PROTOCOL AIO-001-101

OPEN-LABEL, SINGLE DOSE, PARALLEL GROUP, PHASE 1 STUDY IN HEALTHY VOLUNTEERS EVALUATING SAFETY, TOLERABILITY, PHARMACOKINETICS, AND IMMUNOGENICITY OF A 400 MG DOSE OF AIO-001 ADMINISTERED BY SUBCUTANEOUS INJECTIONS WITH TWO FORMULATIONS

Sponsor:

Aiolos Bio, Inc.
c/o Latham & Watkins LLP
200 Clarendon Street
Boston, MA 02116
USA

Contract Research Organization:

Protocol Version: Amendment 2

Date: 29-NOV-2023

CONFIDENTIAL

Protocol Historical File

Version	Brief description/summary of changes	Date
Final	Version submitted to the Independent Ethics Committee (IEC).	20-OCT-2023
Amendment 1	Secondary endpoints were slightly modified to be more concise and avoid repetition. To give some flexibility surrounding Public Holidays for outpatient visits, beginning at Day 8, an additional +1 day visit window may be granted with sponsor approval. The justifications of the dose and risks have been updated. The clinical laboratory assessments to be performed now include IgE. More detail about statistical PK population will be included in the PK analysis plan instead to be described in the protocol.	27-OCT-2023
Amendment 2	See summary of changes below.	29-NOV-2023

Summary of Changes for Amendment 2

In line with local regulations, tubal ligation is noted as an effective contraceptive method, therefore it is not considered a form of sterilization and has been updated. This implies changes in the following sections of the protocol:

- Section 5.2 Inclusion Criteria #7
- Section 7.1 Contraception

Contraception restriction duration has been changed from 5 months to 5 half-lives +1 month. This implies changes in the following section of the protocol:

- Section 7.1 Contraception

Contact for safety reporting has been changed. This implies changes in the following section of the protocol:

- Section 8.3.6.1 Serious Adverse Event Reporting to the Sponsor

Total blood volume to be collected in the study has been corrected to be 550 mL. This implies a change in the following section of the protocol:

- Section 8.1.1 Pharmacokinetic and Immunogenic Blood Sample Collection and Processing

Unscheduled visit will be allowed as needed. This implies a change in the following section of the protocol:

- Table 1 Schedule of Assessments.

Tympanic temperature will be taken instead of oral temperature. Changes were made throughout the document.

Finally, minor typographical edits and corrections were made throughout the document.

Sponsor Signature Page
Aiolos Bio, Inc.
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200 Clarendon Street
Boston, MA 02116
USA

Sponsor's representative:

_____ _____

Name: Tony Adamis Date
Title: Chief Medical Officer

Investigator Signature Page

Study Title	Open-Label, Single Dose, parallel group, Phase 1 Study in Healthy Volunteers Evaluating Safety, Tolerability, Pharmacokinetics, and Immunogenicity of a 400 mg Dose of AIO-001 Administered by Subcutaneous Injections with Two Formulations
Protocol Number	AIO-001-101
Version	Amendment 2; November 29, 2023

I have carefully read this study protocol and agree that it contains all necessary information required to conduct this study. I agree to conduct the study according to this protocol (including any amendments) and in accordance with the clinical site's Standard Operating Procedures (SOPs), ICH Good Clinical Practice (GCP), all other applicable regulations, and the recommendations laid down in the most recent version of the Declaration of Helsinki.

_____ _____
Investigator signature Date

_____
Investigator name

_____
Name of clinical facility

_____
Location of facility

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List of Abbreviations

ACT	Asthma Control Test
ADA	Anti-drug antibody
AE	adverse event
ALT	alanine aminotransferase
ANOVA	analysis of variance
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
$AUC_{0\text{-}inf}$	area under the concentration-time curve from time zero to infinity (extrapolated)
$AUC_{0\text{-}t}$	area under the concentration-time curve from time zero until the last observed concentration
$AUC_{0\text{-}\tau}$	area under the concentration-time curve for one dosing interval (τ) at steady-state
BMI	body mass index
CDER	Center for Drug Evaluation and Research
CFR	Code of Federal Regulations
CHMP	Committee for Medicinal Products for Human Use
CI	confidence interval
CK	creatine kinase
Cl/F	apparent clearance
Cl_R	renal clearance
C_{max}	maximal observed concentration
C_{min}	minimal observed concentration
CRF	case report form
CRO	contract research organization
CV	coefficient of variation
DMP	data management plan
ECG	electrocardiogram
eGFR	estimated glomerular filtration rate
EMA	European Medicines Agency
EOS	end of study
ET	early termination
FDA	Food and Drug Administration
FeNO	fractional exhaled nitric oxide
FEV1	forced expiratory volume

FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GLM	general linear model
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HEENT	head, eyes, ears, nose, and throat
HIV	human immunodeficiency virus
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IgE	immunoglobulin E
INR	international normalized ratio
IRB	Institutional Review Board
IV	intravenous
K_{el}	terminal elimination rate constant
LLN	lower limit of normal
MAD	multiple ascending dose
Max	maximum
MCH	mean cell hemoglobin
MCHC	mean cell hemoglobin concentration
MCV	mean cell volume
MDMA	3,4-methylenedioxymethamphetamine
MDRD	modification of diet in renal disease
MedDRA	Medical Dictionary for Regulatory Activities
Min	minimum
NOAEL	no observed adverse effect level
OTC	over-the-counter
PCP	phencyclidine
PK	pharmacokinetic(s)
PR	PR interval
PRN	*Pro re nata* (as needed)

PT	prothrombin time
QA	quality assurance
QC	quality control
QD	*quaque die* (once a day)
QT	QT interval
QTcF	Fridericia's corrected QT interval
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan
SAS	statistical analysis system
SC	subcutaneous
SD	standard deviation
SOP	standard operation procedure
SUSAR	suspected, unexpected, serious adverse reaction
$T_{½\ el}$	terminal elimination half-life
T3	triiodothyronine
T4	thyroxine
TEAE	treatment-emergent adverse event
THC	tetrahydrocannabinol
T_{max}	time when the maximal concentration is observed
TSH	thyroid-stimulating hormone
TSLP	thymic stromal lymphopoietin
ULN	upper limit of normal
V_z/F	apparent volume of distribution
WBC	white blood cell

Synopsis of Protocol

Project Number:	Aiolos Bio, Inc. Project Number: AIO-001-101
Title:	Open-Label, Single Dose, Parallel Group, Phase 1 Study in Healthy Volunteers Evaluating Safety, Tolerability, Pharmacokinetics, and Immunogenicity of a 400 mg Dose of AIO-001 Administered by Subcutaneous Injections with Two Formulations
Investigational Products:	AIO-001 solution for subcutaneous (SC) injection at 100 mg/ml (Formulation A) AIO-001 solution for SC injection at 182 mg/ml (Formulation B)
Study Phase and Type:	Phase 1 – Comparative Safety and Pharmacokinetic (PK)
Objectives:	Primary objective: • To compare the safety and tolerability of 400 mg of AIO-001 as the new formulation (Formulation B) with a 400 mg dose of AIO-001 administered as the original formulation (Formulation A) in healthy volunteers. Secondary objectives: • To evaluate the PK of a single SC AIO-001 dose of Formulation A in healthy volunteers. • To evaluate the PK of a single SC AIO-001 dose of Formulation B in healthy volunteers. • To evaluate the immunogenicity of AIO-001 in healthy volunteers.
Endpoints:	Primary endpoints: • Adverse events (AEs), vital signs measurements (blood pressure, heart rate, respiratory rate, and tympanic temperature), 12-lead electrocardiogram (ECG) recordings, physical examinations, and clinical laboratory test results, including hematology, biochemistry, and urinalysis. Secondary endpoints: • PK endpoints may include but are not limited to AIO-001 concentrations, $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$, C_{max}, T_{max}, $T_{½}$ • Incidence of anti-drug antibody (ADA) to AIO-001
Study Design:	This is an open-label single dose, parallel group, 24-week, Phase 1 study in 16 healthy subjects. The study is designed to evaluate and compare the safety, tolerability, PK, and immunogenicity of SC AIO-001 400 mg using two different formulations (original Formulation A and new Formulation B) in 16 healthy volunteers (8 receiving each formulation). The study will include a screening visit from Day -28 to Day -2 (Visit 1). Eligible subjects will be admitted to the clinical site on Day -1 and will be confined until completion of the assessments on Day 3 (Visit 2). Subjects will return to the clinical site for outpatient visits for study assessments and laboratory tests as described in the Table 1. Schedule of Assessments. A staggered dosing schedule will be used for dosing of each cohort and will include 2 sentinel subjects (1 for each treatment) dosed initially, and the remaining 14 subjects dosed at least 24 hours later.

