part D Extension

Part D Extension subjects who previously participated in Part D and had a last Part D visit within 60 days before Day 1 of the extension do not need a screening visit for the extension.

Subjects will be evaluated at the screening visit to determine their eligibility to enroll in the study. The following procedures will be done at screening (home health visit or in-clinic visit):

- Obtain signed informed consent from the subject and from the subject's caregiver/LAR (if applicable)

  The informed consent documents will be discussed with each potential subject and caregiver/LAR (if applicable), and each potential subject (and caregiver/LAR if applicable) will sign the informed consent form(s), as appropriate, for the study prior to any study-specific procedures being performed.
- Review inclusion/exclusion criteria
- Obtain medical history (including current medication)
- Obtain demographic data (not necessary for Part D subjects being re-screened for Part D Extension)

- Perform physical examination including height (unless already taken at prescreening) and weight
- Measure vital signs
- Conduct 12-lead ECG
- Collect fasting blood sample(s) for clinical laboratory testing (hematology, coagulation and biochemistry); serology testing (screening for HIV, hepatitis B, and hepatitis C); and, for female subjects of childbearing potential only, serum pregnancy test (also FSH test for female subjects claiming postmenopausal status) (Note: The clinical laboratory testing and serology testing do not need to be repeated if already done at prescreening and prescreening was within 60 days before the first dose of study drug in Part D Extension.)
- Collect fasting urine sample for urinalysis, drugs of abuse screen (also cotinine screen for subjects in Parts A, B, and C only), and for the following urinary markers of renal function: Urine protein-to-creatinine ratio (UPCR), urine albumin-to- creatinine ratio (UACR).

For Parts D, E, and D Extension only, the following procedures will be done in addition to the ones listed above:

- Collect blood sample for assessment of GALT or GALK activity in red blood cells and GALT or GALK gene analysis (GALT for Parts D and D Extension and GALK for Part E) (if not already done during prescreening)
- Collect blood sample for assessment of aldose reductase activity
- Collect blood sample for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p
- Collect urine sample (spot collection) for analysis of galactitol (if not already done during prescreening)

Note: For Part D Extension subjects who were previously screened for Part D but had the last Part D visit >60 days before Day 1 in the extension, GALT and aldose reductase activity assessments do not need to repeated.

Subjects who are taking any disallowed medication(s) (see Section 4.1.2, Section 4.2.2, and Section 4.6) but otherwise eligible for the study may washout their disallowed medications (washout of at least 5 half-lives of the disallowed medication with the longest half-life). After the washout period, they may be rescreened.

Eligible subjects will be contacted to inform them of their eligibility and schedule the start of study drug dosing for those wishing to continue in the study. Subjects (and caregivers if applicable for Part D Extension) in Parts A, B, C, and D Extension will be instructed to arrive at the CRU at approximately 11 am on the day before the scheduled first dose of study drug. Subjects/Caregivers in Parts D and E will be instructed to arrive at the CRU in the morning on the day before the scheduled first dose of study drug (i.e., approximately 24 hours before the first dose).

6.3. Clinical Research Unit (CRU) Check-in (All Parts, Day -1)

Subjects will arrive at the CRU at approximately 11 am (Parts A, B, C, and D Extension) or sometime in the morning (Parts D and E [approximately 24 hours before the first dose]) on the day before the scheduled first dose of study drug. The following procedures will be done:

- For Part D Extension subjects who did not have a Part D Extension screening visit only, obtain signed informed consent from the subject and from the subject's caregiver/LAR (if applicable)
- Review of inclusion/exclusion criteria
- Obtain updates, if any, for medical history
- Perform physical examination including weight
- Measure vital signs
- Conduct 12-lead ECG
- Collect fasting blood sample(s) for clinical laboratory testing (hematology, coagulation and biochemistry); and, for female subjects of childbearing potential only, serum pregnancy test (pregnancy test must be negative for the subject to receive study drug)
    - Hematology, coagulation and biochemistry laboratory tests do not need to be repeated if they were already assessed at the pre-screening or screening visit that took place within 60 days prior the first day of docing on Day 1
- Collect fasting urine sample for urinalysis, drugs of abuse screen (also cotinine screen for subjects in Parts A, B, and C only) and for the following urinary markers of renal function: UPCR and UACR
    - Urinalysis, UPCR and UACR do not need to be repeated if they were already assessed at the pre-screening or screening visit that took place within 60 days prior the first day of docing on Day 1
- Collect AE and concomitant medication data
- Feed the subject (2 meals [lunch and dinner])
- Fast the subject overnight for at least 10 hours

For Parts D and E only, the following procedures will be done in addition to the ones listed above:

- Collect blood sample for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p
- Collect urine over 24 hours (morning of Day -1 to immediately before the first dose [Day 1]) for analysis of galactitol (an aliquot of the total urine collected will be analyzed for creatinine)
- Feed the subject (2 to 3 meals depending on check-in time)

Subjects who no longer fulfill all inclusion criteria and/or now fulfill one or more exclusion criteria will be discontinued from the study and sent home. These subjects will be replaced.

Subjects who continue to fulfill all inclusion criteria and none of the exclusion criteria will continue in the study.

6.4. Day 1 through Penultimate Visit (Part A, Days 1 through 4)

Exact time points and allowable windows for assessments and sample collection are provided in the Schedule of Assessments (Appendix A).

Subjects will have 1 in-clinic stay:

- Subjects will remain at the CRU from Check-in at approximately 11 am of Day -1 until Discharge on Day 3 (Cohorts A1, A2, and A3) or Day 4 (Cohorts A4 and A5).

6.4.1. Day 1 (Study Drug Dosing Day)

Subjects will remain in the CRU on Day 1.

The following procedures will be done before study drug administration on Day 1:

- Measure predose vital signs
- Conduct predose 12-lead ECG
- Collect predose blood sample for PK
- For Cohorts A4 and A5 only, collect predose urine sample for PK
- Randomly assign the subject to either AT-007 or placebo (Note: Unblinded CRU pharmacist will have the randomization schedule.)

Then, study drug (AT-007 or placebo) will be administered to the subject, and the following procedures will be done on Day 1 after the dose:

- For Cohorts A4 and A5 only, collect postdose urine for PK
- Collect postdose blood samples for PK
- Conduct postdose 12-lead ECG
- Measure postdose vital signs
- Feed the subject (3 meals with breakfast provided at ≥2 hours postdose)

Note: Blood/urine collection for PK will continue into Day 2.

AE and concomitant medication data will be collected both before and after dose administration on Day 1.

6.4.2. Days 2 and 3

Subjects will remain in the CRU on Day 2. Subjects in Cohorts A4 and A5 only will also remain in the CRU on Day 3.

The following procedures will be done:

- Measure vital signs on both days
- Collect AE and concomitant medication data on both days
- Collect postdose blood sample(s) for PK on both days
- For Cohorts A4 and A5 only, collect postdose urine for PK on both days (Note: Urine collection for PK will continue into Day 4.)
- Feed the subject (3 meals on Day 2)
- Additional Day 3 Procedures
  - Perform physical examination including weight
  - Conduct 12-lead ECG
  - Collect fasting blood sample(s) for clinical laboratory testing (hematology, coagulation and biochemistry)
  - Collect fasting urine sample for urinalysis and, for female subjects of childbearing potential only, urine pregnancy test
  - Schedule the EOS Visit for Day 28 (±2 days)
  - Discharge subject from the CRU (Cohorts A1, A2, and A3 only) and remind subject/caregiver to fast overnight for at least 10 hours on the day before the EOS Visit and return to the CRU for the EOS Visit before breaking the fast
  - Feed the subject (3 meals) (Cohorts A4 and A5 only)

6.4.3. Day 4

Day 4 procedures apply only to Cohorts A4 and A5.

The following procedures will be done on Day 4:

- Finish the urine collection for PK
- Collect AE and concomitant medications data
- Schedule the EOS Visit for Day 28 (±2 days)
- Discharge subject from the CRU and remind subject/caregiver to fast overnight for at least 10 hours on the day before the EOS Visit and return to the CRU for the EOS Visit before breaking the fast

6.5. Day 1 through Penultimate Visit (Part B, Days 1 through 9)

Exact time points and allowable windows for assessments and sample collection are provided in the Schedule of Assessments (Appendix B).

Subjects will have 1 in-clinic stay:

- Subjects will remain at the CRU from Check-in at approximately 11 am of Day -1 until Discharge on Day 9.

6.5.1. Day 1 (First Study Drug Dosing Day)

The following procedures will be done before study drug administration on Day 1:

- Measure predose vital signs
- Conduct predose 12-lead ECG
- Collect predose blood sample for PK
- Randomly assign the subject to either AT-007 or placebo (Note: Unblinded CRU pharmacist will have the randomization schedule.)

Then, study drug (AT-007 or placebo) will be administered to the subject, and the following procedures will be done on Day 1 after the dose:

- Collect postdose blood samples for PK
- Conduct postdose 12-lead ECG
- Measure postdose vital signs
- Feed the subject (3 meals with breakfast provided at ≥2 hours postdose)
- Fast the subject overnight for at least 10 hours

Note: Blood collection for PK will continue into Day 2.

Adverse event and concomitant medication data will be collected both before and after dose administration on Day 1.

6.5.2. Day 2 (Study Drug Dosing Day)

The following procedures will be done on Day 2:

- Measure vital signs
- Collect predose blood sample for PK (i.e., 24-hour sample after the Day 1 dose)
- Administer study drug at approximately the same time of day as on Day 1
- Feed the subject (3 meals with breakfast provided at ≥2 hours postdose)
- Fast the subject overnight for at least 10 hours
- Collect AE and concomitant medication data throughout the day

6.5.3. Days 3, 4, and 5 (Study Drug Dosing Days)

The subject will take study drug QD at the CRU under fasting conditions (Section 5.3) at approximately the same time of day as on Day 1. The subject will be fed 3 meals a day with breakfast provided at ≥2 hours postdose. The subject will be fasted for at least 10 hours each night before the morning dose. AE and concomitant medication data will be collected.

6.5.4. Day 6 (Study Drug Dosing Day)

The following procedures will be done on Day 6:

- Perform physical examination including weight
- Measure vital signs
- Collect fasting blood sample(s) for clinical laboratory testing (hematology, coagulation and biochemistry)
- Collect fasting urine sample for urinalysis and, for female subjects of childbearing potential only, urine pregnancy test (pregnancy test must be negative for the subject to receive study drug)
- Administer study drug at approximately the same time of day as on Day 1
- Feed the subject (3 meals with breakfast provided at ≥2 hours postdose)
- Fast the subject overnight for at least 10 hours
- Collect AE and concomitant medication data throughout the day

6.5.5. Day 7 (Last Study Drug Dosing Day)

The following procedures will be done on Day 7:

- Measure predose vital signs
- Conduct predose 12-lead ECG
- Collect predose blood sample for PK
- Administer study drug at approximately the same time of day as on Day 1
- Collect postdose blood samples for PK
- Conduct postdose 12-lead ECG
- Measure postdose vital signs
- Collect AE and concomitant medication data throughout the day
- Feed the subject (3 meals with breakfast provided at ≥2 hours postdose)

Note: Blood collection for PK will continue into Days 8 and 9.

6.5.6. Days 8 and 9

The following procedures will be done on Day 8:

- Measure vital signs
- Collect blood samples for PK
- Collect AE and concomitant medications data
- Feed the subject (3 meals)

The following procedures will be done on Day 9:

- Perform physical examination including weight
- Measure vital signs
- Collect blood sample for PK
- Collect AE and concomitant medication data
- Schedule the EOS Visit for Day 35 (±2 days)
- Discharge subject from the CRU and remind subject/caregiver to fast overnight for at least 10 hours on the day before the EOS Visit and return to the CRU for the EOS Visit before breaking the fast

6.6. Day 1 through Penultimate Visit (Part C, Days 1 through 9)

Exact time points and allowable windows for assessments and sample collection are provided in the Schedule of Assessments (Appendix C).

The visit schedule for subjects in Part C is identical to that for subjects in Part B with the following exceptions:

- No randomization. All subjects will receive open-label AT-007.
- Subjects will have a lumbar puncture on Day 7 (see Appendix C for notes on timing of the lumbar puncture relative to the last dose of study drug).

6.7. Day 1 through Penultimate Visit (Parts D and E, Days 1 through 34)

Exact time points and allowable windows for assessments and sample collection are provided in the Schedule of Assessments for Part D (Appendix D) and Part E (Appendix E).

Subjects will have 3 in-clinic stays.

- Subjects will remain at the CRU from Check-in on the morning of Day -1 until Discharge on Day 3 (Cohorts D1 and D2 and Part E) or Day 4 (Cohort D3).
- Subjects will remain at the CRU from Check-in on the morning of Day 12 until Discharge on Day 13.
- Subjects will remain at the CRU from Check-in on the morning of Day 32 until Discharge on Day 34.

