Study Title: A Phase 1, Blinded, Placebo-controlled Study of the Safety, Tolerability, Pharmacokinetics of Single- and Multiple-Ascending Doses of ABI-4334 in Healthy Subjects

NCT Number: NCT05569941

Date of Document: 16 June 2022

CLINICAL STUDY PROTOCOL

PROTOCOL TITLE	A Phase 1, Blinded, Placebo-controlled Study of the Safety, Tolerability, Pharmacokinetics of Single- and Multiple-Ascending Doses of ABI-4334 in Healthy Subjects
PROTOCOL NUMBER	ABI-4334-101
DRUG NAME	ABI-4334
REGULATORY AGENCY IDENTIFIER NUMBER(S)	Not Available
SPONSOR	Assembly Biosciences, Inc. 331 Oyster Point Boulevard, 4th Floor South San Francisco, California 94080, USA (833) 509-4583
PROTOCOL – DATE	Original – 16 June 2022
PROTOCOL HISTORY	Not Applicable

CONFIDENTIALITY STATEMENT

CLINICAL STUDY PROTOCOL SPONSOR APPROVAL FORM

Protocol Title	A Phase 1, Blinded, Placebo-controlled Study of the Safety, Tolerability, Pharmacokinetics of Single- and Multiple-Ascending Doses of ABI-4334 in Healthy Subjects
Protocol Number	ABI-4334-101
Protocol Version – Date	Original – 16 June 2022

This clinical study protocol was subject to critical review and has been approved by the appropriate protocol review personnel of the Sponsor. The information contained in this protocol is consistent with:

- The current risk-benefit evaluation of the investigational product.
- The ethical and scientific standards governing clinical research that are set out in the current International Council for Harmonisation (ICH) guideline (E6) on Good Clinical Practice (GCP), US Title 21 of the Code of Federal Regulations (CFR) Parts 50, 54, 56, and 312, and other applicable local requirements.

The Principal Investigator will be supplied with details of any significant or new findings, including adverse events, relating to treatment with the investigational product.

Sponsor Signatory:

[redacted] Assembly Biosciences Approval Signature and Date	[redacted]

INVESTIGATOR STATEMENT

Protocol Title	A Phase 1, Blinded, Placebo-controlled Study of the Safety, Tolerability, Pharmacokinetics of Single- and Multiple-Ascending Doses of ABI-4334 in Healthy Subjects
Protocol Number	ABI-4334-101
Protocol Version – Date	Original – 16 June 2022

I have read the protocol, including all appendices, and I agree that it contains all of the necessary information for me and my staff to conduct this study as described. I will conduct this study as described herein, in accordance with Good Clinical Practice (GCP) as set out in the current International Council for Harmonisation (ICH) guidelines (E6) and other applicable national or local requirements, and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and any amendments, and access to all information provided by Assembly Biosciences or specified designees. I will discuss the material with them to ensure that they are fully informed about Assembly Biosciences and the study.

Principal Investigator Signature:	
Print Name:	
Date:	

Please keep the original, signed copy of this Investigator signature page in your records and email a copy to your site monitor for archival in the Trial Master File (TMF).

TABLE OF CONTENTS

TABLE OF CONTENTS 4
ABBREVIATIONS AND TERMS 9
PROTOCOL CHANGE HISTORY 13
1 SYNOPSIS 14
2 INTRODUCTION 23
2.1 Chronic Hepatitis B Virus Infection 23
2.2 ABI-4334 24
2.2.1 Description 24
2.2.2 Nonclinical Studies 24
2.2.3 Clinical Studies 25
2.3 Study Rationale 25
2.3.1 Rationale for Dose Selection 25
2.4 Risk-Benefit Profile 26
3 Study Objectives and Endpoints 28
3.1 Study Objectives 28
3.1.1 Primary Objective 28
3.1.2 Secondary Objectives 28
3.1.3 Exploratory Objective 28
3.2 Study Endpoints 28
3.2.1 Primary Endpoint 28
3.2.2 Secondary Endpoints 28
3.2.3 Exploratory Endpoint 29
4 Study Design 30
4.1 Overview 30
4.1.1 Part A (SAD in Healthy Subjects) 30
4.1.2 Part B (MAD in Healthy Subjects) 32
4.2 Study Treatments 33
4.2.1 Part A (SAD in Healthy Subjects) 33
4.2.2 Part B (MAD in Healthy Subjects: 8-Day Dosing Duration) 33
4.3 Duration of Treatment 34
4.4 Duration of Study 34

4.5 End of Study Definition 34
5 Study Population 35
5.1 Number of Subjects 35
5.2 Inclusion Criteria 35
5.3 Exclusion Criteria 35
5.4 Screen Failures 36
5.5 Dietary and Lifestyle Restrictions 37
5.6 Meals 38
5.6.1 Test Meal for Subjects in the Food Effect Cohorts 38
6 Study Drugs 39
6.1 Investigational Medicinal Product(s) (IMPs) 39
6.1.1 ABI-4334 39
6.1.2 Accountability of IMP 41
6.2 Non-Investigational Medicinal Products(s) 41
6.3 Concomitant Medications and Procedures 41
6.3.1 Prohibited Concomitant Therapy 42
6.4 Randomization, Blinding, and Treatment Codes 42
6.4.1 Randomization 42
6.4.2 Blinding 42
7 DISCONTINUATION OF STUDY TREATMENT OR STUDY 43
7.1 Discontinuation of Study Treatment for A Subject 43
7.1.1 Criteria for Discontinuation of Study Treatment for a Subject 43
7.1.2 Subject Discontinuation or Withdrawal from the Study 43
7.1.3 Subject Lost to Follow-Up 44
7.2 Criteria for Discontinuation of Study Treatment for a Cohort 44
7.3 Discontinuation of the Study 45
8 Study Procedures and Assessments 46
8.1 Schedule of Assessments 46
8.2 Study Visit Procedures 46
8.2.1 Screening Assessments 46
8.2.2 Subject Enrollment and Treatment Assignment 46
8.3 Assessment of Safety 46

8.3.1 Physical Examination 46
8.3.2 Vital Signs 47
8.3.3 Electrocardiograms 47
8.3.4 Holter Data Collection 48
8.3.5 Clinical Laboratory Assessments 48
8.4 Assessment of Pharmacokinetics 49
8.5 Unscheduled Visit Procedures 49
8.6 Premature Study Termination Visit Procedures 49
9 Adverse Events and Toxicity Management 50
9.1 Definition of Adverse Event 50
9.2 Documenting Adverse Events 50
9.3 Assessment of Intensity 51
9.4 Assessment of Causality 51
9.5 Adverse Event Follow-up 51
9.6 Serious Adverse Events 52
9.6.1 Definition of Serious Adverse Event 52
9.6.2 Definition of Terms 52
9.6.3 Reporting Serious Adverse Events 53
9.7 Laboratory Abnormalities and Other Abnormal Assessments as AEs or SAEs 53
9.8 Overdose 53
9.9 Pregnancy 54
9.9.1 Female Subjects Who Become Pregnant 54
9.9.2 Male Subjects With Partners Who Become Pregnant 54
9.10 Toxicity Management 54
9.10.1 Grades 1 and 2 Adverse Event 55
9.10.2 Grade 3 or Grade 4 Adverse Event 55
10 Statistical Considerations 56
10.1 General Considerations 56
10.2 Determination of Sample Size 56
10.3 Analysis Populations 56
10.4 Planned Analyses 56
10.4.1 Disposition of the Study Subjects 57

10.4.2 Demographics 57
10.4.3 Extent of Exposure 57
10.4.4 Analysis of Safety Endpoints 57
10.4.5 Analysis of Pharmacokinetic Endpoints 57
10.5 Interim Analysis 60
10.6 Data Review Committee 60
10.6.1 Overview of Data Review Committee 60
10.6.2 Data Review Committee Procedures 61
11 Responsibilities 62
11.1 Investigator Responsibilities 62
11.1.1 Good Clinical Practice 62
11.1.2 Institutional Review Board (IRB)/Independent Ethics Committee (IEC) 62
11.1.3 Informed Consent 62
11.1.4 Confidentiality 63
11.1.5 Study Files and Retention of Records 63
11.1.6 Audits and Inspections 63
11.1.7 Protocol Compliance 64
11.2 Sponsor Responsibilities 64
11.2.1 Protocol Amendments and Modifications 64
11.2.2 Data Management 64
11.2.3 Study Report and Publications 64
REFERENCES 66
APPENDICES 68
APPENDIX 1a. SCHEDULE OF ASSESSMENTS FOR SCHEDULED VISITS: PART A – SAD (COHORTS A1-A5, COHORT A7 Period 1) IN HEALTHY SUBJECTS 69
APPENDIX 1b. SCHEDULE OF ASSESSMENTS FOR SCHEDULED VISITS: PART A – SAD (COHORT A6, COHORT A7 Period 2) 71
APPENDIX 2. SCHEDULE OF ASSESSMENTS FOR SCHEDULED VISITS: PART B – MAD IN HEALTHY SUBJECTS 73
APPENDIX 3. SCHEDULE OF ASSESSMENTS FOR OTHER VISITS 76
APPENDIX 4. TOXICITY GRADING SCALE FOR ADVERSE EVENTS AND LABORATORY ABNORMALITIES 77

APPENDIX 5. PREGNANCY PRECAUTIONS AND THE DEFINITION OF CHILDBEARING POTENTIAL AND CONTRACEPTIVE REQUIREMENTS..... 93

APPENDIX 6. PROTOCOL AMENDMENT SUMMARY OF CHANGES 95

LIST OF TABLES

Table 1. **Assumed NO(A)EL Doses in Nonclinical Species and Proposed Clinical Dose** 26
Table 2. **Clinical Laboratory Tests**..... 48
Table 3. **Plasma Pharmacokinetic Parameters: Parts A and B; Day 1**..... 58
Table 4. **Plasma Pharmacokinetic Parameters: Part B (MAD) Day 8**..... 58
Table 5. **Urine Pharmacokinetic Parameters: Part B (MAD) Day 8** 59

LIST OF FIGURES

Figure 1. **Overview of Study Design – Parts A & B (SAD and MAD)**..... 30
Figure 2. **Study Design Schematic – Part A (SAD in Healthy Subjects)** 31
Figure 3. **Study Design Schematic – Part B (MAD in Healthy Subjects)**..... 33

ABBREVIATIONS AND TERMS

Abbreviation	Definition
Ab(s)	Antibody(ies)
AE	Adverse event
ALT	Alanine aminotransferase
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
BID	Twice daily
BMI	Body mass index
cccDNA	Covalently closed circular DNA
CFR	Code of Federal Regulations
cHBV	Chronic hepatitis B virus infection
C_{max}	Maximum observed plasma concentration
CRO	Clinical Research Organization
CSR	Clinical Study Report
CV	Coefficient of variation
DAIDS	Division of AIDS
DNA	Deoxyribonucleic acid
DRC	Data Review Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EMA	European Medicines Agency
EOS	End of study
ETV	Entecavir
EU	European Union
FDA	Food and Drug Administration

Abbreviation	Definition
FIH	First-in-human
GCP	Good Clinical Practices
GGT	Gamma-glutamyl transferase
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HBcAb	Antibody to the HBV core protein
HBcrAg	Hepatitis B core-related antigen
HBeAb	Antibody to hepatitis B e antigen
HBeAg	Hepatitis B e antigen
HBsAb	Antibody to hepatitis B surface antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HDPE	High-density polyethylene
HED	Human equivalent dose
HIV	Human immunodeficiency virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation
ICMJE	International Committee of Medical Journal Editors
IEC	Independent Ethics Committee
IFNα	Interferon alpha
IgM	Immunoglobulin M
IMPs	Investigational medicinal product(s)

Abbreviation	Definition
IRB	Institutional Review Board
ISMPP	International Society for Medical Publication Professionals
IUD	Intrauterine device
LDD	Last day of dosing
LDH	Lactate dehydrogenase
LLN	Lower limit of normal
LLOQ	Lower limit of quantification
MCV	Mean corpuscular volume
MRSD	Maximum recommended starting dose
NA	Not applicable
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NrtI	Nucleos(t)ide/reverse transcriptase inhibitor
OTC	Over-the-Counter
PBO	Placebo
pgRNA	Pregenomic ribonucleic acid
PK	Pharmacokinetic(s)
PKAP	Pharmacokinetics Analysis Plan
PO	Orally by mouth
PRN	As needed
QD	Once daily
RBC	Red blood cell
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard deviation

Abbreviation	Definition
SDD	Spray Dried Dispersion
SVR	Sustained virologic response
TBD	To be determined
TEAE	Treatment-emergent adverse event
TFV	Tenofovir
TMF	Trial Master File
ULN	Upper limit of normal
US	United States
WHO	World Health Organization

PROTOCOL CHANGE HISTORY

Not Applicable (Appendix 6).

1 SYNOPSIS

Protocol Title	A Phase 1, Blinded, Placebo-controlled Study of the Safety, Tolerability, Pharmacokinetics of Single- and Multiple-Ascending Doses of ABI-4334 in Healthy Subjects
Protocol Number	ABI-4334-101
Test Product, Dose, and Mode of Administration	ABI-4334 will be provided as 10 mg and 50 mg tablets for oral, by mouth (PO) administration. **Part A (Single-ascending dose [SAD] in Healthy Subjects)** • Cohort A1: 30 mg ABI-4334 or matching placebo (PBO), PO, single dose, fasted • Cohort A2: to be determined (TBD) mg ABI-4334 or PBO, PO, single dose, fasted • Cohort A3: TBD mg ABI-4334 or PBO, PO, single dose, fasted • Cohort A4: TBD mg ABI-4334 or PBO, PO, single dose, fasted • Cohort A5: TBD mg ABI-4334 or PBO, PO, single dose, fasted • Cohort A6: Food effect cohort with subjects from a previous fasted cohort, TBD mg ABI-4334 or PBO, PO, single dose, fed • Cohort A7: Additional food effect cohort (if needed), TBD mg ABI-4334, PO, single dose, 2-period crossover (fasted & fed) **Part B (Multiple-ascending dose [MAD] in Healthy Subjects: 8-Day Dosing Duration)** • Cohort B1: TBD mg ABI-4334 or PBO, PO, multiple doses • Cohort B2: TBD mg ABI-4334 or PBO, PO, multiple doses The dose level for Part A (SAD) Cohort A1 is defined. The dose levels for subsequent cohorts and once daily (QD) or twice daily (BID) dosing schedule for Part B (MAD) will be selected based on evaluation of safety, tolerability, and available pharmacokinetics (PK) from the previous cohorts. In no case would each individual dose escalation be greater than 3.3-fold compared with the previous dose. Some prespecified cohorts may not be enrolled.
Reference Therapy, Dose, and Mode of Administration	Matching placebo tablets for oral administration will be supplied for each ABI-4334 dosage strength. The dosing schedule will follow that of ABI-4334 for the respective cohort.
Target Population	Healthy male or female subjects
Phase	1
Number of Subjects Planned	Part A (SAD): Up to 46 (approximately 8 subjects/Cohorts A1-A6; 6 subjects/Cohort A7 [Note: Cohort A6 will enroll returning subjects from a previous SAD cohort])

	Part B (MAD): Up to 16 (approximately 8 subjects/cohort)
Study Sites and Location	A single site in New Zealand
Treatment Duration	Part A (SAD): 1 day Part B (MAD): 8 days
Study Duration	Part A (SAD): Up to 36 days per cohort, including 28 days of screening, 1 day of treatment, 7 days of follow up. The duration of the study will be longer for subjects in the food effect cohort(s) (see Study Design). Part B (MAD): Up to 43 days, including 28 days of screening, 8 days of treatment, and 7 days of follow up. Note: For each subject, end of study (EOS) is defined as completion of the last visit in the follow-up period or premature study termination, or subject is lost-to-follow-up. The study will end when the last subject completes the last visit of the follow-up period or is considered "lost to follow-up," whichever is later.
Rationale	Chronic hepatitis B virus infection is a major cause of liver-related morbidity and mortality affecting approximately 296 million people worldwide. While therapy with nucleos(t)ide reverse transcriptase inhibitors (NrtI) is able to achieve adequate viral suppression in most HBeAg negative patients and three quarters of HBeAg positive patients after 1-year, sustained virologic response (SVR) and loss of hepatitis B surface antigen (HBsAg) are rare (< 5%). Novel agents, administered alone or in combination with other therapies, are likely required to provide durable off-treatment virologic responses. ABI-4334 is one such agent, which following oral administration, provides potent and selective inhibition of the HBV core protein. Through interference with the essential functions of the HBV core protein, ABI-4334 inhibits HBV replication by orthogonal mechanisms distinct from NrtIs or IFNα. Thus, when used in combination with currently approved or other investigational HBV antivirals or immune modulators, ABI-4334 offers the potential to improve therapy for cHBV and provide patients with enhanced rates of durable off-treatment virologic responses following a finite treatment period. Study of ABI-4334-101 is a first-in-human (FIH) study assessing the safety, tolerability, and pharmacokinetics (PK) of single- (Part A, SAD) and multiple-ascending doses (Part B, MAD) of ABI-4334 in healthy subjects.
Objectives	**Primary Objectives** The primary objective of this study is: • To assess the safety and tolerability of orally administered ABI-4334 in healthy subjects following single (Part A, SAD) and 8-day multiple (Part B, MAD) oral doses **Secondary Objectives** The secondary objectives of this study are:

	• To characterize the PK of ABI-4334 in plasma following single doses and 8-day multiple doses in healthy subjects • To evaluate the effect of food on the PK of ABI-4334 following a single dose in healthy subjects (Part A food-effect cohort(s) only) **Exploratory Objective** The exploratory objective of this study is: • To evaluate the PK of ABI-4334 in urine following 8-day multiple doses in healthy subjects (Part B, MAD)
Endpoints	**Primary Endpoints** The primary endpoint is: • Proportion of subjects with adverse events (AEs), premature treatment and study discontinuation due to AEs, and abnormal laboratory results (for each part) **Secondary Endpoints** The secondary endpoints are: • Noncompartmental plasma PK parameters such as AUC_{last}, $AUC_{0\text{-}inf}$, AUC_{tau}, C_{max}, T_{max}, C_{trough}, k_{el}, $t_{½}$, CL/F, Vz/F, dose normalized AUCs and C_{max}, accumulation ratios and linearity factor (as applicable for each part) • Comparison of AUC and C_{max} between fasted and fed treatments (Part A, SAD, food effect cohort(s) only) **Exploratory Endpoints** The exploratory endpoint is: • Noncompartmental urine PK parameters such as Ae, $Ae_{0\text{-}24}$, CLr, %Fe (Part B, MAD)
Study Design	This is a Phase 1, FIH, randomized, blinded, PBO-controlled, single- and multiple-dose dose escalation study of ABI-4334 in healthy subjects. Part A is designed to assess the safety, tolerability, and PK profile of ABI-4334 following SAD in healthy subjects, as well as the effect of food on ABI-4334. Part B is designed to assess the safety, tolerability, and PK profile of ABI-4334 following MAD in healthy subjects. The following figure provides an overview of the 2-Part study design.

Overview of Study Design - Parts A & B (SAD and MAD)

- Cohort A1: 30 mg ABI-4334 or PBO, orally fasted
- Cohort A2: TBD mg fasted
- Cohort A3: TBD mg fasted
- Cohort A4: TBD mg fasted
- Cohort A5: TBD mg fasted
- Cohort A6: Food effect cohort, TBD mg, fed
- Cohort A7: Food effect cohort, TBD mg (2-period crossover: fasted & fed)

- Cohort B1: TBD mg
- Cohort B2: TBD mg

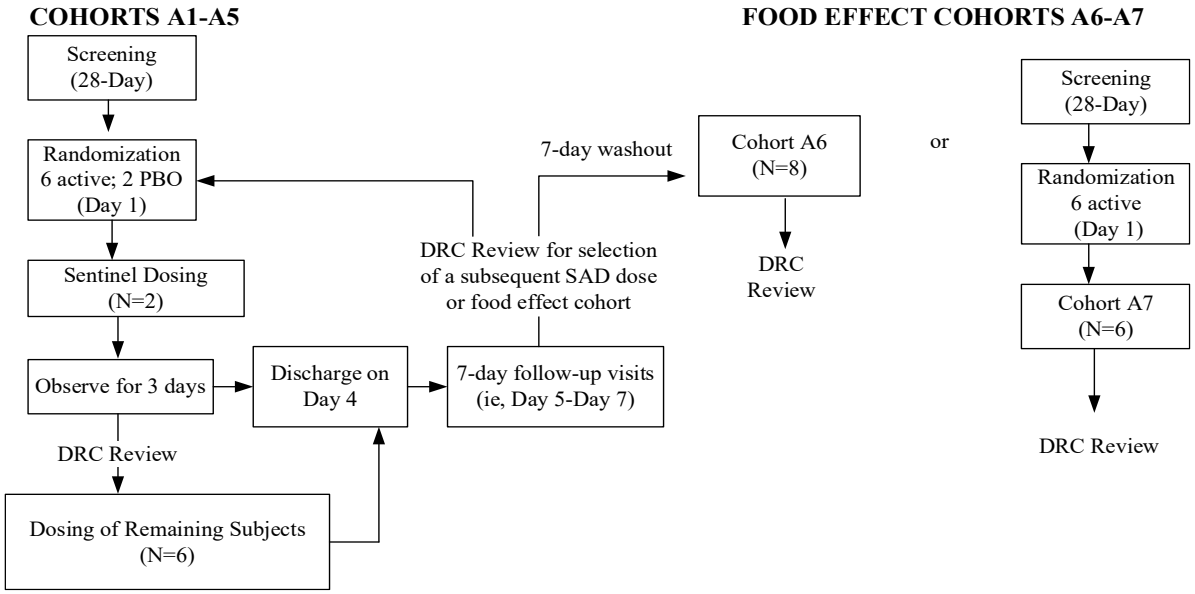
Abbreviations: BID=twice daily; MAD=multiple ascending dose; PBO=placebo; PO=orally by mouth; QD=once daily; SAD=single ascending dose; TBD=to be determined
*Applies to SAD cohorts, except Cohort A7, which will only have 6 active subjects (ie, randomized to ABI-4334), no PBO subjects.
**Part B dosing may commence prior to Part A being completed

Part A (SAD in Healthy Subjects)

Part A of the study will enroll up to 5 fasted cohorts, ie, Cohorts A1-A5, and up to 2 fed cohorts to evaluate food effect, ie, Cohort A6 (with subjects from a previous fasted cohort), and an additional cohort, ie, Cohort A7, if needed (with new subjects).

The first cohort of Part A, ie, Cohort A1, will evaluate a single dose of 30 mg ABI-4334 (see Study Design Schematic for Part A). Dose levels for subsequent cohorts will be determined based on interim cohort review performed by an unblinded Data Review Committee (DRC) between SAD cohorts for purposes of dose escalation. Study data will include safety data from Day 1 through Day 4 for the present cohort and all available safety data for preceding cohorts. In addition, PK data through 24 hours after dosing for the present cohort and available PK data for preceding cohorts will be reviewed. In no case would a dose escalation be greater than 3.3-fold compared with the previous dose. The maximum dose of ABI-4334 to be evaluated in Part A (SAD) will not exceed the exposures observed in dogs at the no observed effect level (NOEL) dose of 30 mg/kg/day, or 1000 mg/day, whichever is lower.

Study Design Schematic – Part A (SAD in Healthy Subjects)



Abbreviations: DRC=Data Review Committee; PBO=placebo; SAD=single ascending dose

Each of the Cohorts A1-A5 will enroll 8 healthy subjects who will be randomized in a 6:2 ratio to receive a single oral dose of the study drug, ie, ABI-4334 (6 subjects) or PBO (2 subjects) in a fasted state, followed by a 7-day follow-up period. Subjects will remain confined to the study clinic from Days -1 to 4, with outpatient follow-up visits, as specified in Appendix 1. Dosing of a subsequent SAD cohort can begin at least 8 days after dosing of the sentinel subjects from the previous cohort (see below).

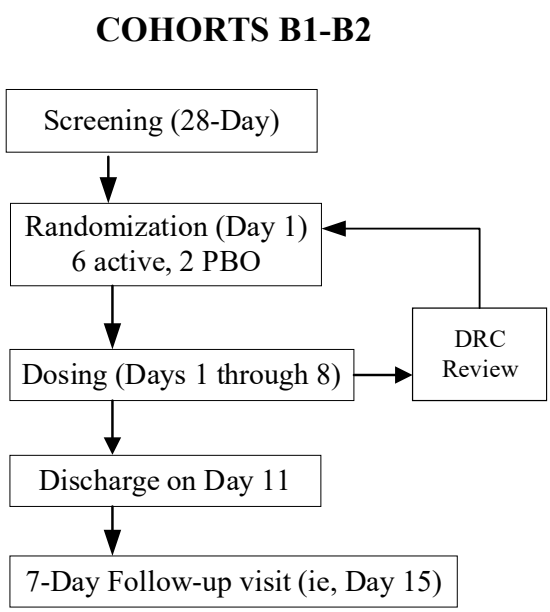
Because this is a FIH study, a sentinel dosing approach will be utilized for each Cohort A1-A5, where initially only 2 subjects per cohort will be dosed (one receiving ABI-4334 and one receiving PBO). These sentinel subjects will be observed for a minimum of 3 days postdose to ensure acceptable tolerability of the study drug, as determined by the DRC, before dosing of the remaining subjects within the cohort can proceed.

The DRC will select a single dose level from Cohorts A1-A5 for evaluation of food effect. The selection of this dose level can be made prior to completion of all fasted SAD cohorts and will be based on a preliminary evaluation by the DRC of the available safety, tolerability, and PK data from Cohorts A1-A5. Subjects who complete participation in this selected cohort will be asked to return to the study site to be enrolled into the food effect cohort (ie, Cohort A6), following a minimum of 7-day washout period since their last study drug dose. While Baseline evaluations will be conducted prior to dosing in Cohort A6, repeat assessment of all study eligibility criteria will not be performed. Returning subjects will retain their original subject number and randomized treatment assignment. After the Day 7 visit in the original cohort (from Cohort A1-A5), the study drug will be administered 30 minutes after the complete consumption of a high fat meal (approximately 50% calories from fat).