Study Population:	It is planned to enroll up to 16 healthy subjects.
Inclusion/Exclusion Criteria:	**Inclusion Criteria** Subjects must meet all of the following criteria to be included in the study: 1. Able to understand the study procedures and provide signed informed consent to participate in the study. 2. Male or female. 3. Non-smokers. Light smokers (no more than 5 cigarettes daily [approximately 50 to 60 mg of nicotine per day], or products with equivalent amount of nicotine within 3 months prior to screening) may be permitted. 4. ≥18 and ≤55 years of age. 5. BMI >18.5 and <32.0 kg/m^2 and body weight ≥45.0 kg. 6. Healthy subjects as defined by: a. the absence of clinically significant illness and surgery within 4 weeks prior to dosing. b. the absence of clinically significant history of neurological, endocrine, cardiovascular, respiratory, hematological, immunological, psychiatric, gastrointestinal, renal, hepatic, and metabolic disease. 7. Female subjects of non-childbearing potential must be: a. post-menopausal (spontaneous amenorrhea for at least 12 months prior to dosing) with confirmation by documented FSH levels ≥ 40 mIU/mL; or b. surgically sterile (bilateral oophorectoy, bilateral salpingectomy, or hysterectomy) at least 3 months prior to dosing. 8. Sexually active female subjects of childbearing potential who are sexually active with the opposite sex and non-sterile male subjects must be willing to use an acceptable contraceptive method throughout the study as detailed in section 7.1 **Exclusion Criteria** Subjects to whom any of the following applies will be excluded from the study: 1. Any clinically significant abnormal finding at physical examination at screening. 2. Clinically significant abnormal laboratory test results or positive serology test results for HBsAg, HCV antibody, or HIV antigen and antibody, or QuantiFERON®-TB test at screening. 3. Positive pregnancy test or lactating female subject. 4. Positive urine drug screen or alcohol breath test. 5. History of anaphylaxis, or severe allergy.

	6. Previous exposure to thymic stromal lymphopoietin antibody. 7. Known allergic reactions to thymic stromal lymphopoietin antibody drugs and their excipients, or other biologic products or to any excipient in the formulation. 8. Estimated Glomerular Filtration Rate (eGFR, using CKD-EPI Creatinine Equation) < 90 ml/min/1.73m^2 during screening or baseline visit. 9. Clinically significant ECG abnormalities (e.g., For male QTcF > 450msec, for female QTcF > 470msec) or vital signs abnormalities (systolic blood pressure lower than 90 or over 140 mmHg, diastolic blood pressure lower than 40 or over 90 mmHg, or heart rate less than 40 or over 100 bpm) at screening. 10. History of drug abuse within 1 year prior to screening. 11. History of alcohol abuse within 1 year prior to screening or regular use of alcohol within 6 months prior to screening that exceeds 10 units for women or 15 units for men of alcohol per week (1 unit = 375 mL of beer 3.5%, or 100 mL of wine 13.5%, or 30 mL of distilled alcohol 40%). 12. Use of medications within the timeframes specified in section 7.2. 13. Previous participation in a clinical trial of AIO-001. 14. Dosed in a clinical research study involving the administration of an investigational or marketed drug or device within 30 days prior to dosing, administration of a biological product in the context of a clinical research study within 90 days or 5 half-lives (whichever is longer) prior to dosing, concomitant participation in an investigational study involving no drug or device administration. 15. Donation of plasma within 7 days prior to dosing or donation or loss of 500 mL or more of whole blood within 4 weeks prior to dosing. 16. Any reason which, in the opinion of the Investigator, would prevent the subject from participating in the study. 17. Intolerance to venipuncture. 18. Any scars, tattoos, etc., at the injection site. 19. More than 5 cigarettes daily (or products with equivalent amount of nicotine) for 3 months prior to screening and unable to abstain from smoking during the trial.
Study Treatments:	Sixteen healthy volunteers (8 receiving each formulation) will receive one of the following treatments, according to the randomization scheme: Group A: 400 mg of 100 mg/ml AIO-001 as Formulation A, given as 4 X 1.0 ml subcutaneous injections administered within approximately 5 minutes. Group B: 400 mg of 182 mg/ml AIO-001 as Formulation B, given as 2 X 1.1 ml subcutaneous injections administered within approximately 5 minutes.

Study Procedures:	Blood samples for PK and ADA analysis will be collected and safety procedures will be performed at pre-defined times throughout the study as specified in Table 1. Schedule of Assessments. Subjects will be monitored throughout the study by the clinical staff for AEs and concomitant medication use.
Statistical Analyses:	Safety and tolerability analysis: Safety and tolerability of AIO-001 will be evaluated through the assessment of AEs (i.e., seriousness, severity, relationship to the study drug, outcome, duration, and management), vital signs, 12-lead ECGs, clinical laboratory tests, and physical examinations. Treatment-emergent adverse events (TEAEs) will be tabulated by treatment. Safety and tolerability data will be reported using descriptive statistics. PK analysis: The pharmacokinetic profile of AIO-001 will be established using non-compartmental analysis of serum concentration data obtained at the timepoints outlined in the Schedule of Assessments. PK parameters including but not limited to $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$, C_{max}, T_{max}, and $T_{½}$ will be calculated if sufficient data are available for estimation, at the discretion of the pharmacokineticist. AIO-001 serum concentrations and PK parameters will be presented in summary tables for each dose cohort and in listings, as appropriate. Individual and mean AIO-001 serum concentrations over time will be presented graphically. In addition, population pharmacokinetic modeling may be applied for PK analysis and will be reported separately. Immunogenicity: The incidence of ADA to AIO-001 will be summarized. As feasible, the impact on PK will be assessed graphically and may be reported separately. Interim analyses: Interim analyses of preliminary data will be performed at Sponsor discretion.

Table 1. Schedule of Assessments

Study Stage	Screening	Baseline	Inpatient[1]							Visit[2]														EOS/ ET[3]
Day	**-28 to -2**	**-1**	**1**					**2**	**3**	**4**	**5**	**6**	**8**	**11**	**15**	**22**	**29**	**43**	**57**	**71**	**85**	**113**	**141**	**169**
Hour			**0**	**2**	**4**	**8**	**12**	**24**	**48**	**72**	**96**	**120**	**168**											
Visit Number	**1**	**2**								**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Time Window ±			Within 60 min	30 min	30 min	30 min	30 min	30 min	30 min	2 h	2 h	2 h	4 h	8 h	8 h	1 d	1 d	2 d	2 d	2 d	2 d	2 d	2 d	2 d
Informed consent	X																							
Inclusion/exclusion criteria	X	X	X																					
Demographic data	X																							
Medical and medication history	X	X																						
Admission to CRU		X																						
Confinement			X[1]	X[1]	X[1]	X[1]	X[1]	X[1]																
Discharge									X[1]															
Outpatient visits										X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety																								
Physical examination[4]	X	X																			X			X
Body measurements (height, weight, BMI)	X	X[5]																						X[5]
Vital signs[6]	X	X	Pre-dose	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG	X	X						X					X		X			X			X	X		X
QuantiFERON-TB	X																							
Serology tests[7]	X																							
COVID 19 test[8]		X																						
Hematology	X	X					X	X			X				X		X	X			X	X	X	X

Study Stage	Screening	Baseline	Inpatient[1]							Visit[2]														EOS/ ET[3]
Day	**-28 to -2**	**-1**	**1**					**2**	**3**	**4**	**5**	**6**	**8**	**11**	**15**	**22**	**29**	**43**	**57**	**71**	**85**	**113**	**141**	**169**
Hour			**0**	**2**	**4**	**8**	**12**	**24**	**48**	**72**	**96**	**120**	**168**											
Visit Number	**1**	**2**								**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Time Window ±			Within 60 min	30 min	30 min	30 min	30 min	30 min	30 min	2 h	2 h	2 h	4 h	8 h	8 h	1 d	1 d	2 d	2 d	2 d	2 d	2 d	2 d	2 d
Clinical laboratory tests[9]	X	X						X			X		X		X		X	X			X	X		X
FSH (for post-menopausal females)[10]	X																							
Pregnancy tests[11]	X	X																						X
Drug and alcohol screens	X	X																						
Monitoring and recording of AEs and prior/concomitant medication use	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pharmacokinetics																								
Blood samples for PK analysis[12]			Pre-dose				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunogenicity																								
Blood samples for ADA			Pre-dose										X		X	X	X		X		X		X	X
Study drug administration																								
Treatment Group Assignment for Healthy Volunteers (Group A or Group B)			Pre-dose																					
AIO-001 injection[13]			X																					
Injection site evaluation			Pre-dose				X	X	X	X	X													

Abbreviations: ADA=, anti-drug antibody; AE = adverse event; BMI = body mass index; d = Day; ECG = electrocardiogram; EOS = end of study; ET = early termination; FSH = follicle-stimulating hormone; h = hour; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; PK = pharmacokinetic; TB= tuberculosis;

1. Subjects will remain in the investigational study center from check-in on Day -1 until after the 48 hours PK samples and all other required assessments are completed on Day 3 (Visit 2).
2. Beginning at Day 8 (Visit 6), for operational feasibility surrounding Public Holidays, an additional +1day visit window may be granted with sponsor approval.
3. In case of ET, EOS procedures will be performed as soon as possible.
4. A complete physical examination will be performed at screening. A brief physical examination will be performed on Day -1 (Visit 2), at Day 85 (Visit 14), and at Day 169 (Visit 17) (EOS/ET).
5. Weight and BMI only.
6. Includes blood pressure, heart rate, respiration rate, and tympanic temperature.
7. Serology tests include HBsAg, HCV antibody, and HIV antigen and antibody.
8. COVID-19 screening will be performed according to clinical site's procedure.
9. Standard biochemistry, coagulation, lipid profile, thyroid function, and urinalysis tests will be performed.

   Note: IgE is not an eligibility determining assessment and therefore results are not needed prior to eligibility confirmation.
10. FSH levels will be measured at screening to confirm the post-menopausal status.
11. A serum pregnancy test will be performed at screening and at EOS/ET, and a urine pregnancy test will be performed on Day -1 (Visit 2).
12. Refer to Table 2 for more details.
13. Group A: 400 mg of 100 mg/ml AIO-001 as Formulation A, given as 4 X 1.0 ml subcutaneous injections administered within approximately 5 minutes.