6.7.1. Day 1 (Single Dose)

Subjects will remain in the CRU on Day 1.

The following procedures will be done before study drug administration on Day 1:

- Measure predose vital signs
- Conduct predose 12-lead ECG
- Collect predose blood sample for PK

- Collect predose blood sample for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p
- For Cohort D3 only, collect predose urine sample for PK
- Finish 24-hour predose urine collection for analysis of galactitol
- Randomly assign the subject to either AT-007 or placebo (Note: Obtain randomized treatment assignment by contacting the Interactive Voice/Web Randomization System [IVRS/IWRS].)

Then, study drug (AT-007 or placebo) will be administered to the subject, and the following procedures will be done on Day 1 after the dose:

- Collect 24 hours of postdose urine for analysis of galactitol (an aliquot of the total urine collected will be analyzed for creatinine)
- For Cohort D3 only, collect postdose urine for PK
- Collect postdose blood samples for PK
- Collect postdose blood samples for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p
- Conduct postdose 12-lead ECG
- Measure postdose vital signs
- Feed the subject (3 meals with breakfast provided at ≥2 hours postdose)

Note: Blood/urine collection for PK and/or galactose metabolism will continue into Day 2.

Adverse event and concomitant medication data will be collected both before and after dose administration on Day 1.

6.7.2. Non-dosing Days Between Single and Multiple Dosing (Days 2 through 5)

Between the single and multiple dosing periods in Part D, there is a 5-day washout period that starts immediately after the Day 1 dose and ends immediately before the Day 6 dose (first dose of the multiple dosing period).

6.7.2.1. Day 2

Subjects will remain in the CRU on Day 2.

The following procedures will be done on Day 2:

- Finish collecting postdose urine through 24 hours after the dose for analysis of galactitol (an aliquot of the total urine collected will be analyzed for creatinine)
- For Cohort D3 only, collect postdose urine for PK
- Collect postdose blood samples for PK
- Collect postdose blood samples for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p

- Measure vital signs
- Collect AE and concomitant medications data
- Feed the subject (3 meals)

Note: Blood collection for PK and biomarkers (and urine collection for PK for Cohort D3 only) will continue into Day 3.

6.7.2.2. Day 3

Subjects in Cohort D3 only will remain in the CRU on Day 3. Subjects in Cohorts D1 and D2 and Part E will be discharged on Day 3.

The following procedures will be done on Day 3:

- For Cohort D3 only, collect postdose urine for PK
- Collect postdose blood sample for PK
- Collect postdose blood sample for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p
- Measure vital signs
- Collect AE and concomitant medications data
- Discharge subject (Cohorts D1 and D2 and Part E only) from the CRU with a reminder to return on the morning of Day 6 after an overnight fast of at least 10 hours
- Feed the subject (3 meals) (Cohort D3 only)

Note: For Cohort D3 only, urine collection for PK will continue into Day 4.

6.7.2.3. Day 4 (Cohort D3 Only)

The following procedures will be done on Day 4:

- Finish collecting postdose urine for PK
- Measure vital signs
- Collect AE and concomitant medications data
- Discharge subject from the CRU with a reminder to return on the morning of Day 6 after an overnight fast of at least 10 hours

6.7.3. Day 6 (First Study Drug Dosing Day for Multiple Dosing Period)

Subjects will arrive in the morning (preferably in time for pre-dose procedures and 8 am dosing).

The following procedures will be done before study drug administration on Day 6:

- Perform physical examination including weight
- Measure predose vital signs
- Conduct predose 12-lead ECG

- Collect fasting blood sample(s) for clinical laboratory testing (hematology, coagulation and biochemistry)
- Collect fasting urine sample for urinalysis and, for female subjects of childbearing potential only, urine pregnancy test (pregnancy test must be negative for the subject to receive study drug)

Then, study drug (AT-007 or placebo) will be administered to the subject, and the following procedures will be done on Day 6 after the dose:

- Conduct postdose 12-lead ECG
- Measure postdose vital signs
- Feed the subject (breakfast provided at ≥2 hours postdose)
- Dispense study drug for Days 7 through 11
- Remind the subject/caregiver that study drug should be taken QD under fasting conditions at approximately the same time of day as on Day 6 and to record the date/time of each dose on the study drug diary card (Section 5.3)
- Remind the subject/caregiver to fast overnight for at least 10 hours and return to the CRU (before either taking study drug or breaking the fast) on the morning of Day 12

Adverse event and concomitant medication data will be collected both before and after dose administration on Day 6.

6.7.4. Day 12 (Check-in for Second In-clinic Period)

Subjects will arrive in the morning and check into the clinic.

The following procedures will be done before study drug administration on Day 12:

- Collect and record unused study drug and review study drug diary card
- Perform physical examination including weight
- Measure predose vital signs
- Conduct predose 12-lead ECG
- Collect predose blood sample for PK
- Collect predose blood sample for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p
- For female subjects of childbearing potential only, urine pregnancy test (pregnancy test must be negative for the subject to receive study drug)

Then, study drug (AT-007 or placebo) will be administered to the subject, and the following procedures will be done on Day 12 after the dose:

- Start 24-hour postdose urine collection for analysis of galactitol (an aliquot of the total urine collected will be analyzed for creatinine)

- Collect fasting blood sample(s) for clinical laboratory testing (hematology, coagulation and biochemistry)
- Collect fasting urine sample for urinalysis
- Collect postdose blood samples for PK
- Collect postdose blood samples for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p
- Conduct postdose 12-lead ECG
- Measure postdose vital signs
- Feed the subject (3 meals with breakfast provided at ≥2 hours postdose)
- Fast the subject overnight for at least 10 hours

Note: Blood collection for PK and galactose metabolism and urine collection for galactitol analysis will continue into Day 13.

Adverse event and concomitant medication data will be collected both before and after dose administration on Day 12.

6.7.5. Day 13 (Discharge from Second In-clinic Period)

The following procedures will be done on Day 13:

- Finish 24-hour postdose (relative to Day 12 dose) urine collection for analysis of galactitol (an aliquot of the total urine collected will be analyzed for creatinine)
- Collect 24-hour postdose (relative to Day 12 dose) blood sample for PK
- Collect 24-hour postdose (relative to Day 12 dose) blood sample for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p
- Measure vital signs
- Administer study drug at approximately 24 hours after the dose on Day 12
- Collect AE and concomitant medications data
- Dispense study drug for Days 14 through 19
- Remind the subject/caregiver that study drug should be taken QD under fasting conditions at approximately the same time of day as on Day 6 and to record the date/time of each dose on the study drug diary card (Section 5.3)
- Discharge subject from the CRU and remind the subject/caregiver to fast overnight for at least 10 hours and return to the CRU (before either taking study drug or breaking the fast) on the morning of Day 20

6.7.6. Day 20 (Out-patient Visit in the Middle of the Multiple Dosing Period)

Subjects will arrive in the morning.

The following procedures will be done before study drug administration on Day 20:

- Collect and record unused study drug and review study drug diary card
- Perform physical examination including weight
- Measure predose vital signs
- Conduct predose 12-lead ECG
- For female subjects of childbearing potential only, urine pregnancy test (pregnancy test must be negative for the subject to receive study drug)

Then, study drug (AT-007 or placebo) will be administered to the subject, and the following procedures will be done on Day 20 after the dose:

- Collect fasting blood sample(s) for clinical laboratory testing (hematology, coagulation and biochemistry)
- Collect fasting urine sample for urinalysis
- Conduct postdose 12-lead ECG
- Measure postdose vital signs
- Dispense study drug for Days 21 through 31
- Remind the subject/caregiver that study drug should be taken QD under fasting conditions at approximately the same time of day as on Day 6 and to record the date/time of each dose on the study drug diary card (Section 5.3)
- Remind the subject/caregiver to fast overnight for at least 10 hours and return to the CRU (before either taking study drug or breaking the fast) on the morning of Day 32

Adverse event and concomitant medication data will be collected both before and after dose administration on Day 20.

6.7.7. Day 32 (Check-in for Third In-clinic Period and Last Study Drug Dosing Day of the Multiple Dosing Period)

Subjects will arrive in the morning.

Subjects will remain in the CRU on Day 32.

The following procedures will be done before study drug administration on Day 32:

- Collect and record unused study drug and review study drug diary card
- Perform physical examination including weight
- Measure predose vital signs
- Conduct predose 12-lead ECG
- Collect predose blood sample for PK
- Collect predose blood sample for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p

- For female subjects of childbearing potential only, urine pregnancy test (pregnancy test must be negative for the subject to receive study drug)

Then, study drug (AT-007 or placebo) will be administered to the subject, and the following procedures will be done on Day 32 after the dose:

- Start 24-hour postdose urine collection for analysis of galactitol (an aliquot of the total urine collected will be analyzed for creatinine)
- Collect fasting blood sample(s) for clinical laboratory testing (hematology, coagulation and biochemistry)
- Collect fasting urine sample for urinalysis
- Collect postdose blood samples for PK
- Collect postdose blood samples for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p
- Conduct postdose 12-lead ECG
- Measure postdose vital signs
- Feed the subject (3 meals with breakfast provided at ≥2 hours postdose)

Note: Blood collection for PK and galactose metabolism and urine collection for galactitol analysis will continue into Day 33.

Adverse event and concomitant medication data will be collected both before and after dose administration on Day 32.

6.7.8. Day 33

Subjects will remain in the CRU on Day 33.

The following procedures will be done:

- Finish 24-hour postdose urine collection for analysis of galactitol (an aliquot of the total urine collected will be analyzed for creatinine)
- Collect postdose blood samples for PK
- Collect postdose blood samples for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p
- Measure vital signs
- Collect AE and concomitant medications data
- Feed the subject (3 meals)

Note: Blood collection for PK and galactose metabolism will continue into Day 34.

6.7.9. Day 34 (Discharge from Third In-clinic Period)

The following procedures will be done:

- Collect postdose blood sample for PK

- Collect postdose blood sample for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p
- Perform physical examination including weight
- Measure vital signs
- Collect AE and concomitant medications data
- Schedule the EOS Visit for Day 60 (±2 days)
- Discharge subject from the CRU and remind subject/caregiver to fast overnight for at least 10 hours on the day before the EOS Visit and return to the CRU for the EOS Visit before breaking the fast

6.8. Day 1 through Penultimate Visit (Part D Extension, Days 1 through 91)

Exact time points and allowable windows for assessments and sample collection are provided in the Schedule of Assessments for Part D Extension (Appendix F).

Subjects will have 2 in-clinic stays.

- Subjects will remain at the CRU from Check-in on the morning of Day -1 until Discharge on Day 2.
- Subjects will remain at the CRU from Check-in on the morning of Day 90 until Discharge on Day 91.

In lieu of being overnight in the CRU, subjects have the option of staying at a local hotel for Day -1 into Day 1 and Day 1 into Day 2. Subjects will abide by all study parameters including overnight fasting with all study procedures in the CRU.

An interview with the patients and their caregivers (wnen appropriate) to assess the impact of disease will be performed at any time during Part D Extension. Detailed processes will be described in a separate Interview Guide.

6.8.1. Day 1

Subjects will remain in the CRU on Day 1.

The following procedures will be done before study drug administration on Day 1:

- Measure predose vital signs
- Conduct predose 12-lead ECG
- Collect predose blood sample for PK
- Collect predose blood sample for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p
- Randomly assign the subject to either AT-007 or placebo (Note: Obtain randomized treatment assignment by contacting the IVRS/IWRS.)

Then, study drug (AT-007 or placebo) will be administered to the subject, and the following procedures will be done on Day 1 after the dose:

- Conduct postdose 12-lead ECG
- Measure postdose vital signs
- Feed the subject (3 meals with breakfast provided at ≥2 hours postdose)
- Fast the subject overnight for at least 10 hours

Adverse event and concomitant medication data will be collected both before and after dose administration on Day 1.

6.8.2. Day 2

Subjects will be discharged on Day 2.

The following procedures will be done on Day 2:

- Measure vital signs
- Administer study drug at approximately 24 hours after the dose on Day 1
- Collect AE and concomitant medications data
- Dispense study drug for Days 3 through 29
- Remind the subject/caregiver that study drug should be taken QD under fasting conditions at approximately the same time of day as on Day 1 and to record the date/time of each dose on the study drug diary card (Section 5.3)
- Discharge subject from the CRU and remind the subject/caregiver to fast overnight for at least 10 hours and return to the CRU (before either taking study drug or breaking the fast) on the morning of Day 30

6.8.3. Day 30, and Day 60

These visits may be done as either home health visits or as in-clinic visits. The visits should start in the morning. Procedures for these 2 visits are the same.