If there is an insufficient number of subjects from previous fasted SAD cohorts (ie, A1-A5), who are willing to participate in Cohort A6, then a separate Cohort A7

	may be enrolled, comprising 6 new subjects meeting the study eligibility criteria, who will all receive ABI-4334. Cohort A7 will involve a two-period crossover assessment of food effect (ie, Period 1: dosing under fasted or high fat-fed conditions; Period 2: dosing under condition opposite of Period 1), with a minimum 7-day washout period between dosing in Period 1 and Period 2, and a 7-day postdose follow-up in Period 2. **Part B (MAD in Healthy Subjects)** Part B dosing may commence prior to Part A being completed. The dose level and dosing schedule for the first cohort of Part B, ie, Cohort B1, will be determined based on emergent safety, tolerability, and PK data from completed Part A cohorts, with the daily dose at least 2-fold lower than a Part A dose level deemed safe and tolerable. Both cohorts planned for Part B, ie, Cohort B1 and Cohort B2, will enroll 8 healthy subjects who will be randomized in a 6:2 ratio to receive oral daily doses of the study drug, ie, ABI-4334 (6 subjects) or PBO (2 subjects), for 8 consecutive days, followed by a 7-day follow-up period (see Study Design Schematic for Part B). Dosing may occur under fasted or fed conditions, depending on the results from the food effect cohorts in Part A. Subjects will remain confined to the study site from Day -1 to Day 11 (ie, 3 days post last day of dosing), with outpatient follow-up visits, as specified in Appendix 2. The dose level and dosing schedule for Cohort B2 will be based on an evaluation by the DRC of safety data from Day 1 through Day 8 for Cohort B1 and all available safety data for preceding cohorts. In addition, PK data through 24 hours after dosing on Day 8 for Cohort B1 and available PK data for preceding cohorts will be reviewed. In no case would the dose escalation be greater than 3.3-fold compared with the previous dose. Dosing of Cohort B2 can begin at least 11 days after the first day dosing of subjects in Cohort B1. The maximum dose of ABI-4334 to be evaluated in Part B (MAD) will not exceed the exposures observed in dogs at the NOEL dose of 30 mg/kg/day, or 1000 mg/day, whichever is lower.

Study Design Schematic – Part B (MAD in Healthy Subjects)



Abbreviations: DRC=Data Review Committee; PBO=placebo

Study Population	**Inclusion Criteria** Subjects must meet all the following inclusion criteria in order to be eligible for the study: 1. Is willing and able to provide informed consent prior to Screening. 2. Male or female ≥ 18 and ≤ 65 years of age at Screening. 3. Subject has a body mass index (BMI) between 18.0 and 30.0 kg/m^2 (inclusive) at Screening. 4. Subject is in good health (as determined by the Investigator) based on medical history, physical examination, 12-lead electrocardiogram (ECG), and clinical laboratory results. Medical history and physical examination must be without major or clinically significant findings. 5. Subject has serum chemistry (fasting), hematology, coagulation, and urinalysis values within reference range or has values outside the reference range, which are deemed not clinically significant deviations from the reference range, during the screening period (as determined by the Investigator). 6. Female subjects of childbearing potential (defined in Appendix 5) must be nonpregnant and have a negative serum pregnancy test at Screening and Day -1 and a negative urine pregnancy test on Day 1, obtained prior to dosing. 7. Agreement to comply with protocol-specified contraceptive requirements (Appendix 5).

	8. Male subjects must agree not to donate sperm from Screening through at least 90 days after administration of the last dose of study drug (Appendix 5).
	Exclusion Criteria Subjects who meet any of the following exclusion criteria will not be eligible for the study: 1. Subject has positive results for any of the following serology tests: HBsAg, hepatitis B core antibody (HBcAb IgM), hepatitis C virus antibody (HCV Ab), or HIV-1 or -2 antibody. 2. Females who are pregnant, lactating, or wish to become pregnant during the course of the study. 3. Subject has a history of any illness that, in the opinion of the Investigator, might confound the results of the study, pose an additional risk in administering study drug to the subject, or a condition known to interfere with the absorption/ distribution/elimination of drugs. This may include, but is not limited to, a history of relevant food allergies, history of cardiovascular or central nervous system disease, history or presence of clinically significant pathology, or history of mental disease. 4. Subject has a history of malignancy in the last 5 years, with the exception of nonmetastatic basal cell or squamous cell carcinoma of the skin or localized carcinoma of the cervix. 5. Subject has had an illness within 5 days before receiving the first dose of study drug ("illness" is defined as a recent nonserious, nonacute condition such as the flu or the common cold). 6. Subject has hypersensitivity or history of idiosyncratic reaction to any components or excipients of the study drug (ABI-4334 or PBO). 7. Subject has a history of any significant (as determined by the Investigator) drug-related allergic reactions such as anaphylaxis, Stevens-Johnson syndrome, urticaria, or multiple drug allergies. 8. Subject has a history of persistent alcohol abuse (alcohol consumption exceeding 2 standard drinks per day on average [1 standard drink=14 grams of alcohol]) or illicit substance/drug use within 3 years prior to Screening. 9. Unwillingness to abstain from the use of marijuana from Screening through end of study. 10. Unwillingness to abstain from alcoholic beverage consumption 48 hours prior to Day 1 dosing through end of study. 11. Unwillingness to abstain from consumption of grapefruit, pomelo, or Seville oranges whole fruits or juices for 7 days prior to Day 1 dosing through end of study. 12. Subject has a smoking habit, uses, or has used tobacco or nicotine-containing products (eg, including but not limited to cigarettes, e-cigarettes, pipes, cigars, chewing tobacco, nicotine patches, nicotine lozenges, or nicotine gum) within 3

	months before Day 1 dosing AND is unwilling to abstain from tobacco or nicotine-containing products through end of study. 13. Unwillingness to abstain from caffeine in any form, including tea, cola, and caffeinated beverages consumption 48 hours prior to Day 1 dosing through end of study. 14. Subject must be willing to stop herbal/dietary supplements (eg, vitamins, St. John's Wort, ginkgo biloba, garlic supplements), over-the-counter (OTC) medications (except for paracetamol), or any prescription medication beginning 14 days prior to the Day 1 dosing or 5 half-lives, whichever is longer, and through end of study. 15. Subject has participated in a clinical study involving administration of either an investigational or a marketed drug within 2 months or 5 half-lives (whichever is longer) before Screening. 16. Subject has donated or lost more than 1 unit of blood (500 mL) within 60 days prior to Screening, or plasma donation within 7 days prior to Screening, or plans to donate blood or plasma during the study.
Statistical Considerations	This is a FIH study. The sample size is similar to that previously utilized for this type of study and is not based upon statistical considerations. All safety and PK endpoints will be summarized using descriptive statistics by treatment. Continuous endpoints will be summarized using the mean, standard deviation, median, minimum, and maximum. Categorical endpoints will be described using the number and percent of subjects who meet the endpoint criterion. Due to sample size limitations, no formal statistical inference is planned.

2 INTRODUCTION

2.1 Chronic Hepatitis B Virus Infection

Worldwide approximately 296 million people are chronically infected with the hepatitis B virus (HBV), and chronic hepatitis B virus infection (cHBV) is a major cause of severe liver morbidity and liver-related mortality (WHO 2021). An estimated 600,000 to 1 million people die each year due to cirrhosis and hepatocellular carcinoma (HCC), the end-stage complications of cHBV (Colvin 2010, El-Serag 2012, Lampertico 2017, Terrault 2018, WHO 2019). The global prevalence of cHBV shows wide geographic variation, with a prevalence of more than 8% of people in highly endemic regions (eg, East Asia and equatorial Africa), 2% to 7% of people in moderately endemic regions (eg, the Middle East and the Indian subcontinent), and less than 2% in locales of low endemicity (eg, North America and Europe) (Schweitzer 2015). Despite broad implementation of HBV vaccination programs, new cases of HBV infection are still common. The World Health Organization (WHO) estimates that there are greater than 4 million acute HBV infections worldwide each year (WHO 2021).

The clinical stages of cHBV represent different risks for ongoing liver injury depending on the degree of HBV replication and individuals' concurrent immune responses (Yim 2006, Hoofnagle 2007, Lok 2009, Sorrell 2009, Pungpapong 2013, Gish 2015, Lampertico 2017). The standard virologic and serologic markers for HBV infection include HBV deoxyribonucleic acid (DNA), hepatitis B surface antigen (HBsAg), antibody to the hepatitis B surface antigen (HBsAb), hepatitis B e antigen (HBeAg), antibody to the hepatitis B e antigen (HBeAb), and in almost all patients, antibody to the HBV core protein (HBcAb). More recently, HBV pregenomic ribonucleic acid (pgRNA) and HBV core-related antigen (HBcrAg) have also been used as markers of infection. In particular, HBV pgRNA is recognized as a direct measure of HBV covalently closed circular DNA (cccDNA) transcriptional activity, and the number of infected cells (Fanning 2019). Historically, treatment goals include prevention of HBV-related liver injury through suppression of HBV DNA, achievement of HBeAg loss and seroconversion, and loss of HBsAg and seroconversion. A transition to an HBsAg negative, minimally replicative state is rare (occurring spontaneously in only 1-3% of patients per year, and < 5% per year in NrtI-treated patients) but usually durable and, if it precedes the development of cirrhosis and HCC, is associated with improved long-term clinical outcomes (Lampertico 2017). As such, HBsAg seroconversion is considered a "functional cure" and a potential endpoint for cHBV therapy. However, as HBsAg is derived from both HBV cccDNA as well as integrated HBV DNA, sustained undetectable HBV DNA with or without HBsAg loss after stopping treatment ("off-therapy sustained virologic response [SVR]") is considered an intermediate goal (Lampertico 2017, Cornberg 2019).

Currently, there are 2 clinically accepted, first-line treatment options for cHBV: nucleos(t)ide reverse transcriptase inhibitors (NrtIs) of the HBV polymerase and interferon alpha (IFNα). The NrtIs are orally administered, potent, direct-acting antivirals targeting the reverse transcription of HBV pgRNA to HBV DNA. They are broadly used, generally safe and well-tolerated and have demonstrated success in achieving and maintaining viral suppression in most patients (Lampertico 2012). However, despite suppression of HBV DNA for extended periods of time with NrtIs,

HBeAg and HBsAg loss is uncommon and the template for ongoing viral replication, HBV cccDNA, is not eliminated in most patients. As a result, off therapy SVR with NrtIs are rare, necessitating long-term chronic suppressive treatment. In contrast, IFNα is an approved, subcutaneously administered protein with a finite treatment duration of 48 weeks. While the specific mechanism of action is not fully understood, it is thought that IFNα directly and indirectly targets HBV by inhibiting multiple steps in the viral lifecycle resulting in modest antiviral activity (Konerman 2016). In addition, IFNα augments innate immunity and triggers HBV-specific T cell responses. Pegylated (Peg)-IFNα is the preferred formulation due to increased pharmacokinetic (PK) and pharmacodynamic half-life supporting weekly dosing. Compared with NrtIs, IFNα is poorly tolerated due to common side effects, including flu-like symptoms, mood disturbances, and cytopenias. After 48 weeks of treatment, although IFNα may provide higher rates of durable HBV DNA suppression compared to NrtIs, rates of HBeAg and HBsAg loss remain low (Marcellin 2016). Consequently, despite the incremental improvements in virologic response of Peg-IFNα monotherapy, this regimen remains suboptimal and is offset by its safety and tolerability profile.

There is a need for improved, novel HBV therapies that further reduce HBV replication and result in a higher proportion of patients achieving SVR (HBV DNA < LLOQ with or without HBsAg loss) with a subsequent improvement in long-term patient outcomes, ie, reduction of HBV associated hepatic inflammation leading to reduced morbidity and mortality from end-stage liver disease and HCC. Additionally, such deeper virologic responses may enable patients to achieve durable virologic and clinical outcomes following finite treatment duration.

2.2 ABI-4334

2.2.1 Description

ABI-4334 is an orally administered, potent and selective, next-generation, small molecule inhibitor of HBV core protein. ABI-4334 interferes with essential functions of the HBV core protein, and therefore inhibits HBV replication by different mechanisms than NrtIs or IFNα. ABI-4334 binds to the HBV core protein, and induces altered, non-functional core protein assembly in preclinical models. Thus, inhibition of HBV core protein functions by ABI-4334, when used in combination with currently approved HBV antivirals, offers the potential to improve therapy for cHBV and provide patients with enhanced rates of durable off-treatment virologic responses following a finite treatment period.

Refer to the Investigator's Brochure (IB) for detailed information on ABI-4334.

2.2.2 Nonclinical Studies

2.2.2.1 In-vitro Activity

In primary human hepatocytes, ABI-4334 exhibited potent antiviral activity against HBV replication (0.5 nM). Furthermore, ABI-4334 potently inhibited the formation of HBV cccDNA (2.4 nM), which is distinct from the mechanisms of action of NrtIs. ABI-4334 is pan-genotypic, with activity across HBV genotypes A-J. No cytotoxicity was observed with ABI-4334 across

multiple cell types and cell lines and the activity is specific to HBV. ABI-4334 remains fully active against a panel of NrtI-resistant viruses. A panel of 16 core inhibitor binding pocket variants were evaluated for sensitivity to ABI-4334; 1 of 16 variants exhibited > 10-fold reduced susceptibility to ABI-4334. Combination studies with ABI-4334 and NrtI (ie, tenofovir [TFV] or entecavir [ETV]) demonstrated additive effects.

Refer to the IB for results from other nonclinical studies, including pharmacology, pharmacokinetics, and toxicology.

2.2.3 Clinical Studies

No clinical studies have been conducted with ABI-4334.

2.3 Study Rationale

Chronic hepatitis B virus infection is a major cause of liver-related morbidity and mortality affecting approximately 296 million people worldwide. While therapy with NrtI is able to achieve adequate viral suppression in most HBeAg negative patients and three quarters of HBeAg positive patients after 1 year, SVR and loss of HBsAg are rare (< 5%). Novel agents, administered alone or in combination with other therapies, are likely required to provide durable off-treatment virologic responses.

ABI-4334 is one such agent, which following oral administration, provides potent and selective inhibition of the HBV core protein. Through interference with the essential functions of the HBV core protein, ABI-4334 inhibits HBV replication by orthogonal mechanisms distinct from NrtIs or IFNα. Thus, when used in combination with currently approved or other investigational HBV antivirals or immune modulators, ABI-4334 offers the potential to improve therapy for cHBV and provide patients with enhanced rates of durable off-treatment virologic responses following a finite treatment period.

Study of ABI-4334-101 is a first-in-human (FIH) study assessing the safety, tolerability, and pharmacokinetics (PK) of single- (Part A; SAD) and multiple-ascending doses (Part B; MAD) of ABI-4334 in healthy subjects.

2.3.1 Rationale for Dose Selection

The starting dose for the proposed Phase 1 FIH clinical study has been determined based on the United States (US) Food and Drug Administration (FDA) guideline on estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers (FDA 2005) and the European Medicines Agency's (EMA) guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products (EMA 2018).

The conclusions from the 28-day repeat-dose Good Laboratory Practices (GLP) toxicology studies were that the no observed adverse effect level (NOAEL) in rats was 300 mg/kg/day, and the no observed effect level (NOEL) in dogs was 30 mg/kg/day (the highest doses tested). Defining the "most appropriate species" as the one that produces the lowest HED, the dog NOEL of

30 mg/kg/day and corresponding HED of 16.7 mg/kg, or approximately 1000 mg for a 60 kg human subject, have been used to calculate the starting dose for the proposed FIH clinical study. Using the standard safety factor of 10 discussed in the FDA guideline (FDA 2005) results in a maximum recommended starting dose of 100 mg. To provide an additional margin of safety, a starting dose of 30 mg is proposed for the initial cohort of healthy subjects in Part A of this study. Thus, the actual safety factor for the proposed starting dose in this study is approximately 33. The ratio of the HED to the proposed starting dose is shown in Table 1.

Table 1. Assumed NO(A)EL Doses in Nonclinical Species and Proposed Clinical Dose

Species	NO(A)EL (mg/kg/day)	ABI-4334 PK Parameter		HED[b] (mg/kg)	HED[c] (mg)	MRSD[d] (mg)	Proposed Starting Dose	Safety Factor for Proposed Starting Human Dose[f]
		C_{max}[a] (ng/mL)	AUC_{0-24}[a] (ng*hour/mL)					
Rat	300	36,100	424,000	48.4	2900	290	NA	NA
Dog	30	15,900	183,500	16.7	1000	100	30[e]	33

Abbreviations: HED = human equivalent dose; MRSD = maximum recommended starting dose; NA = not applicable; NO(A)EL = no observed (adverse) effect level; PK = pharmacokinetics

[a] Day 28 parameters, averaged for males and females

[b] Determined according to the FDA guidance, Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Subjects; conversion factor of 1.8 for dog and 6.2 for the rat.

[c] Assumes 60 kg human.

[d] Using the standard safety factor of 10.

[e] The dog was considered the most appropriate species for calculating the starting dose in this FIH study.

[f] Ratio of the HED to the proposed starting dose for this FIH study.

The maximum dose in the study will not exceed the exposures in dogs at the NOEL dose of 30 mg/kg/day (maximum observed plasma concentration [C_{max}] of 15900 ng/mL or area under the concentration-time curve [AUC] of 183,500 ng*hour/mL). Although the maximum doses correspond to approximately 1800 mg/day based on C_{max} and 2400 mg/day based on AUC using current human predictions, doses higher than 1000 mg/day will not be administered in this study. Therefore, the maximum dose of ABI-4334 to be evaluated in this study will not exceed the exposures observed in dogs at the NOEL dose of 30 mg/kg/day, or 1000 mg/day, whichever is lower.

2.4 Risk-Benefit Profile

ABI-4334 is being developed for the treatment of cHBV. Since this is a FIH study of ABI-4334, the therapeutic potential of ABI-4334 is yet to be determined. Nonclinical data generated with ABI-4334 as well as clinical data from other core inhibitors in development suggest that, as compared to monotherapy with NrtIs, there may be several potential benefits of ABI-4334 either alone or in combination with NrtIs for the treatment of cHBV. These include:

- Greater on-treatment antiviral activity in combination (more rapid suppression of HBV replication) compared to current NrtI alone
- Greater inhibition of HBV cccDNA establishment and thus greater loss of HBV cccDNA over time as compared to current NrtI alone
- Potential for finite therapy as compared to NrtIs which are often lifelong therapies

In the 28-day GLP-compliant oral repeat-dose toxicity studies, ABI-4334 was well-tolerated at doses up to 300 mg/kg/day in rats, and in dogs up to 30 mg/kg/day, the highest doses tested. In rats, there were no drug-related clinical signs or changes in body weights, food consumption, nor changes in clinical pathology data. Non-adverse ABI-4334-related findings were limited to the adrenal gland. In dogs, there were no ABI-4334-related findings at any dose tested. There were no drug-related clinical signs or changes in body weights, food consumption, nor changes in clinical, gross, or histopathology data. Therefore, following daily oral doses for 28 days, the NO(A)ELs for the rat and the dog are 300 mg/kg/day (AUC $\leq$ 455,000 ng*hour/mL) and 30 mg/kg/day (AUC $\leq$ 185,000 ng*hour/mL), respectively (the highest doses tested).

During the conduct of this study to help ensure subject safety, a data review committee (DRC) and the enrolling study Investigator will perform ongoing safety data reviews of the frequent clinical and laboratory assessments, as defined in the DRC charter.

No therapeutic benefit is anticipated for the healthy subjects participating in this study. Any potential benefit is anticipated to be limited to the no-cost clinical and laboratory evaluations associated with the study, and the possibility that ABI-4334 could potentially benefit patients with cHBV in the future if additional studies establish ABI-4334 as a therapeutic advance for patients with cHBV.

Overall, the risk-benefit profile of ABI-4334 is favorable.

3 STUDY OBJECTIVES AND ENDPOINTS

3.1 Study Objectives

3.1.1 Primary Objective

The primary objective of this study is:

- To assess the safety and tolerability of orally administered ABI-4334 in healthy subjects following single (Part A, SAD) and 8-day multiple (Part B, MAD) oral doses

3.1.2 Secondary Objectives

The secondary objectives of this study are:

- To characterize the PK of ABI-4334 in plasma following single doses and 8-day multiple doses in healthy subjects
- To evaluate the effect of food on the PK of ABI-4334 following a single dose in healthy subjects (Part A food-effect cohort(s) only)

3.1.3 Exploratory Objective

The exploratory objective of this study is:

- To evaluate the PK of ABI-4334 in urine following 8-day multiple doses in healthy subjects (Part B, MAD)

3.2 Study Endpoints

3.2.1 Primary Endpoint

The primary endpoint is:

- Proportion of subjects with adverse events (AEs), premature treatment and study discontinuation due to AEs, and abnormal laboratory results (for each part)

3.2.2 Secondary Endpoints

The secondary endpoints are:

- Noncompartmental plasma PK parameters such as AUC_{last}, $AUC_{0\text{-}inf}$, AUC_{tau}, C_{max}, T_{max}, C_{trough}, k_{el}, $t_{½}$, CL/F, Vz/F, dose normalized AUCs and C_{max}, accumulation ratios and linearity factor (as applicable for each part)
- Comparison of AUC and C_{max} between fasted and fed treatments (Part A, SAD, food effect cohorts only)

3.2.3 Exploratory Endpoint

The exploratory endpoint is:

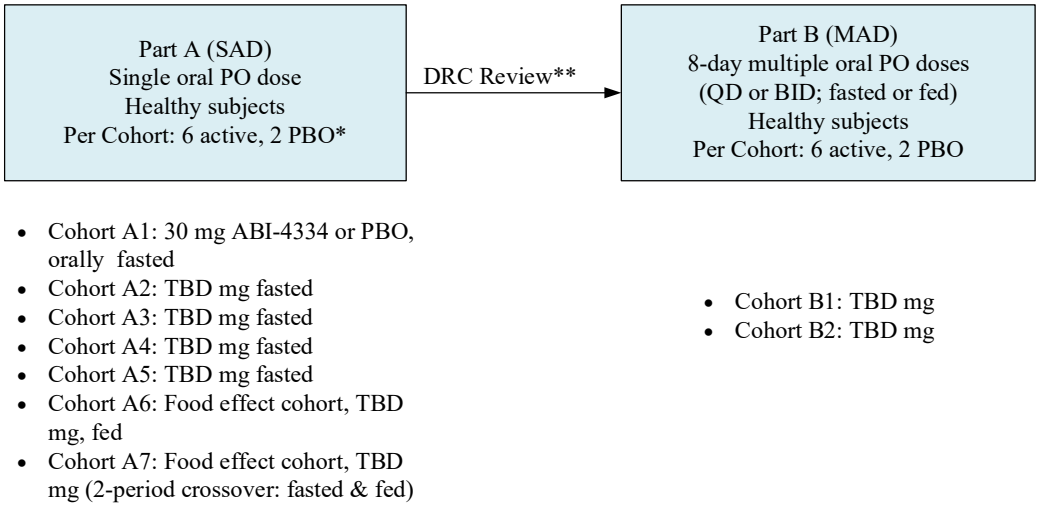
- Noncompartmental urine PK parameters such as Ae, Ae_{0-24}, CLr, %Fe (Part B, MAD)

4 STUDY DESIGN

4.1 Overview

This is a Phase 1, FIH, randomized, blinded, placebo (PBO)-controlled, single- and multiple-dose, dose escalation study of ABI-4334 in healthy subjects. Part A is designed to assess the safety, tolerability, and PK profile of ABI-4334 following SAD in healthy subjects, as well as the effect of food on ABI-4334. Part B is designed to assess the safety, tolerability, and PK profile of ABI-4334 following MAD in healthy subjects. Figure 1 provides an overview of the 2-Part study design.

Figure 1. Overview of Study Design – Parts A & B (SAD and MAD)



Abbreviations: BID=twice daily; MAD=multiple ascending dose; PBO=placebo; PO=orally by mouth; QD=once daily; SAD=single ascending dose; TBD=to be determined
*Applies to all SAD cohorts, except Cohort A7, which will only have 6 active subjects (ie, randomized to ABI-4334), no PBO subjects.
**Part B dosing may commence prior to Part A being completed.

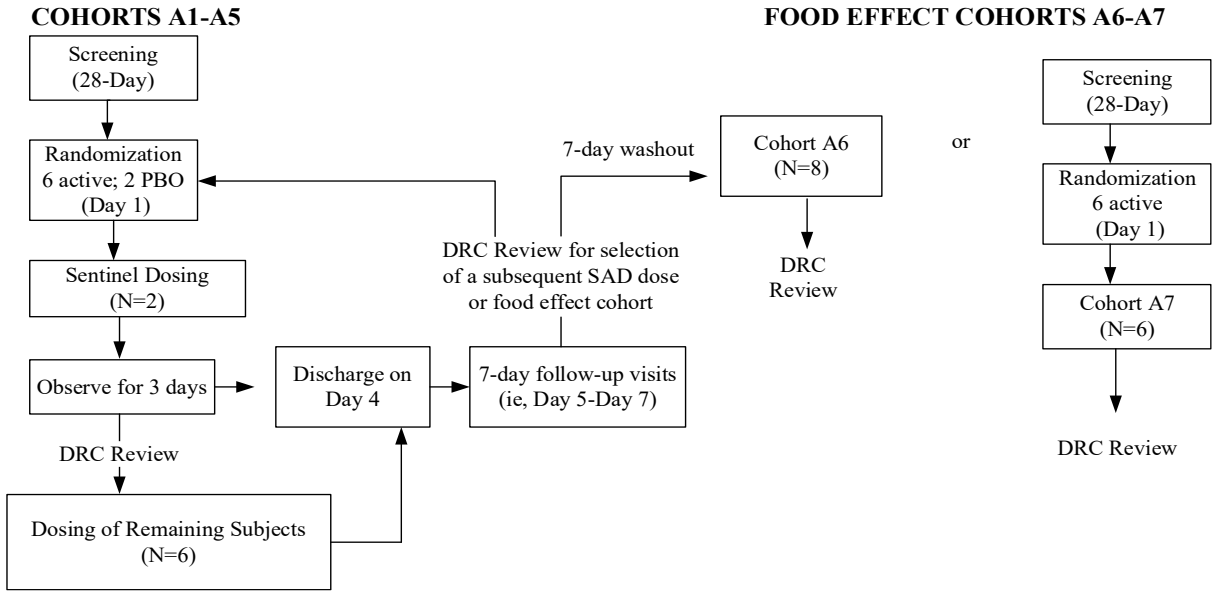
4.1.1 Part A (SAD in Healthy Subjects)

Part A of the study will enroll up to 5 fasted cohorts, ie, Cohorts A1-A5, and up to 2 fed cohorts to evaluate food effect, ie, Cohort A6 (with subjects from a previous fasted cohort), and an additional cohort, ie, Cohort A7, if needed (with new subjects).