    Group B: 400 mg of 182 mg/ml AIO-001 as Formulation B, given as 2 X 1.1 ml subcutaneous injections administered within approximately 5 minutes.

Note:

- When the timing of multiple procedures coincides, the following priority order should be adhered to, whenever possible: vital signs, ECGs, blood samples for PK/ADA analysis, clinical laboratory samples, physical examination.

-Unscheduled visits are allowed, as needed.

1. Introduction

AIO-001 (formally SHR-1905) is a humanized anti-thymic stromal lymphopoietin (TSLP) monoclonal antibody (IgG1 subtype) developed by Hengrui Pharmaceuticals Co., Ltd, and acquired by Aiolos Bio, Inc. as of August 14, 2023. AIO-001 binds to TSLP and block the interaction between TSLP and receptor complex, thereby preventing TSLP-targeted immune cells from releasing proinflammatory cytokines to prevent asthma attack and improve asthma control. In non-clinical studies conducted to date, AIO-001 injection has strong *in vitro* activity and was well tolerated in toxicity studies up to 26-weeks duration in cynomolgus monkeys. Two clinical studies evaluating AIO-001 have been completed, one in China and one in Australia. In addition, one is ongoing in China. The successful development of AIO-001 injection may provide new treatment options for a wide range of patients with severe uncontrolled asthma, including those with non-type 2 immune responses (Th2)-driven asthma.[1]

1.1 Background Information

Asthma is a common chronic respiratory disease worldwide. The main symptoms include airway stenosis, airway hyperresponsiveness, inflammatory cell infiltration, increased mucus discharge, as well as symptoms caused by airway remodeling such as wheezing, tachypnea, chest tightness, and paroxysmal cough. There are approximately 339 million asthma patients in the world, of which 5-10% have severe asthma. These patients receive high-dose standard care, but their conditions are still poorly controlled. The persistence of symptoms and frequent life-threatening deterioration seriously affect the quality of life. For 20-60% of patients with poorly controlled asthma, chronic oral corticosteroid treatment is required, but there is also a series of infectious, cardiovascular, metabolic, mental, and gastrointestinal side effects. [1]

Inflammation is the main component of asthma pathology. Thus, anti-inflammation has always been an important direction for research and development of asthma treatment. However, the progress is relatively slow. One-half of patients with severe asthma are Th2 inflammation-driven. It is characterized by increased Th2 inflammatory biomarkers, infiltration of inflammatory cells such as eosinophils, mast cells, and activated CD4+ T cells, as well as increased Th2 cytokines such as interleukin-4 (IL-4), IL-5, and IL-13. Antigen-specific Th2 cells interact with Th2 cytokines, eventually leading to pathological conditions such as mucosal thickening of airway tissue, tracheal stenosis, and airway hyperresponsiveness, resulting in severe asthma and even glucocorticoid tolerance. Therefore, blocking the Th2 inflammatory pathway is a direction for the treatment of severe asthma. However, asthma is a complex disease that exhibits different phenotypes due to the interaction of multiple molecules. In addition to Th2 inflammation, innate immune inflammation also plays certain pathogenic role. Currently marketed biologics for the treatment of asthma, including omalizumab for asthma pathogenic immunoglobulin E (IgE), Mepolizumab, Reslizumab, and Benralizumab targeting IL-5/IL-5R, as well as dupilumab targeting IL-4Rα, only act on specific inflammatory. molecules that drive asthma inflammation and are only suitable for certain types of patients with severe asthma, i.e., subgroup patients, such as eosinophilic asthma and allergic asthma. [1]

The mechanism of TSLP inducing innate and Th2 immune inflammation has only recently been elucidated. TSLP is an IL-7-like cytokine first discovered in the conditioned medium of mouse thymic stromal cells. TSLP is mainly expressed in lung, skin, and intestinal epithelial cells. TSLP

receptor is a complex consisting of two parts: TSLPR and IL-7Rα. TSLP first binds to TSLPR with a relatively low affinity, then recruits IL-7Rα with high affinity, and finally induces the activation of signal pathways such as signal transducer and activator of transcription 5 (STAT5), leading to the maturation of dendritic cells (DCs) and the differentiation of T cells. [1]

Bone marrow-derived dendritic cells (mDCs) are the most important effector cells of TSLP. TSLP acts on immature mDCs such that mDCs secrete cytokines including IL-8, eotaxin-2, thymus and activation-regulated chemokine (TARC), and macrophage-derived chemokine (MDC), and highly express ligand OX40 (OX40L). In the absence of IL-12, OX40L binds to naïve CD4+ T cells, making them differentiate into Th2 cells, promoting Th2 cells to secrete Th2 cytokines such as IL-5, IL-4, IL-13, and TNF, and inducing Th2 inflammatory response in the body. In addition, TSLP can induce DCs to produce IL-8 to recruit neutrophils, resulting in an innate inflammatory state of neutrophils. TSLP also induces DCs to produce eotaxin-2 to recruit eosinophils and acts together with IL-5 to quickly enter the inflammatory state of eosinophil infiltration. TSLP acts on mast cells and natural killer cells, and mediates innate inflammation by producing IL-4, IL-6, IgE, etc. In summary, TSLP can induce innate inflammation and Th2 inflammation simultaneously, thereby increasing tissue mucus, airway remodeling, and severe cell fibrosis, gradually evolving into asthma.[1]

However, there is also a short form TSLP (sf TSLP) in the human body sf TSLP is expressed in oral, skin and intestinal epithelial cells and salivary glands, and the expression of sf TSLP is down-regulated under inflammation. sf TSLP can inhibit the production of cytokines by DC cells and maintain immune homeostasis. The specific receptor of sf TSLP is still unclear, although this specific receptor does not bind to TSLPR. Therefore, it is a potentially effective strategy for the treatment of asthma by selectively blocking TSLP without blocking sf TSLP. [1]

Tezepelumab, an anti-TSLP antibody, has recently been approved for all moderate-to-severe patients with poorly controlled severe asthma. The phase 2b trial (PATHWAY) showed that compared with patients receiving placebo, the annual asthma attack rate of patients who received Tezepelumab was significantly reduced, and their lung function was significantly improved. Moreover, unlike IL-5, which only targets patients with eosinophilic asthma, Tezepelumab was effective in both patients with or without elevated eosinophil counts, suggesting that Tezepelumab is suitable for a wider range of patients. Because of this feature, Tezepelumab was granted the breakthrough therapy designation by the FDA in 2018 for the treatment of severe asthma patients with no eosinophilic phenotype. Tezepelumab's phase 3 trial (NAVIGATOR) reached the primary endpoint in patients with severe uncontrolled asthma. Compared with the combination of placebo and standard of care (SoC), the combination of Tezepelumab and SoC significantly decreased patients' annual asthma exacerbation rate (AAER) at Week 52 (with statistical significance and clinical significance). In the subgroup of patients with baseline eosinophil count less than 300 cells/μL, Tezepelumab also reached the primary endpoint. A similar decrease in AAER was also observed in the subgroup of patients with baseline eosinophil count less than 150 cells/μL, indicating that Tezepelumab can significantly reduce the acute exacerbation of severe asthma in a wide range of patients. Also, there was no clinically significant difference in the tolerability of Tezepelumab compared with the safety of the placebo group.[1]

In November 2020, AZ and Amgen announced that Tezepelumab reached the primary endpoint in the phase 3 study (NAVIGATOR) in patients with severe uncontrolled asthma. Compared with the combination of placebo and standard of care (SoC), the combination of Tezepelumab and SoC significantly decreased patients' annual asthma exacerbation rate (AAER) at Week 52 (with statistical significance and clinical significance). In the subgroup of patients with baseline eosinophil count less than 300 cells/μL, Tezepelumab also reached the primary endpoint. A similar decrease in AAER was also observed in the subgroup of patients with baseline eosinophil count less than 150 cells/μL, indicating that Tezepelumab can significantly reduce the acute exacerbation of severe asthma in a wide range of patients. Also, there was no clinically significant difference in the tolerability of Tezepelumab compared with the safety of the placebo group. [1]

1.2 Summary of Nonclinical Studies

1.2.1 Nonclinical Pharmacology

CCI

[redacted]

1.2.2 Nonclinical Safety Pharmacology

CCI

[redacted]

CCI

[redacted]