The following procedures will be done before study drug administration:

- Collect and record unused study drug and review study drug diary card
- Perform physical examination including weight
- Measure predose vital signs
- Conduct predose 12-lead ECG
- Collect predose blood sample for PK
    - This should be done 24 hours (±15 minutes) after the dose administered on the previous day (Day 29 and Day 59, respectively).
- Collect predose blood sample for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p

  - This should be done 24 hours (±15 minutes) after the dose administered on the previous day (Day 29 and Day 59, respectively).
- For female subjects of childbearing potential only, urine pregnancy test (pregnancy test must be negative for the subject to receive study drug)

Then, study drug (AT-007 or placebo) will be administered to the subject, and the following procedures will be done after the dose:

- Collect fasting blood sample(s) for clinical laboratory testing (hematology, coagulation and biochemistry)
- Collect fasting urine sample for urinalysis, UACR and UPCR
- Conduct postdose 12-lead ECG
- Measure postdose vital signs
- Collect postdose blood samples for PK
- Collect postdose blood samples for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p
- Schedule the next visit
- Dispense study drug for the time between this visit and the next visit
- Remind the subject/caregiver that study drug should be taken QD under fasting conditions at approximately the same time of day as on Day 1 and to record the date/time of each dose on the study drug diary card (Section 5.3)
- Remind the subject/caregiver to fast overnight for at least 10 hours and return to the CRU (before either taking study drug or breaking the fast) on the morning of the next visit

Adverse event and concomitant medication data will be collected both before and after dose administration.

6.8.4. Day 90 (Check-in for Second In-clinic Period and Last Study Drug Dosing Day)

Subjects will arrive in the morning and will remain in the CRU on Day 90.

The following procedures will be done before study drug administration:

- Collect and record unused study drug and review study drug diary card
- Perform physical examination including weight
- Measure predose vital signs
- Conduct predose 12-lead ECG
- Collect predose blood sample for PK
  - This should be done 24 hours (±15 minutes) after the dose administered on the previous day.

- Collect predose blood sample for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p
    - This should be done 24 hours (±15 minutes) after the dose administered on the previous day.
- For female subjects of childbearing potential only, urine pregnancy test (pregnancy test must be negative for the subject to receive study drug)

Then, study drug (AT-007 or placebo) will be administered to the subject, and the following procedures will be done after the dose:

- Collect fasting blood sample(s) for clinical laboratory testing (hematology, coagulation and biochemistry)
- Collect fasting urine sample for urinalysis, UACR and UPCR
- Conduct postdose 12-lead ECG
- Measure postdose vital signs
- Collect postdose blood samples for PK
- Collect postdose blood samples for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p

Adverse event and concomitant medication data will be collected both before and after dose administration.

6.8.5. Day 91 (Discharge from Second In-clinic Period)

The following procedures will be done:

- Collect postdose blood sample for PK (approximately 24 hours [±15 minutes] after the last [Day 90] dose)
- Collect postdose blood sample for analysis of biomarkers of galactose metabolism including galactose, galactitol, and galactose-1-p (approximately 24 hours [±15 minutes] after the last [Day 90] dose)
- Collect AE and concomitant medications data
- Schedule the EOE Visit for Day 118 (±2 days) [Only if subject is not directly entering the separate Open-Label Extenstion study]
- Discharge subject from the CRU and remind subject/caregiver to fast overnight for at least 10 hours on the day before the EOE Visit and have the EOE Visit before breaking the fast

6.9. EOS or EOE Visit (28 Days After the Last Dose)

All subjects will return to the CRU for an out-patient EOS Visit at 28 ± 2 days (Part A [Day 28]; Parts B and C [Day 35]; Parts D and E [Day 60];) after the last dose. For the Part D Extension, the EOE Visit (Day 118 ± 2 days) can be done either as a home health visit or as an out-patient visit to the site. The EOE Visit will not be performed if subjects transition to the separate Open-

Label Extenstion Study. The study completion for these subjects will be the completion of the Treatment Period at Day 91. The following procedures will be done:

- Update medical history (including current medications)
- Perform physical examination including weight
- Collect fasting blood sample(s) for clinical laboratory testing (hematology, coagulation and biochemistry); and, for female subjects of childbearing potential only, serum pregnancy test
- Collect fasting urine sample for urinalysis, UACR and UPCR
- Measure vital signs
- Conduct 12-lead ECG
- Collect AE and concomitant medication data

6.10. Unscheduled Visit

An unscheduled visit may be performed at any time during the study at the subject's request or as deemed necessary by the Investigator/designee. At a minimum, concomitant medication data, AEs, and the date and reason for the unscheduled visit will be recorded. Other tests may be performed per Investigator/designee judgement.

7. STUDY ASSESSMENTS

Schedules of Assessments for Parts A, B, C, D, E, and D Extension are presented in Appendix A, Appendix B, Appendix C, Appendix D, Appendix E, and Appendix F, respectively. Procedures will be completed as specified in this protocol unless contraindicated due to a reported AE.

Assessments will be conducted within the time frames specified in the footnotes in the Schedules of Assessments.

7.1. Safety Assessments

7.1.1. Adverse Events

AEs will be monitored throughout the study. AEs will be collected for each subject from the time of informed consent through the EOS (or EOE) Visit. Subjects will be queried in a nonleading manner, without specific prompting (e.g., "Has there been any change in your health status since your last visit?").

Safety reporting requirements are provided in Section 8.

7.1.2. Clinical Laboratory Tests

Blood and urine samples for routine clinical laboratory tests will be collected at the visits as indicated in the Schedules of Assessments for Parts A, B, C, D, E, and D Extension (Appendix A, Appendix B, Appendix C, Appendix D, Appendix E, and Appendix F, respectively).

A Clinical Laboratory Improvement Amendments (CLIA) certified laboratory will perform the following clinical laboratory tests:

- Hematology: hemoglobin, hematocrit, total and differential leukocyte count, RBC, and platelet count
- Serum Chemistry: albumin, blood urea nitrogen (BUN), creatinine, total bilirubin, alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), sodium (Na+), potassium (K+), chloride (Cl-), lactate dehydrogenase (LDH), calcium (Ca), uric acid, glucose, direct bilirubin (DB), and gamma-glutamyl transferase (GGT)
- Coagulation: prothrombin time (PT) and international normalized ratio (INR)
- Serology: blood tests for hepatitis B surface antigen, hepatitis C antibody, and HIV
- Urinalysis by an automated or manual urine "dipstick" method: pH, specific gravity, protein, glucose, ketones, bilirubin, blood, nitrite, leukocyte esterase, and urobilinogen. If protein, occult blood, nitrite, or leukocyte esterase values are out of range, a microscopic examination will be performed.
- Urine Drug Screen: drugs of abuse (amphetamines, benzodiazepines, barbiturates, cannabinoids, cocaine, opiates)
- Urinary markers of renal function: UACR and UPCR
- Urine cotinine screen (subjects in Parts A, B, and C only)

- Pregnancy test (all female subjects of childbearing potential)
- FSH (for female subjects claiming postmenopausal status)

For Parts D, E, and D Extension only, the following tests will be done in addition to the ones listed above:

- GALT (Part D and Part D Extension but only done once for subjects participating in both Parts) or GALK (Part E) activity in red blood cells
- GALT (Part D and Part D Extension but only done once for subjects participating in both Parts) or GALK (Part E) gene analysis
- Aldose Reductase activity (only done once for subjects participating in both Parts D and D Extension)

7.1.3. Physical Examinations

Physical examinations will be performed at the visits as indicated in the Schedules of Assessments for Parts A, B, C, D, E, and D Extension (Appendix A, Appendix B, Appendix C, Appendix D, Appendix E, and Appendix F, respectively). Height will be measured at the Screening Visit only. Weight will be measured as part of all physical examinations. Any time during the study after the first dose of study drug, abnormal findings considered to be clinically significant by the Investigator/designee will be reported as AEs.

7.1.4. Vital Signs

Vital signs (blood pressure, pulse rate, respiration rate, and temperature) will be measured at the visits as indicated in the Schedules of Assessments for Parts A, B, C, D, E, and D Extension (Appendix A, Appendix B, Appendix C, Appendix D, Appendix E, and Appendix F, respectively). Additional vital signs measurements may be taken as deemed medically necessary by the Investigator/designee. For purposes of qualifying any given subject for study participation, out of range vital signs may be repeated once.

All vital signs measurements will be taken with the subject in a seated position and after the subject has been resting in that seated position for at least 5 minutes. Any time during the study after the first dose of study drug, abnormal vital sign measurements considered to be clinically significant by the Investigator/designee will be reported as AEs.

7.1.5. Electrocardiograms (ECGs)

ECGs will be performed at the visits as indicated in the Schedules of Assessments for Parts A, B, C, D, E, and D Extension (Appendix A, Appendix B, Appendix C, Appendix D, Appendix E, and Appendix F, respectively). All ECGs will be done with the subject in a supine position and after the subject has been resting in that supine position for at least 5 minutes.

7.1.6. Prior and Concomitant Medications

Prior medications and concomitant medications will be collected throughout the study from the signing of informed consent through the EOS (or EOE) Visit. For each medication (prescription and OTC medication [including nutritional or dietary supplements, herbal preparations, or

vitamins]), start and stop dates, dosage regimen (dose plus route and frequency of administration), and indication will be recorded.

7.1.7. Pregnancy

Pregnancies will not be documented or reported as AEs. However, if at any time the pregnancy falls under the scope and definition of a SAE (Section 8.1.2), it will then be reported as such.

7.1.8. Other Safety Measures

Medical emergency personnel trained in advanced cardiac life support will be on site to monitor subjects during the confinement period in the CRU. Emergency medical equipment including, but not limited to, intubation equipment and pulse oximetry shall be maintained on site to administer appropriate medical care should it be required.

Appropriate drugs and medical equipment to treat acute [hypotensive, bronchoconstrictive, or anaphylactic] reactions must be immediately available at study centers. Study personnel must be trained to recognize and treat these reactions.

The reason for any use of these emergency personnel/equipment or measures to treat acute reactions must be reported as an AE (or SAE, if appropriate).

7.2. Pharmacokinetic (PK) Assessments

Blood and/or urine samples for PK assessments will be collected at the visits as indicated in the Schedules of Assessments for Parts A, B, C, D, E, and D Extension (Appendix A, Appendix B, Appendix C, Appendix D, Appendix E, and Appendix F, respectively). Instructions for collecting and processing blood and urine samples for AT-007 and possible AT-007 metabolite analysis will be provided in a separate laboratory manual. Plasma samples will be analyzed for AT-007 using a validated method. PK samples will be stored for possible future analysis of AT-007 metabolites.

Also, for subjects in Part C, a Day 7 lumbar puncture will be done to collect CSF for AT-007 measurement (Appendix C) using a qualified assay.

The PK parameters in Table 8 will be determined for the SAD parts of the study (i.e., Part A, the single dose period of Parts D and E).

Table 8: PK Parameters of AT-007 After a Single Dose in Healthy Subjects (Part A) and Subjects with Classic Galactosemia (SAD Period of Parts D and E)

PK Parameter	Definition
C_{max}	Maximum concentration, determined directly from individual concentration time data
T_{max}	Time of the maximum concentration
λ_z	The observed terminal rate constant; estimated by linear regression through at least 3 data points in the terminal phase of the log concentration-time profile
$T_{1/2}$	The observed terminal half-life, calculated as: $T_{1/2} = \frac{\ln(2)}{\lambda_z}$
AUC_{last}	Area under the concentration-time curve from time-zero to the time of the last quantifiable concentration; calculated using the linear trapezoidal rule
AUC_{inf}	Area under the concentration-time curve from time-zero extrapolated to infinity; calculated as: $AUC_{inf} = AUC_{last} + \frac{C_{last}}{\lambda_z}$
AUC_{Extrap} (%)	The percentage of AUC_{inf} based on extrapolation
C_{last}	The last quantifiable concentration determined directly from individual concentration-time data
T_{last}	Time of the last quantifiable concentration
CL/F	Clearance after extravascular administration, calculated as: $CL/F = Dose/AUC_{inf}$
Vz/F	Volume of distribution in the terminal phase, calculated as: $Vz/F = (CL/F)/\lambda_z$

The PK parameters in Table 9 will be determined for the multiple dosing parts of the study (i.e., Part B, Part C, MAD period in Parts D and E, and Part D Extension).