The first cohort of Part A, ie, Cohort A1, will evaluate a single dose of 30 mg ABI-4334 (Figure 2). Dose levels for subsequent cohorts will be determined based on interim cohort review performed by an unblinded DRC (see Section 10.6 for details on DRC procedures) between SAD cohorts for purposes of dose escalation. Study data will include safety data from Day 1 through Day 4 for the present cohort and all available safety data for preceding cohorts. In addition, PK

data through 24 hours after dosing for the present cohort and available PK data for preceding cohorts will be reviewed. In no case would a dose escalation be greater than 3.3-fold compared with the previous dose. The maximum dose of ABI-4334 to be evaluated in Part A (SAD) will not exceed the exposures observed in dogs at the NOEL dose of 30 mg/kg/day, or 1000 mg/day, whichever is lower.

Figure 2. Study Design Schematic – Part A (SAD in Healthy Subjects)



Abbreviations: DRC=Data Review Committee; PBO=placebo; SAD=single ascending dose

Each of the Cohorts A1-A5 will enroll 8 healthy subjects who will be randomized in a 6:2 ratio to receive a single oral dose of the study drug, ie, ABI-4334 (6 subjects) or PBO (2 subjects) in a fasted state, followed by a 7-day follow-up period. Subjects will remain confined to the study clinic from Days -1 to 4, with outpatient follow-up visits, as specified in Appendix 1. Dosing of a subsequent SAD cohort can begin at least 8 days after dosing of the sentinel subjects from the previous cohort (see below).

Because this is a FIH study, a sentinel dosing approach will be utilized for each Cohort A1-A5, where initially only 2 subjects per cohort will be dosed (one receiving ABI-4334 and one receiving PBO). These sentinel subjects will be observed for a minimum of 3 days postdose to ensure acceptable tolerability of the study drug, as determined by the DRC, before dosing of the remaining subjects within the cohort can proceed.

The DRC will select a single dose level from Cohorts A1-A5 for evaluation of food effect. The selection of this dose level can be made prior to completion of all fasted SAD cohorts and will be based on a preliminary evaluation by the DRC of the available safety, tolerability, and PK data from selected Cohorts A1-A5. Subjects who complete participation in this selected cohort will be asked to return to the study site to be enrolled into the food-effect cohort (ie, Cohort A6), following a minimum of 7-day washout period since their last study drug dose. While Baseline evaluations will be conducted prior to dosing in Cohort A6, repeat assessment of all study eligibility criteria will not be performed. Returning subjects will retain their original subject number and randomized treatment assignment. After the Day 7 visit in the original cohort (from Cohort A1-A5), the study

drug will be administered 30 minutes after the complete consumption of a high fat meal (approximately 50% calories from fat).

If there is an insufficient number of subjects from previous fasted SAD cohorts (ie, A1-A5), who are willing to participate in Cohort A6, then a separate Cohort A7 may be enrolled, comprising 6 new subjects meeting the study eligibility criteria, who will all receive ABI-4334. Cohort A7 will involve a two-period crossover assessment of food effect (ie, Period 1: dosing under fasted or high fat-fed conditions; Period 2: dosing under condition opposite of Period 1), with a minimum 7-day washout period between dosing in Period 1 and Period 2, and a 7-day postdose follow-up in Period 2.

Details on PK sampling for Part A (SAD) are provided in Section 8.4 and Appendix 1.

4.1.2 Part B (MAD in Healthy Subjects)

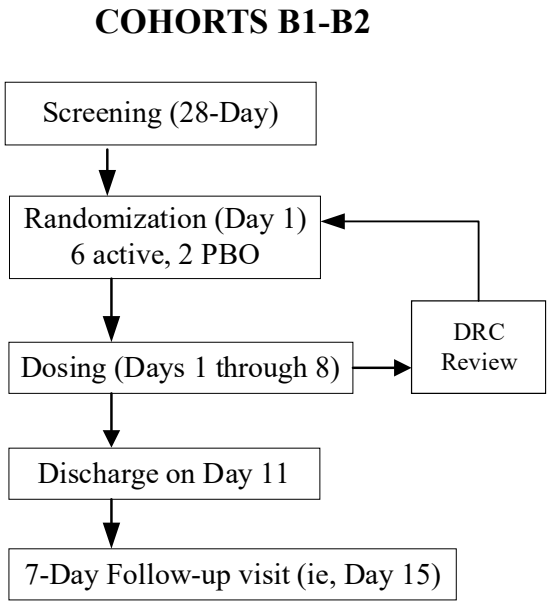
Part B dosing may commence prior to Part A being completed. The dose level and dosing schedule for the first cohort of Part B, ie, Cohort B1, will be determined based on emergent safety, tolerability, and PK data from completed Part A cohorts, with the daily dose at least 2-fold lower than a Part A dose level deemed safe and tolerable.

Both cohorts planned for Part B, ie, Cohort B1 and Cohort B2, will enroll 8 healthy subjects who will be randomized in a 6:2 ratio to receive oral daily doses of the study drug, ie, ABI-4334 (6 subjects) or PBO (2 subjects), for 8 consecutive days, followed by a 7-day follow-up period (Figure 3). Dosing may occur under fasted or fed conditions, depending on the results from the food-effect cohorts in Part A. Subjects will remain confined to the study site from Days -1 to Day 11 (ie, 3 days post last day of dosing), with outpatient follow-up visits, as specified in Appendix 2.

The dose level and dosing schedule for Cohort B2 will be based on an evaluation by the DRC of safety data from Day 1 through Day 8 for Cohort B1 and all available safety data for preceding cohorts. In addition, PK data through 24 hours after dosing on Day 8 for Cohort B1 and available PK data for preceding cohorts will be reviewed. In no case would the dose escalation be greater than 3.3-fold compared with the previous dose. Dosing of Cohort B2 can begin at least 11 days after the first day dosing of subjects in Cohort B1. The maximum dose of ABI-4334 to be evaluated in Part B (MAD) will not exceed the exposures observed in dogs at the NOEL dose of 30 mg/kg/day, or 1000 mg/day, whichever is lower.

Details on PK sampling for Part B (MAD) are provided in Section 8.4 and Appendix 2.

Figure 3. Study Design Schematic – Part B (MAD in Healthy Subjects)



Abbreviations: DRC=Data Review Committee; PBO=placebo

4.2 Study Treatments

As previously described, the starting dose of 30 mg for SAD Cohort A1 is based on nonclinical safety findings and regulatory guidelines for selection of starting doses for FIH studies (see Section 2.3.1). For all subsequent parts of the study described below, the decision to enroll a cohort and its dose level and once daily (QD) or twice daily (BID) dosing schedule will be based on the assessment of available data, and some prespecified cohorts may not be enrolled.

4.2.1 Part A (SAD in Healthy Subjects)

- Cohort A1: 30 mg ABI-4334 or PBO, orally (PO), single dose, fasted
- Cohort A2: to be determined (TBD) mg ABI-4334 or PBO, PO, single dose, fasted
- Cohort A3: TBD mg ABI-4334 or PBO, PO, single dose, fasted
- Cohort A4: TBD mg ABI-4334 or PBO, PO, single dose, fasted
- Cohort A5: TBD mg ABI-4334 or PBO, PO, single dose, fasted
- Cohort A6: Food effect cohort with subjects from a previous fasted cohort, TBD mg ABI-4334 or PBO, PO, single dose, fed
- Cohort A7: Additional food effect cohort (if needed), TBD mg ABI-4334, PO, single dose, 2-period crossover (fasted & fed)

4.2.2 Part B (MAD in Healthy Subjects: 8-Day Dosing Duration)

- Cohort B1: TBD mg ABI-4334 or PBO, PO, multiple doses

- Cohort B2: TBD mg ABI-4334 or PBO, PO, multiple doses

For detailed information regarding dosing and administration, refer to Section 6.1.1.4.

4.3 Duration of Treatment

The duration of treatment for each part of the study is provided below:

- Part A (SAD): 1 day
- Part B (MAD): 8 days

4.4 Duration of Study

- Part A (SAD): Up to 36 days per cohort, including 28 days of screening, 1 day of treatment, 7 days of follow up. The duration of the study will be longer for subjects in the food effect cohort(s).
- Part B (MAD): Up to 43 days, including 28 days of screening, 8 days of treatment, and 7 days of follow up.

4.5 End of Study Definition

For each subject, EOS is defined as completion of the last visit in the follow-up period or premature study termination, or subject is lost-to-follow-up.

The study will end when the last subject completes the last visit of the follow-up period or is considered “lost to follow-up,” whichever is later.

5 STUDY POPULATION

5.1 Number of Subjects

- Part A: Up to 46 (approximately 8 subjects/Cohorts A1-A6; 6 subjects/Cohort A7 [Note: Cohort A6 will enroll returning subjects from a previous SAD cohort])
- Part B: Up to 16 (approximately 8 subjects/cohort)

5.2 Inclusion Criteria

Subjects must meet all of the following inclusion criteria in order to be eligible for the study:

1. Is willing and able to provide informed consent prior to Screening.
2. Male or female ≥ 18 and ≤ 65 years of age at Screening.
3. Subject has a body mass index (BMI) between 18.0 and 30.0 kg/m^2 (inclusive) at Screening.
4. Subject is in good health (as determined by the Investigator) based on medical history, physical examination, 12-lead electrocardiogram (ECG), and clinical laboratory results. Medical history and physical examination must be without major or clinically significant findings.
5. Subject has serum chemistry (fasting), hematology, coagulation, and urinalysis values within reference range or has values outside the reference range, which are deemed not clinically significant deviations from the reference range, during the screening period (as determined by the Investigator).
6. Female subjects of childbearing potential (defined in Appendix 5) must be nonpregnant and have a negative serum pregnancy test at Screening and Day-1 and a negative urine pregnancy test on Day 1, obtained prior to dosing.
7. Agreement to comply with protocol-specified contraceptive requirements (Appendix 5).
8. Male subjects must agree not to donate sperm from Screening through at least 90 days after administration of the last dose of study drug (Appendix 5).

5.3 Exclusion Criteria

Subjects who meet any of the following exclusion criteria will not be eligible for the study:

1. Subject has positive results for any of the following serology tests: HBsAg, hepatitis B core antibody (HBcAb IgM), hepatitis C virus antibody (HCV Ab), or HIV-1 or -2 antibody.
2. Females who are pregnant, lactating, or wish to become pregnant during the course of the study.
3. Subject has a history of any illness that, in the opinion of the Investigator, might confound the results of the study, pose an additional risk in administering study drug to the subject, or a condition known to interfere with the absorption/distribution/elimination of drugs. This may include, but is not limited to, a history of relevant food allergies, history of cardiovascular or

central nervous system disease, history or presence of clinically significant pathology, or history of mental disease.

4. Subject has a history of malignancy in the last 5 years, with the exception of nonmetastatic basal cell or squamous cell carcinoma of the skin or localized carcinoma of the cervix.
5. Subject has had an illness within 5 days before receiving the first dose of study drug ("illness" is defined as a recent nonserious, nonacute condition such as the flu or the common cold).
6. Subject has hypersensitivity or history of idiosyncratic reaction to any components or excipients of the study drug (ABI-4334 or PBO).
7. Subject has a history of any significant (as determined by the Investigator) drug-related allergic reactions such as anaphylaxis, Stevens-Johnson syndrome, urticaria, or multiple drug allergies.
8. Subject has a history of persistent alcohol abuse (alcohol consumption exceeding 2 standard drinks per day on average [1 standard drink=14 grams of alcohol]) or illicit substance/drug use within 3 years prior to Screening.
9. Unwillingness to abstain from the use of marijuana from Screening through end of study.
10. Unwillingness to abstain from alcoholic beverage consumption 48 hours prior to Day 1 dosing through end of study.
11. Unwillingness to abstain from consumption of grapefruit, pomelo, or Seville oranges whole fruits or juices for 7 days prior to Day 1 dosing through end of study.
12. Subject has a smoking habit, uses, or has used tobacco or nicotine-containing products (eg, including but not limited to cigarettes, e-cigarettes, pipes, cigars, chewing tobacco, nicotine patches, nicotine lozenges, or nicotine gum) within 3 months before Day 1 dosing AND is unwilling to abstain from tobacco or nicotine-containing products through end of study.
13. Unwillingness to abstain from caffeine in any form, including tea, cola, and caffeinated beverages consumption 48 hours prior to Day 1 dosing through end of study.
14. Subject must be willing to stop herbal/dietary supplements (eg, vitamins, St. John's Wort, ginkgo biloba, garlic supplements), over-the-counter (OTC) medications (except for paracetamol), or any prescription medication beginning 14 days prior to the Day 1 dosing or 5 half-lives, whichever is longer, and through end of study.
15. Subject has participated in a clinical study involving administration of either an investigational or a marketed drug within 2 months or 5 half-lives (whichever is longer) before Screening.
16. Subject has donated or lost more than 1 unit of blood (500 mL) within 60 days prior to Screening, or plasma donation within 7 days prior to Screening, or plans to donate blood or plasma during the study.

5.4 Screen Failures

Screen failures are defined as subjects who sign the Informed Consent Form (ICF), are assigned a subject number, complete screening procedures but are not subsequently randomized to study

treatment. Minimal information to be retained on all screen failures includes demography, screen failure details, eligibility criteria, and any SAE information.

Individuals who do not meet the protocol eligibility criteria for participation in this study (screen failure) may be allowed to rescreen (once). Rescreening may occur following resolution of acute exclusionary conditions or stabilization of conditions that were exclusionary and reversible (eg, unstable hypothyroidism, electrolyte abnormalities) and with the approval of the study Medical Monitor.

A single retest for a laboratory parameter(s) is permitted if there is a specific issue related to the collection, shipping, processing or analysis of a sample (eg, receipt of a hemolyzed sample at the testing laboratory, or samples received by the testing laboratory outside of the acceptable time window or temperature range) or related to a resolved condition.

If more than 28 days are required to obtain a result of a screening procedure and/or test conducted within the permitted window, the screening period may be extended until results are obtained. However, the clinical safety laboratory tests may need to be repeated under the instruction of the study Medical Monitor if the screening period is beyond 28 days.

5.5 Dietary and Lifestyle Restrictions

Subjects will be required to comply with the following restrictions during study participation:

- Subjects will not engage in strenuous exercise beyond their accustomed daily level of activity from Day 1 dosing through end of study.
- Subjects are prohibited from illicit substance/drug use and the use of marijuana before dosing on Day 1 through end of study.
- Subjects are prohibited from consuming any amount of alcohol 48 hours before dosing on Day 1 through end of study.
- Subjects are prohibited from nicotine consumption from Screening through end of study.
- Subjects are prohibited from consuming caffeine in any form, including tea, cola, and caffeinated beverages 48 hours before dosing on Day 1 through end of study.
- Subjects are prohibited from consuming grapefruit, pomelo, or Seville orange whole fruits or juice for 7 days before dosing on Day 1 through end of study.
- Subjects will be advised to take measures to minimize exposure to ultraviolet light from Day 1 through 7 days after the last dose of study drug, as an initial assessment for the potential for phototoxicity has not yet been conducted.

- Subjects who are women of childbearing potential should not plan to become pregnant during the study and all subjects should adhere to the contraception guidelines specified in Appendix 5.

- Subjects must be fasting for at least 8 hours before chemistry assessments (Note fasting requirements for assessment of PK are provided in Sections 6.1.1.4.1 and 6.1.1.4.2).

5.6 Meals

To reduce the potential impact of meal composition on safety and PK parameters, it is recommended standardized meals be provided while subjects are confined to the clinical study site: Part A (SAD) Day -1 to Day 4; Part B (MAD) Day -1 to Day 11 (ie, 3 days post last day of dosing). The meals will be consistent in calorie count and nutritional content (eg, similar amounts of protein, carbohydrate, and fat) and will be scheduled around required fasting/fed periods for study dosing and procedures. The meal timing and consumption will be documented in the source document. Other than the 240 mL of water administered with dosing, water and other fluids will be restricted from 1 hour before dosing to 1 hour after dosing. All food and beverages will be provided by the study site and no additional food, snacks, sweets, drinks, or any similar items will be allowed during the clinic visits.

5.6.1 Test Meal for Subjects in the Food Effect Cohorts

Subjects in the food effect Cohort A6 (and additional Cohort A7 if needed) in Part A (SAD) will consume a high-fat, high-calorie test meal, containing approximately 800 to 1000 calories, 30 minutes prior to dosing on Day 1. Subjects will be instructed to consume the entire test meal within 30 minutes. This test meal will derive approximately 150, 250, and 500 to 600 calories from protein, carbohydrate, and fat, respectively. Approximately 50 percent of total caloric content will be fat. An example high-fat, high-calorie test meal would be two eggs fried in butter, two strips of bacon, two slices of toast with butter, four ounces of hash brown potatoes, and eight ounces of whole milk (FDA 2019). Substitutions to this test meal can be made if the substituted test meal provides a similar number of calories from protein, carbohydrate, and fat and has comparable volume and viscosity.

6 STUDY DRUGS

6.1 Investigational Medicinal Product(s) (IMPs)

The investigational medicinal products (IMPs) to be used in this study are ABI-4334 and PBO.

6.1.1 ABI-4334

6.1.1.1 Formulation

The drug product is a tablet containing approximately 10 mg or 50 mg of ABI-4334 and standard pharmaceutical excipients, such as microcrystalline cellulose, lactose, croscarmellose sodium, sodium lauryl sulfate, hypromellose, and magnesium stearate that are compressed into a tablet according to Good Manufacturing Practices (GMP).

Matching placebo tablets contain standard compendial excipients and are manufactured in conformance with GMP. Matching placebo tablets have the same appearance as the ABI-4334 active tablets.

6.1.1.2 Packaging and Labeling

The study drugs are packaged in high-density polyethylene (HDPE) bottles, induction sealed with child-resistant screw caps. Study drug will be labelled in accordance with the US FDA requirements and the European Union (EU) GMP Annex 13, Investigational Medicinal Products. Additional local labelling requirements in countries of study conduct will be incorporated in the study drug label(s).

6.1.1.3 Storage and Handling

Bottled study drug tablets at the study site should be stored at controlled room temperature (15 °C to 30 °C [59 °F to 86 °F]) in a secure, locked location at the sites, accessible only to authorized study personnel.

6.1.1.4 Dosage and Administration

Study drug doses will be administered with 240 mL of water in the clinic under supervision. Subjects will remain in an upright or sitting position for at least 30 minutes after ingestion unless a semi-recumbent or recumbent position is required for ECGs or vital signs. Water, other than for drug administration, will be restricted for 1 hour before and 1 hour after each oral dose. After study drug administration, site personnel will perform a hand and mouth check to verify that the administered dose was swallowed.

If the PK results for Part A Cohorts A1-A5 indicate that BID dosing should be assessed, then subjects in one or both of the Part B Cohorts ie, B1-B2, may receive their study drug as two divided doses, separated by approximately 12 hours on the dosing days.

Fasting requirements for dosing are described below.

6.1.1.4.1 Fasting Requirements

The fasting requirements for all subjects in Part A (SAD) Cohorts A1-A5, for subjects in Cohort A7 during the fasting period, and for subjects in Part B (MAD) Cohorts B1 and B2 are described in this section.

On Day 1 for Part A and Days 1 and 8 for Part B, study drug doses will be administered in the morning after an overnight fast of at least 10 hours. Subjects will continue fasting for 4 hours after dosing, and will receive standardized lunch, dinner, and an evening snack at approximately 4 hours, 10 hours, and 12 hours after dosing, respectively. On Days 2 to 7 for Part B, study drug will be administered in the morning after an overnight fast of at least 10 hours. Subjects will continue fasting for 1 hour after dosing, and will receive standardized snack, lunch, dinner, and an evening snack at approximately 1 hour, 4 hours, 10 hours, and 12 hours after dosing, respectively. Subjects will have approximately 30 minutes to consume the meals.

On all other days (Days 2 to 4 for Part A and Days 9 (ie, 1 day post last day of dosing) to Day 11 (ie, 3 days post last day of dosing) for Part B, subjects will receive a standardized breakfast at approximately the same time as dosing time on dosing days, and lunch, dinner, and an evening snack approximately 4 hours, 10 hours, and 12 hours later. Subjects will have approximately 30 minutes to consume the meals. Meals on Day 4 Part A and Day 11 (ie, 3 days post last day of dosing) Part B are optional due to discharge from the clinic.

If a BID dosing schedule is indicated, the requirements will be the same for the morning doses. Evening doses should be taken on an empty stomach, at least 2 hours after a meal and 1 hour before the next meal.

If it is determined subjects in Part B should take their study drug doses in a fed status, all morning doses will be administered 30 minutes after start of a standardized breakfast. Subjects will receive standardized lunch, dinner, and an evening snack at approximately 4 hours, 10 hours, and 12 hours after dosing, respectively. Subjects will have approximately 30 minutes to consume the meals. If a BID dosing schedule is indicated, the requirements will be the same for the morning doses. Evening doses should be taken 30 minutes after start of a meal.

6.1.1.4.2 Fasting Requirements for Part A (SAD) Food Effect Cohorts

The fasting requirements for subjects in Part A (SAD) Cohort A6 and the fed period of Cohort A7 are provided in this section.

On Day 1, subjects in the food effect Cohort A6 (and the fed period for the Cohort A7), will fast for at least 10 hours until 30 minutes before dosing when they will receive a high-fat, high-calorie test meal and will continue fasting for 4 hours after dosing. Subjects will receive standardized lunch, dinner, and an evening snack at approximately 4 hours, 10 hours, and 12 hours after dosing, respectively. Subjects will have approximately 30 minutes to consume the meals.

On all other days (Days 2 to 4), subjects will receive a standardized breakfast at approximately the same time as dosing time on dosing days, and lunch, dinner, and an evening snack approximately

4 hours, 10 hours, and 12 hours later. Subjects will have approximately 30 minutes to consume the meals. Meals on Day 4 are optional due to discharge from the clinic.

6.1.2 Accountability of IMP

Regulatory requirements stipulate accounting of all IMPs received by the study site. Records of drug disposition must include the date received by the site, date administered, quantity administered, and the subject to whom IMP was administered. The Investigator is responsible for the accountability of all IMPs at their site. The study site is to use an IMP accountability record to document study drug disposition. All items on this form are to be fully completed. The Sponsor or the clinical research organization (CRO) will confirm if the method of recording study drug accountability by the site and the location of IMP records at the site is appropriate.

Each time designated site personnel dispense IMP for a subject, he or she is to record the date dispensed, the quantity of study drug dispensed, and his or her initials. Study site personnel are to monitor the inventory of study drug and maintain a count of all used and unused study drug. The site monitor will review the study drug accountability records during monitoring visits. The site pharmacist or designated staff member will keep accurate records of drug dispensation routinely during the study.

6.1.2.1 Return or Disposal of IMP

Procedures for the return of IMP or provisions for onsite destruction (where approved prospectively by Assembly or designee) are described separately in the Pharmacy Manual.

6.2 Non-Investigational Medicinal Products(s)

Not applicable.

6.3 Concomitant Medications and Procedures

A concomitant medication is defined as any prescription or OTC preparation, including vitamins, medications, vaccinations, herbal preparations, and supplements (including traditional Chinese medicine) that is taken by a subject during the conduct of the clinical trial.

All concomitant medications taken during the study from the date the ICF is signed through the end of study participation (Section 4.4) (ie, completion of the follow-up period or the subject is "lost to follow-up") must be recorded in the subject's source documentation. This information should include the name of the medication, the start and stop dates, the dosage, route of administration, and the indication for which the concomitant medication was administered. Medications taken for a procedure will also be included. Concomitant medications will be coded using the World Health Organization Drug Reference List.

Elective procedures should not be performed during the study.

6.3.1 Prohibited Concomitant Therapy

As the potential for drug-drug interactions between ABI-4334 and other compounds has not yet been fully evaluated, all concomitant medications (defined in Section 6.3) being taken at the time of Screening, except for paracetamol (also known as acetaminophen), should be washed out beginning 14 days or 5 half-lives whichever is longer prior to Day 1 dosing. Paracetamol (up to 4 g/day) and topical agents are allowed, as needed (PRN) during the course of the study at the Investigator's discretion. All other concomitant medications are prohibited through end of study.

6.4 Randomization, Blinding, and Treatment Codes

6.4.1 Randomization

All subjects will be assigned a unique subject number at Screening and will be randomized upon meeting all protocol eligibility requirements. Randomization will be performed by an unblinded pharmacist.

6.4.2 Blinding

All study team members (including Sponsor team members, Investigator, site personnel administering the study drug, performing the clinical assessments, and handling data on the study subjects), except the members of the DRC (specified in the DRC charter), will be blinded to individual subject treatment assignments throughout the duration of the study. The study subjects will also be blinded to their treatment assignment throughout the duration of the study.

The DRC will be unblinded to individual subjects' treatment assignments throughout the study to ensure timely analysis of any emergent safety and tolerability issues, and completion of the prespecified safety and PK data reviews. The unblinded individuals will be identified in the DRC charter along with their specific roles and responsibilities. All other Sponsor personnel will remain blinded until completion of the study. The CRO's Medical Monitor may be unblinded to individual subjects' treatment assignments where required for assessment of emergent safety/tolerability issues.

6.4.2.1 Unblinding in Emergency Situations

During the study, the randomization code must not be broken except in emergency situations where the identification of a subject's study treatment is required by the Investigator for further treatment to the subject. Randomization information will be held by designated individual(s). When possible, the Investigator should discuss the emergency with the Sponsor prior to unblinding. The date and reason for breaking the blind must be recorded.

The unblinded pharmacist will be used to unblind a subject's assigned treatment. In the event that an individual subject's treatment assignment is unblinded, the date and reason that the blind was broken must be recorded in the source documentation, as applicable. Any treatment assignments that are unblinded must be immediately reported to the Sponsor and Medical Monitor.

7 DISCONTINUATION OF STUDY TREATMENT OR STUDY

7.1 Discontinuation of Study Treatment for A Subject

The Investigator (upon consultation with the Sponsor, if possible) may discontinue subject treatment with study drug if any of the treatment discontinuation criteria listed in Section 7.1.1 are met. Discontinuation from treatment does not mean discontinuation from the study.

Any subject in Part B who prematurely discontinues their assigned treatment regimen, will enter a 7-day follow-up period, during which the subject will undergo the assessments listed under the follow-up visits specified in Appendix 2.