1.2.3 Nonclinical Pharmacokinetics and Product Metabolism in Animals

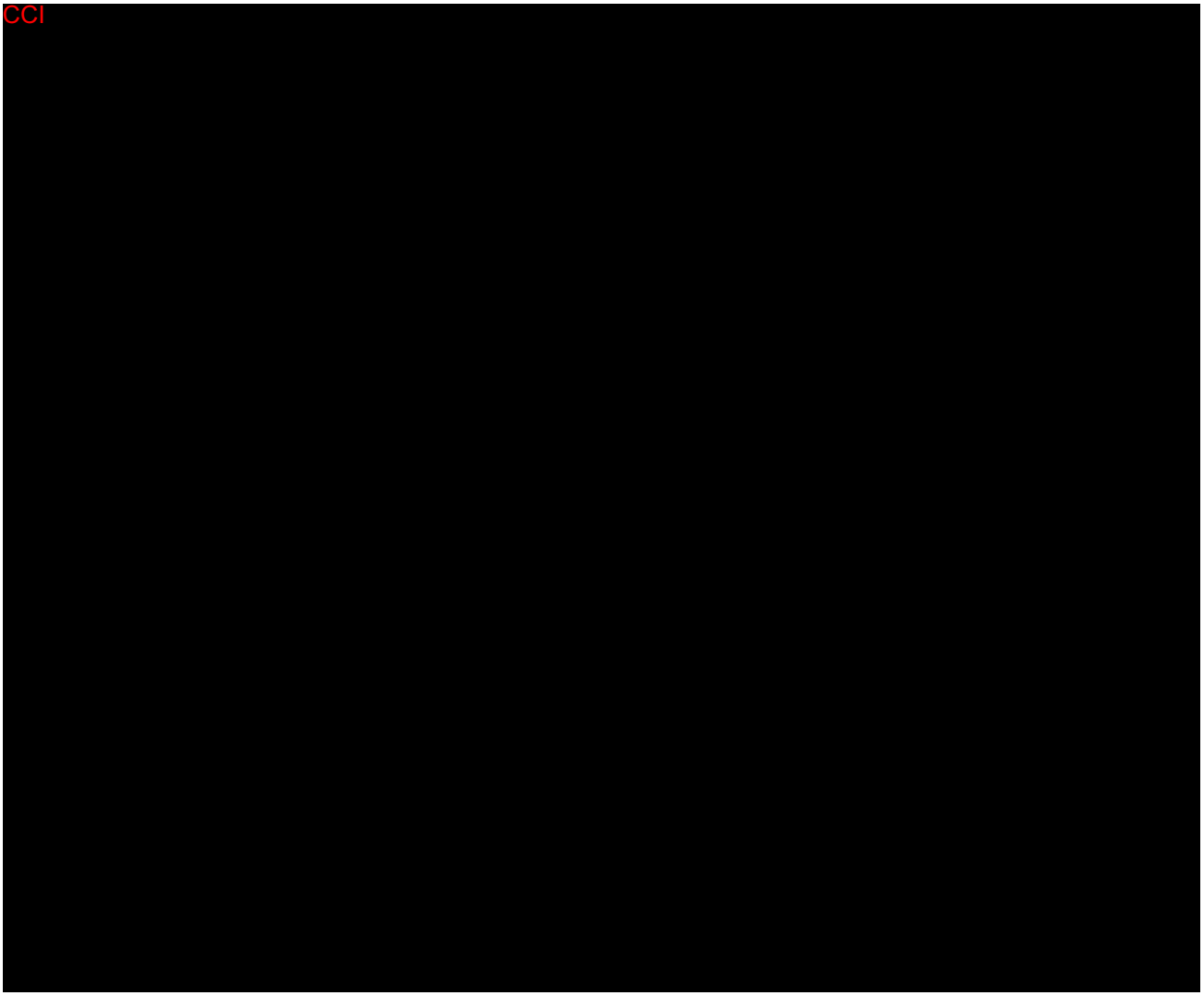
CCI

[redacted]

1.2.4 Nonclinical Toxicology

CCI

[redacted]



1.3 Summary of Clinical Experience

1.3.1 Clinical Safety

As of 09 May 2023 (data cut-off date), Hengrui Pharmaceuticals Co., Ltd has initiated 3 clinical studies (two Phase I and one Phase II) of AIO-001 (SHR-1905). Two clinical studies (SHR-1905-101 and SHR-1905-201) were performed in China, and the other one (SHR-1905-I-101-AUS) was conducted in Australia. A total of 147 subjects received AIO-001, including 100 healthy subjects were exposed to AIO-001 (n=80) or placebo (n=20) at a single dose of 50 - 600 mg, 12 patients with mild asthma received a single subcutaneous dose of 200 mg AIO-001 (n=8) or placebo (n=4), 24 patients with moderate or severe asthma received a single subcutaneous dose of 100/600 mg AIO-001 (n=16) or placebo (n=8), and 11 patients with severe asthma received multiple subcutaneous doses of CCI AIO-001 or placebo.[1]

Among 100 healthy subjects exposed to AIO-001/placebo, 63 subjects (63.0%) experienced TEAEs, which occurred in ≥5% of the subjects included blood thyroid stimulating hormone increased, blood uric acid increased and blood bilirubin increased (7.0% each), bilirubin conjugated increased, aspartate aminotransferase increased, headache and alanine

aminotransferase increased (6.0% each), and haemoglobin decreased and blood creatine phosphokinase increased (5.0% each).[1]

Among 47 patients with asthma who were exposed to AIO-001/placebo, the incidence of TEAEs was 31.9% (15/47), including alanine aminotransferase increased, blood creatine phosphokinase increased, blood uric acid increased and triiodothyronine free increased (2 subjects each), and the others were seen in single subjects.[1]

All TEAEs above were mild or moderate in severity, which can be controlled with or without medical intervention. No subjects experienced serious adverse events (SAEs) or fatal TEAEs. No subjects withdrew from studies due to TEAEs.[1]

1.3.2 Pharmacokinetics and Immunogenicity in Humans

Following a single SC injection of AIO-001 from 50 mg to 600 mg in Australian healthy subjects (SHR-1905-I-101-AUS) (N=40), the serum concentrations of AIO-001 increased along with the increased dose level. CCI [redacted]

To explore the dose proportionality of AIO-001 in tested dose range (50 mg to 600 mg) in healthy subjects, C_{max}, $AUC_{0\text{-last}}$, and $AUC_{0\text{-inf}}$ were further examined against dose using power model. The slopes of CCI [redacted] indicating that the exposure of AIO-001rs to increase in a slightly greater-than-dose-proportional manner with increased dose from 50 mg to 600 mg. The slopes of CCI [redacted], indicating that the exposure of AIO-001 appears to increase in a slightly greater-than-dose-proportional manner with increased dose from 50 mg to 600 mg.[1]

In all the Australian healthy subjects (N=50) in this study, treatment-induced ADA positive subjects were reported in both placebo group CCI [redacted] and AIO-001 treatment group CCI [redacted]. Treatment-induced ADA positive subjects with AIO-001 were: CCI [redacted]. There was CCI [redacted] subject in the placebo group who had a pre-existing ADA sample at baseline. The observed earliest time point of treatment-induced ADA after AIO-001 Treatment started from CCI [redacted] in the 200 mg and 600 mg group, and CCI [redacted] in the 400 mg group. There was no obvious effect observed in corresponding PK concentrations in all the ADA positive subjects.[1]

1.4 Study Rationale

Existing safety data from China and Australia Phase 1 single ascending dose studies of AIO-001 are from subjects who received SC injections with a 100 mg/ml formulation requiring multiple 1 ml injections to achieve doses up to 600 mg. The study in China also included subjects with asthma.

In a proposed phase 2 trial, Aiolos Bio will be utilizing a new formulation and subjects will receive two SC injections of 1.1 ml at AIO-001 concentration of 182 mg/ml to achieve a 400 mg dose. The primary purpose of the present study is to compare the safety, tolerability, and PK parameters of 400 mg of AIO-001 as the new formulation (Formulation B) with a 400 mg dose of AIO-001

administered as the original formulation (Formulation A) in healthy volunteers. Pharmacokinetic parameters and other primary and secondary endpoints will be described and compared quantitatively.

1.4.1 Rationale for the Dose

CCI

[redacted]

1.4.2 Rationale for the Study Population

A healthy volunteer population has been selected for the study because healthy subjects with no concomitant diseases and using no concomitant medications represent a homogenous population allowing for proper evaluation of the PK, safety, and tolerability of a drug without confounding factors.

CCI

[redacted]

The effects of AIO-001 on fertility, pregnancy and lactation in human have not been investigated so far. Since there have been no studies with pregnant women, it is uncertain whether there is human fetal risk associated with the use of AIO-001. Therefore, only male and non-pregnant, non-lactating female subjects will be included in the study. Non-sterile males and females of childbearing potential will be included if they use appropriate methods of contraception.

1.5 Benefit/Risk Assessment

The inclusion and exclusion criteria have been chosen to select subjects who are known to be free from any significant illness, history of autoimmune diseases, and from any condition that could impact their safety or interfere with meeting the study objectives. The proposed safety screening and monitoring assessments are deemed to be sufficient to monitor potential risks of AIO-001 administration. There is no anticipated therapeutic benefit for the healthy subjects in this study.

So far, as mentioned before, a total of 147 subjects received AIO-001/placebo, including 100 healthy subjects were exposed to AIO-001 or placebo (n=20) at a single dose of 50 – 600 mg, 12 patients with mild asthma received a single subcutaneous dose of 200 mg AIO-001 (n=8) AIO-001 or placebo (n=4), 24 patients with moderate or severe asthma received a single subcutaneous dose of 100/600 mg AIO-001 (n=16) or placebo (n=8), and 11 patients with severe asthma received multiple subcutaneous doses of CCI mg AIO-001 or placebo. CCI

All the safety data obtained so far was with AIO-001 using Formulation A. Although this is the first time the new Formulation (Formulation B) will be tested in humans, this study will evaluate Formulation B, and a similar safety profile is expected. All excipients are generally recognised as safe. The information obtained in this study may help in the treatment of future patients with severe, uncontrolled asthma.

2. Objectives

Primary objective:

- To compare the safety and tolerability of 400 mg of AIO-001 as the new formulation (Formulation B) with a 400 mg dose of AIO-001 administered as the original formulation (Formulation A) in healthy volunteers.