Table 9: PK Parameters of AT-007 During Multiple Dosing in Healthy Subjects (Parts B and C) and Subjects with Galactosemia (MAD Period of Parts D and E and Part D Extension)

PK Parameter	Definition
C_{max}	Maximum concentration, determined directly from individual concentration time data (Day 1 and Day of Last Dose)
T_{max}	Time of the maximum concentration (Day 1 and Day of Last Dose)
λ_z	The observed terminal rate constant; estimated by linear regression through at least 3 data points in the terminal phase of the log concentration-time profile (Day of Last Dose only)
$T_{1/2}$	The observed terminal half-life, calculated as: $T_{1/2} = \frac{\ln(2)}{\lambda_z}$ (Day of Last Dose only)
AUC_{tau}	Area under the concentration-time curve during the 24-hour dosing interval; calculated using the linear trapezoidal rule (Day 1 and Day of Last Dose) Note: If quantifiable data are not observed through 24 h postdose, AUC_{tau} will be estimated using extrapolation (from the time of the last reported concentration to 24 h) and AUC_{last} will also be reported.
AUC_{last}	Area under the concentration-time curve from time-zero to the time of the last quantifiable concentration; calculated using the linear trapezoidal rule (Day of Last Dose; Day 1 if quantifiable data are not observed through 24 h postdose)
AUC_{inf}	Area under the concentration-time curve from time-zero extrapolated to infinity; calculated as: $AUC_{inf} = AUC_{last} + \frac{C_{last}}{\lambda_z}$ (Day of Last Dose only)
AUC_{Extrap} (%)	The percentage of AUC_{inf} based on extrapolation (Day of Last Dose only)
C_{last}	The last quantifiable concentration determined directly from individual concentration-time data (Day 1 and Day of Last Dose)
T_{last}	Time of the last quantifiable concentration (Day 1 and Day of Last Dose)
CL/F	Clearance after extravascular administration, calculated as: $CL/F = Dose/AUC_{tau\ (Day\ X)}$, where Day X = Day of Last Dose and Dose is the actual dose administered (mg/kg x kg). (Day of Last Dose only) Note: CL/F/kg may also be reported
Vz/F	Volume of distribution in the terminal phase, calculated as: $Vz/F = (CL/F)/\lambda_z$ (Day of Last Dose only) Note: Vz/F/kg may also be reported
Rac	The accumulation ratio during multiple dose administration, based on C_{max} and AUC_{tau}; calculated as: Rac (AUC_{tau}) = AUC_{tau} (Day X) / AUC_{tau} (Day 1) Rac (C_{max}) = C_{max} (Day X) / AUC_{tau} (Day 1) Note: Rac will be tabulated with the pharmacokinetic parameters on Day X. Note: Day X = Day of Last Dose

Note: Day of Last Dose is Day 7 in Parts B and C, Day 32 in the MAD portion of Parts D and E, and Day 90 in Part D Extension.

7.3. Assessments of Biomarkers of Galactose Metabolism

In Parts D, E, and D Extension, blood samples for the analysis of galactose and galactose metabolites including galactitol and galactose-1-phosphate, and urine samples for the analysis of galactitol will be collected at the visits as indicated in the Schedules of Assessment (Appendix D, Appendix E, and Appendix F, respectively). Instructions for collecting and processing blood and urine samples for these biomarkers will be provided in a separate laboratory manual. Blood and urine samples will be analyzed for the levels of galactose (in plasma and urine), and galactose metabolites including galactitol (in plasma and urine), and galactose-1-phosphate (erythrocytes) using qualified or validated assays.

8. ADVERSE EVENTS

Subjects will be monitored for AEs from the signing of informed consent through the EOS (or EOE) Visit. The Investigator or a medically qualified designee will review each event. The Investigator or designee will assess its relationship to the study drug. Each AE will be graded for severity, and the date and time of onset and cessation will be recorded along with any treatment given for the AE and any action taken with regard to the study drug. Treatment of any AE will be evaluated and managed by a physician, either at the study site or at a nearby hospital or emergency room, as appropriate. The AE resolution or outcome will be recorded. All non-serious AEs will be reported on a regular basis or as specified by the Sponsor.

8.1. Definitions

8.1.1. Adverse Event (AE)

An AE is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without judgment to causality. An AE can arise from any use of the drug (e.g., off-label, use in combinations with another drug) and from any route of administration, formulation, or dose, including an overdose. AEs may include the onset of new illness and the exacerbation of pre-existing conditions. New signs and symptoms of underlying disease, or signs and symptoms of emerging disease must be recorded as AEs.

The Investigator/designee should attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE and not the individual signs/symptoms.

8.1.2. Serious Adverse Event (SAE)

A SAE is an AE that meets one or more of the following criteria:

- Results in death
- Is life-threatening (i.e., a subject is at immediate risk of death at the time of the event, not an event where occurrence in a more severe form might have caused death)
- Requires hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event that may jeopardize the subject or may require a medical or surgical intervention to prevent one of the outcomes listed above

Examples of “important medical events” that are SAEs include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, or convulsions that do not result in in-patient hospitalization.

The term "severe" is often used to describe the severity of a specific event (as in mild, moderate, or severe); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning as defined by the criteria above.

During the study, if a subject has a hospitalization or procedure (e.g., elective surgery) that was scheduled before the study entry (i.e., before informed consent for an event/condition that occurred before the study), then the hospitalization is considered a therapeutic intervention and not the result of a SAE. However, if the event/condition worsens during the study, it should be reported as an AE (or SAE, if the event/condition results in a serious outcome such as prolongation of hospitalization).

8.1.3. Expectedness

An AE is considered “unexpected” if the AE is not listed in the Investigator Brochure or is not listed with the observed specificity or severity.

8.2. AE Reporting

All AEs must be collected and recorded in the subject’s study records/source documents, in accordance with the Investigator’s normal clinical practice, and on the CRF.

Each AE will be evaluated by the Investigator or designee for duration (onset/cessation), severity, causal relationship to the study drug, seriousness, action taken with the study drug, and resolution or outcome. Treatment given for an AE should be recorded as a concomitant medication with the AE as the indication for the treatment. Definitions for severity and causal relationship to the study drug are presented in Section 8.2.1 and Section 8.2.2, respectively. The definition for serious is provided in Section 8.1.2.

8.2.1. AE Severity

The Investigator or designee will assess the severity of each AE according to the following definitions:

- Mild - Transient symptoms that do not influence performance of the subject’s daily activities. Other treatment is not indicated.
- Moderate - Marked symptoms sufficient to make the subject uncomfortable. Moderate influence on performance of the subject’s daily activities. Other treatment may be necessary.
- Severe - Symptoms cause considerable discomfort. Substantial influence on subject’s daily activities. May be unable to continue the study, and other treatment may be necessary.

8.2.2. AE Relationship to Study Drug

The Investigator or designee will assess the relationship of each AE to study drug using his/her clinical judgment and according to the following definitions:

- Probably Related: An adverse event that is probably related to the use of the drug
- Possibly Related: An adverse event that might be due to the use of the drug. An alternative explanation (e.g., concomitant drug, concomitant disease) is inconclusive. The relationship in time is reasonable; therefore, the casual relationship cannot be excluded.
- Unlikely Related: An adverse event that might not be due to the use of the drug. An adverse event for which an alternative explanation (e.g., concomitant drug, concomitant disease) is more likely or the relationship in time suggests that a causal relationship is unlikely.
- Not Related: An adverse event that is not related to the use of the drug.

For the purposes of regulatory reporting, a casuality of probably or possibly related will be mapped to related, while a causality of unlikely or not related will be mapped to unrelated.

8.3. SAE Reporting

The Investigator or designee will notify the appropriate Sponsor contact immediately after the SAE detection, observation, or report of occurrence (regardless of the relationship to study drug). The Sponsor contact information for SAE reporting is provided below:

Primary Contact

Chief Medical Officer: Riccardo Perfetti, MD, PhD
Phone office: +1 212-220-9227
Phone mobile: +1 347-702-3018

Back-up Contact

Head of Development: Francesca Lawson, MD
Phone mobile: +1 610-787-2998

SAE reports to be submitted to Contract Research Organization (CRO) ICON Safety Services

Email: ICON-Safety-CentralReceipt@iconplc.com

These SAE reports must contain the following information:

- Study name/number
- Study drug
- Investigator details (name, phone, fax, email)
- Subject number
- Subject initials
- Subject demographics

- Clinical event:
  - Description
  - Date of onset
  - Treatment (drug, dose, dosage form)
  - Adverse event relationship to study drug
  - Action taken regarding study drug in direct relationship to the AE If the AE was fatal or life-threatening
  - If applicable, cause of death (whether or not the death was related to study drug)
  - If applicable, autopsy findings (if available)

The person responsible for the study shall take care that the study has been carried out in accordance with pharmacovigilance regional/local regulations.

All SAE reporting will adhere to US Code of Federal Regulations (21 CFR Part 312.32) for IND drugs and 21 CFR 314.80 for marketed drugs (15-day alerts) or other regional/local regulations as applicable. The IRB(s)/IEC(s) will be notified of the alert reports per applicable regulations.

All AEs, including SAEs, will be followed to resolution when possible. All AEs and treatments administered for these AEs will be recorded on the CRF.

9. STATISTICS

9.1. Sample Size Determination

No formal sample size calculation is required. The approximate sample size (up to 40 healthy subjects in Part A, up to 40 healthy subjects in Parts B and C combined, up to 46 subjects with CG in Part D and Part D Extension combined if no subject participates in both, and up to 18 subjects with GALK-deficient Galactosemia in Part E) is not based on statistical considerations. The number of subjects is typical for Phase 1 studies of this type and is adequate to meet study objectives.

Note: The number of cohorts and/or total number of subjects may increase if the PK data reveal an insufficient exposure at 40 mg/kg (see Section 1.4). In Parts D and E, the overall number of subjects per cohort may be increased up to 8 subjects to address any potential lost-to-follow-up among subjects treated with either placebo or AT-007. In Parts D and E, subjects treated with study drug (placebo or AT-007) in a cohort may be randomized to treatment in another cohort (separate consent required) within the same Part (assuming subject had no safety or tolerability issues in the prior cohort) as long as there is a washout period ≥5 days between the last dose in a cohort and the first dose in the next cohort. Subject to certain timing considerations (see Section 3.3.6), Part D subjects may also participate in Part D Extension (separate consent required).

9.2. Statistical Methods

Detailed statistical methods will be provided in a Statistical Analysis Plan (SAP) that will be finalized before database lock.

NOTE: Part E and Part D Extension results might not be included in the CSR. If the Part E and/or Part D Extension results are not included in the CSR, they will be summarized and presented in a separate report or CSR addendum.

9.2.1. General Considerations

Safety measures including the baseline data will be summarized descriptively within each part (Parts A, B, C, D, E, and D Extension) of the study by each cohort (dose level of AT-007 and placebo), pooled AT-007 group, and pooled placebo group. Within each part of the study, PK data will be summarized by AT-007 dose group using descriptive statistics. In case of repeat cohorts, data from all cohorts of the same dose level will be combined for analysis.

For continuous outcomes, descriptive statistics will include the number of subjects, mean, 95% confidence interval (CI) for the mean, standard deviation (SD), median, minimum (Min), maximum (Max), 25th percentile, and 75th percentile. For categorical outcomes, the number and percentage of subjects will be presented. Descriptive statistics will generally be presented by AT-group. For summaries of continuous AT-007 PK concentration and parameter data, the coefficient of variation will also be presented.

For analyses of change from baseline, baseline will generally be defined as the predose assessment on Day 1 or Day -1, as scheduled. If this value is unavailable, the last nonmissing value prior to dosing will be used. Otherwise, missing observations will be treated as missing at

random, and no data imputation will be performed. All data from the CRFs, as well as any derived variables, will be presented in data listings.

SAS® version 9.4 or later will be used for all analyses.

9.2.2. Analysis Populations

Two analysis populations will be defined:

- Safety population: The safety population will consist of all subjects who are randomized and receive at least 1 dose of study drug. All analyses will be performed according to treatment received. This population will be used for all analyses of safety data.
- Pharmacokinetic (PK) population: The PK population will consist of all subjects who receive at least 1 dose of AT-007 and have sufficient concentration-time data to estimate at least one of the planned PK parameters, as determined by the study pharmacokineticist. This population will be used for all analyses of PK and biomarker data.

For Parts D and E, these populations may be defined separately for the SAD and MAD periods.

9.2.3. Subject Disposition

The number and percentage of subjects screened, screen failed, enrolled, randomized, dosed, and completing the study will be summarized. The number and percentage of subjects who prematurely discontinue the study will also be summarized along with reasons for discontinuation.

9.2.4. Drug Exposure and Compliance

A data listing, by subject, containing study drug dosing and dosing errors, if any, will be provided. Treatment compliance will be summarized using descriptive statistics.

9.2.5. Important Protocol Deviations

Important protocol deviations (IPDs) will be identified and documented based on a review of potential IPDs. The potential IPDs will be identified through programmatic checks of study data, as well as through review of selected data listings. The potential IPDs to be reviewed include, but are not limited to, subjects who:

- Did not meet inclusion/exclusion criteria
- Received disallowed concomitant medication
- Informed consent date obtained after date of first study procedure

Additional IPDs may be identified from clinical review of Investigator/designee comments or other data. Individual IPDs will be presented in a data listing.

9.2.6. Demographic and Baseline Characteristics

Demographic and baseline characteristics (including age, sex, race, ethnicity, weight, height, and body mass index) will be summarized using descriptive statistics. No formal statistical analyses will be performed.

