Clinical Study Protocol

A Phase I Study to Evaluate the Tolerance, Safety and Pharmacokinetics of ALT-BB4 in Healthy Volunteers

Investigational Product (IP)	ALT-BB4 (Recombinant Hyaluronidase)
Protocol No.	ALT-BB4-01
Protocol Version	3.2
Study Phase	Phase 1
Sponsor	Alteogen, Inc.
Protocol Preparation Date	2022-06-10

CONFIDENTIAL

▣ Term Definition and Abbreviation

ADR	Adverse drug reaction
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
AVG	Average
BLQ	Below the limit of quantification
BPM	Beats per minute
BUN	Blood urea nitrogen
CFR	Code of federal regulations
CIOMS	Council for international organizations of medical sciences
Cl	Clearance
Co-mix	Concomitant
CS	Clinically significant
DB	Database
ECM	Extracellular matrix
ECG	Electrocardiogram
EDC	Electronic data capture
eCRF	Electronic case report form
FDA	Food and drug administration
GCP	Good clinical practice
HA	Hyaluronic acid
HBV	Hepatitis B virus
hCG	human Chorionic gonadotrophin
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HYALP1	Hyaluronoglucosaminidase pseudogene 1
IB	Investigator's brochure
IgE	Immunoglobulin E
IRB	Institutional review board
K_{cat}	Catalytic rate constant
KGCP	Korean good clinical practice
MedDRA	Medical dictionary for regulatory activities

NCS	Not clinically significant
NOAEL	No observed adverse effect level
NRS	Numeric rating scale
NSAIDs	Non-steroidal anti-inflammatory drugs
RBC	Red blood cell
RPM	Revolutions per minute
SADR	Serious adverse drug reaction
SAE	Serious adverse event
SEM	Standard error of the mean
Seq	Sequential
SI	Stimulation Index
SOC	System organ class
SPAM 1	Sperm adhesion molecule 1
SUSAR	Suspected unexpected serious adverse reaction
OTC	Over-the-counter
PK	Pharmacokinetics
PT	Preferred term
USV	Unscheduled visit
VDRL	Venereal disease research laboratory
WBC	White blood cell
WOCBP	Women of childbearing potential

▣ Table of Contents

1. Study Title, Phase, Protocol Identification No. and Creation/Revision History

1.1. Title

A Phase I Study to Evaluate the Safety, Tolerability and Pharmacokinetics of ALT-BB4 (Recombinant Hyaluronidase) in Healthy Volunteers

1.2. Phase

- Part I (Allergy assessment): Multicenter, 2-arm (study drug and comparator), randomized (randomization of dosing site of the study drug and comparator), double-blinded, placebo-controlled, intradermal injection, Phase I
- Part II-A (PK assessment): Single center, open-labeled, subcutaneous injection, Phase I
- Part II-B (Safety assessment): Multicenter, 2-arm (study group and control group), randomized, double-blinded, placebo-controlled, subcutaneous injection, Phase I

1.3. Protocol Identification No. and Creation/Revision History

- Protocol Identification No.: ALT-BB4-01
- Creation/Revision History

Version	Date	Major Changes
1.0	2020-12-18	Created.
2.0	2021-07-16	- Design changed (2 arms → 3 arms). - Sample size changed. - Institutions/Principal Investigators added.
2.1	2021-07-30	- Section 3.4: Rationale for dose selection added. - Section 5.3.1: Change of the inclusion criteria (in the contraception, exclude 'concomitant use of male condom and female condom' from double barrier methods) - Section 5.3.2: Change of the exclusion criteria ('Past history of drug abuse' added) - Section 10.3.1: Photographing of administration site at Visit 4 (2D) in Part I becomes mandatory. - Section 10.3.3: Photographing of administration site at Visit 4 (0D) and Visit 5 (2D) in Part II-B becomes mandatory. A measure to collect AEs is added. - Section 10.1.13: A measure to document rationale for photographing of the administration site is added. - Section 11.1.1: Definition of safety set is modified. - Section 11.2.1: Imputation method of missing data is added - Section 11.2.4.1: Statistical analysis method of primary endpoints is added - Others: Terms modified, description clarification, etc.
3.0	2021-12-28	- Section 2.2.1: Timing of vital signs and laboratory tests at baseline is clarified before the ID injection of the IP). - Section 2.2.2 and Section 2.2.3: Corrections are made to vital signs and laboratory tests at Visit 4: they are to be performed before the ID injection of the IP and may be replaced with the results obtained at Visit 4 of Part I. - Section 5.3.2: Change of the exclusion criteria 1) Common 1. Prohibited concomitant medications before