7.1.1 Criteria for Discontinuation of Study Treatment for a Subject

Study drug treatment may be discontinued in the following instances:

- Unacceptable tolerance of study drug treatment due to AE, in the judgement of the Investigator
- Intercurrent illness that would, in the judgement of the Investigator, affect assessments of clinical status to a significant degree
- Unacceptable toxicity as defined in Section 9.10 (Toxicity Management), or toxicity that, in the judgment of the Investigator, compromises the ability to continue study-specific procedures or is considered to not be in the subject's best interest
- Subject noncompliance
- Pregnancy during the study
- Discontinuation of the study at the request of the Sponsor, a regulatory agency or an Institutional Review Board (IRB) or Independent Ethics Committee (IEC)

7.1.2 Subject Discontinuation or Withdrawal from the Study

A subject may withdraw from the study at any time at his/her own request. The subject may also be withdrawn from the study at any time at the discretion of the Investigator or Sponsor for safety, behavioral, compliance, or administrative reasons. At the time of discontinuing from the study, if possible, a Premature Study Termination visit should be conducted, as soon as feasibly possible (Section 8.6; Appendix 3). If the subject withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before withdrawal of consent. If a subject withdraws from the study, they may request destruction of any samples in storage, and the Investigator must document this in the site study record and inform the Sponsor.

Subjects withdrawn due to a study drug-related AE will not be replaced. Subjects who are withdrawn for other reasons may be replaced as required by agreement between the Investigator and Sponsor to ensure a sufficient number of subjects for each cohort.

7.1.3 Subject Lost to Follow-Up

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site must attempt to contact the subject, reschedule the missed visit as soon as possible, and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study

- Before a subject is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record

Should the subject continue to be unreachable, they will be considered lost to follow-up.

7.2 Criteria for Discontinuation of Study Treatment for a Cohort

Study enrollment and drug administration in the current cohort will be stopped pending a full safety review for any the following:

- Any SAE considered as at least possibly related to study drug by the Investigator and/or the Sponsor's Medical Monitor

- Any Grade 4 AE considered as at least possibly related to the study drug by the Investigator and/or the Sponsor's Medical Monitor

- Two Grade 3 AEs that are similar in nature and considered as at least possibly related to the study drug by the Investigator and/or the Sponsor's Medical Monitor

- A pattern of significant symptoms, physical findings, or confirmed laboratory abnormalities (AEs) that, although individually minor, collectively represent a safety concern in the opinion of the Investigator and/or the Sponsor's Medical Monitor and are judged to be at least possibly related to study drug

If any of the above predefined criteria are met, the Sponsor will rapidly inform the study sites that the study drug should not be administered to additional subjects at the same or higher doses until the safety concern is reviewed and considered resolved by the DRC and the Investigator. A cohort with lower dose might be considered subsequently to continue to explore the study objectives.

If the study is stopped or suspended for reasons of safety, the Sponsor will notify the relevant regulatory agencies.

7.3 Discontinuation of the Study

The Sponsor or designee reserves the right to terminate the study at any time for any reason at the sole discretion of the Sponsor. If the study is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigator, the IECs/IRBs, the regulatory authorities, and any CROs used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the study subjects and should ensure appropriate follow-up.

8 STUDY PROCEDURES AND ASSESSMENTS

8.1 Schedule of Assessments

All procedures and assessments of this study and associated timepoints are outlined in the Schedule of Assessments for scheduled visits in Appendix 1 for Part A, Appendix 2 for Part B, and for all other visits in Appendix 3.

8.2 Study Visit Procedures

8.2.1 Screening Assessments

Subjects who agree to participate will sign the ICF prior to undergoing any other Screening procedure. The Screening period begins once the ICF has been signed. The ICFs for enrolled subjects and for subjects who are screened but not enrolled will be maintained at the study site.

Screening evaluations must be completed and reviewed to confirm that a subject meets all eligibility criteria before randomization. Refer to Section 5.4 for instructions regarding screen failure.

The Investigator will maintain a Screening log to record details of all subjects screened and to confirm eligibility or record reasons for screen failure, as applicable.

8.2.2 Subject Enrollment and Treatment Assignment

The Investigator will assess and confirm the eligibility of each subject. All Screening procedure results, and relevant medical history must be available before eligibility can be determined. All inclusion criteria must be met and none of the exclusion criteria may apply. No eligibility waivers will be granted.

Following provision of informed consent and completion of all Screening and Day 1 predose assessments, if a subject meets all protocol eligibility requirements the Investigator or designee will randomize the subject as described in Section 6.4.1.

8.3 Assessment of Safety

8.3.1 Physical Examination

A complete or symptom-directed physical examination will be performed at timepoints indicated in Appendix 1, Appendix 2, and Appendix 3. Height and weight will also be recorded.

The complete physical examination will consist of the following body systems: head, eyes, ears, nose, and throat; cardiovascular; respiratory; gastrointestinal; dermatologic; musculoskeletal; nervous; extremities; and lymph nodes. Additional body systems may be evaluated at the Investigator's discretion. Examination of the breast and genitalia are not required unless clinically indicated.

Additional symptom-directed or complete physical examinations may be performed at the Investigator's discretion throughout the course of the study. If the subject reports feeling unwell or has ongoing AEs, the Investigator or qualified subinvestigator will examine the appropriate body system(s).

8.3.2 Vital Signs

Vital sign assessments will include temperature, heart rate, respiration rate, and blood pressure at timepoints specified in Appendix 1, Appendix 2, and Appendix 3.

8.3.3 Electrocardiograms

A standard 12-lead ECG will be obtained as indicated in Appendix 1, Appendix 2, and Appendix 3. The ECGs will be measured in triplicates at 3 minutes apart only at Screening. Single ECGs will be collected thereafter, unless required by the Investigator.

Prior to the conduct of 12-lead ECGs, subjects should rest in a supine position for 10 minutes. Electrocardiograms should be conducted in accordance with local practice and equipment. The ECG assessment will include interpretation of the tracings (eg, rhythm, presence of arrhythmia or conduction defects, morphology, any evidence of myocardial infarction, or ST segment, T-wave, and Uwave abnormalities). The Investigator or a physician subinvestigator is responsible for reviewing and overreading the ECG interpretation, for assessing whether the ECG machine interpretation findings are accurate, appropriate, normal or abnormal, and for providing corrected interpretations as appropriate. In addition, any abnormal ECGs will be assessed for clinical significance.

Additional ECGs may be obtained if clinically indicated and will be obtained if abnormal and clinically significant or thought to be an error (eg, lead placement error, movement artifact, etc). Any additional relevant data obtained by the Investigator during the course of this study will be recorded in the subject's source documentation. The Investigator or a physician subinvestigator will review all ECGs, evaluate the results, and sign/date the tracing or report. For any ECG abnormality that the Investigator considers clinically significant, the Investigator will:

- Repeat the ECG
- Obtain follow-up ECG(s) if any significant abnormalities are detected after study drug administration to document resolution and as clinically indicated
- Record as an AE any ECG abnormality that: (1) is confirmed and the Investigator considers clinically significant; or (2) requires a subject to be discontinued from the study; or (3) the abnormality requires a subject to receive treatment

8.3.4 Holter Data Collection

Holter data collection will be performed at the timepoints indicated in Appendix 1 and Appendix 2 and will be performed on Day 1 for Part A (except for the food effect Cohorts A6 and A7) and on Days 1 and 8 for Part B. The ECG extraction will be performed by the central ECG laboratory with replicate (minimum of 3) recordings extracted according to the central laboratory's standard practice. At the timepoints for ECG extraction, subjects will be in the supine or semirecumbent position for at least 10 minutes prior to and 5 minutes after. Note: Subjects must be in the upright position for dosing and then return to the supine or semirecumbent position for ECG monitoring.

Holter data will be collected and downloaded by the clinical site and subsequently transferred to the Sponsor and stored for possible future analysis.

8.3.5 Clinical Laboratory Assessments

Clinical laboratory tests will be performed at the designated central laboratories at the timepoints indicated in Appendix 1, Appendix 2, and Appendix 3. Should any laboratory parameter require urgent testing to support eligibility determination or immediate medical care of a subject, samples can be collected for local laboratory testing. However, only the results from the central laboratories will be reported in the study database for analysis purposes.

The specific components of the clinical laboratory tests are listed below in Table 2.

Table 2. Clinical Laboratory Tests

Panel	Tests
Chemistry	Glucose, sodium, potassium, chloride, bicarbonate, calcium, blood urea nitrogen, creatinine, creatine kinase, uric acid, total and direct bilirubin, ALT, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase, lactate dehydrogenase (LDH), triglycerides, total cholesterol, phosphorous (inorganic), total protein, albumin, amylase, lipase
Hematology	Hemoglobin, hematocrit, red blood cell count, RBC indices (MCV), reticulocyte count, white blood cell count including, lymphocytes, monocytes, neutrophils, eosinophils, basophils, platelet counts
Coagulation	Prothrombin time/INR, aPTT
Urinalysis	pH, specific gravity, protein, glucose, ketones, occult blood
Pregnancy tests for female subjects of childbearing potential	Serum pregnancy test and/or urine dipstick pregnancy test
Drug Screen	Dipstick
Serology	HIV-1 Ab, HIV-2 Ab, HCV Ab, HBcAb IgM, HBsAg Note: For antibody tests, if antibody is positive, reflex to DNA or RNA, as applicable
Alcohol	Urine dipstick or breathalyzer

Panel	Tests
Other	FSH for female subjects < 54 years of age with no spontaneous menses > 12 months

Abbreviations: Ab=antibody; ALT=alanine aminotransferase; aPTT=activated partial thromboplastin time; AST=aspartate aminotransferase; DNA=deoxynucleic acid; FSH=follicle stimulating hormone; GGT=gamma-glutamyl transferase; HBcAb=antibody to the HBV core protein; HBsAg= hepatitis B surface antigen; HCV=hepatitis C virus; HIV=human immunodeficiency virus; IgM=immunoglobulin M; INR=international normalized ratio; LDH=lactate dehydrogenase; MCV=mean corpuscular volume; RBC=red blood cell; RNA=ribonucleic acid

8.4 Assessment of Pharmacokinetics

Plasma PK samples will be collected at time points specified in the PK sampling schedules provided in Appendix 1 (Part A [SAD]) and Appendix 2 (Part B [MAD]). In Part B (MAD), urine will also be collected as specified in Appendix 2. Blood and urine sample collection, processing, and shipping details will be outlined in a separate lab manual or instructions.

Plasma and urine concentrations of ABI-4334 will be determined using a validated assay at a contracted bioanalytical laboratory. Plasma and urine concentrations of any potential ABI-4334 metabolites may also be determined. Furthermore, blood and urine samples will be stored for potential future bioanalytical testing of the parent drug or any metabolites and to support the validity of the study.

8.5 Unscheduled Visit Procedures

An Unscheduled Visit may be performed at any time at the discretion of the Investigator in order to further evaluate a subject. The specific assessments to be performed at these visits would be determined by the Investigator according to the nature of the subject-specific follow-up required. Suggested assessments, as applicable, are indicated in Appendix 3.

Assessments performed should be documented in the subject's source documentation. Refer to Section 8.3.5 for clinical laboratory assessments.

8.6 Premature Study Termination Visit Procedures

Should a subject prematurely discontinue from the study (Section 7.1.2), a Premature Study Termination Visit should be scheduled, as feasible. While the Investigator may include additional assessments and evaluations determined by the status of the individual subject, at a minimum, the assessments indicated in Appendix 3 should be performed as soon as feasibly possible.

9 ADVERSE EVENTS AND TOXICITY MANAGEMENT

9.1 Definition of Adverse Event

An AE is any untoward medical occurrence in a study subject administered a study drug regardless of the causal relationship with treatment.

An AE, therefore, can be any unfavorable and unintended sign, symptom, or disease temporally associated with participation in an investigational study, whether or not considered study drug related. In addition to new events, any increase in the severity or frequency of a pre-existing condition that occurs after the subject signs the ICF for participation is considered an adverse event. This includes any side effect, injury, toxicity, or sensitivity reaction. AEs may also include complications associated with protocol mandated procedures.

An AE does not include the following:

- Medical or surgical procedures such as surgery, endoscopy, tooth extraction, and transfusion. The condition that led to the procedure may be an AE and must be reported
- Pre-existing diseases, conditions, or clinically significant laboratory abnormalities present or detected before the screening visit that do not worsen during the study
- Situations where an untoward medical occurrence has not occurred (eg, hospitalization for elective surgery, social and/or convenience admissions)
- Overdose without clinical sequelae
- Any medical condition or clinically significant laboratory abnormality with an onset date before the consent form is signed and not related to a protocol-associated procedure is not an AE. It is considered to be pre-existing and should be documented on the medical history CRF

9.2 Documenting Adverse Events

Adverse events will be monitored and recorded from the written informed consent obtained through end of study (completion of follow-up period or “lost to follow-up”). The Investigator will ask the subject at each visit if they have experienced any untoward effects since the last study visit. All AEs will be entered in the electronic case report forms (eCRFs); refer to the eCRF Completion Guidelines for additional information.

For the follow-up period for AEs, see Section 9.5. For the definition of TEAEs, see Section 10.4.4.

9.3 Assessment of Intensity

The severity of each AE is to be assessed by the Investigator according to the modified Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric AEs (Appendix 4), which grades the severity of clinical AEs and laboratory abnormalities in a 4-category system.

For AEs not included in Appendix 4, the following guidelines will be used to describe severity:

- Grade 1 (Mild): Mild symptoms causing no or minimal interference with usual social and functional activities with intervention not indicated
- Grade 2 (Moderate): Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated
- Grade 3 (Severe): Severe symptoms causing inability to perform usual social and functional activities with intervention or hospitalization indicated (Of note, the term "severe" does not necessarily equate to "serious")
- Grade 4 (Life-Threatening): Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

9.4 Assessment of Causality

All AEs must have their relationship to study intervention assessed by the clinician who examines and evaluates the subject based on temporal relationship and his/her clinical judgment. Each AE must be recorded in the source documentation, whether serious or not serious. For the purposes of this study, each event is to be assessed with regard to the following causality categorizations, in the Investigator's considered judgment:

- Not related: An AE with sufficient evidence to accept that there was no causal relationship to administration of study drug (eg, no temporal relationship because the study drug was administered after the onset of the event, an investigation showed that study drug was not administered, another cause was proven)
- Related: An AE occurred in a plausible time relationship to administration of study drug and that could not be explained by a concurrent disease or other drugs or events. The response to withdrawal of the drug (dechallenge) was clinically reasonable

9.5 Adverse Event Follow-up

The Investigator is responsible to follow all AEs should through resolution, unless the event is considered by the Investigator to be unlikely to resolve due to the subject's underlying disease, or the subject is lost to follow-up.

9.6 Serious Adverse Events

9.6.1 Definition of Serious Adverse Event

An SAE is any event that meets any of the following criteria:

- Death
- Life-threatening
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect in the offspring of a subject
- Other: Important medical events that may not result in death, be life-threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. Examples of such events are:
    - Intensive treatment in an emergency room or at home for allergic bronchospasm
    - Blood dyscrasias or convulsions that do not result in inpatient hospitalization
    - Development of drug dependency or drug abuse

9.6.2 Definition of Terms

- Life threatening: An AE is life-threatening if the subject was at immediate risk of death from the event as it occurred; ie, it does not include a reaction that if it had occurred in a more serious form might have caused death. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life threatening even though drug induced hepatitis can be fatal
- Hospitalization: AEs requiring hospitalization should be considered SAEs. Hospitalization for elective surgery or routine clinical procedures that are not the result of AEs (eg, elective surgery for a pre-existing condition that has not worsened) need not be considered AEs or SAEs. If anything untoward is reported during the procedure, that occurrence must be reported as an AE, either “serious” or “non-serious” according to the usual criteria
- In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. When in doubt as to whether “hospitalization” occurred or was necessary, the AE should be considered serious

- Disability/incapacitating: An AE is incapacitating or disabling if the experience results in a substantial and/or permanent disruption of the subject's ability to carry out normal life functions

9.6.3 Reporting Serious Adverse Events

All SAEs must be reported by the investigational staff within 24 hours of learning about the event using the SAE Report form to the Sponsor or its designee (eg, CRO Pharmacovigilance group).

The initial report should be promptly followed by detailed, written reports, which will include copies of relevant hospital case reports, autopsy reports, and other documents when requested and applicable.

For a follow-up report to the authorities, the monitor may be required to collect further information for a final evaluation of the case. Reporting to the respective country Health Authorities will be the responsibility of the Sponsor and the CRO.

The CRO will be responsible for informing all central Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs) of SAEs as required. It will be the responsibility of the individual Investigators to inform any local IRBs/IECs of SAEs as required. Correspondence with the IRB(s)/IEC(s) relating to the reporting of SAEs will be retained in the study file.

9.7 Laboratory Abnormalities and Other Abnormal Assessments as AEs or SAEs

Laboratory abnormalities (defined as outside of reference range) are usually not recorded as AEs or SAEs, unless they are clinically significant (see definition of AEs in Section 9.1), as determined by the Investigator. Clinical laboratory abnormalities that require medical or surgical intervention or lead to study drug interruption or discontinuation must be recorded as an AE or SAE, if applicable. The modified DAIDS Table for Grading the Severity of Adult and Pediatric AEs (Appendix 4) will be used to assess the severity of laboratory abnormalities. In addition, laboratory or other abnormal assessments (eg, ECG and vital signs) that are associated with signs and/or symptoms must be recorded as an AE or SAE if they meet the definition of an AE or SAE described in Sections 9.1 and 9.6. If the laboratory abnormality or abnormal assessment is part of a syndrome or diagnosis, record the syndrome or diagnosis (eg, anemia), not the laboratory result (ie, decreased hemoglobin).

9.8 Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product which is above the maximum recommended dose as per protocol or in the product labelling (as it applies to the daily dose of the subject in question). The Medical Monitor may be contacted for questions concerning potential cases of overdose. An overdose is not in and of itself considered to be an AE unless it results in untoward medical effects. Any AE associated with an overdose or incorrect administration of study drug should be entered in the subject’s source documentation and

Adverse Event eCRF. If the associated AE fulfills the criteria of an SAE, then the event should be reported to the Sponsor or CRO within 24 hours after the site learns of the event.

9.9 Pregnancy

9.9.1 Female Subjects Who Become Pregnant

The Investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study (see Appendix 5 for contraceptive requirements). The initial information will be recorded on the Pregnancy Reporting form and submitted to the Sponsor or its designee within 24 hours of learning of a subject's pregnancy.

The subject will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the subject and the neonate and the information will be forwarded to the Sponsor or its designee. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE. A spontaneous abortion (occurring at < 22 weeks gestational age) or still birth (occurring at > 22 weeks gestational age) is always considered to be an SAE and will be reported as such.

Any female subject who becomes pregnant while participating in the study will discontinue the study drug(s) immediately.

9.9.2 Male Subjects With Partners Who Become Pregnant

The Investigator will attempt to collect pregnancy information on any male subject's female partner who becomes pregnant while the male subject is participating in this study (see Appendix 5 for contraceptive requirements).

After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the Pregnancy Reporting form and submit it to the Sponsor or its designee within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

9.10 Toxicity Management

Administration of study drug may be discontinued due to an adverse event. The Medical Monitor should be consulted prior to dose interruption or discontinuation of study drug, if possible, unless

the Investigator believes that immediate action is warranted to ensure the continued safety of subject, in which case the Medical Monitor should be notified immediately after.

Grade 3 and 4 clinically significant laboratory abnormalities (reported as AEs) should be confirmed by repeat testing within 3 days of receipt of results and before study drug discontinuation, unless such a delay is not consistent with good medical practice.

Any questions regarding toxicity management should be directed to the Medical Monitor.

9.10.1 Grades 1 and 2 Adverse Event

- Continue study drug at the discretion of the Investigator

9.10.2 Grade 3 or Grade 4 Adverse Event

- For Grade 3 or Grade 4 AE, the study drug may be continued if considered to be unrelated to the study drug
- For a Grade 3 or Grade 4 AE that is considered to be related to the study drug, the study drug should be discontinued
- For a Grade 4 laboratory abnormality (reported as an AE) confirmed by repeat testing that is considered related to the study drug, the subject should be followed as clinically indicated until the laboratory abnormality returns to Baseline (as defined in Section 10.1) or is otherwise explained, whichever occurs first

10 STATISTICAL CONSIDERATIONS

10.1 General Considerations

This section provides the key details of the statistical analyses to be performed using data captured according to this protocol. A complete Statistical Analysis Plan (SAP) describing all planned analyses will be finalized prior to database lock. For all analyses, Baseline will be defined as predose, Day 1 or Screening closest to Day 1 (predose) if Day 1 testing is not available.

10.2 Determination of Sample Size

This is a first in human study. The sample size is similar to that previously utilized for this type of study and is not based upon statistical considerations.

10.3 Analysis Populations

The following populations will be considered for analysis of various endpoints:

- Randomized Set: It includes all randomized subjects, classified according to the treatment to which they were randomized, regardless of the actual treatment received.

- Full Analysis Set: It includes all randomized subjects, classified according to the treatment to which they were randomized, regardless of the actual treatment received, who took at least 1 dose of study drug.

- Safety Analysis Set: It includes all subjects, classified according to the actual treatment received regardless of random assignment, who took at least one dose of study drug. This is the main analysis population for all safety analyses.

- Pharmacokinetic-Evaluable Set: It consists of all subjects, classified according to the actual treatment received regardless of random assignment, who receive at least 1 dose of study drug. At least 1 evaluable pharmacokinetic blood sample following a dose of study treatment is required for inclusion in this analysis. This is the main analysis population for all pharmacokinetic analyses.

Based on the actual deviations, the criteria for exclusion of subjects from the different data sets will be specified and updated, if necessary, prior to database lock.

10.4 Planned Analyses

All safety and PK endpoints will be summarized using descriptive statistics by treatment. Continuous endpoints will be described using the mean, standard deviation, median, minimum, and maximum. Categorical endpoints will be described using the number and percent of subjects who meet the endpoint criterion. The safety and PK analyses will be presented according to the treatment received. Due to sample size limitations, no formal statistical inference is planned.

10.4.1 Disposition of the Study Subjects

The disposition of subjects will be described with summaries by treatment of the number of subjects in each analysis set described above, the number of subjects who completed the study, and the number of subjects for whom study drug was permanently discontinued (including the reasons for discontinuation). Randomized Analysis Set will be used to produce this analysis.

10.4.2 Demographics

The demographics will be summarized using standard descriptive methods by treatment and overall. Demographic summaries will include age, sex, race, ethnicity, body weight, and body mass index.

10.4.3 Extent of Exposure

Extent of exposure to study drug will be examined by assessing the total duration of study drug exposure and the level of adherence to the study drug by treatment for the Safety Analysis Set.

10.4.4 Analysis of Safety Endpoints

The safety parameters to be assessed are described in Section 8.3. Displays for safety results will utilize descriptive statistics. No formal hypothesis testing of safety data is planned.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). A treatment-emergent adverse event (TEAE) will be defined as any AE that has an onset date or worsening in severity from Baseline on or after the first dose of study drug through end of study (defined in Section 4.5). Incidence of each body system and preferred term will be tabulated for each treatment. Summaries will include all TEAEs, TEAEs considered related to study treatment by the Investigator, graded TEAEs, serious TEAEs, TEAEs leading to premature discontinuation from the study intervention. All AEs, emergent or nonemergent, will be listed by subject.

Clinical laboratory results will be summarized descriptively by treatment including values, changes from Baseline, and incidence of laboratory abnormalities. Laboratory results will be listed for each subject.

Vital signs data and ECG findings reported at each visit will be displayed by treatment, using descriptive statistics for observed and change from Baseline values.

10.4.5 Analysis of Pharmacokinetic Endpoints

The PK parameters as shown in Table 3 and Table 4 will be estimated from the plasma concentrations of ABI-4334 (and potential metabolites) using noncompartmental methods in Phoenix® WinNonlin® as applicable. Additional parameters may be estimated, as appropriate.

Table 3. Plasma Pharmacokinetic Parameters: Parts A and B; Day 1

Parameter	Definition
$AUC_{0\text{-last}}$	AUC from time 0 to the time of the last quantifiable concentration (time t)
$AUC_{0\text{-}24}$	AUC from time 0 to 24 hours postdose
$AUC_{0\text{-inf}}$*	AUC from time 0 extrapolated to infinity
$\%AUC_{ext}$*	Percent of $AUC_{0\text{-inf}}$ that is extrapolated to infinity
C_{max}	Maximum observed plasma concentration
T_{max}	Time to C_{max}
k_{el}*	Apparent first-order terminal elimination rate constant
$t_{1/2}$*	Apparent terminal elimination half-life
V_z/F*	Apparent volume of distribution
CL/F*	Apparent systemic clearance
C_{max}_DN	Dose normalized C_{max}
$AUC_{0\text{-}24}$_DN	Dose-normalized $AUC_{0\text{-}24}$
$AUC_{0\text{-last}}$_DN	Dose-normalized $AUC_{0\text{-last}}$
$AUC_{0\text{-inf}}$_DN*	Dose-normalized $AUC_{0\text{-inf}}$

* Parameters will be reported only if the apparent terminal phase can be adequately characterized.

Table 4. Plasma Pharmacokinetic Parameters: Part B (MAD) Day 8

Parameter	Definition
AUC_{tau}	AUC from time 0 to the end of the dosing interval
$C_{max,ss}$	Maximum observed plasma concentration during the dosing interval
C_{av}	Average plasma concentration over the dosing interval
$T_{max,ss}$	Time to $C_{max,ss}$
k_{el}*	Apparent first order terminal elimination rate constant
$t_{1/2}$*	Apparent terminal elimination half-life
C_{24hr}	Observed plasma concentration at 24 hours postdose
V_z/F	Apparent volume of distribution
CL_{ss}/F	Apparent systemic clearance
$C_{max,ss}$_DN	Dose-normalized $C_{max,ss}$
AUC_{tau}_DN	Dose-normalized AUC_{tau}
RA_$C_{max,ss}$	Accumulation ratio based on $C_{max,ss}$
RA_AUC_{tau}	Accumulation ratio based on AUC
Linearity factor**	Ratio of AUC_{tau} (Day 8) to $AUC_{0\text{-inf}}$ (Day 1)

* Parameters will be reported only if the apparent terminal phase can be adequately characterized.

** Parameter will be reported only if $AUC_{0\text{-}inf}$ on Day 1 is reported.

The PK parameters as shown in Table 5 will be estimated from the urine concentrations of ABI-4334 (and potential metabolites) using noncompartmental methods in Phoenix® WinNonlin®, as applicable. Additional parameters may be estimated, as appropriate.