The medical history of all subjects as well as the neurological history of subjects with CG and subjects with GALK-deficient Galactosemia will be coded by system organ class (SOC) and preferred term (PT) using the Medical Dictionary for Regulatory Activities (MedDRA) version 22.0 or higher. The number and percentage of subjects with abnormal findings in each SOC and each PT will be summarized.

9.2.7. Safety Analyses

The evaluation of safety will be based on the occurrence of treatment-emergent adverse events (TEAEs); discontinuations from study drug and/or the study because of AEs; clinical laboratory test results, physical examination findings, vital sign evaluations, and 12-lead ECGs results. All safety endpoints will be summarized descriptively. No statistical inference will be applied to the safety endpoints. Descriptive statistics will be calculated for quantitative safety data and frequency counts will be compiled for classification of qualitative safety data.

AEs will be coded by SOC and PT using MedDRA, version 22.0 or higher.

TEAEs are defined as AEs with onset on or after the date/time of the first dose of study drug.

Safety analyses will be described in detail in the SAP.

9.2.8. Pharmacokinetic (PK) Analyses

PK calculations will be performed using appropriate software, e.g., Phoenix™ WinNonlin® (Version 6.3 or higher, Pharsight Corporation) and/or SAS® (Version 9.4 or higher, SAS Institute Inc.).

PK parameters will be calculated using noncompartmental methods, using an appropriate model. Concentrations below the lower limit of quantitation (BLQ) will be treated as zero from the time of the first quantifiable concentration; embedded and terminal BLQ concentrations will be treated as "missing." Actual sample times, if available, will be used in the PK analysis.

AT-007 concentration-time data and PK parameters will be summarized by study part and cohort (dose level) using descriptive statistics (n, mean, SD, median, minimum, maximum, percent coefficient of variation [CV%]).

Dose proportionality, the proportionality of a change in systemic exposure with a change in dose, may be assessed using a power model or other appropriate methods.

Details of the PK analyses, including any additional exploratory analyses, will be described in the SAP.

9.2.9. Biomarker Analyses

For Parts D, E, and D Extension, blood and urine levels of galactose, galactitol, and its other metabolites will be summarized using descriptive statistics. Details of these analyses will be provided in the SAP.

9.2.10. Efficacy Analyses

Not applicable.

10. GENERAL CONSIDERATIONS

10.1. Ethical and Regulatory Considerations

10.1.1. Study Conduct

This research will be carried out in accordance with the protocol, the International Council for Harmonisation (ICH), Guideline for GCP: Consolidated Guidance (E6), and applicable regulatory requirements including clinical research guidelines established by the Basic Principles defined in the US 21 CFR Parts 50, 56, and 312; other applicable regional/local regulations, and the principles enunciated in the Declaration of Helsinki (revised version Fortaleza 2013).

10.1.2. Institutional Review Boards (IRBs) and Independent Ethics Committees (IECs)

For all study centers, this protocol will be reviewed by an appropriate IRB/IEC and study enrollment will not commence until the IRB/IEC has approved the protocol or a modification thereof. The IRB/IEC will be constituted and operated in accordance with the principles and requirements in applicable regional/local regulations.

10.1.3. Informed Consent

Written informed consent will be obtained from each subject (and caregiver/LAR if applicable) prior to performing any study-specific procedures. The informed consent document is prepared by the Investigator or designee, subject to review and approval by the Sponsor, and forwarded to a qualified IRB/IEC for final review and approval. The IRB/IEC-approved consent document must contain, at minimum, the 8 basic elements of informed consent. Only the most recently IRB/IEC-approved Informed Consent Form (ICF) must be used to consent prospective study subjects (and caregivers/LARs if applicable).

One copy of the signed and dated consent form(s) will be given to the subject (and caregiver/LAR if applicable) and the original(s) retained by the Investigator/site.

10.2. Data Collection, Monitoring, Management, and Quality Assurance

The results from screening and data collected during the study (except clinical laboratory test results) will be recorded in the subject's CRF.

CRO will be responsible for monitoring the study to ensure compliance with the protocol and GCP. Compliance may be verified by one or more of the following methods: on site visits, frequent communication with the Investigator, and/or review of CRFs and source documents. On site review of CRFs will include a review of forms for completeness and clarity, and consistency with source documents available for each subject. The Investigator agrees to permit such monitoring as well as audits or reviews by regulatory authorities and the IRB/IEC.

The study may be subject to audit by the Sponsor/designee and/or Regulatory Agency. If such an audit occurs, the Investigator must agree to allow access to required subject records. This is dependent on the subject (and caregiver/LAR if applicable) granting consent by signing the ICF. By signing this protocol, the Investigator grants permission to personnel from the Sponsor or its representatives for on-site monitoring and auditing of all appropriate study documentation, as

well as on-site review of the procedures employed in CRF generation, where clinically appropriate.

10.3. Study Documentation

Study records are comprised of source documents, CRFs, and all other administrative documents, e.g., IRB/IEC correspondence, clinical study materials and supplies shipment manifests, monitoring logs, Sponsor and CRO correspondence, etc.

A source document is defined as any hand-written or computer-generated document that contains medical information or test results that have been collected for or are in support of the protocol specifications, e.g., clinical laboratory reports, clinic notes, drug disbursement log, subject sign-in sheets, subject-completed questionnaires if applicable, telephone logs, ECGs, etc. All draft, preliminary and pre-final iterations of a final report are also considered to be source documents, e.g., faxed laboratory reports and hard-copy laboratory reports, faxed initial results and hard-copy, final report.

All documents pertaining to the study, including a copy of the approved protocol, copy of the informed consent document and Health Insurance Portability and Accountability Act (HIPAA) documents, completed CRFs (where applicable), drug accountability and retention records, and other study related documents will be retained in the permanent archives of the study site. These will be available for inspection at any time by the Sponsor or the FDA or other applicable regulatory agency. Per 21 CFR 312, record retention for this study is required for a period of 2 years following the date on which this study drug is approved by the FDA for the marketing purposes that were the subject of this investigation; or, if no application is to be filed or if the application is not approved for such indication, until 2 years following the date on which the entire study is completed, terminated, or discontinued, and the FDA is notified. If ex-US, then applicable regional/local regulations for records retention will be followed.

Subject records will be kept private except when ordered by law. The following individuals will have access to study subject records: Principal Investigator and designees, study Sponsor, monitors, and auditors, the FDA, other applicable regulatory agencies, other government offices, and the IRB/IEC.

10.4. Miscellaneous Administrative Information

The Investigator is referred to the Investigator Brochure or information provided during the study initiation visit, information provided by the study monitor, and ICH Guidelines for GCP for information regarding the study drug, details, or general considerations to be followed during the course of this study.

Reimbursement, indemnity, and insurance shall be addressed in a separate agreement on terms agreed upon by the parties.

10.5. Facilities

For Parts A, B, and C, which will be conducted at the same CRU, the laboratory at the CRU will perform all safety tests.

For Parts D, E, and D Extension, which will be conducted in multiple CRUs, local laboratories will perform the serum and urine pregnancy tests. A local or central laboratory will perform all remaining safety tests.

Measurements of AT-007 as well as biomarkers of galactose metabolism will be performed by a specialized bioanalytical laboratory.

Instructions for the collection, processing, and/or shipping of various samples are provided in the study laboratory manual(s).

11. REFERENCES

Augustin R. The protein family of glucose transport facilitators: It's not only about glucose after all. IUBMB Life. 2010;62:NA-NA. doi:10.1002/iub.315.

Berry GT, Palmieri M, Gross KC, et al. The effect of dietary fruits and vegetables on urinary galactitol excretion in galactose1-phosphate uridyltransferase deficiency. J Inherit Metab Dis. 1993;16:91-100.

Berry GT. Classic Galactosemia and clinical variant Galactosemia. 04 Feb 2000 [Updated 09 Mar 2017]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1518/.

Berry GT, Nissim I, Gibson JB, et al. Quantitative assessment of whole-body galactose metabolism in galactosemic patients. Eur J Pediatr. 1997;156 Suppl 1:S43-S49.

Berry GT, Hunter JV, Wang Z, et al. In vivo evidence of brain galactitol accumulation in an infant with Galactosemia and encephalopathy. J Pediatr. 2001;138:260–262.

Daenzer JMI, Jumbo-Lucioni PP, Hopson ML, et al. Acute and long-term outcomes in a Drosophila Melanogaster model of Classical Galactosemia occur independently of galaqctose-1-phosphate accumulation. Dis Model Mech. 2016;9:1375-1382.

Demirbas D, Coelho AI, Rubio-Gozalbo ME, Berry GT. Hereditary Galactosemia. Y Meta. 2018. https://doi.org/10.1016/j.metabol.2018.01.025 [unpublished].

Fridovich-Keil JL, Gambello MJ, Singh RH, et al. Duarte variant Galactosemia. 04 Dec 2014. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018. Available from: https://www.ncbi.nlm.nih.gov/books/NBK258640/.

Hennermann JB, Schadewaldt P, Vetter B, et al. Features and outcome of galactokinase deficiency in children diagnosed by newborn screening. J Inherit Metab Dis. 2011;34:399–407.

Martineli D, Bernardi B, Napolitano A, et al. Teaching neuro-images: Galactitol peak and fatal cerebral edema in Classic Galactosemia. Neurology. 2016;86:e32-e33.

Meyer WR, Doyle MB, Grifo JA, et al. Aldose reductase inhibition prevents galactose-induced ovarian dysfunction in the Sprague- Dawley rat. Am J Obst Gynecol. 1992;167(6):1837-1843.

Otaduy MCG, Leite CC, Lacerda MTC, et al. Proton MR spectroscopy and imaging of a galactosemic patient before and after dietary treatment. AJNR. 2006;27:204–207.

Palmieri M, Mazur A, Berry GT, et al. Urine and plasma galactitol in patients with galactose-l-phosphate uridyltransferase deficiency Galactosemia. Metabolism. 1999;48(10):1294-1302.

Rubio-Gozalbo ME, Haskovic M, Bosch AM, et al. The natural history of Classic Galactosemia: Lessons from the GalNet registry. Orphanet J of Rare Dis. 2019;14:86 https://doi.org/10.1186/s13023-019-1047-z.

Pyhtila BM, Shaw KA, Neumann SE, Fridovich-Keil JL. Newborn screening for Galactosemia in the United States: Looking back, looking around, and looking ahead. JIMD Reports. 2014. doi:10.1007/8904_2014_302.

Waisbren SE, Potter NL, Gordon CM, et al. The adult galactosemic phenotype. J Inherit Metab Dis. 2012;35:279–86.

Yager CT, Chen J, Reynolds R, Segal S. Galactitol and galactonate in red blood cells of galactosemic patients. Mol Gen Metab. 2003;80:283–289.

APPENDIX A. SCHEDULE OF ASSESSMENTS FOR PART A (SAD IN HEALTHY SUBJECTS)

Schedule of Assessments for SAD in Healthy Subjects (Part A)

PROCEDURE	Screening (Days -28 to -1)	Day -1[a] (Check-in)	Day 1	Day 2	Day 3[a] (Check-out)	Day 4[a] (Check-out)	EOS Visit[b] (Day 28±2)
Check-in		X[a]					
Discharge					X[a]	X[a]	
Informed consent	X						
I/E criteria	X	X					
Medical history/updates	X	X					X
Demographic data	X						
Physical examination[c]	X	X			X		X
Vital signs[d]	X	X	X	X	X		X
Electrocardiogram[e]	X	X	X		X		X
Clinical laboratory testing (biochemistry, hematology, coagulation, urinalysis, UACR, UPCR)[f]	X	X			X		X
HIV and hepatitis B & C testing	X						
Urine drug/cotinine screen	X	X					
Pregnancy test (female subjects)[g]	X	X			X		X
Drug administration			X				
Blood sample collection for PK analysis[h]			X	X	X		
Urine collection for PK analysis[i] (Cohorts A4 and A5 only)[j]			X	X	X	X	
Adverse Events		Throughout Study					
Prior/Concomitant medications	X	Throughout Study					

EOS = end-of-study; FSH = follicle-stimulating hormone; HIV = human immunodeficiency virus; I/E = inclusion/exclusion; PK = pharmacokinetic; SAD = single ascending dose; UACR – urinary albumin to creatinine ratio; UPCR = urinary protein to creatinine ratio

a: Check-in will occur around 11 am on Day -1. For Cohorts A1, A2, and A3, discharge from the clinic will occur on Day 3 after all Day 3 procedures are done and blood samples for PK are collected through 48 hours after the dose. For Cohorts A4 and A5, discharge from the clinic will occur on Day 4 after urine for PK is collected through 72 hours after the dose.

b: The EOS visit will be scheduled for 28 (±2) days after the dose of study drug.

c: All physical examinations will include weight; however, height will be measured only at screening.

d: Vital signs will be measured at Screening, Day -1 check-in, predose (within 60 min before the dose) on Day 1, at 4 hours (±10 minutes) after the dose on Day 1, once daily on Days 2 and 3, and at the EOS visit.

e: Electrocardiograms will be performed at Screening, Day -1 check-in, predose (within 60 min before the dose) on Day 1, at 2 hours (±10 minutes) after the dose on Day 1, on Day 3, and at the EOS visit.

f: Samples for clinical safety laboratory tests will be collected under fasting conditions.

g: Serum pregnancy tests at Screening, Day -1, and EOS visits; urine pregnancy tests at other time points. Also, female subjects claiming postmenopausal status will have that status verified with an FSH test at Screening. Once a female subject is verified as postmenopausal or otherwise not of childbearing potential, the subject is exempt for further pregnancy tests.

h: Blood samples for PK will be collected predose (within 60 min before the dose) and at 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, and 48 hours (±5 minutes for all time points) after the dose.

i: Urine for PK will be collected predose (spot collection within 60 min before the dose) and in 4-hour intervals (0 to 4, >4 to 8, >8 to 12, >12 to 16, >16 to 20, and >20 to 24) through the first 24 hours after the dose followed by 12-hour intervals (>24 to 36, >36 to 48, >48 to 60, and >60 to 72) from 24 hours postdose to 72 hours postdose. Allowable windows for urine collection container changes will be ±30 minutes through the first 24 hours and ±1 hour thereafter.

j: Cohort A4 = highest originally planned dose level cohort in Part A. Cohort A5 = highest planned dose level cohort in Part A after the protocol amendment resulting in protocol version 4.0.