Secondary objectives:

- To evaluate the PK of a single SC AIO-001 dose of Formulation A in healthy volunteers.
- To evaluate the PK of a single SC AIO-001 dose of Formulation B in healthy volunteers.
- To evaluate the immunogenicity of AIO-001 in healthy volunteers.

3. Endpoints

Primary endpoints:

- Adverse events (AEs), vital signs measurements (blood pressure, heart rate, respiratory rate, and tympanic temperature), 12-lead electrocardiogram (ECG) recordings, physical examinations, and clinical laboratory test results, including hematology, biochemistry, and urinalysis.

Secondary endpoints:

- PK endpoints may include but are not limited to AIO-001 concentrations, $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$, C_{max}, T_{max}, $T_{½}$.
- Incidence of anti-drug antibody (ADA) to AIO-001.

4. Study Design

This is an open-label single dose, parallel group, 24-week phase 1 study in 16 healthy subjects.

The study is designed to evaluate and compare the safety, tolerability, pharmacokinetics, and immunogenicity of subcutaneously (SC) administered AIO-001 at a total dose of 400 mg using two different formulations (original Formulation A and new Formulation B) in 16 healthy volunteers (8 receiving each formulation).

The study will include a screening visit from Day -28 to Day -2 (Visit 1). Eligible subjects will be admitted to the clinical site on Day -1 and will be confined until completion of the assessments on Day 3 (Visit 2). Subjects will return to the clinical site for outpatient visits for study assessments and laboratory tests as described in the Table 1. Schedule of Assessments.

A staggered dosing schedule will be used for dosing of each cohort and will include 2 sentinel subjects (1 for each formulation) dosed initially, and the remaining 14 subjects dosed at least 24 hours later.

The total duration of study participation for each subject from screening through the EOS is anticipated to be approximately 28 weeks.

5. Study Population

5.1 Number of Subjects

It is planned to enroll up to 16 healthy subjects for participation in this study. The proposed number of subjects is in line with the sample sizes commonly used in clinical studies of this nature and is considered sufficient to achieve the study objectives.

5.2 Inclusion Criteria

Subjects must meet all of the following criteria to be included in the study:

1. Able to understand the study procedures and provide signed informed consent to participate in the study.
2. Male or female.
3. Non-smokers. Light smokers (no more than 5 cigarettes daily [approximately 50 to 60 mg of nicotine per day], or products with equivalent amount of nicotine within 3 months prior to screening) may be permitted.
4. ≥18 and ≤55 years of age.
5. BMI >18.5 and <32.0 kg/m^2 and body weight ≥45.0 kg.
6. Healthy subjects as defined by:
   a. the absence of clinically significant illness and surgery within 4 weeks prior to dosing.
   b. the absence of clinically significant history of neurological, endocrine, cardiovascular, respiratory, hematological, immunological, psychiatric, gastrointestinal, renal, hepatic, and metabolic disease.
7. Female subjects of non-childbearing potential must be:
   a. post-menopausal (spontaneous amenorrhea for at least 12 months prior to dosing) with confirmation by documented FSH levels ≥40 mIU/mL; or
   b. surgically sterile (bilateral oophorectoy, bilateral salpingectomy, or hysterectomy) at least 3 months prior to dosing.
8. Sexually active female subjects of childbearing potential who are sexually active with the opposite sex and non-sterile male subjects must be willing to use an acceptable contraceptive method throughout the study as detailed in section 7.1.

5.3 Exclusion Criteria

Subjects to whom any of the following applies will be excluded from the study:

1. Any clinically significant abnormal finding at physical examination at screening.

2. Clinically significant abnormal laboratory test results or positive serology test results for HBsAg, HCV antibody, or HIV antigen and antibody, or QuantiFERON®-TB test at screening.
3. Positive pregnancy test or lactating female subject.
4. Positive urine drug screen or alcohol breath test.
5. History of anaphylaxis, or severe allergy.
6. Previous exposure to thymic stromal lymphopoietin antibody.
7. Known allergic reactions to thymic stromal lymphopoietin antibody drugs and their excipients, or other biologic products or to any excipient in the formulation.
8. Estimated Glomerular Filtration Rate (eGFR, using CKD-EPI Creatinine Equation) < 90 ml/min/1.73m^2 during screening or baseline visit.
9. Clinically significant ECG abnormalities (e.g., For male QTcF > 450msec, for female QTcF > 470msec) or vital signs abnormalities (systolic blood pressure lower than 90 or over 140 mmHg, diastolic blood pressure lower than 40 or over 90 mmHg, or heart rate less than 40 or over 100 bpm) at screening.
10. History of drug abuse within 1 year prior to screening.
11. History of alcohol abuse within 1 year prior to screening or regular use of alcohol within 6 months prior to screening that exceeds 10 units for women or 15 units for men of alcohol per week (1 unit = 375 mL of beer 3.5%, or 100 mL of wine 13.5%, or 30 mL of distilled alcohol 40%).
12. Use of medications within the timeframes specified in section 7.2.
13. Previous participation in a clinical trial of AIO-001.
14. Dosed in a clinical research study involving the administration of an investigational or marketed drug or device within 30 days prior to dosing, administration of a biological product in the context of a clinical research study within 90 days or 5 half-lives (whichever is longer) prior to dosing, concomitant participation in an investigational study involving no drug or device administration.
15. Donation of plasma within 7 days prior to dosing or donation or loss of 500 mL or more of whole blood within 4 weeks prior to dosing.
16. Any reason which, in the opinion of the Investigator, would prevent the subject from participating in the study.
17. Intolerance to venipuncture.
18. Any scars, tattoos, etc., at the injection site.
19. More than 5 cigarettes daily (or products with equivalent amount of nicotine) for 3 months prior to screening and unable to abstain from smoking during the trial.

5.4 Subject Withdrawal and Replacement

Subjects will be advised that they are free to withdraw from the study at any time. Over the course of the study, the Sponsor and the Investigator or designee may withdraw any subject from the study for one of the reasons described below; subject withdrawal will be done in accordance with the local guidance at the clinical site:

- Safety reason;
- Non-compliance with protocol requirements;
- Significant protocol deviation;
- Positive pregnancy test, drug screen or alcohol breath test.

Subjects who withdraw prior to dosing may be replaced automatically. In the event that the number of drop-outs exceeds initial expectations, subjects who withdraw or are withdrawn from the study after dosing, for reasons other than safety and tolerability, may be replaced after consultation between the Investigator and the Sponsor. In such case, the total number of subjects dosed will remain within a maximum of 10 subjects for Groups A and B.

Subjects who withdraw or are withdrawn on Day-1 to Day 3 will be asked to remain at the clinic until the Investigator or designee agrees that the subject is fine and can be discharged. A PK blood sample may be collected at the time of withdrawal if deemed required by the Investigator. End of study EOS/ET procedures will be performed at the time of withdrawal from the study or as soon as possible thereafter.

6. Study Treatments

6.1 Drug Supplies and Accountability

It is the responsibility of the Sponsor to ensure that study drugs provided for this study are manufactured under Good Manufacturing Practice (GMP) and are suitable for human use. The Sponsor is responsible to ship a sufficient amount of dosage units to allow the clinical site to maintain an appropriate supply for the study.

The study drugs will be stored at the clinical site as per GCP and local requirements in a locked, temperature-controlled room with restricted access. IP vials and any secondary packaging (if applicable) will be labeled according to applicable local regulations.

For full detail on study drug dispensation, storage, handling, and disposal, please refer to the Pharmacy Manual.

6.2 Identification of Treatments

Sixteen healthy volunteers (8 receiving each formulation) will receive one of the following treatments, according to the randomization scheme:

Group A: 400 mg of 100 mg/ml AIO-001 (as Formulation A), AIO-001 antibody as the active ingredient [redacted]

Group B: 400 mg of 182 mg/ml AIO-001 (as Formulation B): AIO-001 antibody as the active ingredient CCI [redacted]

6.3 AIO-001 Randomization and Blinding

This study will be an open-label study due to the objective nature of the data. Subjects will be administered each treatment according to the block randomization scheme. Randomization will be performed on Day 1 by the pharmacy personnel as per site standard process and will be conducted manually via a printed randomization schedule/scheme provided to site.

6.4 Study Drug Administration

The study drug (AIO-001 injection Formulation A or B) will be injected subcutaneously on Day 1. For safety reasons, subjects will be required to remain seated or semi-reclined during dosing.

- Formulation A: Subjects from Group A will receive 400 mg of 100 mg/ml AIO-001, given as 4 X 1.0 ml subcutaneous injections administered within approximately 5 minutes. The four injection sites are two injection sites on each upper arm separated from each other by at least 5 cm.

- Formulation B: Subjects from Group B will receive 400 mg of 182 mg/ml AIO-001 given as 2 X 1.1 ml subcutaneous injections administered within approximately 5 minutes. The 2 injection sites are one on each upper arm.

IP should not be administered on skin disfigurements or lesions such as scars, tattoos, wounds, bruises, redness and hardening. Please refer to Pharmacy Manual for more details.

The start time of the first injection will be called “0” hour.

7. Study Restrictions

7.1 Contraception

Female subjects who are of non-childbearing potential will not be required to use contraception.