Table 5. Urine Pharmacokinetic Parameters: Part B (MAD) Day 8

Parameter	Definition
Ae	Amount of drug excreted in urine over a collection interval
Ae_{tau}	Amount of drug excreted in urine over the last dosing interval
%Fe	Fraction of the dose excreted in urine as unchanged ABI-4334 over the last dosing interval
CLr	Renal Clearance

Concentrations will be used for the PK analysis as reported by the bioanalytical laboratory. Urine volumes will be presented and used as supplied from the clinical data base. The handling of values that are below the limit of quantitation will be addressed in the SAP (or the Pharmacokinetics Analysis Plan [PKAP], if needed).

Plasma pharmacokinetic analysis will be carried out using actual postdose blood draw times relative to dosing times recorded in the raw data. If an actual blood draw time or a dosing time is missing, nominal time may be used. Interim analysis may be performed using nominal blood draw times.

10.4.5.1 Statistical Analysis of PK Parameters

Summary statistics (number, arithmetic mean, standard deviation [SD], percent coefficient of variation [%CV], minimum, median, maximum, geometric mean, and %CV for the geometric mean) will be presented for plasma concentrations by nominal PK sampling time and for the plasma PK parameters by cohort and day. Individual subject and mean plasma ABI-4334 concentrations versus time will be plotted on linear and semi-logarithmic axes. Mean (SD) trough concentrations will be plotted on a linear axis for visual assessment of steady-state conditions.

Dose proportionality of PK exposure parameters (C_{max} and AUC parameters) across dose levels may be explored using dose-normalized C_{max} and AUC, calculated for each subject by dividing AUC and C_{max} by dose. Scatter plots of natural log (ln)-transformed PK parameters versus ln-transformed ABI-4334 dose will be presented. A power model may also be used to further evaluate dose proportionality. This assessment will be performed on the Day 1 PK for the Part A (SAD), on the Day 8 PK for the Part B (MAD).

The effect of food on the steady state pharmacokinetics of ABI-4334 will be explored in the food effect cohorts of Part A (SAD), ie, Cohorts A6 and A7, as appropriate. The PK parameters after administration under fed (test) compared with fasted (reference) conditions will be presented, and the ratios (fed to fasted) of the geometric least squares means for AUC and C_{max} will be presented for the PK evaluable population.

An assessment of time to achieve steady state will be performed on the ln-transformed predose concentrations of ABI-4334 obtained in Part B of the study on Days 3 through 8, for each cohort.

Details of the PK and associated statistical analyses will be included in the SAP (or PKAP, if needed).

10.5 Interim Analysis

The DRC will review the cumulative safety and PK data from each previous cohorts to determine dose level and dosing schedule for the subsequent cohorts (see Section 10.6).

10.6 Data Review Committee

10.6.1 Overview of Data Review Committee

The DRC will review data as described in Section 10.6.2. Other designated unblinded team members may participate in the DRC review meetings, as required.

For each interim cohort review, study data will include safety data from Day 1 through Day 4 (Part A, SAD cohorts) or Day 1 through Day 8 (Part B, MAD cohorts) for the present cohort and all available safety data for preceding cohorts. In addition, PK data through 24 hours after dosing (SAD cohorts) or from the first 24 hours after dosing on Day 8 (MAD cohorts) for the present cohort and available PK data for preceding cohorts will be reviewed.

The DRC will make recommendations for dose level and dosing schedule, as per below:

- Continue with the next cohort at a higher or lower dose, OR
- Repeat a dose, OR
- Modify the next cohort(s), including addition of assessments or procedures, OR
- Discontinue a cohort, OR
- Discontinue the study

A decision to reduce the dose in the next cohort may be made for either safety findings (if there is a concern for a dose related toxicity), or to fully evaluate the PK.

A decision to increase the dose in the next cohort may be made if the dose under evaluation is found to be sufficiently safe and well tolerated, and a higher dose is required to fully evaluate the PK.

10.6.2 Data Review Committee Procedures

For the purposes of the DRC safety reviews, any of the following observations in an ongoing or recently completed study cohort will be defined as a "potential safety concern" requiring a specific assessment by the DRC:

- A single SAE or Grade 4 AE regardless of relatedness to study treatment
- Two or more Grade 3 AEs that are similar in nature, affecting 2 or more subjects in the given cohort

Using the criteria described above, if no potential safety concerns are apparent in the safety observations for a given dosing cohort, then the tabulated treatment blinded safety data summary for the cohort combined with a Sponsor recommendation for treatment in the next cohort will be circulated to the Investigator by email for their review and approval of dose level and dosing schedule modification. This recommendation email should be forwarded to the clinical site IEC by the Investigator.

Additionally, if deemed appropriate, the DRC may also elect to terminate a cohort early based on ongoing analysis of safety or PK data.

Any such recommendations by the DRC will be documented in writing via email and circulated to the Investigator. Any DRC recommendations or safety issues engendering changes in the study conduct will be communicated to the Investigator's IEC. Also, any safety-related change in study dosing levels, changes in safety-related clinical or laboratory assessments, or changes in study design, will be submitted as protocol amendments to the IEC and as required, to the national regulatory authorities for approval prior to implementation.

11 RESPONSIBILITIES

11.1 Investigator Responsibilities

11.1.1 Good Clinical Practice

This study will be conducted in compliance with IRB/IEC and International Council for Harmonisation (ICH) Good Clinical Practices (GCP) Guidelines; Title 21 Part 56 of the US Code of Federal Regulations (CFR) relating to IRBs/IECs and GCP as described in the US FDA CFR (21 CFR § 50, 56, 312; China GCP; applicable ICH guidelines regarding clinical safety data management (E2A, E2B(R3)); European Community directives 2001/20, 2001/83, 2003/94 and 2005/28 as enacted into local law, and with ICH guidelines regarding scientific integrity (E4, E8, E9, and E10). In addition, this study will adhere to all local regulatory requirements, and requirements for data protection.

11.1.2 Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

Before initiating a trial/study, the Investigator/institution must have written and dated approval/favorable opinion from the IRB/IEC for the study protocol/amendment(s), written ICF, any consent form updates, subject recruitment procedures (eg, advertisements), and any written information to be provided to subjects and a statement from the IRB/IEC that they comply with GCP requirements. The IRB/IEC approval must identify the protocol version as well as the documents reviewed.

11.1.3 Informed Consent

The Investigator will explain the benefits and risks of participation in the study to each subject or the subject's legally acceptable representative and obtain written informed consent. Written informed consent must be obtained prior to the subject entering the study and before initiation of any study-related procedure (including administration of investigational product).

The Sponsor or its designee will provide a sample ICF. The final, version dated, ICF must be agreed to by the Sponsor or its designees and the IRB/IEC and will contain all elements in the sample form, in language readily understood by the subject. Each subject's original consent form must be personally signed and dated by the subject or by the subject's legally acceptable representative, and by the person who conducted the informed consent discussion. The original, signed ICF will be retained by the Investigator. The Investigator will supply all enrolled subjects with a copy of their signed ICF.

The ICF may need to be revised during the study should important new information become available that may be relevant to the safety of the subject. In this instance approval should always be given by the IRB/IEC and existing subjects informed of the changes and reconsented. This is documented in the same way as previously described.

The Investigator should encourage subjects to inform their primary physician about their participation in the clinical study.

11.1.4 Confidentiality

All information generated in this study is considered confidential and must not be disclosed to any person or entity not directly involved with the study unless prior written consent is gained from the Sponsor. However, authorized regulatory officials, IRB/IEC personnel, and the Sponsor and the Sponsor's authorized representatives are allowed full access to the records.

Identification of subjects and eCRFs shall be by Subject Number only. If required, the subject's full name may be made known to an authorized regulatory agency or other authorized official.

11.1.5 Study Files and Retention of Records

Records must be retained in accordance with the current ICH guidelines on GCP. All essential study documents including records of subjects, source documents, eCRFs, and investigational product inventory must be kept on file.

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational products. However, essential documents may be retained for a longer period if required by the applicable regulatory requirements or by agreement with the Sponsor. The Sponsor is responsible for informing the Investigator when these documents need no longer be retained.

The Investigator will not dispose of any records relevant to this study without written permission from the Sponsor and will give the Sponsor the opportunity to collect such records. The Investigator shall take responsibility for maintaining adequate and accurate hard copy source documents of all observations and data generated during this study. Such documentation is subject to inspection by the Sponsor, its representatives, and regulatory authorities.

If an Investigator moves, withdraws from an investigation, or retires, responsibility for maintaining the records may be transferred to another person who will accept responsibility. Notice of transfer must be made to and agreed by the Sponsor.

11.1.6 Audits and Inspections

The Sponsor or their designee, the CRO, may conduct audits at the investigative sites including, but not limited to, drug supply, presence of required documents, the informed consent process, and comparison of eCRFs with source documents. All medical records (progress notes) must be available for audit. The Investigator agrees to participate with audits conducted at a convenient time in a reasonable manner.

Government regulatory authorities may also inspect the site during or after the study. The Investigator or designee should contact the Sponsor and/or the CRO, immediately if this occurs. The site must cooperate fully with regulatory authorities or other audits conducted at a convenient time in a reasonable manner.

The purpose of an audit is to assess whether ethical, regulatory, and quality requirements are fulfilled.

11.1.7 Protocol Compliance

It is the responsibility of the Investigator to ensure that the study is conducted at their respective site in accordance with this protocol. Protocol compliance assessments will be conducted during routine site monitoring visits.

11.2 Sponsor Responsibilities

11.2.1 Protocol Amendments and Modifications

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made only by the Sponsor. A protocol change intended to eliminate an apparent immediate hazard to subjects may be implemented immediately, provided the IRB/IEC is notified within 5 days.

Any permanent, significant change to the protocol must be handled as a protocol amendment. The written amendment must be submitted to the IRB/IEC and the Investigator must await approval before implementing the changes. The Sponsor will submit protocol amendments to the appropriate regulatory authorities for approval.

If, in the judgment of the IRB/IEC, the Investigator, and/or the Sponsor, the amendment to the protocol substantially changes the study design and/or increases the potential risk to the subject and/or has an impact on the subject's involvement as a study subject, the currently approved written ICF will require similar modification. In such cases, subjects will be required for to sign the amended informed consent prior to continued participation in the study.

11.2.2 Data Management

A set of data management documents will be created under the responsibility of the Sponsor, or designated CRO, to describe the processes being used to ensure data quality.

The data management plan, and other associated documentations, will specify data capture methods, who is authorized to enter data, decisions about ownership of data, source data storage, which data will be transferred (including timing of transfers), and the origin/destination of data.

11.2.3 Study Report and Publications

The Sponsor or its designee is responsible for preparing and providing the appropriate regulatory authorities with the Clinical Study Report (CSR) according to the applicable regulatory requirements. CSR will be developed in accordance with the ICH E3 Guideline on the ‘Structure and Content of Clinical Study Reports’. Local country requirements will be considered during CSR preparation.

The Sponsor or its designee is responsible for posting clinical trial information for public access according to country-specific requirements. At a minimum, clinical trial information will be posted according to US NIH CT.gov regulations.

The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all proposed manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments. The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.

Assembly Biosciences follows the guidelines and recommendations of the International Committee of Medical Journal Editors (ICMJE) and the International Society for Medical Publication Professionals (ISMPP) when preparing publications associated with clinical studies (Battisti 2015, ICMJE 2019).

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APPENDICES

APPENDIX 1a. SCHEDULE OF ASSESSMENTS FOR SCHEDULED VISITS: PART A – SAD (COHORTS A1-A5, COHORT A7 Period 1) IN HEALTHY SUBJECTS

Period of Visit	Screening		Treatment	Follow-Up					
Study Day	Day -28 to Day -2	Day -1	Day 1 (LDD)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 (EOS)
Visit Windows (days)	NA	NA	NA	NA	NA	NA	NA	NA	NA
In-Clinic Assessments									
Informed Consent(s)	X								
Confined to Study Clinic		X	X	X	X				
Discharge						X			
Demographics	X								
Medical History	X	X							
Height	X								
Weight	X	X							X
Complete Physical Exam	X	X				X [h]			
Symptom-Directed Physical Exam			X	X	X		X	X	X
Vital Signs [a]	X	X	X	X	X	X	X	X	X
12-Lead ECG [a]	X	X	X	X		X			X
Holter Data Collection [b]			X	X					
Concomitant Medications & Adverse Events	X	X	X	X	X	X	X	X	X
Alcohol Testing	X	X							
Urine Drug Test	X	X							
FSH Testing [c]	X								
Urine Pregnancy Test (females of childbearing potential) [d]			X			X			
Confirm Subject Eligibility		X	X						
Randomization (predose)			X						

Period of Visit	Screening		Treatment	Follow-Up					
Study Day	Day -28 to Day -2	Day -1	Day 1 (LDD)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 (EOS)
Visit Windows (days)	NA	NA	NA	NA	NA	NA	NA	NA	NA
In Clinic Study Drug Dosing			X						
Laboratory Assessments									
HIV-1 Ab, HIV-2 Ab, HCV Ab, HBcAb IgM, HBsAg [e]	X								
Chemistry	X	X	X [f]	X	X		X		X
Hematology	X	X	X [f]	X	X		X		X
Coagulation	X	X	X [f]	X	X				X
Urinalysis	X	X	X [f]	X	X				X
Serum Pregnancy Test (females of childbearing potential) [d]	X	X							
Pharmacokinetics Assessments									
PK Sampling [g]			X	X	X	X	X	X	X

Abbreviations Ab=antibody; ECG=electrocardiogram; EOS=end of study; FSH=follicle-stimulating hormone; HBcAb=antibody to the HBV core protein; HBsAg=hepatitis B surface antigen; HCV=hepatitis C virus; HIV=human immunodeficiency virus; IgM=immunoglobulin M; LDD=last day of dosing; NA=not applicable; PK=pharmacokinetic

Note: When multiple procedures are scheduled at the same time, the order of events will be: Holter extraction, followed by 12-lead ECGs, vital signs, PK blood sample collection (to be collected on the scheduled time point), and meals. The only exception will be at 2 and 4 hours postdose, when the 12-lead ECG will be performed before the Holter extraction

[a] Collection of vital signs and ECG will be repeated throughout the day. Specifically, vital signs will be collected predose (within 2 hours before dosing) on Day 1, and at 2, 4, 8, 12, 24, 48, 72, 96, 120, and 144 hours after dosing on Day 1. Collection of ECG will be performed predose on Day 1, and at 2, 4, 12, and 24 hours (ie, Day 2) after dosing on Day 1; and anytime on Days 4 and 7. The window for collection of vital signs and ECGs will be ±15 minutes.

[b] Holter data collection timepoints on Day 1 are -60, -45, and -30 minutes predose and 0.5, 1, 2, 3, 4, 5, 6, 9, 12, 18, and 24 hours (ie, Day 2) postdose (time matched to PK assessments). Holter monitoring will not be performed for Cohort A7.

[c] Only for female subjects < 54 years of age with no spontaneous menses for > 12 months.

[d] A serum pregnancy test is required at Screening and Day -1 for female subjects of childbearing potential. On Day 1, a urine pregnancy test will be performed for females of childbearing potential, prior to dosing. All post Day 1 pregnancy tests may be conducted by urine dipstick. If positive on dipstick, reflex to serum.

[e] For antibody tests, if antibody is positive, reflex to DNA or RNA (as applicable).

[f] On Day 1, samples for clinical laboratory tests will be taken predose.

[g] Blood samples for PK analysis of ABI-4334 in plasma will be collected at the following time points: predose (within 0.5 hours before dosing) and 0.5, 1, 2, 3, 4, 5, 6, 9, 12, 18, 24, 36, 48, 72, 96, 120, and 144 hours after dosing on Day 1; Time point windows are ±2 minutes for 0.5- and 1-hour postdose samples and ±5 minutes for samples from 2 through 18 hours postdose and ±30 minutes for samples 24 hours postdose and after.

[h] A complete physical exam will be done before the subject is discharged from the study site.

APPENDIX 1b. SCHEDULE OF ASSESSMENTS FOR SCHEDULED VISITS: PART A – SAD (COHORT A6, COHORT A7 Period 2)

Period of Visit		Treatment	Follow-Up					
Study Day	Day -1	Day 1 (LDD)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 (EOS)
Visit Windows (days)	NA	NA	NA	NA	NA	NA	NA	NA
In-Clinic Assessments								
Confined to Study Clinic	X	X	X	X				
Discharge					X			
Complete Physical Exam	X				X [e]			
Symptom-Directed Physical Exam		X	X	X		X	X	X
Vital Signs [a]	X	X	X	X	X	X	X	X
12-Lead ECG [a]		X	X		X			X
Weight	X							X
Concomitant Medications & Adverse Events	X	X	X	X	X	X	X	X
Alcohol Testing	X							
Urine Drug Test	X							
Urine pregnancy test (females of childbearing potential) [b]		X			X			
Confirm Subject Eligibility	X	X						
In Clinic Study Drug Dosing		X						
Laboratory Assessments								
Chemistry		X [c]	X	X		X		X
Hematology		X [c]	X	X		X		X
Coagulation		X [c]	X	X				X
Urinalysis		X [c]	X	X				X
Serum Pregnancy Test (females of childbearing potential) [b]	X							
Pharmacokinetics Assessments								
PK Sampling [d]		X	X	X	X	X	X	X

Abbreviations: ECG=electrocardiogram; EOS=end of study LDD=last day of dosing; NA=not applicable; PK=pharmacokinetic

Note: When multiple procedures are scheduled at the same time, the order of events will be: 12-lead ECGs, vital signs, PK blood sample collection (to be collected on the scheduled time point), and meals.

[a] Collection of vital signs and ECG will be repeated throughout the day. Specifically, vital signs will be collected predose (within 2 hours before dosing) on Day 1, and at 2, 4, 8, 12, 24, 48, 72, 96, 120, and 144 hours after dosing on Day 1. Collection of ECG will be performed predose on Day 1, and at 2, 4, 12, and 24 hours (ie, Day 2) after dosing on Day 1; and anytime on Days 4 and 7. The window for collection of vital signs and ECGs will be ±15 minutes.

[b] A serum pregnancy test is required at Day -1 for female subjects of childbearing potential. On Day 1, a urine pregnancy test will be performed for females of childbearing potential, prior to dosing. All post Day 1 pregnancy tests may be conducted by urine dipstick. If positive on dipstick, reflex to serum.

[c] On Day 1, samples for clinical laboratory tests will be taken predose.

[d] Blood samples for PK analysis of ABI-4334 in plasma will be collected at the following time points: predose (within 0.5 hours before dosing) and 0.5, 1, 2, 3, 4, 5, 6, 9, 12, 18, 24, 36, 48, 72, 96, 120, and 144 hours after dosing on Day 1; Time point windows are ±2 minutes for 0.5- and 1-hour postdose samples and ±5 minutes for samples from 2 through 18 hours postdose and ±30 minutes for samples 24 hours postdose and after.

[e] A complete physical exam will be done before the subject is discharged from the study site.

APPENDIX 2. SCHEDULE OF ASSESSMENTS FOR SCHEDULED VISITS: PART B – MAD IN HEALTHY SUBJECTS

Period of Visit	Screening		Treatment								Follow-Up					
Study Day	Day -28 to Day -2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8 (LDD)	1 Day Post LDD	2 Days Post LDD	3 Days Post LDD	4 Days Post LDD	5 Days Post LDD	7 Days Post LDD (EOS)
Visit Windows (days)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
In-Clinic Assessments																
Informed Consent(s)	X															
Confined to Study Clinic		X	X	X	X	X	X	X	X	X	X	X				
Discharge													X			
Demographics	X															
Medical History	X	X														
Height	X															
Weight	X	X														X
Complete Physical Exam	X	X											X [i]			
Symptom-Directed Physical Exam			X	X	X		X		X	X	X	X		X	X	X
Vital Signs [a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
12-Lead ECG [a]	X	X	X		X		X			X			X			X
Holter Data Collection [b]			X							X						
Concomitant Medications & Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Alcohol Testing	X	X														
Urine Drug Test	X	X														
Urine Pregnancy Test (females of childbearing potential) [c]			X										X			
FSH Testing [d]	X															
Confirm Subject Eligibility		X	X													
Randomization (predose)			X													
In Clinic Study Drug Dosing [e]			X	X	X	X	X	X	X	X						
Laboratory Assessments																

Period of Visit	Screening		Treatment								Follow-Up					
Study Day	Day -28 to Day -2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8 (LDD)	1 Day Post LDD	2 Days Post LDD	3 Days Post LDD	4 Days Post LDD	5 Days Post LDD	7 Days Post LDD (EOS)
Visit Windows (days)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
HIV-1 Ab, HIV-2 Ab, HCV Ab, HBcAb IgM, HBsAg [f]	X															
Chemistry	X	X	X	X		X		X		X			X			X
Hematology	X	X	X	X		X		X		X			X			X
Coagulation	X	X	X	X		X				X			X			X
Urinalysis	X	X	X	X		X				X						X
Serum Pregnancy Test (females of childbearing potential) [c]	X	X														
Pharmacokinetics Assessments																
PK Sampling (Blood) [g]			X	X	X	X	X	X	X	X	X	X	X	X	X	
PK Sampling (Urine) [h]										X	X					

Abbreviations Ab=antibody; ECG=electrocardiogram; EOS=end of study; FSH=follicle-stimulating hormone; HBcAb=antibody to the HBV core protein; HBsAg=hepatitis B surface antigen; HCV=hepatitis C virus; HIV=human immunodeficiency virus; IgM=immunoglobulin M; LDD=last day of dosing; NA=not applicable; PK=pharmacokinetic

Note: When multiple procedures are scheduled at the same time, the order of events will be: Holter extraction, followed by 12-lead ECGs, vital signs, PK blood sample collection (to be collected on the scheduled time point), and meals. The only exception will be at 2 and 4 hours postdose, when the 12-lead ECG will be performed before the Holter extraction.

[a] From Day 1 through Day 8, vital signs will be collected predose (within 2 hours before dosing) and at 2, 4, 8, and 12 hours postdose. After Day 8, vital signs will be collected every 24 hours through 5 days post last day of dosing, and anytime on EOS. Collection of ECGs will be performed predose on Day 1, and on Days 1, 3, 5, and 8 at 2, 4, and 12 hours postdose; anytime on 3 days and 7 days post last day of dosing. The window for collection of vital signs and ECGs will be ±15 minutes.

[b] Holter data collection timepoints on Days 1 and 8 are -60, -45, and -30 minutes predose and 0.5, 1, 2, 3, 4, 5, 6, 9, 12, 18, and 24 hours postdose (time matched to PK assessments).

If a BID dosing schedule is indicated for Part B, Holter data will be collected on Day 1: -60, -45, and -30 minutes predose and then post morning dose at 0.5, 1, 2, 3, 4, 5, 6, 9, and 12 hours. On Day 8: -60, -45, and -30 minutes predose and then postdose at 0.5, 1, 2, 3, 4, 5, 6, 9, 12, 18, and 24 hours.

[c] A serum pregnancy test is required at Screening and Day -1 for female subjects of childbearing potential. On Day 1, a urine pregnancy test will be performed for females of childbearing potential, prior to dosing. All post Day 1 pregnancy tests may be conducted by urine dipstick. If positive on dipstick, reflex to serum.

[d] Only for female subjects < 54 years of age with no spontaneous menses for > 12 months.

[e] Study drug dosing (Days 1-8) will be administered with 240 mL of water in the clinic under supervision.

If a BID dosing schedule is indicated for Part B, study drug will be administered twice a day, 12 hours apart, from Day 1 to Day 7. On Day 8, subjects will receive their last dose in the morning.

[f] For antibody tests, if antibody is positive, reflex to DNA or RNA (as applicable).

[g] Blood samples for PK analysis of ABI-4334 in plasma will be collected at the following time points:

- Day 1: predose (within 0.5 hours before dosing) and 0.5, 1, 2, 3, 4, 5, 6, 9, 12, 18, and 24 hours after dosing on Day 1. Note: The 24-hour sample after the Day 1 dose is the predose sample on Day 2.
- Day 8: predose (within 0.5 hours before dosing) and 0.5, 1, 2, 3, 4, 5, 6, 9, 12, 18, 24, 36, 48, 72, 96, and 120 hours after dosing on Day 8.
- Days 2, 3, 4, 5, 6, and 7: predose (within 0.5 hours before dosing).

If a BID dosing schedule is indicated for Part B, blood samples for PK analysis of ABI-4334 in plasma will be collected at the following time points:

- *Day 1: predose (within 0.5 hours before morning dosing) and 0.5, 1, 2, 3, 4, 5, 6, 9, and 12 hours after morning dosing on Day 1. Note: The 12-hours sample should be taken prior to the evening dose on Day 1.*
- *Day 8: predose (within 0.5 hours before dosing) and 0.5, 1, 2, 3, 4, 5, 6, 9, 12, 18, 24, 36, 48, 72, 96, and 120 hours after dosing on Day 8.*
- *Days 3, 4, 5, 6, and 7: predose (within 0.5 hours before morning dosing)*

Time point windows are ±2 minutes for 0.5- and 1-hour postdose samples and ±5 minutes for samples from 2 through 18 hours postdose and ±30 minutes for samples 24 hours postdose and after.

[h] Urine for PK assessment will be collected over 24-hours and will include predose on Day 8 (void and discard), and at times 0-4, 4-8, 8-12, and 12-24 hours postdose. All excreted urine will be collected during each time interval.

If a BID dosing schedule is indicated for Part B, the same schedule of urine sample collection should be followed. All excreted urine will be collected during each time interval.

[i] A complete physical exam will be done before the subject is discharged from the study site.

APPENDIX 3. SCHEDULE OF ASSESSMENTS FOR OTHER VISITS

Study Visit	**Premature Study Termination**	**Unscheduled**
Visit Timing or Frequency [a]	As soon as feasibly possible	As Applicable
Weight	X	
Vital Signs	X	X
Complete Physical Exam	X	
Symptom-Directed Physical Exam		X
12-Lead ECG	X	
Concomitant Medications & Adverse Events	X	X
Urinalysis	X	X
Urine Pregnancy Test (females of childbearing potential) [b]	X	X
Chemistry, Hematology, Coagulation, Alcohol testing	X	X
Any time PK Sample	X	X

Abbreviations: ECG=electrocardiogram PK=pharmacokinetic

[a] Tests to be conducted as applicable, based on Investigator discretion, and may include tests not specified in the Schedule of Assessments.

[b] Pregnancy tests for females of childbearing potential to be conducted by urine dipstick. If positive on dipstick, reflex to serum.