APPENDIX B. SCHEDULE OF ASSESSMENTS FOR PART B (MAD IN HEALTHY SUBJECTS)

Schedule of Assessments for MAD in Healthy Subjects (Part B)

PROCEDURE	Screening (Days -28 to -1)	Day -1[a] (Check-in)	Day 1	Day 2	Days 3, 4, & 5	Day 6	Day 7	Day 8	Day 9[a] (Check-out)	EOS Visit[b] (Day 35±2)
Check-in		X[a]								
Discharge									X[a]	
Informed consent	X									
I/E criteria	X	X								
Medical history/updates	X	X								X
Demographic data	X									
Physical examination[c]	X	X				X			X	X
Vital signs[d]	X	X	X	X		X	X	X	X	X
Electrocardiogram[e]	X	X	X				X			X
Clinical laboratory testing (biochemistry, hematology, coagulation, urinalysis)[f]	X	X				X				X
HIV and hepatitis B & C testing	X									
Urine drug/cotinine screen	X	X								
Pregnancy test (female subjects)[g]	X	X				X				X
Drug administration[h]			X	X	X	X	X			
Blood sample collection for PK analysis[i]			X	X			X	X	X	
Adverse events		Throughout Study								
Prior/Concomitant medications	X	Throughout Study								

CSF = cerebrospinal fluid; EOS = end-of-study; FSH = follicle-stimulating hormone; HIV = human immunodeficiency virus; I/E = inclusion/exclusion; MAD = multiple ascending dose; PK = pharmacokinetic; QD = once daily.

a: Check-in will occur around 11 am on Day -1 and Discharge will occur on Day 9 after the blood sample for PK is collected at 48 hours after the Day 7 dose and all Day 9 procedures are done.

b: The EOS visit will be scheduled for 28 (±2) days after the last dose of study drug.

c: All physical examinations will include weight; however, height will be measured only at screening.

d: Vital signs will be measured at Screening, check-in on Days -1 and 6, predose (within 60 min before the dose) on Days 1 and 7, at 4 hours (±10 minutes) after the dose on Days 1 and 7, once daily on Days 2, 8, and 9, and at the EOS visit.
e: Electrocardiograms will be performed at Screening, Day -1 check-in, predose (within 60 min before the dose) on Days 1 and 7, at 2 hours (±10 minutes) after the dose on Days 1 and 7, and at the EOS visit.
f: Samples for clinical safety laboratory tests will be collected under fasting conditions.
g: Serum pregnancy tests at Screening, Day -1, and EOS visits; urine pregnancy tests at other time points. Also, female subjects claiming postmenopausal status will have that status verified with an FSH test at Screening. Once a female subject is verified as postmenopausal or otherwise not of childbearing potential, the subject is exempt for further pregnancy tests. On Day 6, the urine pregnancy test must be done (and the result negative) before study drug is administered.
h: All doses of study drug will be administered at the clinic.
i: On Day 1, blood samples for PK will be collected predose (within 60 minutes before the dose) and at 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours (±5 minutes for all time points) after the dose. On Day 2, blood samples for PK will be collected predose (i.e., at 24 hours ±5 minutes after the Day 1 dose and before the Day 2 dose). On Day 7, blood samples for PK will be collected predose (within 60 minutes before the dose) and at 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours (±5 minutes for all time points) after the dose. Also, blood samples for PK will be collected at 24, 36, and 48 hours (±5 minutes for all time points) after the Day 7 dose; these collections will be taken on Day 8 and Day 9.

APPENDIX C. SCHEDULE OF ASSESSMENTS FOR PART C (MAD IN HEALTHY SUBJECTS)

Schedule of Assessments for MAD in Healthy Subjects (Part C)

PROCEDURE	Screening (Days -28 to -1)	Day -1[a] (Check-in)	Day 1	Day 2	Days 3, 4, & 5	Day 6	Day 7	Day 8	Day 9[a] (Check-out)	EOS Visit[b] (Day 35±2)
Check-in		X[a]								
Discharge									X[a]	
Informed consent	X									
I/E criteria	X	X								
Medical history/updates	X	X								X
Demographic data	X									
Physical examination[c]	X	X				X			X	X
Vital signs[d]	X	X	X	X		X	X	X	X	X
Electrocardiogram[e]	X	X	X				X			X
Clinical laboratory testing (biochemistry, hematology, coagulation, urinalysis)[f]	X	X				X				X
HIV and hepatitis B & C testing	X									
Urine drug/cotinine screen	X	X								
Pregnancy test (female subjects)[g]	X	X				X				X
Drug administration[h]			X	X	X	X	X			
Blood sample collection for PK analysis[i]			X	X			X	X	X	
Lumbar puncture[j]							X			
Adverse events		Throughout Study								
Prior/Concomitant medications	X	Throughout Study								

CSF = cerebrospinal fluid; EOS = end-of-study; FSH = follicle-stimulating hormone; HIV = human immunodeficiency virus; I/E = inclusion/exclusion; MAD = multiple ascending dose; PK = pharmacokinetic; QD = once daily.

a: Check-in will occur around 11 am on Day -1 and Discharge will occur on Day 9 after the blood sample for PK is collected at 48 hours after the Day 7 dose and all Day 9 procedures are done.

b: The EOS visit will be scheduled for 28 (±2) days after the last dose of study drug.

c: All physical examinations will include weight; however, height will be measured only at screening.
d: Vital signs will be measured at Screening, check-in on Days -1 and 6, predose (within 60 min before the dose) on Days 1 and 7, at 4 hours (±10 minutes) after the dose on Days 1 and 7, once daily on Days 2, 8, and 9, and at the EOS visit.
e: Electrocardiograms will be performed at Screening, Day -1 check-in, predose (within 60 min before the dose) on Days 1 and 7, at 2 hours (±10 minutes) after the dose on Days 1 and 7, and at the EOS visit.
f: Samples for clinical safety laboratory tests will be collected under fasting conditions.
g: Serum pregnancy tests at Screening, Day -1, and EOS visits; urine pregnancy tests at other time points. Also, female subjects claiming postmenopausal status will have that status verified with an FSH test at Screening. Once a female subject is verified as postmenopausal or otherwise not of childbearing potential, the subject is exempt for further pregnancy tests. On Day 6, the urine pregnancy test must be done (and the result negative) before study drug is administered.
h: All doses of study drug will be administered at the clinic.
i: On Day 1, blood samples for PK will be collected predose (within 60 minutes before the dose) and at 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours (±5 minutes for all time points) after the dose. On Day 2, blood samples for PK will be collected predose (i.e., at 24 hours ±5 minutes after the Day 1 dose and before the Day 2 dose). On Day 7, blood samples for PK will be collected predose (within 60 minutes before the dose) and at 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours (±5 minutes for all time points) after the dose. Also, blood samples for PK will be collected at 24, 36, and 48 hours (±5 minutes for all time points) after the Day 7 dose; these collections will be taken on Day 8 and Day 9.
j: A lumbar puncture will be done after the Day 7 dose to collect CSF for analysis of AT-007. For Cohort C1, the timing (hours after the dose) of the lumbar puncture was based on PK results from Cohorts B1 and B2. For Cohort C2, the timing of the lumbar puncture was based on PK results from Cohorts B1, B2, and B3. For Cohort C2, the timing of the lumbar puncture will be based on PK results from Cohorts B1, B2, B3, and B4. The intent is to perform the lumbar puncture at the time of maximum observed concentration (T_{max}) after the Day 7 dose.

Note: The Schedule for Part C will differ from that for Part B only by the lack of randomization on Day 1 and the addition of a Day 7 lumbar puncture.

APPENDIX D. SCHEDULE OF ASSESSMENTS FOR PART D (SAD AND MAD IN SUBJECTS WITH CLASSIC GALACTOSEMIA)

Schedule of Assessments for SAD and MAD in Subjects with Classic Galactosemia (Part D) [q]

PROCEDURE	Screening (Days -40 to -1[a])	Day 1	Day 2	Day 3[b]	Day 4[b]	Day 5	Day 6[a]	Day 12[a]	Day 13[b]	Day 20[a]	Day 32[a]	Day 33	Day 34[b]	EOS Visit Day 60 (±2)
Check-in	X[a] (Day -1)							X[a]			X[a]			
Discharge				X[b]	X[b]				X[b]				X[b]	
Informed consent	X													
I/E criteria	X[r]													
Medical history/updates	X[r]													X
Demographic data	X													
Physical examination[d]	X[r]						X	X		X	X		X	X
Vital signs[e]	X[r]	X	X	X	X		X	X	X	X	X	X	X	X
Electrocardiogram[f]	X[r]	X					X	X		X	X			X
Clinical laboratory testing[g]	X[r]						X	X		X	X			X
GALT activity in red blood cells and GALT gene analysis	X													
Aldose Reductase activity	X													
HIV and hepatitis B & C testing	X													
Urine drug screen	X[r]													
Pregnancy test (female subjects)[h]	X[r]						X	X		X	X			X
Drug administration[j]		X					QD for 27 days							
5-day washout[k]		X												
Blood sample collection for PK analysis[l]		X	X	X				X	X		X	X	X	
Urine collection for PK analysis[m] (Cohort D3 only)[n]		X	X	X	X									
Blood sample collection[o] for galactose, galactitol, galactose-1-p and other galactose metabolites	X[r]	X	X	X				X	X		X	X	X	

PROCEDURE	Screening (Days -40 to -1[a])	Day 1	Day 2	Day 3[b]	Day 4[b]	Day 5	Day 6[a]	Day 12[a]	Day 13[b]	Day 20[a]	Day 32[a]	Day 33	Day 34[b]	EOS Visit Day 60 (±2)
Urine sample collection[p] for galactitol	X[r]	X	X					X	X		X	X		
Adverse events		Throughout Study												
Prior/Concomitant medications	X[r]	Throughout Study												

CRU = clinical research unit; EOS = end-of-study; FSH = follicle-stimulating hormone; HIV = human immunodeficiency virus; I/E = inclusion/exclusion; MAD = multiple ascending dose; PK = pharmacokinetic; QD = once daily; SAD = single ascending dose.

a: Subjects will arrive at the CRU in the morning on Day -1 (approximately 24 hours before the planned first dose). For the Days 12, 20, and 32 check-ins/visits, the subject should arrive after an overnight fast of at least 10 hours (and before breaking the fast) and before taking study drug. On Days 6 and 60, the subject should not have any study drug in his/her possession and should arrive (in a fasted condition) in the morning (before the planned time of study drug dosing to be given in the clinic on Day 6 [no study drug dosing on Day 60]).

b: Discharge from the 1st, 2nd, and 3rd (last) in-clinic periods of the required portions of Part D will occur on Days 3 (Cohorts D1 and D2) or 4 (Cohort D3), 13, and 34, respectively, after all procedures for that day are done and the last blood sample or urine collection for that in-clinic period is collected.

c: The EOS visit will be scheduled for 28 (±2) days after the last dose of study drug in the MAD portion.

d: All physical examinations will include weight; however, height will be measured only at screening.

e: Vital signs will be measured at Screening, every check-in, every check-out, predose (within 60 min before the dose) and 4 hours (±10 minutes) after the dose on Days 1, 6, 12, 20, and 32; once daily on all other in-clinic days; and at the EOS visit.