Version	Date	Major Changes
		study participation should be checked relative to baseline, and the timeframe for NSAIDs is changed to within 14 days from baseline since 14 days are enough to wash out for NSAIDs. 2) The criteria is clarified to exclude current clinically significant diseases. 3) Common 4. The criteria is clarified to exclude subjects who are diagnosed with hypertension/hypotension or subjects who have clinically significant blood pressure. 4) Part II-A 13, 14, 15, and 16. The exclusion criteria should be checked relative to Visit 4 (baseline of Part II-A). - Section 9.3.2: It is stated that whole blood donation, apheresis, and transfusion are prohibited only for subjects in Part II-A. - Section 10.1.2: Height, weight, and BMI are deleted since they are measured in the physical examination, and history of whole blood donation, apheresis, and transfusion are to be investigated only for subjects considering participating in Part II-A. - Section 10.1.5: ECG parameters are modified. - Section 10.1.7: It is allowed to replace test results for baseline (Visit 2) with the results obtained within 7 days, if available. - Section 10.1.13: The time window for observation of immediate allergic reactions is changed. - Section 10.3.1.2 and Section 10.3.2.2: The statement that elevation considered due to an injection is not considered wheal is added. - Section 11.2.4.1: The statistical analysis method is corrected to one that is appropriate for result analysis of the primary and secondary endpoints. - Section 11.2.4.2: Details description of the comparison time points within a group and comparative analysis between the groups at each time point after IP administration are added. - Other: Errors corrected, description clarification, etc.
3.1	2022-02-22	- Section 11.2.4.1: Corrected the statistical analysis method to the method suitable for the analysis of primary endpoint results - Section 11.2.4.2: Corrected the analysis for comparison between the groups by dividing into Part 1 and Part 2 for the analysis of secondary endpoint results
3.2	2022-06-10	- Section 5: Changed the total number of subjects - Section 6.4.2: Corrected the unblinding method

2. Synopsis, Table of Schedule, and Diagram

2.1 Synopsis

Study Title	A Phase I Study to Evaluate the Safety, Tolerability and Pharmacokinetics of ALT-BB4 (Recombinant Hyaluronidase) in Healthy Volunteers
Phase and Design	• Part I (Allergy assessment): Multicenter, 2-arm (study drug and comparator), randomized (randomization of dosing site of the study drug and comparator), double-blinded, placebo-controlled, intradermal (ID) injection, Phase I • Part II-A (PK assessment): Single center, single arm, open-labeled, subcutaneous (SC) injection, Phase I • Part II-B (Safety assessment): Multicenter, 2-arm (study group and control group), randomized, double-blinded, placebo-controlled, SC injection, Phase I
Institutions and Principal Investigators	• See Annex 1.
Coordinating Investigator	• Professor Dong Hoon Lee, Dept. of Dermatology, Seoul National University Hospital
Sponsor	Alteogen, Inc.
Study Objective	**Primary Objective** To evaluate the allergic reactivity, safety and tolerability of ALT-BB4 (recombinant hyaluronidase) by checking for drug allergy reactivity following single ID injection of ALT-BB4 and monitoring systemic reactions and adverse events following its single SC injection **Secondary Objective** To evaluate the PK profile of single dose of ALT-BB4 **Exploratory Objective** To evaluate the immunogenicity of ALT-BB4 (Visit 1 and End of Study visit)
Study Population	Healthy volunteers aged ≥19 years
Inclusion Criteria	**Common** 1. Healthy volunteers aged ≥19 years at the time of Screening (Visit 1) 2. Female subjects or male subjects' female partner must be menopausal or should have received a sterilization procedure or have agreed to use contraceptive methods during the study period, as defined below: • Post-menopausal female subjects or male subjects' female partners (non-drug induced amenorrhea for at least 12 months or confirmed diagnosis with menopause) • Female subjects or male subjects' female partners who have received a sterilization procedure (removal of ovary and/or uterus) • Subjects who have agreed to practice total abstinence during the study period [For female subjects, periodic abstinence (e.g., ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception.] • When female subjects or male subjects' female partners are