APPENDIX 4. TOXICITY GRADING SCALE FOR ADVERSE EVENTS AND LABORATORY ABNORMALITIES

Adapted from the U.S. National Institutes of Health (Division of AIDS) Table for Grading Severity of Adult Adverse Experiences (Corrected Version 2.1, July 2017) [https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf]

MAJOR CLINICAL CONDITIONS

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life -threatening
CARDIOVASCULAR				
Arrhythmia (by ECG or physical examination) Specify type, if applicable	No symptoms AND No intervention indicated	No symptoms AND Non-urgent intervention indicated	Non-life-threatening symptoms AND Non-urgent intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Blood Pressure Abnormalities Hypertension (with the lowest reading taken after repeat testing during a visit) ≥ 18 years of age	140 to < 160 mmHg systolic OR 90 to < 100 mmHg diastolic	≥ 160 to < 180 mmHg systolic OR ≥ 100 to < 110 mmHg diastolic	≥ 180 mmHg systolic OR ≥ 110 mmHg diastolic	Life-threatening consequences in a participant not previously diagnosed with hypertension (e.g., malignant hypertension) OR Hospitalization indicated
Hypotension	No symptoms	Symptoms corrected with oral fluid replacement	Symptoms AND IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
Cardiac Ischemia or Infarction *Report only one*	NA	NA	New symptoms with ischemia (stable angina) OR New testing consistent with ischemia	Unstable angina OR Acute myocardial infarction
Heart Failure	No symptoms AND Laboratory or cardiac imaging abnormalities	Symptoms with mild to moderate activity or exertion	Symptoms at rest or with minimal activity or exertion (e.g., hypoxemia) OR Intervention indicated (e.g., oxygen)	Life-threatening consequences OR Urgent intervention indicated (e.g., vasoactive medications, ventricular assist device, heart transplant)

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life -threatening
Hemorrhage (with significant acute blood loss)	NA	Symptoms AND No transfusion indicated	Symptoms AND Transfusion of ≤ 2 units packed RBCs indicated	Life-threatening hypotension OR Transfusion of > 2 units packed RBCs (for children, packed RBCs > 10 cc/kg) indicated
Prolonged PR Interval or AV Block *Report only one* > 16 years of age	PR interval 0.21 to < 0.25 seconds	PR interval ≥ 0.25 seconds OR Type I 2nd degree AV block	Type II 2nd degree AV block OR Ventricular pause ≥ 3.0 seconds	Complete AV block
Prolonged QTc Interval [2]	0.45 to 0.47 seconds	> 0.47 to 0.50 seconds	> 0.50 seconds OR ≥ 0.06 seconds above baseline	Life-threatening consequences (e.g., Torsade de pointes, other associated serious ventricular dysrhythmia)
Thrombosis or Embolism *Report only one*	NA	Symptoms AND No intervention indicated	Symptoms AND Intervention indicated	Life-threatening embolic event (e.g., pulmonary embolism, thrombus)
DERMATOLOGIC				
Alopecia (scalp only)	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	NA	NA
Bruising	Localized to one area	Localized to more than one area	Generalized	NA
Cellulitis	NA	Non-parenteral treatment indicated (e.g., oral antibiotics, antifungals, antivirals)	IV treatment indicated (e.g., IV antibiotics, antifungals, antivirals)	Life-threatening consequences (e.g., sepsis, tissue necrosis)
Hyperpigmentation	Slight or localized causing no or minimal interference with	Marked or generalized causing greater than minimal interference with	NA	NA

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life -threatening
	usual social & functional activities	usual social & functional activities		
Hypopigmentation	Slight or localized causing no or minimal interference with usual social & functional activities	Marked or generalized causing greater than minimal interference with usual social & functional activities	NA	NA
Petechiae	Localized to one area	Localized to more than one area	Generalized	NA
Pruritus [3] (without skin lesions)	Itching causing no or minimal interference with usual social & functional activities	Itching causing greater than minimal interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities	NA
Rash Specify type, if applicable	Localized rash	Diffuse rash OR Target lesions	Diffuse rash AND Vesicles or limited number of bullae or superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Stevens-Johnson syndrome OR Toxic epidermal necrolysis
ENDOCRINE AND METABOLIC				
Diabetes Mellitus	Controlled without medication	Controlled with medication OR Modification of current medication regimen	Uncontrolled despite treatment modification OR Hospitalization for immediate glucose control indicated	Life-threatening consequences (e.g., ketoacidosis, hyperosmolar nonketotic coma, end organ failure)
Gynecomastia	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing pain with greater than minimal interference with usual social & functional activities	Disfiguring changes AND Symptoms requiring intervention or causing inability to perform usual social & functional activities	NA

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life -threatening
Hyperthyroidism	No symptoms AND Abnormal laboratory value	Symptoms causing greater than minimal interference with usual social & functional activities OR Thyroid suppression therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., thyroid storm)
Hypothyroidism	No symptoms AND Abnormal laboratory value	Symptoms causing greater than minimal interference with usual social & functional activities OR Thyroid replacement therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., myxedema coma)
Lipoatrophy[4]	Detectable by study participant, caregiver, or physician AND Causing no or Minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	Disfiguring changes	NA
Lipohypertrophy[5]	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	Disfiguring changes	NA
GASTROINTESTINAL				
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated (e.g., tube feeding, total parenteral nutrition)
Ascites	No symptoms	Symptoms AND Intervention indicated (e.g.,	Symptoms recur or persist despite intervention	Life-threatening consequences

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life -threatening
		diuretics, therapeutic paracentesis)		
Bloating or Distension *Report only one*	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Cholecystitis	NA	Symptoms AND Medical intervention indicated	Radiologic, endoscopic, or operative intervention indicated	Life-threatening consequences (e.g., sepsis, perforation)
Constipation	NA	Persistent Constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated	Life-threatening consequences (e.g., obstruction)
Diarrhea ≥ 1 year of age	Transient or Intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 to 6 stools over baseline per 24-hour period	Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
Dysphagia or Odynophagia *Report only one and specify location*	Symptoms but able to eat usual diet	Symptoms causing altered dietary intake with no intervention indicated	Symptoms causing severely altered dietary intake with intervention indicated	Life-threatening reduction in oral intake
Gastrointestinal Bleeding	Not requiring intervention other than iron supplement	Endoscopic intervention indicated	Transfusion indicated	Life-threatening consequences (e.g., hypotensive shock)
Mucositis or Stomatitis *Report only one and specify location*	Mucosal erythema	Patchy pseudomembranes or ulcerations	Confluent pseudomembranes or ulcerations OR Mucosal bleeding with minor trauma	Life-threatening consequences (e.g., aspiration, choking) OR Tissue necrosis OR Diffuse spontaneous mucosal bleeding
Nausea	Transient (< 24 hours) or intermittent AND No or minimal	Persistent nausea resulting in decreased oral intake for 24 to 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Rehydration	Life-threatening consequences (e.g., hypotensive shock)

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life -threatening
	interference with oral intake		indicated (e.g., IV fluids)	
Pancreatitis	NA	Symptoms with hospitalization not indicated	Symptoms with hospitalization indicated	Life-threatening consequences (e.g., circulatory failure, hemorrhage, sepsis)
Perforation (colon or rectum)	NA	NA	Intervention indicated	Life-threatening consequences
Proctitis	Rectal discomfort with no intervention indicated	Symptoms causing greater than minimal interference with usual social & functional activities OR Medical intervention indicated	Symptoms causing inability to perform usual social & functional activities OR Operative intervention indicated	Life-threatening consequences (e.g., perforation)
Rectal Discharge	Visible discharge	Discharge requiring the use of pads	NA	NA
Vomiting	Transient or intermittent AND No or minimal interference with oral intake	Frequent episodes with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
MUSCULOSKELETAL				
Arthralgia	Joint pain causing no or minimal interference with usual social & functional activities	Joint pain causing greater than minimal interference with usual social & functional activities	Joint pain causing inability to perform usual social & functional activities	Disabling joint pain causing inability to perform basic self-care functions
Arthritis	Stiffness or joint swelling causing no or minimal interference with usual social & functional activities	Stiffness or joint swelling causing greater than minimal interference with usual social & functional activities	Stiffness or joint swelling causing inability to perform usual social & functional activities	Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Myalgia (generalized)	Muscle pain causing no or Minimal interference with usual social & functional activities	Muscle pain causing greater than minimal interference with usual social & functional activities	Muscle pain causing inability to perform usual social & functional activities	Disabling muscle pain causing inability to perform basic self-care functions

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life -threatening
Osteonecrosis	NA	No symptoms but with radiographic findings AND No operative intervention indicated	Bone pain with radiographic findings OR Operative intervention indicated	Disabling bone pain with radiographic findings causing inability to perform basic self-care functions
Osteopenia[6] ≥ 30 years of age < 30 years of age	BMD t-score -2.5 to -1 BMD z-score -2 to -1	NA NA	NA NA	NA NA
Osteoporosis[6] ≥ 30 years of age < 30 years of age	NA NA	BMD t-score < -2.5 BMD z-score < -2	Pathologic fracture (e.g., compression fracture causing loss of vertebral height) Pathologic fracture (e.g., compression fracture causing loss of vertebral height)	Pathologic fracture causing life-threatening consequences Pathologic fracture causing life-threatening consequences
NEUROLOGIC				
Acute CNS Ischemia	NA	NA	Transient ischemic attack	Cerebral vascular accident (e.g., stroke with neurological deficit)
Altered Mental Status *(for Dementia, see Cognitive, Behavioral, or Attentional Disturbance below)*	Changes causing no or minimal interference with usual social & functional activities	Mild lethargy or somnolence causing greater than minimal interference with usual social & functional activities	Confusion, memory impairment, lethargy, or somnolence causing inability to perform usual social & functional activities	Delirium OR Obtundation OR Coma
Ataxia	Symptoms causing no or minimal interference with usual social & functional activities OR No symptoms with ataxia detected on examination	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Disabling symptoms causing inability to perform basic self-care functions
Cognitive, Behavioral, or Attentional Disturbance (includes dementia and attention deficit disorder) Specify type, if applicable	Disability causing no or minimal interference with usual social & functional activities OR Specialized resources not indicated	Disability causing greater than minimal interference with usual social & functional activities OR Specialized resources on	Disability causing inability to perform usual social & functional activities OR Specialized resources on a fulltime basis indicated	Disability causing inability to perform basic self-care functions OR Institutionalization indicated

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life -threatening
		parttime basis indicated		
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated OR Headache with significant impairment of alertness or other neurologic function
Neuromuscular Weakness (includes myopathy and neuropathy) Specify type, if applicable	Minimal muscle weakness causing no or minimal interference with usual social & functional activities OR No symptoms with decreased strength on examination	Muscle weakness causing greater than minimal interference with usual social & functional activities	Muscle weakness causing inability to perform usual social & functional activities	Disabling muscle weakness causing inability to perform basic self-care functions OR Respiratory muscle weakness impairing ventilation
Neurosensory Alteration (includes paresthesia and painful neuropathy) Specify type, if applicable	Minimal paresthesia causing no or minimal interference with usual social & functional activities OR No symptoms with sensory alteration on examination	Sensory alteration or paresthesia causing greater than minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing inability to perform usual social & functional activities	Disabling sensory alteration or paresthesia causing inability to perform basic self-care functions
Seizures New Onset Seizure ≥ 18 years of age	NA	NA	1 to 3 seizures	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
Pre-existing Seizure	NA	Increased frequency from previous level of control without change in seizure character	Change in seizure character either in duration or quality (e.g., severity or focality)	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life -threatening
Syncope	Near syncope without loss of consciousness (e.g., pre-syncope)	Loss of consciousness with no intervention indicated	Loss of consciousness AND Hospitalization or intervention required	NA
PREGNANCY, PUERPERIUM, AND PERINATAL				
Stillbirth (report using mother's participant ID) *Report only one*	NA	NA	Fetal death occurring at ≥ 20 weeks gestation	NA
Preterm Birth (report using mother's participant ID)	Live birth at 34 to < 37 weeks gestational age	Live birth at 28 to < 34 weeks gestational age	Live birth at 24 to < 28 weeks gestational age	Live birth at < 24 weeks gestational age
Spontaneous Abortion or Miscarriage[7] (report using mother's participant ID) *Report only one*	Chemical pregnancy	Uncomplicated spontaneous abortion or miscarriage	Complicated spontaneous abortion or miscarriage	NA
PSYCHIATRIC				
Insomnia	Mild difficulty falling asleep, staying asleep, or waking up early causing no or minimal interference with usual social & functional activities	Moderate difficulty falling asleep, staying asleep, or waking up early causing more than minimal interference with usual social & functional activities	Severe difficulty falling asleep, staying asleep, or waking up early causing inability to perform usual social & functional activities requiring intervention or hospitalization	NA
Psychiatric Disorders (includes anxiety, depression, mania, and psychosis) Specify disorder	Symptoms with intervention not indicated OR Behavior causing no or minimal interference with usual social & functional activities	Symptoms with Intervention indicated OR Behavior causing greater than minimal interference with usual social & functional activities	Symptoms with Hospitalization indicated OR Behavior causing inability to perform usual social & functional activities	Threatens harm to self or others OR Acute psychosis OR Behavior causing inability to perform basic self-care functions
Suicidal Ideation or Attempt *Report only one*	Preoccupied with thoughts of death AND No wish to kill oneself	Preoccupied with thoughts of death AND Wish to kill oneself with no specific plan or intent	Thoughts of killing oneself with partial or complete plans but no attempt to do so OR Hospitalization indicated	Suicide attempted

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life -threatening
RESPIRATORY				
Acute Bronchospasm	Forced expiratory volume in 1 second or peak flow reduced to ≥ 70 to < 80% OR Mild symptoms with intervention not indicated	Forced expiratory volume in 1 second or peak flow 50 to < 70% OR Symptoms with intervention indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Forced expiratory volume in 1 second or peak flow 25 to < 50% OR Symptoms causing inability to perform usual social & functional activities	Forced expiratory volume in 1 second or peak flow < 25% OR Life-threatening respiratory or hemodynamic compromise OR Intubation
Dyspnea or Respiratory Distress *Report only one*	Dyspnea on exertion with no or minimal interference with usual social & functional activities OR Wheezing OR Minimal increase in respiratory rate for age	Dyspnea on exertion causing greater than minimal interference with usual social & functional activities OR Nasal flaring OR Intercostal retractions OR Pulse oximetry 90 to < 95%	Dyspnea at rest causing inability to perform usual social & functional activities OR Pulse oximetry < 90%	Respiratory failure with ventilator support indicated (e.g., CPAP, BPAP, intubation)
SENSORY				
Hearing Loss ≥ 12 years of age	NA	Hearing aid or intervention not indicated	Hearing aid or intervention indicated	Profound bilateral hearing loss (>80 dB at 2 kHz and above) OR Non-serviceable hearing (i.e., >50 dB audiogram and <50% speech discrimination)
Tinnitus	Symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Symptoms causing inability to perform usual social & functional activities	NA
Uveitis	No symptoms AND Detectable on examination	Anterior uveitis with symptoms OR Medical	Posterior or panuveitis OR Operative	Disabling visual loss in affected eye(s)

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life -threatening
		intervention indicated	Intervention indicated	
Vertigo	Vertigo causing no or minimal interference with usual social & functional activities	Vertigo causing greater than minimal interference with usual social & functional activities	Vertigo causing inability to perform usual social & functional activities	Disabling vertigo causing inability to perform basic self-care functions
Visual Changes (assessed from baseline)	Visual changes causing no or minimal interference with usual social & functional activities	Visual changes causing greater than minimal interference with usual social & functional activities	Visual changes causing inability to perform usual social & functional activities	Disabling visual loss in affected eye(s)
SYSTEMIC				
Acute Allergic Reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with intervention indicated OR Mild angioedema with no intervention indicated	Generalized urticaria OR Angioedema with intervention indicated OR Symptoms of mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR Laryngeal edema
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Cytokine Release Syndrome[8]	Mild signs and symptoms AND Therapy (i.e., antibody infusion) interruption not indicated	Therapy (i.e., antibody infusion) interruption indicated AND Responds promptly to symptomatic treatment OR Prophylactic medications indicated for ≤ 24 hours	Prolonged severe signs and symptoms OR Recurrence of symptoms following initial improvement	Life-threatening consequences (e.g., requiring pressor or ventilator support)
Fatigue or Malaise Report only one	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating symptoms of fatigue or malaise causing inability to perform

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life -threatening
				basic self-care functions
Fever (non-axillary temperatures only)	38.0 to < 38.6°C or 100.4 to < 101.5°F	≥ 38.6 to < 39.3°C or ≥ 101.5 to < 102.7°F	≥ 39.3 to < 40.0°C or ≥ 102.7 to < 104.0°F	≥ 40.0°C or ≥ 104.0°F
Pain[9] (not associated with study agent injections and not specified elsewhere) Specify location	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities	Pain causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Hospitalization indicated
Serum Sickness[10]	Mild signs and symptoms	Moderate signs and symptoms AND Intervention indicated (e.g., antihistamines)	Severe signs and symptoms AND Higher level intervention indicated (e.g., steroids or IV fluids)	Life-threatening consequences (e.g., requiring pressor or ventilator support)
Underweight > 5 to 19 years of age	WHO BMI z-score < -1 to -2	WHO BMI z-score < -2 to -3	WHO BMI z-score < -3	WHO BMI z-score < -3 with life-threatening consequences
Unintentional Weight Loss (excludes postpartum weight loss)	NA	5 to < 9% loss in body weight from baseline	≥ 9 to < 20% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated (e.g., tube feeding, total parenteral nutrition)
URINARY				
Urinary Tract Obstruction	NA	Signs or symptoms of urinary tract obstruction without hydronephrosis or renal dysfunction	Signs or symptoms of urinary tract obstruction with hydronephrosis or renal dysfunction	Obstruction causing life-threatening consequences

2 As per Bazett's formula.
3 For pruritus associated with injections or infusions, see the Site Reactions to Injections and Infusions section.
4 Definition: A disorder characterized by fat loss in the face, extremities, and buttocks.
5 Definition: A disorder characterized by abnormal fat accumulation on the back of the neck, breasts, and abdomen.
6 BMD t and z scores can be found in: Kanis JA on behalf of the World Health Organization Scientific Group (2007). Assessment of osteoporosis at the primary health-care level. Technical Report. World Health Organization Collaborating Centre for Metabolic Bone Diseases, University of Sheffield, UK. 2007: Printed by the University of Sheffield.
7 Definition: A pregnancy loss occurring at < 20 weeks gestational age.
8 Definition: A disorder characterized by nausea, headache, tachycardia, hypotension, rash, and/or shortness of breath.

9 For pain associated with injections or infusions, see the Site Reactions to Injections and Infusions section.
10 Definition: A disorder characterized by fever, arthralgia, myalgia, skin eruptions, lymphadenopathy, marked discomfort, and/or dyspnea.

LABORATORY VALUES* CHEMISTRIES

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life-threatening
Acidosis	NA	pH ≥ 7.3 to < LLN	pH < 7.3 without life-threatening consequences	pH < 7.3 with life-threatening consequences
Albumin, Low (g/dL; g/L)	3.0 to < LLN 30 to < LLN	≥ 2.0 to < 3.0 ≥ 20 to < 30	< 2.0 < 20	NA
Alkaline Phosphatase, High	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Alkalosis	NA	pH > ULN to ≤ 7.5	pH > 7.5 without life-threatening consequences	pH > 7.5 with life-threatening consequences
ALT or SGPT, High *Report only one*	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Amylase (Pancreatic) or Amylase (Total), High *Report only one*	1.1 to < 1.5 x ULN	1.5 to < 3.0 x ULN	3.0 to < 5.0 x ULN	≥ 5.0 x ULN
AST or SGOT, High Report only one	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Bicarbonate, Low (mEq/L; mmol/L)	16.0 to < LLN 16.0 to < LLN	11.0 to < 16.0 11.0 to < 16.0	8.0 to < 11.0 8.0 to < 11.0	< 8.0 < 8.0
Bilirubin Direct Bilirubin[13], High > 28 days of age	NA	NA	> ULN with other signs and symptoms of hepatotoxicity	> ULN with life-threatening consequences (e.g., signs and symptoms of liver failure)
Total Bilirubin, High > 28 days of age	1.1 to < 1.6 x ULN	1.6 to < 2.6 x ULN	2.6 to < 5.0 x ULN	≥ 5.0 x ULN
Calcium, High (mg/dL; mmol/L) ≥ 7 days of age	10.6 to < 11.5 2.65 to < 2.88	11.5 to < 12.5 2.88 to < 3.13	12.5 to < 13.5 3.13 to < 3.38	≥ 13.5 ≥ 3.38
Calcium (Ionized), High (mg/dL; mmol/L)	> ULN to < 6.0 > ULN to < 1.5	6.0 to < 6.4 1.5 to < 1.6	6.4 to < 7.2 1.6 to < 1.8	≥ 7.2 ≥ 1.8

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life-threatening
Calcium, Low (mg/dL; mmol/L) ≥ 7 days of age	7.8 to < 8.4 1.95 to < 2.10	7.0 to < 7.8 1.75 to < 1.95	6.1 to < 7.0 1.53 to < 1.75	< 6.1 < 1.53
Calcium (Ionized), Low (mg/dL; mmol/L)	< LLN to 4.0 < LLN to 1.0	3.6 to < 4.0 0.9 to < 1.0	3.2 to < 3.6 0.8 to < 0.9	< 3.2 < 0.8
Cardiac Troponin I, High	NA	NA	NA	Levels consistent with myocardial infarction or unstable angina as defined by the local laboratory
Creatine Kinase, High	3 to < 6 x ULN	6 to < 10x ULN	10 to < 20 x ULN	≥ 20 x ULN
Creatinine, High ***Report only one*	1.1 to 1.3 x ULN	> 1.3 to 1.8 x ULN OR Increase to 1.3 to < 1.5 x participant's baseline	> 1.8 to < 3.5 x ULN OR Increase to 1.5 to < 2.0 x participant's baseline	≥ 3.5 x ULN OR Increase of ≥ 2.0 x participant's baseline
Creatinine Clearance [14] or eGFR, Low [14]***Report only one*	NA	< 90 to 60 ml/min or ml/min/1.73 m^2 OR 10 to < 30% decrease from participant's baseline	< 60 to 30 ml/min or ml/min/1.73 m^2 OR 30 to < 50% decrease from participant's baseline	< 30 ml/min or ml/min/1.73 m^2 OR ≥ 50% decrease from participant's baseline or dialysis needed
Glucose (mg/dL; mmol/L) Fasting, High Nonfasting, High	110 to 125 6.11 to < 6.95 116 to 160 6.44 to < 8.89	> 125 to 250 6.95 to < 13.89 > 160 to 250 8.89 to < 13.89	> 250 to 500 13.89 to < 27.75 > 250 to 500 13.89 to < 27.75	≥ 500 ≥ 27.75 ≥ 500 ≥ 27.75
Glucose, Low (mg/dL; mmol/L) ≥ 1 month of age	55 to 64 3.05 to <3.55	40 to < 55 2.22 to < 3.05	30 to < 40 1.67 to < 2.22	< 30 < 1.67
Lactate, High	ULN to < 2.0 x ULN without acidosis	≥ 2.0 x ULN without acidosis	Increased lactate with pH < 7.3 without life-threatening consequences	Increased lactate with pH < 7.3 with life-threatening consequences
Lipase, High	1.1 to < 1.5 x ULN	1.5 to < 3.0 x ULN	3.0 to < 5.0 x ULN	≥ 5.0 x ULN
Lipid Disorders (mg/dL; mmol/L) Cholesterol, Fasting, High ≥ 18 years of age	200 to < 240 5.18 to < 6.19	240 to < 300 6.19 to < 7.77	≥ 300 ≥ 7.77	NA

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life-threatening
LDL, Fasting, High ≥ 18 years of age	130 to < 160 3.37 to < 4.12	160 to < 190 4.12 to < 4.90	≥ 190 ≥ 4.90	NA
Triglycerides, Fasting, High	150 to 300 1.71 to 3.42	>300 to 500 >3.42 to 5.7	>500 to < 1,000 >5.7 to 11.4	> 1,000 > 11.4
Magnesium[15], Low (mEq/L; mmol/L)	1.2 to < 1.4 0.60 to < 0.70	0.9 to < 1.2 0.45 to < 0.60	0.6 to < 0.9 0.30 to < 0.45	< 0.6 < 0.30
Phosphate, Low (mg/dL; mmol/L) > 14 years of age	2.0 to < LLN 0.65 to < LLN	1.4 to < 2.0 0.45 to < 0.65	1.0 to < 1.4 0.32 to < 0.45	< 1.0 < 0.32
Potassium, High (mEq/L; mmol/L)	5.6 to < 6.0 5.6 to < 6.0	6.0 to < 6.5 6.0 to < 6.5	6.5 to < 7.0 6.5 to < 7.0	≥ 7.0 ≥ 7.0
Potassium, Low (mEq/L; mmol/L)	3.0 to < 3.4 3.0 to < 3.4	2.5 to < 3.0 2.5 to < 3.0	2.0 to < 2.5 2.0 to < 2.5	< 2.0 < 2.0
Sodium, High (mEq/L; mmol/L)	146 to < 150 146 to < 150	150 to < 154 150 to < 154	154 to < 160 154 to < 160	≥ 160 ≥ 160
Sodium, Low (mEq/L; mmol/L)	130 to < 135 130 to < 135	125 to < 130 125 to < 130	121 to < 125 121 to < 125	≤ 120 ≤ 120
Uric Acid, High (mg/dL; mmol/L)	7.5 to < 10.0 0.45 to < 0.59	10.0 to < 12.0 0.59 to < 0.71	12.0 to < 15.0 0.71 to < 0.89	≥ 15.0 ≥ 0.89
HEMATOLOGY				
Absolute CD4+ Count, Low (cell/mm^3; cells/L) > 5 years of age (not HIV infected)	300 to < 400 300 to < 400	200 to < 300 200 to < 300	100 to < 200 100 to < 200	< 100 < 100
Absolute Lymphocyte Count, Low (cell/mm^3; cells/L) > 5 years of age (not HIV infected)	600 to < 650 0.600 x 10^9 to < 0.650 x 10^9	500 to < 600 0.500 x 10^9 to < 0.600 x 10^9	350 to < 500 0.350 x 10^9 to < 0.500 x 10^9	< 350 < 0.350 x 10^9
Absolute Neutrophil Count (ANC), Low (cells/mm^3; cells/L) > 7 days of age	800 to 1,000 0.800 x 10^9 to 1.000 x 10^9	600 to 799 0.600 x 10^9 to 0.799 x 10^9	400 to 599 0.400 x 10^9 to 0.599 x 10^9	< 400 < 0.400 x 10^9
Fibrinogen, Decreased (mg/dL; g/L)	100 to < 200 1.00 to < 2.00 OR 0.75 to < 1.00 x LLN	75 to < 100 0.75 to < 1.00 OR ≥ 0.50 to < 0.75 x LLN	50 to < 75 0.50 to < 0.75 OR 0.25 to < 0.50 x LLN	< 50 < 0.50 OR < 0.25 x LLN OR Associated with gross bleeding
Hemoglobin[16], Low (g/dL; mmol/L)[17] ≥ 13 years of age (male only)	10.0 to 10.9 6.19 to 6.76	9.0 to < 10.0 5.57 to < 6.19	7.0 to < 9.0 4.34 to < 5.57	< 7.0 < 4.34
≥ 13 years of age (female only)	9.5 to 10.4 5.88 to 6.48	8.5 to < 9.5 5.25 to < 5.88	6.5 to < 8.5 4.03 to < 5.25	< 6.5 < 4.03

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life-threatening
INR, High (not on anticoagulation therapy)	1.1 to < 1.5 x ULN	1.5 to < 2.0 x ULN	2.0 to < 3.0 x ULN	≥ 3.0 x ULN
Methemoglobin (% hemoglobin)	5.0 to < 10.0%	10.0 to < 15.0%	15.0 to < 20.0%	≥ 20.0%
PTT, High (not on anticoagulation therapy)	1.1 to < 1.66 x ULN	1.66 to < 2.33 x ULN	2.33 to < 3.00 x ULN	≥ 3.00 x ULN
Platelets, Decreased (cells/mm^3; cells/L)	100,000 to < 125,000 100.000 x 10^9 to < 125.000 x 10^9	50,000 to < 100,000 50.000 x 10^9 to < 100.000 x 10^9	25,000 to < 50,000 25.000 x 10^9 to < 50.000 x 10^9	< 25,000 < 25.000 x 10^9
PT, High (not on anticoagulation therapy	1.1 to < 1.25 x ULN	1.25 to < 1.50 x ULN	1.50 to < 3.00 x ULN	≥ 3.00 x ULN
WBC, Decreased (cells/mm^3; cells/L) > 7 days of age	2,000 to 2,499 2.000 x 10^9 to 2.499 x 10^9	1,500 to 1,999 1.500 x 10^9 to 1.999 x 10^9	1,000 to 1,499 1.000 x 10^9 to 1.499 x 10^9	< 1,000 < 1.000 x 10^9
URINALYSIS				
Glycosuria (random collection tested by dipstick)	Trace to 1+ or ≤ 250 mg	2+ or > 250 to ≤ 500 mg	> 2+ or > 500 mg	NA
Hematuria (not to be reported based on dipstick findings or on blood believed to be of menstrual origin)	6 to < 10 RBCs per high power field	≥ 10 RBCs per high power field	Gross, with or without clots OR With RBC casts OR Intervention indicated	Life-threatening consequences
Proteinuria (random collection tested by dipstick)	1+	2+	3+ or higher	NA

* Reminder: An asymptomatic abnormal laboratory finding without an accompanying AE should not be reported to DAIDS in an expedited time frame unless it meets protocol-specific reporting requirements.