f: Electrocardiograms will be performed at Screening, Day -1 check-in, predose (within 60 min before the dose) and 2 hours (±10 minutes) after the dose on Days 1, 6, 12, 20, and 32 and at the EOS visit.

g: Samples for clinical safety laboratory tests (hematology, chemistry, coagulation, and urinalysis) will be collected under fasting conditions.

h: Serum pregnancy tests at Screening, Day -1, and EOS visits; urine pregnancy tests at other time points. Also, female subjects claiming postmenopausal status will have that status verified with an FSH test at Screening. Once a female subject is verified as postmenopausal or otherwise not of childbearing potential, the subject is exempt for further pregnancy tests. On Days 6, 12, 20, and 32, the urine pregnancy test must be done (and the result negative) before study drug is administered.

j: On Days 1, 6, 12, 13, 20, and 32, study drug will be administered at the clinic. All other doses of study drug will be taken at home QD with water in the morning (preferably after an overnight fast of ≥10 hours with the fast broken ≥2 hours after the dose). Study drug administration should be at approximately the same time of day (preferably 8 am) throughout the multiple dosing period. See Section 6.7 for when study drug will be dispensed and/or collected.

k: The 5-day washout between single and multiple dosing will start immediately after the Day 1 dose and end immediately before the Day 6 dose. There is no visit on Day 5; it is in the Schedule of Assessments only for depiction of the 5-day washout.

l: For the Day 1 dose, blood samples for PK will be collected predose (within 60 minutes before the dose) and at 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, and 48 hours (±5 minutes for all time points) after the dose. On Days 12 and 32, blood samples for PK will be collected predose (within 60 minutes before the dose) and at 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours (±5 minutes for all time points) after the dose. On Day 13, a blood sample for PK will be collected predose (i.e., at 24 hours ±5 minutes after the Day 12 dose). On Day 33, blood samples for PK will be collected at 24 and 36 hours (±5 minutes for both time points) after the Day 32 dose. On Day 34, a blood sample for PK will be collected at 48 hours (±5 minutes) after the Day 32 dose.

m: For Cohort D3 only, urine for PK will be collected predose (spot collection within 60 minutes before the Day 1 dose) and in 4-hour intervals (0 to 4, >4 to 8, >8 to 12, >12 to 16, >16 to 20, and >20 to 24) through the first 24 hours after the Day 1 dose followed by 12-hour intervals (>24 to 36, >36 to 48, >48 to 60, and >60 to 72) from 24 to 72 hours after the Day 1 dose. Allowable windows for urine collection container changes will be ±30 minutes through the first 24 hours and ±1 hour thereafter.
n: Cohort D3 = highest planned dose level cohort in Part D.
o: With the exception of the blood samples for biomarkers taken at Screening and Day -1, the time points and allowable windows for the blood samples for biomarkers will match those for the blood samples for PK.
p: Urine for galactitol measurement will be collected over the 24 hours immediately before and the 24 hours immediately after the dose on Day 1. Urine for galactitol measurement will be collected over the 24 hours immediately after the dose on Days 12 and 32 during the MAD period. For each 24-hour collection of urine for galactitol measurement, an aliquot of the total urine collected will be analyzed for creatinine. Also, a spot collection for urine galactitol measurement will be taken at screening.
q: Visit window +/- 1 day of scheduled visit for all visits except the EOS visit which has a window of +/- 2 days.
r: These assessments must be performed on Day -1 even if they were already performed earlier during the screening period.

APPENDIX E. SCHEDULE OF ASSESSMENTS FOR PART E (SAD AND MAD IN SUBJECTS WITH GALK-DEFICIENT GALACTOSEMIA)

Schedule of Assessments for SAD and MAD in Subjects with GALK-deficient Galactosemia (PART E)[o]

PROCEDURE	Screening (Days -40 to -1[a])	Day 1	Day 2	Day 3[b]	Day 5	Day 6[a]	Day 12[a]	Day 13[b]	Day 20[a]	Day 32[a]	Day 33	Day 34[b]	EOS Visit[c] Day 60 (±2)
Check-in	X[a] (Day -1)						X[a]			X[a]			
Discharge				X[b]				X[b]				X[b]	
Informed consent	X												
I/E criteria	X[p]												
Medical history/updates	X[p]												X
Demographic data	X												
Physical examination[d]	X[p]					X	X		X	X		X	X
Vital signs[e]	X[p]	X	X	X		X	X	X	X	X	X	X	X
Electrocardiogram[f]	X[p]	X				X	X		X	X			X
Clinical laboratory testing[g]	X[p]					X	X		X	X			X
GALK activity in red blood cells and GALK gene analysis	X												
Aldose Reductase activity	X												
HIV and hepatitis B & C testing	X												
Urine drug screen	X[p]												
Pregnancy test (female subjects)[h]	X[p]					X	X		X	X			X
Drug administration[j]		X				QD for 27 days							
5-day washout[k]		X											
Blood sample collection for PK analysis[l]		X	X	X			X	X		X	X	X	
Blood sample collection[m] for galactose, galactitol, galactose-1-p and other galactose metabolites	X[p]	X	X	X			X	X		X	X	X	

PROCEDURE	Screening (Days -40 to -1[a])	Day 1	Day 2	Day 3[b]	Day 5	Day 6[a]	Day 12[a]	Day 13[b]	Day 20[a]	Day 32[a]	Day 33	Day 34[b]	EOS Visit[c] Day 60 (±2)
Urine sample collection[n] for galactitol	X[p]	X	X				X	X		X	X		
Adverse events		Throughout Study											
Prior/Concomitant medications	X[p]	Throughout Study											

CRU = clinical research unit; EOS = end-of-study; FSH = follicle-stimulating hormone; HIV = human immunodeficiency virus; I/E = inclusion/exclusion; MAD = multiple ascending dose; PK = pharmacokinetic; QD = once daily; SAD = single ascending dose.

a: Subjects will arrive at the CRU in the morning on Day -1 (approximately 24 hours before the planned first dose). For the Days 12, 20, and 32 check-ins/visits, the subject should arrive after an overnight fast of at least 10 hours (and before breaking the fast) and before taking study drug. On Days 6 and 60, the subject should not have any study drug in his/her possession and should arrive (in a fasted condition) in the morning (before the planned time of study drug dosing to be given in the clinic on Day 6 [no study drug dosing on Day 60]).

b: Discharge from the 1st, 2nd, and 3rd (last) in-clinic periods will occur on Days 3, 13, and 34, respectively, after all procedures for that day are done and the last blood sample or urine collection for that in-clinic period is collected.

c: The EOS visit will be scheduled for 28 (±2) days after the last dose of study drug in the MAD portion.

d: All physical examinations will include weight; however, height will be measured only at screening.

e: Vital signs will be measured at Screening, every check-in, every check-out, predose (within 60 min before the dose) and 4 hours (±10 minutes) after the dose on Days 1, 6, 12, 20, and 32, once daily on all other in-clinic days, and at the EOS visit.

f: Electrocardiograms will be performed at Screening, Day -1 check-in, predose (within 60 min before the dose) and 2 hours (±10 minutes) after the dose on Days 1, 6, 12, 20, and 32, and at the EOS visit.

g: Samples for clinical safety laboratory tests (hematology, chemistry, coagulation, and urinalysis) will be collected under fasting conditions.

h: Serum pregnancy tests at Screening, Day -1, and EOS visits; urine pregnancy tests at other time points. Also, female subjects claiming postmenopausal status will have that status verified with an FSH test at Screening. Once a female subject is verified as postmenopausal or otherwise not of childbearing potential, the subject is exempt for further pregnancy tests. On Days 6, 12, 20, and 32, the urine pregnancy test must be done (and the result negative) before study drug is administered.

j: On Days 1, 6, 12, 13, 20, and 32, study drug will be administered at the clinic. All other doses of study drug will be taken at home QD with water in the morning (preferably after an overnight fast of ≥10 hours with the fast broken ≥2 hours after the dose). Study drug administration should be at approximately the same time of day (preferably 8 am) throughout the multiple dosing periods. See Section 6.7 for when study drug will be dispensed and/or collected.

k: The 5-day washout between single and multiple dosing will start immediately after the Day 1 dose and end immediately before the Day 6 dose. There is no visit on Day 5; it is in the Schedule of Assessments only for depiction of the 5-day washout.

l: For the Day 1 dose, blood samples for PK will be collected predose (within 60 minutes before the dose) and at 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, and 48 hours (±5 minutes for all time points) after the dose. On Days 12 and 32, blood samples for PK will be collected predose (within 60 minutes before the dose) and at 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours (±5 minutes for all time points) after the dose. On Day 13, a blood sample for PK will be collected predose (i.e., at 24 hours ±5 minutes after the Day 12 dose). On Day 33, blood samples for PK will be collected at 24 and 36 hours (±5 minutes for both time points) after the Day 32 dose. On Day 34, a blood sample for PK will be collected at 48 hours (±5 minutes) after the Day 32 dose.

m: With the exception of the blood samples for biomarkers taken at Screening and Day -1, the time points and allowable windows for the blood samples for biomarkers will match those for the blood samples for PK.
n: Urine for galactitol measurement will be collected over the 24 hours immediately before and the 24 hours immediately after the dose on Day 1. Urine for galactitol measurement will be collected over the 24 hours immediately after the dose on Days 12 and 32. For each collection of urine for galactitol measurement, an aliquot of the total urine collected will be analyzed for creatinine. Also, a spot collection for urine galactitol measurement will be taken at screening.
o: Visit window +/- 1 day of scheduled visit for all visits except the EOS visit which has a window of +/- 2 days.
p: These assessments must be performed on Day -1 even if they were already performed earlier during the screening period.

APPENDIX F. SCHEDULE OF ASSESSMENTS FOR PART D EXTENSION

Schedule of Assessments for the 90-day Extension in Subjects with Classic Galactosemia (Part D Extension)[a]

PROCEDURE	Prescreening	Screening (Day -60 to -1)[b]	Day -1[c]	Day 1	Day 2[d]	Day 30[c]	Day 60[c]	Day 90[c]	Day 91	EOE Visit[e] Day 118
Check-in			X					X		
Discharge					X				X	
Informed consent		X[f]	X[f]							
I/E criteria		X	X							
Medical history/updates	X (only CG diagnosis)	X	X							X
Demographic data		X[q]								
Physical examination[g]	X	X	X			X	X	X		X
Vital signs[h]		X	X	X	X	X	X	X		X
Electrocardiogram[i]		X	X	X		X	X	X		X
Clinical laboratory testing[j]	X[j]	X[j]	X[j]			X	X	X		X
GALT activity in red blood cells and GALT gene analysis	X[q]	X[q]								
Aldose Reductase activity		X[q]								
HIV and hepatitis B & C testing[j]	X	X								
Urine drug screen		X	X							
Pregnancy test (female subjects)[k]		X[k]	X[k]			X	X	X		X
Drug administration[m]				X						
Blood sample collection for PK analysis[n]				X		X	X	X	X	
Blood sample collection[o] for galactose, galactitol, galactose-1-p and other galactose metabolites		X		X		X	X	X	X	
Urine sample for galactitol[p]	X	X								
Impact-of-disease interview				X						
Adverse events				Throughout Extension						
Prior/Concomitant medications		X		Throughout Extension						

CG = Classic Galactosemia; CRU = clinical research unit; EOE = end-of-extension; EOS = end-of-study; I/E = inclusion/exclusion; MAD = multiple ascending dose; PK = pharmacokinetic; QD = once daily.