Female subjects of childbearing potential who are sexually active with a non-sterile male partner (sterile male partners are defined as men vasectomized for at least 3 months prior to dosing) must be willing to use one of the following acceptable contraceptive methods throughout the study and for 5 half-lives +1 month after the last dose:

- Simultaneous use of hormonal contraceptive (e.g., oral, depot injection, implant, vaginal ring, intrauterine device) or non-hormonal intrauterine device used for at least 4 weeks prior to dosing (must agree to use the same contraceptive throughout the study) and condom for the male partner.

- Bilateral tubal occlusion or ligation, and condom for the male partner, started at least 21 days prior to dosing.
- Abstinence

Female subjects of childbearing potential must not donate ova during the study and for at least 90 days after the dosing.

Male subjects who are not surgically sterile for at least 3 months prior to dosing, and who are sexually active with a female partner of childbearing potential must be willing to use one of the following acceptable contraceptive methods from the first dose and for 5 half-lives +1 month after the last dose:

- Simultaneous use of condom and hormonal contraceptive (e.g., oral, patch, depot injection, implant, vaginal ring, intrauterine device) or non-hormonal intrauterine device used for at least 4 weeks prior to sexual intercourse for the female partner.
- Abstinence

Male subjects (including men who have had a vasectomy) with a pregnant partner must agree to use a condom from the first dose and for 5 half-lives +1 month after the last dose. Male subjects must also be willing not to donate sperm and for 5 half-lives +1 month after the last dose.

Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject.

7.2 Concomitant Medications

Subjects will be required to avoid using any non-study necessitated medications for the timeframes specified below:

- Depot injection or implant of any drug (other than hormonal contraceptives) for 3 months prior to dosing.
- Prescription medications for 4 weeks prior to dosing.
- Any vaccine, including COVID-19 vaccine and live attenuated vaccination, for 30 days prior to dosing.
- Over-the-counter (OTC) medications and natural health products (including herbal remedies such as St. John's wort, homeopathic and traditional medicines, probiotics, food supplements such as vitamins, minerals, amino acids, essential fatty acids, and protein supplements used in sports) for 14 days prior to dosing (with the exception of the occasional use of acetaminophen up to 2 g daily), throughout the study.

No concomitant medications will be allowed during the study, with the exception of hormonal contraceptives, medications required for the medical management of an AE, and medications

exempted by the Investigator on a case-by-case basis that are judged unlikely to affect the PK profile of the study drug or subject safety (e.g., topical drug products without significant systemic absorption).

If a vaccination is required for any reason, it must first be discussed with and exempted by the Investigator on a case-by-case basis to ensure that it does not compromise the PK profile of the study drug or the subject safety.

Medications taken by subjects before dosing will be documented as prior medications and medications taken by subjects after dosing through the last study day will be documented as concomitant medications. Any prior or concomitant medication use, other than the allowed medications stated above, will be reviewed, and evaluated on a case-by-case basis by the Investigator to determine if they affect a subject's eligibility or continued participation in the study, or for potential impact on the study results.

7.3 Drugs, Nicotine, and Alcohol

Subjects will be required to abstain from:

- Drugs of abuse and illicit use of recreational drugs from screening through study end;
- Any nicotine product within 48 hours prior to admission until the end of inpatient stay (Day 3), and 2 hours prior to each outpatient visit. No more than equivalent of 5 cigarettes per day will be allowed.
- Alcohol-based products from 24 hours prior to admission until discharge.

7.4 Diet

Subjects will be required to abstain from:

- Food containing poppy seeds from 24 hours prior to admission.

While confined at the study site, subjects will receive a standardized diet at scheduled times that will not conflict with other study-related activities.

7.5 Posture and Physical Activity

For safety reasons, subjects will be required to remain seated or semi-reclined and avoid lying down or sleeping for the first 4 hours after the start of study treatment administration.

Failure of subjects to comply with these requirements does not constitute a deviation from the protocol if it is medically necessary, required for procedures, or to go to the bathroom. When appropriate, subjects will be accompanied by a staff member while walking.

Subjects will be required to refrain from strenuous exercise at least 3 days before admission and 3 days prior each follow-up visit.

8. Study Procedures

Subjects must provide written informed consent prior to initiation of any study procedures.

Unless otherwise specified, study procedures will be conducted in accordance with local guidance at the clinical site. From screening through the EOS, subjects will undergo study procedures at pre-defined times as specified in Table 1. Schedule of Assessments and as described in sections 8.1 and 8.2.

Every effort will be made to schedule and perform the procedures within the specified time window, giving consideration to appropriate posture conditions, practical restrictions, and other procedures to be performed at the same time point.

Unless otherwise specified or for subject safety, when the timing of multiple procedures coincides, the following priority order should be adhered to, whenever possible: vital signs, ECGs, blood samples for PK/ADA analysis, clinical laboratory samples, physical examination.

8.1 Pharmacokinetic and Immunogenic Assessments

8.1.1 Pharmacokinetic and Immunogenic Blood Sample Collection and Processing

Blood samples for PK and ADA analysis will be collected via an intravenous catheter or by direct venipuncture at the time points indicated in Table 1. Schedule of Assessments. Applicable time windows for PK and ADA blood samples are defined in the table below.

Table 2 Time Windows for PK and ADA Blood Samples

Day	Hour	Visit Number	Timepoints for Serum PK	Timepoints for Serum ADA	Time window
1	Pre-dose	2	X	X	Within 60 minutes
	12 hours post-dose		X		±30 minutes
2	24 hours post-dose		X		±30 minutes
3	48 hours post-dose		X		±30 minutes
4	72 hours post-dose	3	X		±2 hour
5	96 hours post-dose	4	X		±2 hour
6	120 hours post-dose	5	X		±2 hour
8*	168 hours post-dose	6	X	X	± 4 hours
11*	240 hours post-dose	7	X		±8 hours
15*	336 hours post-dose	8	X	X	±8 hours
22*	504 hours post-dose	9	X	X	±1 day
29*	672 hours post-dose	10	X	X	±1 day

43*	1008 hours post-dose	11	X		±2 days
57*	1344 hours post-dose	12	X	X	±2 days
71*	1680 hours post-dose	13	X		±2 days
85*	2016 hours post-dose	14	X	X	±2 days
113*	2688 hours post-dose	15	X		±2 days
141*	3360 hours post-dose	16	X	X	±2 days
169*	4032 hours post-dose	17	X	X	±2 days

* Beginning at Day 8, for operational feasibility surrounding Public Holidays, an additional +1 day visit window may be granted with sponsor approval.

The planned volume of blood to be collected for this study, including that collected for eligibility and safety purposes, should not exceed 550 mL. Additional tests or blood draws could be performed, if deemed required by the Investigator or study staff.

Procedures for collection, processing, and shipping of PK blood samples will be detailed in the Bioanalytical Laboratory Manual.

Serum concentrations of the AIO-001, as well as anti-drug antibodies will be determined using validated analytical methods. Details of the analytical methods will be provided in a separate document.

8.2 Safety and Tolerability Assessments

Subjects will be monitored throughout the study by the clinical staff for AEs. Adequate medical surveillance will be assured during the confinement period and the Investigator or designee will be available on call at all times. If necessary, the Investigator or designee at the clinical site or a healthcare professional in a nearby hospital will administer treatment for any AE(s). A crash cart or emergency bag containing the necessary rescue material and appropriate medications will be available in the clinic to allow rapid intervention in case of emergency.

Safety parameters will be assessed by the Investigator or designee, using the clinical site acceptance ranges as suggested guidelines in making the medical assessment.

For eligibility purposes, abnormal vital signs measurements or clinical laboratory test results may be repeated once if an abnormal result is observed at the initial reading. Moreover, abnormalities found in the ECG may need to be confirmed by repeated measurements. In the event that the participation of a subject in the study is delayed and some screening procedures had been performed outside of the prescribed screening window, outdated screening procedures can be repeated.

Safety assessments scheduled during the study will be repeated according to the clinical site local guidance or upon request from the Investigator or designee. Any abnormal repeated measurement will be evaluated and repeated, if judged necessary. Further action may be taken upon the Investigator or designee's request.

8.2.1 Physical Examination

Physical examinations will be performed at the times specified in Table 1. Schedule of Assessments A complete physical examination will include assessments of the following: head, eyes, ears, nose, throat (HEENT), neck, chest, lungs, abdomen, musculoskeletal, dermatological, cardiovascular/peripheral vascular, and general neurological examination.

A brief physical examination will include assessments of the following: HEENT, chest, lungs, abdomen, dermatological, cardiovascular/peripheral vascular, and areas of note elicited from the subject. In the event of significant skin reactions appropriate informal photographs may be taken of the affected area.

8.2.2 Body Measurements

Body measurements will be performed at the times specified in Table 1. Schedule of Assessments and will include body weight and height measurements, as well as BMI calculation.

8.2.3 Injection Site Evaluation

Local reactions at the injection site will be evaluated by a trained observer before dosing on Visit 2 (Day 1 and approximately 1, 12, 24 hours (Day 2), 48 hours (Day 3)), 72 hours (Day 4, Visit 3) and 96 hours (Day 5, Visit 4) post-dose. A global severity rating for injection site reactions will be included in the assessment of AEs. An injection site examination post-dose will consist of examining the injection site and making note of the presence of any skin reactions, disturbances, or discolorations which will be graded and reported as per the table below:

Table 3. Injection Site Reaction Scores

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest
Erythema/Redness*	2.5-5 cm	5.1-10 cm	> 10 cm
Induration/Swelling**	2.5-5 cm and does not interfere with activity	5.1- 10 cm or interferes with activity	> 10 cm or prevents daily activity

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

8.2.4 Vital Signs

Blood pressure, heart rate, respiratory rate, and tympanic temperature will be measured after the subjects have been resting for at least 5 minutes in a sitting position at the times specified in Table 1. Schedule of Assessments. On Day 1 (Visit 2), vital signs will be performed: within 60 min of dosing, at 2, 4, 8, and 12 hours (±30 min) post-dose.