13 Direct bilirubin >1.5 mg/dL in a participant < 28 days of age should be graded as grade 2, if < 10% of the total bilirubin.

** Reminder: Choose the method that selects for the higher grade.

14 Use the applicable formula (ie, Cockcroft-Gault in mL/min or Schwartz, MDRD, CKD-Epi in mL/min/1.73m^2). Sites should choose the method defined in their study and when not specified, use the method most relevant to the study population.

15 To convert a magnesium value from mg/dL to mmol/L, laboratories should multiply by 0.4114.

16 Male and female sex are defined as sex at birth. For transgender participants ≥13 years of age who have been on hormone therapy for more than 6 consecutive months, grade hemoglobin based on the gender with which they identify (ie, a transgender female should be graded using the female sex at birth hemoglobin laboratory values).

17 The most commonly used conversion factor to convert g/dL to mmol/L is 0.6206. For grading hemoglobin results obtained by an analytic method with a conversion factor other than 0.6206, the result must be converted to g/dL using appropriate conversion factor for the particular laboratory.

APPENDIX 5. PREGNANCY PRECAUTIONS AND THE DEFINITION OF CHILDBEARING POTENTIAL AND CONTRACEPTIVE REQUIREMENTS

Pregnancy Precautions

The effect of ABI-4334 on pregnancy, including preclinical development and reproductive toxicology, has not been assessed. As such, pregnancy precautions are required and contraceptive requirements for female subjects of childbearing potential are described below.

Definition of Female Subjects of Childbearing Potential

As defined in this protocol, "women of childbearing potential" are female subjects who are physiologically capable of becoming pregnant. Conversely, "women of no childbearing potential" are defined as female subjects meeting any of the following criteria:

- Surgically sterile (ie, through bilateral salpingectomy, bilateral oophorectomy, or hysterectomy) or with medically documented ovarian failure
- Postmenopausal, defined as ≥ 54 years of age with no spontaneous menses for ≥ 12 months without alternative medical condition

If female subject is < 54 years of age with no spontaneous menses for ≥ 12 months without alternative medical condition, then follicle-stimulating hormone (FSH) levels should be consistent with menopause at Screening.

Documentation to determine childbearing potential can come from the site personnel's review of the subject's medical records, medical examination, or medical history interview.

Contraceptive Requirements

Note that the drug-drug interaction of ABI-4334 and systemic (oral/injectable/implantable) hormonal contraceptives has not been assessed and so systemic hormonal contraceptives are not considered an effective contraceptive method for female subjects of childbearing potential for the purposes of this study.

Female subjects of childbearing potential must have a negative serum pregnancy test at Screening and Day -1 and a negative urine pregnancy test prior to receiving the first dose of study drug on Day 1. Subjects will be instructed to notify the Investigator if they become pregnant at any time during the study, or if they become pregnant within 28 days of the last dose of study drug. Instructions for reporting pregnancy and pregnancy outcomes are outlined in Section 9.9 of the protocol.

1) Female subjects of childbearing potential (defined above) must agree to use two effective contraceptive methods, or complete abstinence for the duration of the study and follow-up.
    - Effective contraceptive methods (two are required):

- Male OR female condom, but not together due to increased risk of breakage
- Intra-uterine device (hormonal or copper IUD)
- Diaphragm or cervical cap with spermicide (where locally available).
- Vasectomy of male partner at least 6 months prior to the first study drug administration
- Bilateral tubal ligation

- Complete abstinence from heterosexual intercourse of reproductive potential.
  - Note: Abstinence does not require a second contraceptive method and is an acceptable method of contraception only if it is in line with the subject's preferred and usual lifestyle. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

2) Female subjects must avoid egg donation from Screening through at least 28 days after administration of the last dose of study drug. All male subjects that are able to procreate (if their female partner is of childbearing potential) must agree to use two effective contraceptive methods with their female partners, or complete abstinence for the duration of the study and follow-up.
   - Please refer to the contraceptive requirements listed above. Note: In the case of female partners of male subjects, systemic hormonal birth control may be utilized.

Male subjects must avoid sperm donation from Screening through at least 90 days after administration of the last dose of study drug.

APPENDIX 6. PROTOCOL AMENDMENT SUMMARY OF CHANGES

Not Applicable.

Study Title: A Phase 1, Blinded, Placebo-controlled Study of the Safety, Tolerability, Pharmacokinetics of Single- and Multiple-Ascending Doses of ABI-4334 in Healthy Subjects

NCT Number: NCT05569941

Date of Document: 11 May 2023

STATISTICAL ANALYSIS PLAN

Sponsor:	Assembly Biosciences, Inc 331 Oyster Point Blvd South San Francisco, CA 94030
Protocol Number:	ABI-4334-101
Protocol Title:	A Phase 1, Blinded, Placebo-controlled Study of the Safety, Tolerability, Pharmacokinetics of Single- and Multiple-Ascending Doses of ABI-4334 in Healthy Subjects
Product:	ABI-4334
Protocol Version (Date):	Original (16 June 2022)
Indication:	Chronic Hepatitis B Virus Infection
Analysis Type:	Final Analysis
Analysis Plan Version (Date):	Version 2.0 (11 May 2023)
Analysis Plan Author:	[redacted]

CONFIDENTIAL AND PROPRIETARY INFORMATION

STATISTICAL ANALYSIS PLAN APPROVAL FORM

Protocol Title:	A Phase 1, Blinded, Placebo-controlled Study of the Safety, Tolerability, Pharmacokinetics of Single- and Multiple-Ascending Doses of ABI-4334 in Healthy Subjects
Protocol Number:	ABI-4334-101
SAP Version (Date):	Version 2.0 (11 May 2023)

The SAP was subject to critical review and has been approved.

Name and Title	Approval Signature/Date
[redacted] Assembly Biosciences	See e-signature
[redacted] Assembly Biosciences	See e-signature
[redacted] Assembly Biosciences	See e-signature

DOCUMENT HISTORY

Version	Date (DD MMM YYYY)	Summary of Changes
1.0	30 NOV 2022	Original
2.0	11 MAY 2023	Adjusted for study completion and CSR

TABLE OF CONTENTS

DOCUMENT HISTORY 3
TABLE OF CONTENTS 4
LIST OF ABBREVIATIONS 6
1 INTRODUCTION 8
1.1.1 Study Objectives 8
1.2 Study Design 8
1.2.1 Part A (SAD in Healthy Subjects) 9
1.2.2 Part B (MAD in Healthy Subjects) 11
1.3 Sample Size and Power 12
1.4 Methods of Assigning Subjects to Treatment 12
1.4.1 Randomization 12
1.4.2 Blinding 12
2 TYPE OF PLANNED ANALYSIS 14
2.1 DRC Interim Analyses 14
2.2 Final Analysis 14
3 GENERAL CONSIDERATIONS FOR DATA ANALYSES 15
3.1 Analysis Populations 15
3.1.1 Randomized Set 15
3.1.2 Full Analysis Set 15
3.1.3 Safety Analysis Set 15
3.1.4 PK-Related Sets 15
3.2 Subject Grouping 15
3.3 Data Handling Conventions and Transformations 16
3.4 Missing Data and Outliers 16
3.4.1 Missing Data 16
3.4.2 Outliers 16
3.5 Analytic Definitions 16
3.5.1 Definition of Study Drug 16
3.5.2 Definition of Study Period 16
3.5.2.1 All Cohorts Except Cohort A7 16
3.5.2.2 Cohort A7 16
3.5.3 Definition of Study Day 17
3.5.3.1 All Cohorts Except Cohort A7 17
3.5.3.2 Cohort A7 17
3.5.4 Definition of Baseline 18
3.5.5 Analysis Visit Windows 18
3.5.6 Selection of Data in the Event of Multiple Records on Same Analysis Visit Day 18
4 SUBJECT DISPOSITION 19
4.1 Subject Enrollment and Disposition 19
4.2 Protocol Deviations 19
4.3 Extent of Study Drug Exposure and Compliance 20
4.3.1 Duration of Exposure to Study Drug 20
4.3.2 Study Drug On-Treatment Compliance 20
4.4 Meal Times and PK Sample Collection 21
4.5 Medical History 21

4.6 Demographics and Baseline Characteristics 21
5 SAFETY ANALYSES 22
5.1 Adverse Events and Deaths 22
5.1.1 Adverse Event Dictionary 22
5.1.2 Adverse Event Severity 22
5.1.3 Relationship of Adverse Events to Study Drug 22
5.1.4 Serious Adverse Events 22
5.1.5 Treatment-Emergent Adverse Events 22
5.1.5.1 Definition of Treatment-Emergent Adverse Events 22
5.1.5.2 Incomplete Dates 22
5.1.6 Summaries of Adverse Events and Deaths 23
5.2 Laboratory Evaluations 24
5.2.1 Graded Laboratory Values 24
5.2.1.1 Treatment-Emergent Laboratory Abnormalities 25
5.2.1.2 Summaries of Laboratory Abnormalities 25
5.2.2 Ungraded Laboratory Values 25
5.2.3 Summaries of Numeric Laboratory Results 25
5.2.4 Summaries of Categorical Laboratory Results 26
5.3 Body Weight and Vital Signs 26
5.4 Prior and Concomitant Medications 26
5.4.1 Prior Medications 26
5.4.2 Concomitant Medications 27
5.5 Electrocardiogram Results 28
5.6 Other Safety Measures 28
6 PK ANALYSES 29
7 REFERENCES 30
8 SOFTWARE 31
9 APPENDICES 32

LIST OF IN-TEXT FIGURES

Figure 1. Overview of Study Design – Parts A & B (SAD and MAD) 9
Figure 2. Study Design Schematic – Part A (SAD in Healthy Subjects) 10
Figure 3. Study Design Schematic – Part B (MAD in Healthy Subjects) 12

LIST OF ABBREVIATIONS

AE	adverse event
ALT	alanine aminotransferase
ATC	anatomical therapeutic chemical
BMI	body mass index
CRO	clinical research organization
CSR	clinical study report
DAIDS	Division of AIDS
DRC	data review committee
ECG	Electrocardiogram
eCRF	case report form
FAS	full analysis set
FIH	first-in-human
HLGT	high-level group term
HLT	high-level term
IPD	important protocol deviation(s)
LLOQ	lower limit of quantitation
LLT	lower-level term
LOD	limit of detection
LOQ	limit of quantitation
MAD	multiple ascending dose
MedDRA	medical dictionary for regulatory activities
NOEL	No observed effect level
PBO	Placebo
PD	protocol deviation(s)
PK	pharmacokinetics
PKAP	PK analysis plan
PT	preferred term
Q1, Q3	first quartile, third quartile
QD	once daily
SAD	single ascending dose
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SE	standard error
SOC	system organ class
TE	treatment-emergent
TEAE	treatment-emergent adverse event

TFLs	tables, figures, and listings
WHO	world health organization

1 INTRODUCTION

This statistical analysis plan (SAP) describes the statistical analysis methods and data presentations to be used in tables, figures, and listings (TFLs) in the clinical study report (CSR) for Study ABI-4334-101. This SAP is based on the original study protocol version 1.0, dated 16 June 2022 and the electronic case report form (eCRF) version 1.0, dated 18 October 2022. The SAP will be finalized before database lock. Any changes made after the finalization of the SAP will be documented in the CSR.

1.1.1 Study Objectives

The primary objective is:

- To assess the safety and tolerability of orally administered ABI-4334 in healthy subjects following single (Part A, single ascending dose [SAD]) and 8-day multiple (Part B, multiple ascending dose [MAD]) oral doses.

The secondary objectives are:

- To characterize the pharmacokinetics (PK) of ABI-4334 in plasma following single doses and 8-day multiple doses in healthy subjects.
- To evaluate the effect of food on the PK of ABI-4334 following a single dose in healthy subjects (Part A food-effect cohort(s) only).

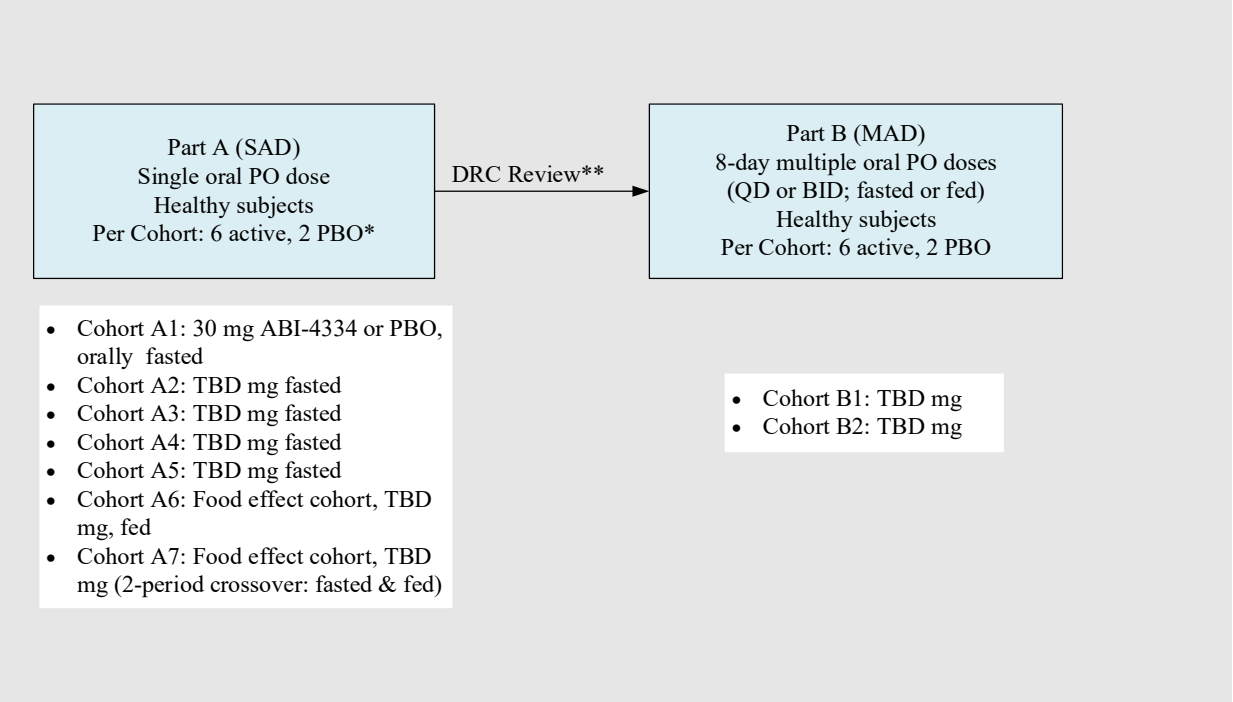
The exploratory objectives are:

- To evaluate the PK of ABI-4334 in urine following 8-day multiple doses in healthy subjects (Part B, MAD).

1.2 Study Design

This is a Phase 1, first-in-human (FIH), randomized, blinded, placebo (PBO)-controlled, single- and multiple-dose, dose escalation study of ABI-4334 in healthy subjects. Part A is designed to assess the safety, tolerability, and PK profile of ABI-4334 following SAD in healthy subjects, as well as the effect of food on ABI-4334. Part B is designed to assess the safety, tolerability, and PK profile of ABI-4334 following MAD in healthy subjects. Figure 1**Figure 1** provides an overview of the 2-Part study design.

Figure 1. Overview of Study Design – Parts A & B (SAD and MAD)



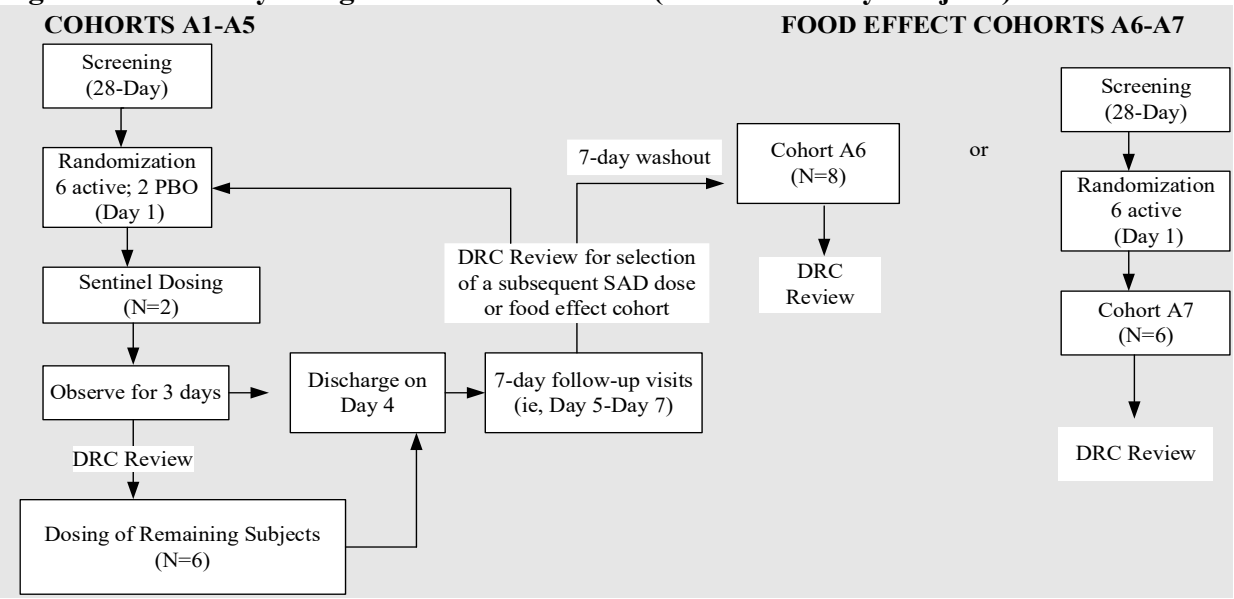
Abbreviations: BID=twice daily; MAD=multiple ascending dose; PBO=placebo; QD=once daily; SAD=single ascending dose; TBD=to be determined
*Applies to all SAD cohorts, except Cohort A7, which will only have 6 active subjects (ie, randomized to ABI-4334), no PBO subjects.
**Part B dosing may commence prior to Part A being completed.

1.2.1 Part A (SAD in Healthy Subjects)

Part A of the study will enroll up to 5 fasted cohorts, ie, Cohorts A1-A5, and up to 2 fed cohorts to evaluate food effect, ie, Cohort A6 (with subjects from a previous fasted cohort), and an additional cohort, ie, Cohort A7, if needed (with new subjects).

The first cohort of Part A, ie, Cohort A1, will evaluate a single dose of 30 mg ABI-4334. Dose levels for subsequent cohorts will be determined based on interim cohort review performed by an unblinded Data Review Committee (DRC) between SAD cohorts for purposes of dose escalation. In no case would a dose escalation be greater than 3.3-fold compared with the previous dose. The maximum dose of ABI-4334 to be evaluated in Part A (SAD) will not exceed the exposures observed in dogs at the no observed effect level (NOEL) dose of 30 mg/kg/day, or 1000 mg/day, whichever is lower. Figure 2 provides an overview of Part A study design.

Figure 2. Study Design Schematic – Part A (SAD in Healthy Subjects)



Abbreviations: DRC=Data Review Committee; PBO=placebo; SAD=single ascending dose

Each of the Cohorts A1-A5 will enroll 8 healthy subjects who will be randomized in a 6:2 ratio to receive a single oral dose of the study drug, ie, ABI-4334 (6 subjects) or PBO (2 subjects) in a fasted state, followed by a 7-day follow-up period. Subjects will remain confined to the study clinic from Days -1 to 4, with outpatient follow-up visits. Dosing of a subsequent SAD cohort can begin at least 8 days after dosing of the sentinel subjects from the previous cohort (see below).

Because this is a FIH study, a sentinel dosing approach will be utilized for each Cohort A1-A5, where initially only 2 subjects per cohort will be dosed (one receiving ABI-4334 and one receiving PBO). These sentinel subjects will be observed for a minimum of 3 days postdose to ensure acceptable tolerability of the study drug, as determined by the DRC, before dosing of the remaining subjects within the cohort can proceed.

The DRC will select a single dose level from Cohorts A1-A5 for evaluation of food effect. The selection of this dose level can be made prior to completion of all fasted SAD cohorts and will be based on a preliminary evaluation by the DRC of the available safety, tolerability, and PK data from selected Cohorts A1-A5. Subjects who complete participation in this selected cohort will be asked to return to the study site to be enrolled into the food-effect cohort (ie, Cohort A6), following a minimum of 7-day washout period since their last study drug dose. While Baseline evaluations will be conducted prior to dosing in Cohort A6, repeat assessment of all study eligibility criteria will not be performed. Returning subjects will retain their original subject number and randomized treatment assignment. After the Day 7 visit in the original cohort (from Cohort A1-A5), the study drug will be administered 30 minutes after the complete consumption of a high fat meal (approximately 50% calories from fat).

If there is an insufficient number of subjects from previous fasted SAD cohorts (ie, A1-A5), who are willing to participate in Cohort A6, then a separate Cohort A7 may be enrolled, comprising 6 new subjects meeting the study eligibility criteria, who will all receive ABI-4334. Cohort A7 will

involve a two-period crossover assessment of food effect (ie, Period 1: dosing under fasted or high fat-fed conditions; Period 2: dosing under condition opposite of Period 1), with a minimum 7-day washout period between dosing in Period 1 and Period 2, and a 7-day postdose follow-up in Period 2.

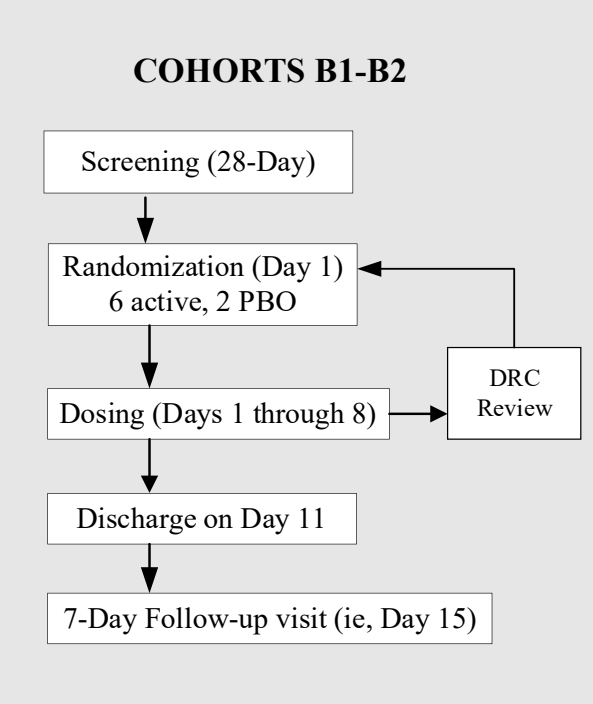
1.2.2 Part B (MAD in Healthy Subjects)

Part B dosing may commence prior to Part A being completed. The dose level and dosing schedule for the first cohort of Part B, ie, Cohort B1, will be determined based on emergent safety, tolerability, and PK data from completed Part A cohorts, with the daily dose at least 2-fold lower than a Part A dose level deemed safe and tolerable.

Both cohorts planned for Part B, ie, Cohort B1 and Cohort B2, will enroll 8 healthy subjects who will be randomized in a 6:2 ratio to receive oral daily doses of the study drug, ie, ABI-4334 (6 subjects) or PBO (2 subjects), for 8 consecutive days, followed by a 7-day follow-up period. Dosing may occur under fasted or fed conditions, depending on the results from the food-effect cohort(s) in Part A. Subjects will remain confined to the study site from Days -1 to Day 11 (ie, 3 days post last day of dosing), with outpatient follow-up visits.