a: All visits beyond Day 2 have visit windows of +/- 2 days. However, Day 1 must be the day of the first dose (in the extension); Day -1 must be the day before the first dose; Day 90 must be the day of the last dose; Days 88 and 89 must be the 2 days before day of the last dose; and Day 91 must be the day after the last dose.
b: Screening visit for the Part D Extension is needed for subjects who have not previously participated in Part D. For subjects who previously participated in Part D, this Screening Visit is only needed if Day 1 of the extension period will be >60 days after the last visit (EOS or otherwise) in Part D.
c: Check-in will occur around 11 am on Day -1. On Day 90, subjects will arrive at the CRU after an overnight fast of at least 10 hours (and before breaking the fast) and before taking study drug. In lieu of overnight stays in the CRU, subjects may stay at a local hotel; subjects will abide by all study guidelines including overnight fasting. The Day 30 and Day 60 visits can be either home health or in-clinic visits.
d: Discharge from the in-clinic stay will occur after all procedures for that day are done and the subject has stayed in the clinic for at least 24 hours after the first dose.
e: The EOE visit will be scheduled for 28 (±2) days after the last dose of study drug. The EOE Visit will not be required if subjects transition to the separate Open-Label Extenstion Study, in which case the study completion will be considered the completion of the Treatment Period at Day 91.
f: The informed consent form needs to be signed at the first visit for the extension which may be either the Screening visit or the Day -1 check-in (see footnote b).
g: All physical examinations will include weight; however, height will be measured only at screening (or prescreening). Prescreening does not include a physical examination (only height and weight).
h: Vital signs will be measured at Screening (if applicable), every check-in, every check-out, predose (within 60 min before the dose) and 4 hours (±10 minutes) after the dose on Days 1, 30, 60, and 90, once daily on all other visits, and at the EOE visit.
i: Electrocardiograms will be performed at Screening (if applicable), Day -1 check-in, predose (within 60 min before the dose) and 2 hours (±10 minutes) after the dose on Days 1, 30, 60, and 90, and at the EOE visit.
j: Samples for clinical safety laboratory tests (hematology, chemistry, coagulation, urinalysis, UACR and UPCR) will be collected under fasting conditions. At the Screening visit only (i.e., only applicable to subjects for whom this visit is required and does not need to be repeated at screening if already done at prescreening), HIV and hepatitis B & C testing will also be done. Hematology, chemistry, and coagulation at the Screening visit (i.e., only applicable to subjects for whom this visit is required) does not need to be repeated if already done at prescreening and prescreening was within 60 days before the first dose of Part D Extension study drug. Clinical safety laboratory tests (hematology, chemistry, coagulation, urinalysis, UACR and UPCR) do not need to be repeated at Day -1 if already done at prescreening or screening and the prescreening/screening visit was done within 60 days before the first dose of Part D Extension study drug.
k: Serum pregnancy tests will be done at Screening (if applicable), Day -1 (also urine test if serum test cannot be processed immediately), and EOE visits; urine pregnancy tests will be done at all other time points. From Days 1 through 90, pregnancy tests must be done (and the result negative) on dosing days before study drug is administered. A pregnancy test must be performed on Day -1 even if already performed earlier during the screening period.
m: On Days 1, 2, and 90, study drug will be administered at the clinic. On Days 30 and 60, study drug will be administered during the visit (home health or in-clinic), All other doses of study drug will be taken at home QD with water in the morning (preferably after an overnight fast of ≥10 hours with the fast broken ≥2 hours after the dose). Study drug administration should be at approximately the same time of day (preferably 8 am) throughout the extension. Drug accountability (including dispensing and/or collecting) will be assessed at all visits during the 90 days of dosing.
n: On Day 1, a predose (within 60 minutes before the dose) blood sample for PK will be collected. On Days 30, 60, and 90, blood samples for PK will be collected predose (24 hours [±15 minutes] after the dose taken the day before and within 60 minutes before the dose administered on that day) and at 2, 4, 8, and 12 hours (±5 minutes for all time points) after the dose. An additional sample will be taken on Day 91 at 24 hour after the Day 90 dose.
o: With the exception of the blood sample for biomarkers taken Screening (for those subjects requiring a screening visit), the time points and allowable windows for the blood samples for biomarkers will match those for the blood samples for PK.

p: Urine spot collection for galactitol analysis to check the study entry criterion based on urine galactitol (applicable only to subjects requiring a screening visit because it is assumed that urine galactitol for subjects not requiring the screening visit remain in the study eligible range and does not need to be repeated if done at prescreening)q:Required only for de novo subjects (i.e., subjects who did not previously participate in Part D SAD and MAD and does not need to be repeated at screening if already done at prescreening).

APPENDIX G. GUIDANCE IN CASE OF INCREASE IN ALT OR AST

1. If ALT or AST > 3x ULN and Total Bilirubin < 2x ULN:

- The event must be reported to the Sponsor within 24 hours.
- The following laboratory tests will be performed: alkaline phosphatase, total and conjugated bilirubin, prothrombin time, international normalized ratio (INR), creatinine phosphokinase (CPK), serum creatinine, complete blood count, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV).
- Recent medical history will be investigated specifically for intercurrent illness, malaise with or without loss of consciousness, dizziness, hypotension, episodes of arrhythmia, and muscular injury.
- Concomitant medications, including recreational drugs, nonprescription medications and herbal and dietary supplement preparations, will be reviewed and any substance potentially responsible for the elevation of liver enzymes will be discontinued, if possible
- Information on potential exposure to environmental chemical agents, alcohol use, and special diets, including adherence to a galactose-restricted diet, will be collected and any factor potentially responsible for the elevation of liver enzymes will be addressed, if possible
- If the patient is asymptomatic and the ALT/AST elevation is not associated with abnormal liver function (e.g. increased bilirubin or abnormal coagulation parameters), the STUDY TREATMENT WILL BE CONTINUED UNDER STRICT MONITORING as below.
- Liver function tests (LFTs) will be monitored every two weeks x2 and then monthly for six months. If ALT or AST decrease to below 3x ULN, then monthly monitoring will continue until resolution. If ALT or AST increase to >5x ULN or if Total Bilirubin increases to >2x ULN, implement the below procedures.
- For ALT or AST >3x ULN lasting six months, perform a hepatic ultrasound and consult with a hepatologist

2. If ALT or AST > 5x ULN OR if ALT or AST >3x ULN and Total Bilirbuin >2x ULN, a retest must be performed within 24 hours

2a. If confirmed ALT or AST > 5x ULN OR if ALT or AST >3x ULN and Total Bilirbuin >2x ULN:

- The event must be reported to the Sponsor within 24 hours.
- STUDY TREATMENT WILL BE DISCONTINUED.
- The following laboratory tests will be performed: alkaline phosphatase, total and conjugated bilirubin, prothrombin time, international normalized ratio (INR), CPK,

serum creatinine, complete blood count, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis E virus (HEV)

- A 2 mL serum sample will be collected and stored frozen for future analysis
- An hepatobiliary ultrasonography will be performed.
- All necessary investigations will be performed to rule out autoimmune or alcoholic hepatitis; non-alcoholic steatohepatitis (NASH); biliary tract disease, and hypoxic/ischemic hepatopathy (possibly due to right heart failure or other cardiovascular disease)
- Recent medical history will be investigated specifically for malaise with or without loss of consciousness, dizziness, hypotension, episodes of arrhythmia, and muscular injury.
- Concomitant medications, including recreational drugs, nonprescription medications and herbal and dietary supplement preparations, will be reviewed
- Information on potential exposure to environmental chemical agents, alcohol use, and special diets, including adherence to a galactose-restricted diet, will be collected
- A consult with an hepatologist will be requested.
- Liver Function tests (LFTs) will continue to be monitored every 48 hours until stabilization and symptoms remission (if any), then every 2 weeks until normalization.

2b. If ALT or AST > 5x ULN OR if ALT or AST >3x ULN and Total Bilirbuin >2x ULN not confirmed and ALT or AST is <5x ULN and > 3x ULN (with Total Bilirbuin <2x ULN) all the procedures listed above under section 1 will be implemented.

2c. ALT or AST < 3x ULN but > 1.5x ULN:

- The event must be reported to the Sponsor within 24 hours.
- LFTs will continue to be monitored every month until normalization or further elevation. If ALT or AST > 3 x ULN, all the procedure above in section 1 will be implemented. If ALT/AST > 5x ULN, OR if ALT or AST >3x ULN and Total Bilirbuin >2x ULN, all the procedures listed above under section 2 will be implemented.

APPENDIX H. GUIDANCE IN CASE OF WORSENING RENAL FUNCTION

Renal function must be closely monitored during the study, particularly during administration of potentially nephrotoxic drugs, such as acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), fluoxetine, antihistamines, antimicrobials, benzodiazepines, proton pump inhibitors, phenytoin, trimethoprim, fenofibrate, cimetidine, cephalosporins, probenecid, aminoglycoside, amphotericin, ketoconazole, and clofibrate

Urine protein-to-creatinine ratio (UPCR), urine albumin-to- creatinine ratio (UACR) and eGFR will be assessed at each visit. In case of increased UPCR ($\geq$ 200 mg/g), increased UACR ($\geq$ 30 mg/g), eGFR < 30 mL/min/1.73 m^2 , or decreased eGFR > 30% compared to baseline, a retest must be performed within 24 hours and if the abnormal laboratory result is confirmed, the following actions will be taken:

- The event must be reported to the Sponsor within 24 hours
- Concomitant medications will be reviewed and any potentially nephrotoxic drug must be discontinued
- Recent medical history will be investigated specifically for significant increase or decrease of blood pressure, plasma volume depletion, flu-like symptoms, urinary tract infection, and abdominal pain suggestive of obstructive uropathy.
- If there is no suspected concomitant medication , or if no improvement of renal function is observed after 72 hours of discontinuation of the potentially nephrotoxic drug, and if there is nothing in recent medical history that is likely to cause the abnormal renal function, the study drug will be temporarily discontinued.
- The following urinary markers of kidney injury must be measured: Clusterin (CLU), Cystatin-C (CysC), Kidney Injury Molecule-1 (KIM-1), N-acetyl-beta-D-glucosaminidase (NAG), Neutrophil gelatinase-associated lipocalin (NGAL), and Osteopontin (OPN)
- A renal ultrasound will be performed.
- A nephrologist will be consulted
- Renal function (eGFR, UPCR and UACR) will continue to be monitored every 48 hours until stabilization, then every 2 weeks until normalization.
- At this time, the Investigator in consultation with the nephrologist will decide if the study drug may resume

APPENDIX I. RESULTS OF IN-VITRO DRUG-DRUG-INTERACTION (DDI) STUDIES WITH AT-007

Recommendations related to the use of co-medications	Potential effect	Rationale/Comments	Reference
CYP1A2 substrates - use with caution with sensitive substrates (Alosetron, caffeine, duloxetine, melatonin, ramelteon, tasimelteon, tizanidine)	Increase in systemic exposure of sensitive substrates of CYP1A2	Reversible inhibition was not significant (AUCR = 1.03 for caffeine). TDI was observed with an IC_{50} of 35.7 μM; however, K_I and k_{inact} could not be determined	Study No. AACM-0014-DV-TB, Study No. Applied-03
CYP3A4 substrates - use with caution with sensitive substrates (Alfentanil, avanafil, buspirone, conivaptan, darifenacin, darunavir, ebastine, everolimus, ibrutinib, lomitapide, lovastatin, midazolam, naloxegol, nisoldipine, saquinavir, simvastatin, sirolimus, tacrolimus, tipranavir, triazolam, vardenafil, budesonide, dasatinib, dronedarone, eletriptan, eplerenone, felodipine, indinavir, lurasidone, maraviroc, quetiapine, sildenafil, ticagrelor, tolvaptan)	Reduction in systemic exposure of sensitive substrates of CYP3A4	CYP3A4 induction AUCR = 0.14, so significant using Mechanistic Static Model	Study No. AACM-0015-DV-DA
	Increase in systemic exposure of sensitive substrates of CYP3A4	There was no TDI effect. AUCR was <1.66 (so >1.25), based on reversible IC_{50} of >100 μM. However, IC_{50} would have to be ~800 μM for AUCR to be <1.25; inhibition at 100 μM was 36.7% for midazolam and 52.5% for testosterone, so there is negligible likelihood that the actual IC_{50} would be near 800 μM	Study No. AACM-0014-DV-TB

Recommendations related to the use of co-medications	**Potential effect**	**Rationale/Comments**	**Reference**
CYP2B6 substrates - use with caution with sensitive substrates (bupropion)	Increase in systemic exposure of sensitive substrates of CYP2B6	Reversible inhibition was not significant (AUCR = 1.04 for bupropion). TDI was observed; AUCR including both reversible and TDI was 10.4 for bupropion, which is highly significant (>1.25).	Study No. **AACM-0014-DV-TB, Study No. Applied-03**
CYP2C19 substrates - use with caution with sensitive substrates (omeprazole, S-mephenytoin)	Increase in systemic exposure of sensitive substrates of CYP2C19	Reversible inhibition was not significant (AUCR = 1.07 for omeprazole). TDI was observed; AUCR including both reversible and TDI was 5.2 for omeprazole, which is highly significant (>1.25).	Study No. AACM-0014-DV-TB, Study No. Applied-03
BCRP substrates. Use with caution with sensitive substrates of BCRP (rosuvastatin, sulfasalazine)	Increase in systemic exposure of sensitive substrates of BCRP	Potentially clinically relevant inhibition of BCRP observed with I_{gut}/IC_{50} of 1200	Study No. Applied-01-11Jun2019
BCRP inhibitors. Use with caution with potent inhibitors of BCRP (cyclosporine A)	Increase in systemic exposure of AT-007	AT007 observed to be a substrate of BCRP	Study No. Applied-01-11Jun2019
OAT1, OAT3 substrates - use with caution with sensitive substrates (adefovir, cefaclor, ceftizoxime, famotidine, furosemide, ganciclovir, methotrexate, oseltamivir carboxylate, penicillin G)	Increase in systemic exposure of sensitive substrates of OAT1 or OAT3	Potentially clinically relevant inhibition of OAT1 (minor) and OAT3 observed with $I_{max,u}/IC_{50}$ of 10.0 for OAT3	Study No. Applied-01-11Jun2019