8.2.5 12-lead ECG

Standard 12-lead ECG will be recorded after the subjects have been resting for at least 5 minutes in a semi-recumbent or supine position at the times specified in Table 1. Schedule of Assessments. The examination includes: heart rate, PR interval, QT interval, corrected QT (QTcF using Fridericia's formula) interval and QRS, and should be performed by qualified personnel.

8.2.6 Laboratory Assessments

Blood and urine samples for clinical laboratory assessments will be collected according to the clinical site SOPs at the times specified in Table 1. Schedule of Assessments. The clinical laboratory assessments to be performed are listed in the table below.

Table 4. Clinical Laboratory Assessments

Biochemistry[1]	**Hematology**	**Urinalysis**
Albumin Alkaline phosphatase ALT AST Calcium Chloride Creatine kinase Creatinine eGFR (calculated using the CKD-EPI equation) GGT Glucose IgE Lactate dehydrogenase Phosphorus Potassium Sodium	Hematocrit Hemoglobin MCH MCHC MCV Platelet count RBC count RBC distribution width WBC count and differential: • Basophils • Eosinophils • Lymphocytes • Monocytes • Neutrophils	Bilirubin Blood (occult) Color and appearance Glucose Ketones Leukocyte esterase Nitrite pH Protein Specific gravity Urobilinogen Microscopic examination (in the event of abnormal findings)
	Coagulation	**Endocrinology**

Total, direct and indirect bilirubin Total protein Troponin-I Urea Uric acid **Lipid profile:** Total cholesterol Triglycerides	aPTT PT INR	FSH TSH T3 T4 (free) Serum pregnancy test Urine pregnancy test
Serology	**Drug and alcohol screens**	**Hormone panel - females only**
HBsAg HCV antibody HIV antigen/antibody QuantiFERON-TB COVID screening[1]	Amphetamines/methamphetamines (MET) Barbiturates Benzodiazepines Cocaine MDMA Methadone Opiates PCP THC Alcohol breath test	FSH (post-menopausal females only) Serum pregnancy test[1] Urine pregnancy test[1]

[1] Note: Biochemistry assessment will be done after 8 hours fasting period. Serum pregnancy test will be performed at screening and at EOS. A urine pregnancy test will be performed on Day -1 (Visit 2). COVID screening will be performed according to clinic site's procedure.

[2] Note: IgE is not an eligibility determining assessment and therefore results are not needed prior to eligibility confirmation.

Abbreviations: ALT = alanine aminotransferase; aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; eGFR = estimated glomerular filtration rate; FSH = follicle-stimulating hormone; GGT = gamma-glutamyl transferase; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; IgE = immunoglobulin E; INR = international normalized ratio; LDH=Lactate dehydrogenase; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; MCV = mean cell volume; MDMA = 3,4- methylenedioxymethamphetamine; MDRD = modification of diet in renal disease; PCP = phencyclidine; PT = prothrombin time; RBC = red blood cell; T3 = triiodothyronine; T4 = thyroxine; THC = tetrahydrocannabinol; TSH = thyroid-stimulating hormone; WBC = white blood cell.

8.3 Adverse Events

8.3.1 Definition of Adverse Event

An AE is defined as any untoward medical occurrence in a patient or clinical trial subject administered a pharmaceutical product and which does not necessarily have a causal relationship with the treatment. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product.

8.3.2 Recording of Adverse Events

AEs will be recorded and evaluated for their seriousness, severity, and relationship to the study drug. AEs will be collected and documented from the time of signing the ICF and throughout the study. AEs will be followed-up until complete resolution, or until the Investigator or designee judges safe to discontinue follow-up. The severity of AEs and relationship to the study drug will be classified according to the sections below.

8.3.3 Assessment of Severity

The severity of AEs will be described and documented using the following definitions:

Table 5. Severity of Adverse Events

Severity	Description
Mild (Grade 1)	Awareness of signs and symptoms but are easily tolerated; are of minor irritant type; causing no limitations of usual activities. Signs or symptoms may require minor action.
Moderate (Grade 2)	Discomfort severe enough to cause some limitations of usual activities and may require action.
Severe (Grade 3)	Incapacitating with inability to carry out usual activities or significantly affects clinical status and requires specific action and/or medical attention.

8.3.4 Assessment of Relationship to Study Drug

Each AE must be classified based on medical judgment and according to the following relationship categories: probable, possible, remote/unlikely, and unrelated. The definitions for the relationship categories are as follows:

Table 6. Adverse Event Relationship Categories

Category	Description
[Related] **Probable** (must have first three points)	This category applies to AEs that are considered, with a high degree of certainty, to be related to the investigational product. An AE may be considered probable, if: 1. It follows a reasonable temporal sequence from the administration of the drug. 2. It cannot be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors or other modes of therapy administered to the subject. 3. It disappears or decreases on cessation or reduction in dose (there are important exceptions when an AE does not disappear upon discontinuation of the drug, yet drug relatedness clearly exists; e.g. (1) bone marrow depression, (2) tardive dyskinesias). 4. It follows a known pattern of response to the suspected drug. 5. It reappears upon re-challenge.
[Related] **Possible** (must have first two points)	This category applies to AEs in which the connection with the investigational product administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possible if, or when: 1. It follows a reasonable temporal sequence from the administration of the drug. 2. It may have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3. It follows a known pattern of response to the suspected drug.
Remote/Unlikely (must have first two points)	In general, this category is applicable to an AE that meets the following criteria: 1. It does not follow a reasonable temporal sequence from the administration of the investigational product. 2. It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3. It does not follow a known pattern of response to the suspected drug. 4. It does not reappear or worsen when the investigational product is re-administered.
Unrelated	This category is applicable to AEs that are judged to be clearly and incontrovertibly due only to extraneous causes (disease, environment, etc.), and do not meet the criteria for medication relationship listed under remote/unlikely, possible, or probable.

8.3.5 Definition of Serious Adverse Event

A SAE is defined as any untoward medical occurrence that, at any dose:

- Results in death;
- Is life-threatening;
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect, or;
- Is otherwise considered to be an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require

intervention to prevent one of the other outcomes listed in the definition above. Medical and scientific judgement should be exercised in deciding whether an event should be considered as an Important Medical Event. Examples of such medical events include:

- Allergic bronchospasm requiring intensive treatment in an emergency room or at home;
- Blood dyscrasias or convulsions that do not result in hospitalization;
- Development of drug dependency or drug abuse.

8.3.6 Reporting of Adverse Events

8.3.6.1 Serious Adverse Event Reporting to the Sponsor

Any SAE will be reported to the Sponsor (or representative) within 24 hours of learning of the event. The notification must be directed to:

Aiolos Bio Clinical Safety
Email: clinical.safety@aiolosbio.com

Information on SAEs will be recorded on the SAE Report Form. Blank copies are included in the study Investigator's file. It is not acceptable for the Investigator to send photocopies of the patient's medical records to the Sponsor company or its representative in lieu of completion of the appropriate AE eCRF page or SAE Report Form. However, there may be instances when copies of medical records for certain cases are requested by the Sponsor or its representative. In this instance, all subject identifiers will be blinded on the copies of the medical records before submission to the Sponsor or its representative.

The completed SAE Form and SAE cover sheet should be sent via e-mail immediately upon completion to Aiolos Bio Clinical Safety. If the Investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before completing and sending the form.

Additional relevant information or clinical follow-up should be sent via e-mail to Aiolos Bio Clinical Safety as soon as it becomes available. The Investigator should follow the subject with the event until resolution or stabilization of the condition. Follow-up reports (as many as required) should be completed and faxed/e-mailed following the same procedure above.

A final report is required once the condition is resolved or stabilized and no more information about the event is expected. The final report should be completed and e-mailed following the same procedure above.

The Investigator must keep a copy of all documentation related to the event in the clinical site files.

If the Investigator learns of any SAE, including death, at any other time after a subject completes the study, and he/she considers the event reasonably related to the study drug, the Investigator will promptly notify the Sponsor/Medical Monitor.

8.3.6.2 Serious Adverse Event Reporting to the IEC and Regulatory Agency(ies)

It is the responsibility of the clinical site to report suspected, unexpected, serious adverse reactions (SUSARs) to the IEC responsible for the study per their policies. Report of fatal or life-threatening SUSARs must be made as soon as possible, but no later than 7 calendar days after first knowledge of the event. Report of SUSARs that are neither fatal nor life-threatening must be made as soon as possible, but no later than 15 calendar days after first knowledge of the event.

The Sponsor (or representative) is responsible for notifying the regulatory agency(ies) of SUSARs observed during the study conduct per their regulations, as soon as possible but no later than 7 calendar days after becoming aware of the information when fatal or life-threatening, or 15 calendar days when neither fatal nor life-threatening. The Sponsor (or representative) is responsible to comply with any other applicable regulatory requirement(s) related to the reporting of SAE to other regulatory authority(ies).