The dose level and dosing schedule for Cohort B2 will be based on an evaluation by the DRC of safety data from Day 1 through Day 8 for Cohort B1 and all available safety data for preceding cohorts. In addition, PK data through 24 hours after dosing on Day 8 for Cohort B1 and available PK data for preceding cohorts will be reviewed. In no case would the dose escalation be greater than 3.3-fold compared with the previous dose. Dosing of Cohort B2 can begin at least 11 days after the first day dosing of subjects in Cohort B1. The maximum dose of ABI-4334 to be evaluated in Part B (MAD) will not exceed the exposures observed in dogs at the NOEL dose of 30 mg/kg/day, or 1000 mg/day, whichever is lower. Figure 3 provides an overview of Part B study design.

Figure 3. Study Design Schematic – Part B (MAD in Healthy Subjects)



Abbreviations: DRC=Data Review Committee; PBO=placebo

1.3 Sample Size and Power

Approximately 8 healthy subjects will be enrolled in each cohort. Approximately 6 subjects will be enrolled in Cohort 7 of Part A if necessary. For this FIH study, the sample sizes are similar to those previously utilized for this type of study and are not based on statistical considerations.

1.4 Methods of Assigning Subjects to Treatment

1.4.1 Randomization

For cohorts A1-A4 and B1-B2, all subjects will be assigned a unique subject number at Screening and will be randomized to treatment with either ABI-4334 or placebo, upon meeting all protocol eligibility requirements. Randomization will be performed by an unblinded pharmacist. Subjects returning as part of cohort A6 will retain their original subject number and randomized treatment assignment.

Subjects in cohort A7 will take only ABI-4334. Subjects will be randomized, with 3 subjects being fed then fasted, and 3 subjects fasted then fed, in each of the two periods.

1.4.2 Blinding

Except for DRC members, unblinded site pharmacists, and other personnel administering treatment randomization, all study team members will be blinded to individual subject treatment assignments for cohorts A1-A4 and cohorts B1-B2 throughout the duration of the study. The

study subjects will also be blinded to their treatment assignment throughout the duration of the study.

The DRC will be unblinded to individual subjects' treatment assignments throughout the study to ensure timely analysis of any emergent safety and tolerability issues, and completion of the prespecified safety and PK data reviews. The unblinded individuals will be identified in the DRC charter along with their specific roles and responsibilities. The clinical research organization's (CRO) Medical Monitor may be unblinded to individual subjects' treatment assignments where required for assessment of emergent safety/tolerability issues.

2 TYPE OF PLANNED ANALYSIS

2.1 DRC Interim Analyses

The DRC will review the cumulative safety and PK data from each previous cohort to determine dose level and dosing schedule for the subsequent cohort(s). Details concerning the DRC will be documented in the DRC charter.

2.2 Final Analysis

The final analysis will be performed after all subjects have completed the study, outstanding data queries have been resolved or adjudicated as unresolvable, and the data have been cleaned and finalized.

3 GENERAL CONSIDERATIONS FOR DATA ANALYSES

Analysis results will be presented using descriptive statistics. For categorical variables, the number and percentage of subjects in each category will be presented; for continuous variables, the number of subjects (n), mean, standard deviation (SD) or standard error (SE), median, first quartile (Q1), third quartile (Q3), minimum, and maximum will be presented.

Summary tables will be presented by cohort and treatment. For cohort A7, summary tables will be presented fasting order (i.e. “fed,fasted” or “fasted,fed”).

No formal inference is planned in this study. Hence, no multiplicity adjustment is required.

3.1 Analysis Populations

Analysis populations or sets define the subjects to be included in an analysis. Analysis sets and their definitions are provided in this section. The analysis set will be identified and included as a subtitle of each table, figure, and listing. For each analysis set, the number and percentage of subjects eligible for inclusion will be summarized by treatment and cohort. A listing of reasons for exclusion from analysis sets will be provided by cohort and subject.

3.1.1 Randomized Set

Randomized Set includes all randomized subjects, classified according to the treatment to which they were randomized, regardless of the actual treatment received.

3.1.2 Full Analysis Set

The Full Analysis Set (FAS) includes all randomized subjects, classified according to the treatment to which they were randomized, regardless of the actual treatment received, who took at least 1 dose of study drug.

3.1.3 Safety Analysis Set

The Safety Analysis Set includes all randomized subjects, classified according to the actual treatment received regardless of randomized assignment, who took at least 1 dose of study drug. This is the main analysis set for all safety analyses.

3.1.4 PK-Related Sets

PK-related sets will be described in a separate PK analysis plan (PKAP).

3.2 Subject Grouping

For analyses based on the Randomized Set and FAS, subjects will be grouped according to the treatment to which they were randomized, regardless of actual treatment received. For analyses based on the Safety Analysis Set, subjects will be grouped according to the actual treatment

received, regardless of random assignment. The actual treatment received will differ from the randomized treatment only when the actual treatment differs from randomized treatment for the entire treatment duration.

3.3 Data Handling Conventions and Transformations

Subject age, collected at the Screening visit, will be used for analyses and will be presented in listings.

Total bilirubin values entered as < 0.2 mg/dL will be analyzed as 0.1 mg/dL; direct bilirubin values entered as < 0.1 mg/dL will be analyzed as 0.05 mg/dL (according to the methods of Nehls and Akland, 1973).

3.4 Missing Data and Outliers

3.4.1 Missing Data

In general, missing data will not be imputed unless methods for handling missing data are specified. Exceptions are presented in this document.

For missing last dose date of study drug, imputation rules are described in Section 4.3.1. The handling of missing or incomplete dates for adverse event (AE) onset is described in Section 5.1.5.2, and for prior and concomitant medications in Section 5.3.

3.4.2 Outliers

Outliers will be identified during the data management review process. No sensitivity analyses will be conducted. All data will be included in the data analysis.

3.5 Analytic Definitions

3.5.1 Definition of Study Drug

The study drugs in this study are ABI-4334 and PBO.

3.5.2 Definition of Study Phase

3.5.2.1 All Cohorts Except Cohort A7

The following periods will be defined for all cohorts except cohort A7:

- On-Treatment Period: From first dose date/time through last dose date/time.
- Follow-Up Period: From last dose date/time +1 through end of study.

3.5.2.2 Cohort A7

The following periods will be defined for cohort A7:

- On-Treatment Period 1: Period 1 dose date
- Follow-Up Period 1: From Period 1 dose date +1 until Period 2 dose date.
- On-Treatment Period 2: Period 2 dose date.
- Follow-Up Period 2: From period 2 dose date +1 through end of study.

For each subject, end of study will be defined as completion of the last follow-up visit or premature study termination, or subject is lost-to-follow-up.

The study will end when the last subject completes the last visit of the follow-up period or is considered "lost to follow-up," whichever is later.

3.5.3 Definition of Study Day

The first dose date of study drug will be calculated where applicable. The last dose date of study drug will be calculated where applicable. The last dose date of study drug will be the end date on study drug administration eCRF.

3.5.3.1 All Cohorts Except Cohort A7

Study Day 1 is defined as the first dose date of study drug. If the first dose date is missing, the date of randomization will be used.

For the On-Treatment Period, study days will be calculated from Study Day 1 and derived as follows:

- For days prior to the first dose: Assessment Date – Study Day 1.
- For postdose study days: Assessment Date – Study Day 1 + 1.

For the Follow-Up Period, the Follow-Up Study Day 1 will be defined as the day after the last dose date of study drug and the follow-up study day will be calculated from the last dose date and derived as Assessment Date − Last Dose Date of study drug.

3.5.3.2 Cohort A7

Period 1 Study Day 1 is defined as the period 1 dose date of study drug. Period 2 Study Day 1 is defined as the period 2 dose date of study drug.

Study days will be calculated for each study period.

In On-Treatment Period 1, for days prior to the first dose, Period 1 study days will be derived as Assessment Date – Period 1 Study Day 1.

For the Follow-Up Period 1, the Follow-Up Period 1 Study Day 1 will be defined as the day after the Period 1 study drug dose date and Follow-Up Period 1 study days will be calculated from Follow-Up Period 1 Study Day 1 and derived as Assessment Date − Follow-Up Period 1 Study Day 1 + 1. Follow-Up Period 2 will be treated similarly.

3.5.4 Definition of Baseline

For all analyses, Baseline will be defined as the last non-missing evaluation prior to the first dose date/time. For cohort A7, Period 2 Baseline will be defined as last non-missing evaluation prior to the dosing on Period 2 Study Day 1.

For all cohorts, except cohort A7 Period 2, Change from Baseline will be defined as the result at desired timepoint minus the result at Baseline. For cohort A7 Period 2, Change from Baseline will be defined as the result at desired timepoint minus the result at Period 2 Baseline.

3.5.5 Analysis Visit Windows

Analysis visit windowing will not be used for this study. Nominal scheduled study visits will be used as analysis visits. Unscheduled visits and premature termination visits that occur on or in place of a scheduled study visit will be mapped to the same analysis visit as the scheduled study visit.

3.5.6 Selection of Data in the Event of Multiple Records on Same Analysis Visit Day

Depending on the statistical analysis method, single values may be required for each analysis visit day. If a single value is needed, but multiple valid, nonmissing measurements exist in an analysis visit day, records will be chosen based on the following:

- For Baseline, the last nonmissing value on or prior to the first dose date of study drug will be selected, unless specified differently. If there are multiple records with the same time or no time recorded on the same day, the record with the lowest accession number will be used.

- For post-Baseline values, the latest record will be selected. If there is more than 1 record with sample collected on the same day with the same time, the retest record will be selected. If there is no clear indication for which record is the retest record, then the record with the lowest accession number will be used.

4 SUBJECT DISPOSITION

4.1 Subject Enrollment and Disposition

A summary of subject enrollment will be provided by treatment and cohort. The summary will present the number and percentage of subjects enrolled. For each column, the denominator for the percentage calculation will be the total number of subjects analyzed for that column.

The randomization schedule used for the study will be provided as an appendix to the CSR.

A summary of subject disposition will be provided by treatment and cohort. This summary will present the number of subjects in each of the categories listed below:

- Randomized Set
- Full Analysis Set
- Safety Analysis Set

The number and percentage of the subjects in the following categories will be summarized using the Safety Analysis Set:

- Completed study drug
- Did not complete study drug and reasons for study drug discontinuation
- Completed study
- Did not complete the study and reasons for study discontinuation

For the status of study drug and study completion and reasons for discontinuation, the number and percentage of subjects in each category will be provided. The denominator for the percentage calculation will be the total number of subjects in the Safety Analysis Set corresponding to that column.

The following by-subject listings will be provided by cohort and subject number in ascending order:

- Reasons for premature study drug or study discontinuation.

4.2 Protocol Deviations

Subjects who did not meet the eligibility criteria for study entry but enrolled in the study will be summarized. The number and percentage of subjects who did not meet at least 1 eligibility criterion will be provided by treatment and cohort for each specific criteria based on the Randomized Set. A by-subject listing will be provided for those subjects who did not meet at least

1 eligibility (inclusion or exclusion) criterion. The listing will present the eligibility criterion (or criteria if more than 1 deviation) that subjects did not meet and related comments, if collected.

Protocol deviations (PDs) occurring after subjects entered the study are documented during routine monitoring. The number and percentage of subjects with important PDs (IPDs) by deviation category will be summarized by treatment and cohort for the Randomized Set. A by-subject listing will be provided for those subjects with any PDs.

A table will be provided to summarize any COVID-19 related PDs with the deviation reason by treatment and cohort. A listing of subjects who had study disruption due to COVID-19 will be provided with a description.

4.3 Extent of Study Drug Exposure and Compliance

Extent of exposure to study drug will be examined by assessing the total duration of exposure to study drug and the level of compliance relative to the study drug regimen specified in the protocol. Summary tables will be provided.

4.3.1 Duration of Exposure to Study Drug

Total duration of exposure to study drug will be summarized. Total duration of exposure will be defined as the last dose date of study drug minus the first dose date plus 1. If the last study drug dose date is missing, the latest date among the study drug end date, clinical visit date, laboratory sample collection date, and vital signs assessment date that occurred during the On-Treatment Period will be used for subjects included in the final analyses or the last available date in the database snapshot for subjects who were still on treatment at the time of an interim analysis.

The total duration of exposure to study drug will be summarized using descriptive statistics. Summaries will be provided by treatment and cohort for the Safety Analysis Set. The expected total duration of exposure is 1 day for subjects in Part A and 8 days for subjects in Part B.

4.3.2 Study Drug On-Treatment Compliance

The level of on-treatment compliance to the study drug will be determined by the total amount of study drug administered relative to the total amount of study drug expected to be administered during a subject's actual on-treatment period.

The level of on-treatment compliance for each study drug will be expressed as a percentage using the following formula:

$$\text{On-Treatment Compliance (\%)} = \left(\frac{\text{Total Amount of Study Drug Administered}}{\text{Total Amount of Study Drug Expected to be Administered on Treatment}}\right) \text{ x } 100$$

For subjects that prematurely discontinue, the denominator will be the total amount of study drug expected to be administered on treatment by the date of premature discontinuation.

Descriptive statistics for the level of on-treatment compliance with the number and percentage of subjects belonging to compliance categories (e.g. ≤ 80%, > 80- 90%, and ≥ 90%) will be provided by treatment and cohort for the Safety Analysis Set.

A by-subject listing of study drug administration will be provided by cohort and subject number in ascending order and visit in chronological order.

4.4 Meal Times and PK Sample Collection

Meal time information is collected in eCRF and will be listed for each subject. Listing will include date, start time, stop time, and percent of meal consumed.

PK plasma sample collection data is collected in eCRF and will be listed for each subject. Listing will include collection date/time, time of collection relative to dosing, and deviation from nominal sample time.

PK urine sample collection data is collected in eCRF and will be listed for each subject. Listing will include collection date/time, interval of collection relative to dosing, and total volume collected.

4.5 Medical History

Medical history collected at screening will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version (v) 25.1.

Medical history will be summarized by preferred term (PT), treatment, and cohort. Subjects who report 2 or more medical history items that are coded to the same PT will be counted only once by the unique coded term in the summary. The summary will be provided for the Safety Analysis Set.

A by-subject listing of medical history will be provided by cohort and subject number in ascending order.

4.6 Demographics and Baseline Characteristics

Subject demographic variables (ie, age, sex, race, and ethnicity), Baseline characteristics (body weight [in kg], height [in m], and body mass index [BMI; in kg/m^2]) will be summarized by treatment and cohort using descriptive statistics for continuous variables and using number and percentage of subjects for categorical variables. The summary of demographic data will be provided for the Safety Analysis Set.

A by-subject demographic listing, including the informed consent date, will be provided by cohort and subject number in ascending order.

5 SAFETY ANALYSES

5.1 Adverse Events and Deaths

5.1.1 Adverse Event Dictionary

Clinical and laboratory adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version (v) 25.1. System organ class (SOC), high-level group term (HLGT), high-level term (HLT), preferred term (PT), and lower-level term (LLT) will be provided in the AE dataset.

5.1.2 Adverse Event Severity

Adverse events are graded by the Investigator as Grade 1, 2, 3, or 4 according to the toxicity criteria specified in the protocol. The severity grade of events for which the investigator did not record severity will be categorized as "missing" for tabular summaries and data listings. The missing category will be listed last in summary presentation.

5.1.3 Relationship of Adverse Events to Study Drug

Related AEs are those for which the investigator selected "Related" on the AE CRF to the question of "Relationship to Study Treatment" based on the his/her clinical assessment. Events for which the investigator did not record relationship to an applicable study drug will be considered related to study drug for summary purposes. However, by-subject data listings will show the relationship as missing.

5.1.4 Serious Adverse Events

Serious adverse events (SAEs) will be identified and captured as SAEs if the AEs met protocol-specified definitions of a SAE.

5.1.5 Treatment-Emergent Adverse Events

5.1.5.1 Definition of Treatment-Emergent Adverse Events

A treatment-emergent adverse event (TEAE) will be defined as any AE that has an onset date/time or worsening in severity from Baseline on or after the first dose date/time of study drug through end of study.

5.1.5.2 Incomplete Dates

If the onset date of the AE is incomplete and the AE stop date is not prior to the first dose date of any study drug, then the month and year (or year alone if month is not recorded) of onset determine whether an AE is treatment emergent. The event is considered treatment emergent if both of the following 2 criteria are met:

- The AE onset is the same as or after the month and year (or year) of the first dose date of study drug, and

- The AE onset date is the same as or before the month and year (or year) of the end of study.

An AE with completely missing onset and stop dates, or with the onset date missing and a stop date later than the first dose date of study drug, will be considered to be treatment emergent. In addition, an AE with the onset date missing and incomplete stop date with the same or later month and year (or year alone if month is not recorded) as the first dose date of any study drug will be considered treatment emergent.

5.1.6 Summaries of Adverse Events and Deaths

Treatment-emergent AEs will be summarized based on the Safety Analysis Set.

A brief, high-level summary of the number and percentage of subjects who experienced at least 1 TEAE in the categories described below will be provided by treatment and cohort. All deaths observed in the study will also be included in this summary. The number and percentage of subjects will be provided by SOC, PT, treatment, and cohort for each AE category and also by PT only in descending order of total frequency:

- TEAEs

- TEAEs by severity grade

- TEAEs related to study drug

- TE SAEs

- TE SAEs related to study drug

- TEAEs leading to study drug discontinuation

- TEAEs leading to study discontinuation

- TEAEs leading to death (ie, outcome of death)

- COVID-19 specific TEAEs

- COVID-19 specific TE SAEs

Multiple events will be counted only once per subject in each summary. Adverse events will be summarized and listed first in alphabetic order of SOC, and then by PT in descending order of total frequency within each SOC. For summary by severity grade, the most severe non-missing grade will be used for those AEs that occurred more than once in an individual subject during the study.

Data listings will be provided for the following:

- All AEs
- All treatment-related AEs
- All SAEs
- All treatment-related SAEs
- All AEs with severity of Grade 3 or higher
- All AEs leading to discontinuation of study drug
- All AEs leading to discontinuation of study
- All AEs leading to death (ie, outcome of death)
- All Deaths

5.2 Laboratory Evaluations

Laboratory data collected during the study will be analyzed and summarized using both quantitative and qualitative methods based on the Safety Analysis Set. The lab values that are below LLOQ or above the upper LOQ will be listed as such, and the closest imputed value will be used for the purpose of calculating summary statistics as specified in Section 3.3.

A by-subject listing for laboratory test results will be provided by cohort, subject number, and visit in chronological order for hematology, serum chemistry, coagulation and urinalysis separately. Values falling outside of the relevant reference range and/or having a United States National Institutes of Health Division of AIDS (DAIDS) severity grade of 1 or higher will be flagged in the data listings, as appropriate.

5.2.1 Graded Laboratory Values

The criteria specified in the study protocol will be used to grade laboratory results as normal (no grade), mild (Grade 1), moderate (Grade 2), severe (Grade 3) or potentially life threatening (Grade 4). See Appendix 4 of the protocol for detailed DAIDS grading criteria on the relevant laboratory tests. For laboratory tests with criteria for both increased and decreased levels, analyses for each direction (ie, increased, decreased) will be presented separately.

Some lab tests require fasting to be graded (i.e. triglycerides and cholesterol). For those lab tests, Baseline will be defined as the last non-missing fasted evaluation prior to the first dose on Day 1.

5.2.1.1 Treatment-Emergent Laboratory Abnormalities

Treatment-emergent laboratory abnormalities will be defined as values that increase at least 1 toxicity grade from Baseline at any post-Baseline time point. If the relevant Baseline laboratory value is missing, any abnormality of at least Grade 1 observed within the time frame specified above will be considered treatment emergent.

5.2.1.2 Summaries of Laboratory Abnormalities

Treatment-emergent graded laboratory abnormalities will be summarized (number and percentage of subjects) by cohort, lab test, and treatment group; subjects will be categorized according to the most severe post-baseline abnormality grade for a given lab test.

For all summaries of laboratory abnormalities, the denominator is the number of subjects with nonmissing post-Baseline values.

The maximum post-Baseline grade observed will be tabulated for each laboratory test, and percentages will be based on the number of subjects with a post-Baseline evaluation of the specific laboratory test. A by-subject listing of graded laboratory abnormalities will be provided by cohort, subject number, and visit in chronological order. This listing will include all test results that were collected throughout the study for the lab test of interest, with all applicable severity grades displayed.

5.2.2 Ungraded Laboratory Values

Treatment-emergent abnormal direct bilirubin will be summarized by cohort and treatment group. A treatment-emergent abnormal direct bilirubin record is defined as a record with value > baseline and >=ULN. The highest direct bilirubin measurement will be counted for each subject. A by-subject listing of visits with abnormal direct bilirubin will be provided.

5.2.3 Summaries of Numeric Laboratory Results

Descriptive statistics will be provided by treatment and cohort for each laboratory test specified in the study protocol as follows:

- Baseline values
- Values at each post-Baseline visit
- Change from Baseline at each post-Baseline visit

A Baseline laboratory value will be defined as the last measurement obtained on or prior to the date/time of first dose of study drug. Change from Baseline to a post-Baseline visit will be calculated for any post-Baseline visit including follow-up visits. For Cohort A7 Period 2, Period 2 Baseline and Change from Period 2 Baseline will be used instead.

In the case of multiple values in a visit day, data will be selected for analysis as described in Section 3.5.6.

5.2.4 Summaries of Categorical Laboratory Results

For categorical urinalysis parameters, the number and percentage of subjects in each category will be presented by treatment and cohort at each visit.

5.3 Body Weight and Vital Signs

Descriptive statistics will be provided by treatment and cohort for body weight, BMI, and vital signs (systolic and diastolic blood pressure [mmHg], pulse [beats/min], respiration [breaths/min], and body temperature [^{o}C]) as follows:

- Baseline value
- Values at each post-Baseline visit
- Change from baseline at each post-Baseline visit

A Baseline value will be defined as the last available value collected on or prior to the date/time of first dose of study drug. Change from Baseline to a post-Baseline visit will be calculated for any post-Baseline visit including follow-up visits.

For Cohort A7 Period 2, Period 2 Baseline and Change from Period 2 Baseline will be used instead.

In the case of multiple values in an analysis window, data will be selected for analysis as described in Section 3.5.6.

A by-subject listing of vital signs will be provided by cohort, subject number, and visit in chronological order. In the same manner, a by-subject listing of body weight, height, and BMI will be provided separately.

5.4 Prior and Concomitant Medications

Medications collected at Screening and during the study will be coded using the current version of the World Health Organization (WHO) Drug dictionary (WHO Drug Global 1SEP2022).

5.4.1 Prior Medications

Prior medications will be defined as any medications taken before a subject took the first study drug. Prior medications will be summarized by Anatomical Therapeutic Chemical (ATC) drug class Level 2 and preferred name using the number and percentage of subjects for each treatment and cohort. A subject reporting the same medication more than once will be counted only once within each ATC drug class. The summary will be ordered alphabetically by ATC medical class

and then by preferred term in order of descending overall frequency within each ATC medical class. For drugs with the same frequency, sorting will be done alphabetically.

A medication will be considered as a prior medication if it meets one of the following criteria:

- A medication with a start date prior to the first dose date of study drug will be included in the prior medication summary regardless of when the stop date is.
- If a partial start date is entered, the medication will be considered prior unless the month and year (if day is missing) or year (if day and month are missing) of the start date are after the first dose date.
- A medication with a completely missing start date will be included in the prior medication summary, unless otherwise specified.

Summaries will be based on the Safety Analysis Set.

5.4.2 Concomitant Medications

Concomitant medications will be defined as any prescription or over-the-counter preparation, including vitamins, medications, vaccinations, herbal preparations, and supplements (including traditional Chinese medicine) taken by a subject during the study. Use of concomitant medications will be summarized by ATC drug class Level 2 and preferred name using the number and percentage of subjects for each treatment and cohort. A subject reporting the same medication more than once within each ATC drug class will be counted only once when calculating the number and percentage of subjects who received that medication. Medications may appear under multiple ATC drug classes. The summary will be ordered alphabetically by ATC medical class and then by preferred term in descending overall frequency within each ATC medical class. For drugs with the same frequency, sorting will be done alphabetically.

A medication will be considered as a concomitant medication if it meets one of the following criteria:

- A medication with a start date prior to or on the first dose date of study drug, and continued to be taken after the first dose date.
- A medication started after the first dose date but prior to or on the last dose date of study drug.
- If a partial start date is entered, any medication with the month and year (if day is missing) or year (if day and month are missing) before the study drug stop date, and with the stop date after the first dose date of study drug. If a partial stop date is entered, any medication with the month and year (if day is missing) or year (if day and month are missing) after the first dose date of study drug.

- A medication started and stopped on the same day as the first dose date or the last dose date of study drug.
- Medications with completely missing start and stop dates, unless otherwise specified.

A medication with a stop date prior to the date of first dose date of study drug or a start date after the last dose date of study drug will be excluded from the summary.

Summaries of prior and concomitant medications will be based on the Safety Analysis Set.

All the prior and concomitant medications will be provided in a by-subject listing sorted by cohort, subject number, and administration date in chronological order.

5.5 Electrocardiogram Results

A shift table of the Investigators' assessment of electrocardiogram (ECG) results at each visit compared with Baseline values will be presented by treatment and cohort using the following categories:

- normal
- abnormal (not clinically significant)
- abnormal (clinically significant)
- missing/not done

The number and percentage of subjects in each cross-classification group of the shift table will be presented. Subjects with a missing value at Baseline or post-Baseline will not be included in the denominator for percentage calculation.

A by-subject listing for ECG assessment results will be provided by cohort, subject number, and visit in chronological order.

5.6 Other Safety Measures

Pregnancy test results, alcohol test results, and urine drug test results will be listed by cohort and subject number.

6 PK ANALYSES

Analysis of PK data will be performed for the interim DRC reviews and at the end of the study and will be outlined in a separate PKAP.

7 REFERENCES

Nehls G, Akland G. Procedures for Handling Aerometric Data. Journal of the Air Pollution Control Association 1973;23 (3):180-4.

8 SOFTWARE

SAS® Software Version 9.4. SAS Institute Inc., Cary, NC, USA.

9 APPENDICES

None.

Signature Page for VV-TMF-57312 v2.0

Reason for signing: Approved	[redacted] Date of signature: 11-May-2023 20:25:14 GMT+0000

Reason for signing: Approved	[redacted] Date of signature: 11-May-2023 23:56:43 GMT+0000

Reason for signing: Approved	[redacted] Date of signature: 12-May-2023 16:30:09 GMT+0000

Signature Page for VV-TMF-57312 v2.0