8.3.7 Pregnancy

In the event a dosed female subject or the female partner of a dosed male subject received at least one dose of the investigational product) becomes pregnant during or shortly after participation in the study, this pregnancy will be reported to the Sponsor (or representative) within 24 hours of first knowledge of the event. Any subject who becomes pregnant during the study will be immediately withdrawn. Follow-up information regarding the course and outcome of the pregnancy will be documented (after obtaining the consent of the female partner) as per site's SOP. If the outcome of the pregnancy meets the criteria of reportable event, reporting of the event to the IEC responsible for the study and/or to applicable regulatory agency(ies) will be performed as per site's SOP.

8.4 Premature Termination of the Study

The study may be prematurely terminated by the Investigator following consultation with the Sponsor, by the Sponsor or by the regulatory authorities. Following a decision to discontinue the trial, the Investigator will promptly inform the active study subjects and the IEC responsible for this trial, stating the reasons for discontinuation of the study. It is the responsibility of the Sponsor (or representative) to report the premature termination of the study to the regulatory authority(ies), when required by the applicable regulatory requirement(s).

9. Statistical Analyses

A complete description of the statistical analyses to be performed on PK and ADA as well as safety and tolerability data will be presented in a statistical analysis plan (SAP).

9.1 Analysis Populations

9.1.1 Safety Population

The safety population is defined as all subjects who receive at least one dose of the study drug.

9.1.2 Pharmacokinetic Population

The PK population will include all subjects who have at least 1 measured PK concentration following dosing. If any participants have incomplete data, protocol deviations, or clinical events that affect PK, a decision will be made on a case-by-case basis as to their inclusion in the analysis.

9.1.3 Immunogenicity Population

The immunogenicity population will include all subjects who have received any amount of AIO-001 and have at least one post-dose ADA measurement.

9.2 Pharmacokinetic Analysis

Serum samples collected at the PK timepoints in Table 1 and Table 2 will be analyzed to determine concentrations of AIO-001. The actual start and end times of AIO-001 administration and the exact timing of blood sampling must be recorded in the CRF. Samples collected for analysis of AIO-001 PK may additionally be used to evaluate safety and efficacy to address concerns arising during or after the study period or for further development and characterization of PK and immunogenicity assays. Instructions for handling of blood samples and shipping of samples for storage or analyses will be provided in the Laboratory Manual (provided separately).

The following PK parameters will be calculated based on the PK population using standard non-compartmental analysis methods and may include but are not limited to:

- $AUC_{0\text{-last}}$: Area under the concentration-time curve from time zero until the last observed concentration
- $AUC_{0\text{-inf}}$: Area under the concentration-time curve from time zero to infinity (extrapolated)
- C_{max}: Maximal observed concentration
- T_{max}: Time when the maximal concentration is observed
- $T_{½}$: Terminal elimination half-life

9.3 Immunogenicity Analysis

Anti-AIO-001 antibodies will be evaluated in serum samples collected from all participants at the timepoints listed in Table 1/Table 2. Serum samples will be screened for antibodies binding to AIO-001 and the titer of confirmed positive samples will be reported. Incidence of treatment-emergent ADA will be reported. Other analyses may be performed to further characterize the immunogenicity of AIO-001. Immunogenicity analyses may be conducted on PK samples collected at other timepoints, if deemed necessary.

9.4 Pharmacokinetic and Immunogenicity Statistical Analysis

The pharmacokinetic profile of AIO-001 will be established using non-compartmental analysis of serum concentration data obtained at the timepoints outlined in the Schedule of Assessments. PK parameters including but not limited to $AUC_{0\text{-last}}$, $AUC_{0\text{-inf}}$, C_{max}, T_{max}, and $T_{½}$ will be calculated if sufficient data are available for estimation, at the discretion of the pharmacokineticist.

AIO-001 serum concentrations and PK parameters will be presented in summary tables for each dose cohort and in listings, as appropriate. Individual and mean AIO-001 serum concentrations over time will be presented graphically.

In addition, population pharmacokinetic modeling may be applied for PK analysis and will be reported separately.

Individual and mean serum concentration versus time curves will be presented for both linear and semi-log scales. Descriptive statistics (arithmetic and geometric means, standard deviation [SD], CV%, minimum [Min], maximum [Max], and median) of the serum concentrations versus time will be presented as well for the PK parameters.

Additional PK statistical analysis may be performed.

9.5 Safety and Tolerability Analysis

Demographic parameters will be summarized descriptively.

Safety and tolerability analysis will be performed for all subjects in the safety population. No inferential statistical analysis of safety data is planned.

Safety and tolerability of AIO-001 will be evaluated through the assessment of AEs (i.e., seriousness, severity, relationship to the study drug, outcome, duration, and management), vital signs, 12-lead ECGs, clinical laboratory tests, and physical examinations. AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA).

TEAEs will be tabulated by treatment. Changes from baseline values in vital signs, 12-lead ECGs, and clinical laboratory tests will be evaluated. Safety and tolerability data will be reported using descriptive statistics.

9.5.1 Interim Analyses

Interim analyses of preliminary data will be performed at Sponsor discretion.

10. Data Collection

The electronic source data capture system is the primary data collection instrument for the study. Collection screen in the electronic source data capture system will be utilized for the collection of all data. Data will be entered using the English language and should be kept current to enable the monitor to review the subjects' status throughout the course of the study.

Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Source documents will be maintained in order to maintain data integrity. The Investigator and/or the clinical staff have the responsibility of ensuring the accuracy, completeness, legibility, and timeliness of the source data.

Details on the data management process will be described in a data management plan (DMP).

11. Regulatory Considerations and Quality Assurance

11.1 IEC Approval of Protocol and Other Study Documents

The Investigator(s) agree to provide the IEC with all appropriate documents, including a copy of the protocol/amendments, ICFs, advertising text (if any), Investigator's Brochure (if any), and any other written information provided to study subjects. The trial will not begin until the Investigators have obtained the IEC favourable written approvals for the above-mentioned study documents. A properly executed written ICF shall be read, signed, and dated by each subject prior to entering the trial or prior to performing any study procedure. The original signed and dated ICF will be kept at the clinical site and a copy will be given to the subject.

In the event that the protocol is amended, the revised protocol must be approved by the IEC prior to its implementation, unless the changes involve only logistical or administrative aspects of the trial. If a revised ICF is introduced during the study, each subject's further consent must be obtained. The new version of the ICF must be approved by the IEC, prior to subsequently obtaining each subject's consent.

The Investigator and the Sponsor's representative must sign the protocol and its amendments (if any) before initiating the study.

It is the Sponsor's responsibility to submit the protocol and its amendments (if any), and the ICFs to regulatory authorities when necessary.

11.2 Compliance

This study will be conducted in compliance with the protocol, GCP, the requirements of Directive 2001/83/EC Annex I, as amended by Directive 2003/63/EC and Directive 2001/20/EC, and all applicable regulations, including the Federal Food, Drug and Cosmetic Act, U.S. applicable Code of Federal Regulations (title 21), and any IEC requirements relative to clinical studies. The study will also be conducted in compliance with the recommendations laid down in the most recent version of the Declaration of Helsinki, with the exception that registration of such Phase 1 trials in a publicly accessible database is not mandatory.

11.3 Quality Assurance and Monitoring

The Sponsor or its designee will perform the quality assurance and quality control activities of this study; however, responsibility for the accuracy, completeness, security, and reliability of the study data presented to the Sponsor lies with the Investigator generating the data. When applicable, quality assurance procedures will be performed according to the site SOPs.

The study will be monitored according to the site monitoring plan and SOP to ensure that it is conducted and documented properly according to the protocol, GCP, and all applicable regulatory requirements. The Sponsor is entitled to access information about the status of the study and to review the original documents of the study.

The Sponsor may arrange audits as part of the implementation of quality assurance to ensure that the study is being conducted in compliance with the protocol, SOPs, GCP, and all applicable

regulatory requirements. In such case, audits will be independent of and separate from the routine monitoring and quality control functions.

11.4 Confidentiality and Retention of Study Records

This document contains trade secrets and commercial information that is confidential and may not be disclosed to third parties. Persons to whom this study protocol is disclosed must be informed that all the information herein is confidential and may not be further divulged. These restrictions will apply as well to all future communications if deemed privileged or confidential. Publication of the study results may only be allowed with written permission from the Sponsor.

All information on a subject obtained during the conduct of the study will be kept confidential. Subjects will be identified by an anonymized identifier on all samples and study records provided to the Sponsor or designee. In compliance with ICH GCP, the Sponsor's authorized representatives, monitor(s), auditor(s), IEC, and regulatory authority(ies) will be granted direct access to the subject's original trial-related records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations. Consent from the subject for disclosure of such information will be obtained in writing in the ICF. In addition, should a subject require medical care or hospitalization during the course of the study, the clinical site may contact the treating physician with the subject's consent, except that consent may not be requested if there is an emergency situation. If the results of the study are published, the subject's identity will remain confidential.

The clinical site will maintain adequate study records according to applicable regulatory requirements. The Sponsor will be notified prior to the destruction of study records.

12. References

1 AIO-001. Investigator's Brochure. Aiolos Bio. Inc . Version No. 3.1, 20 Oct. 2023.

2 van Dijk, B.C.P., Svedsater, H., Heddini, A. et al. Relationship between the Asthma Control Test (ACT) and other outcomes: a targeted literature review. BMC Pulm Med 20, 79 (2020). https://doi.org/10.1186/s12890-020-1090-5