	women of childbearing potential (WOCBP) who have not received a sterilization procedure, they must agree to use of following contraceptive methods: - Hormones (implantable, patch, and oral) - Intrauterine device (IUD) - Double barrier methods (concomitant use of two of following contraceptive methods: male condom, female condom, cervical cap, diaphragm, sponge, spermicide) (However, concomitant use of male condom and female condom is excluded from double barrier methods.) 3. WOCBP or females who have the last menstrual period within 12 months must have a negative serum or urine pregnancy test at Screening (Visit 1). 4. Subjects who have voluntarily decided to take part in the study and able to comply with the study protocol 5. Subjects who have no tattoo, acne, dermatitis, pigmentation or lesion on the administration site and who have no damage in the skin, so that they can receive the investigational product (IP) and allergy test 6. Subjects determined eligible for the study through Screening tests (vital signs, physical examination, medical history and surgery history, electrocardiogram (ECG), and laboratory tests) **Part II-A** 7. Subjects with BMI no less than 18.5 kg/m^2, no greater than 24.9 kg/m^2
Exclusion Criteria	Below are the study exclusion criteria: **Common** 1. Subjects who have received or treated with following medications within the specified timeframe prior to Baseline (Visit 2) or who are expected to receive them during the study period: • Within 1 month: Hyaluronidase, Chemotherapeutic agent, penicillins antibiotics (e.g.: Amoxicillin, Ampicillin, etc.), cephalosporins antibiotics (e.g.: Cefaclor, Cefadroxil, Cefixime, etc.), sulfonamides antibiotics (e.g.: Sulfadiazine, Sulfamethoxazole, etc.), quinolones antibiotics (e.g.: Ciprofloxacin, Levofloxacin, etc.), Glucocorticosteroid, Immunosuppressive agent • Within 14 days: Antihistamine (e.g.: Chlorpheniramine, Hydroxyzine, Ketotifen, etc.), non-steroidal anti-inflammatory drugs (NSAIDs; e.g.: Aspirin, Aceclofenac, etc.) 2. Subjects who have current or prior history of clinically significant liver, kidney, gastrointestinal, cardiovascular, respiratory, endocrine, immune system, psychiatric/nerve system, and blood/oncological disorders 3. Subjects with acute fever > 37.5℃ within 7 days from the expected IP administration or showing symptoms suspecting acute diseases within 14 days from the expected IP administration 4. Subjects who are diagnosed with hypertension/hypotension or subjects who are with clinically significant blood pressure* *Hypertension=systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg

	Hypotension=systolic blood pressure ≤ 90 mmHg and/or diastolic blood pressure ≤ 60 mmHg 5. Subjects who persistently drink more than the weekly recommended alcohol units * 14 g of alcohol content per unit is applied, which corresponds to 1 can (small bottle) of beer (5%), 350 mL of draft beer (5%), 1 cup (300 mL) of makgeoli, 1 glass (150 mL) of wine (12%), 1/4 bottle (90 mL) of soju (20%), and 1 shot (45 mL) of liquor (40%). Moderate amount of alcohol by age and sex is provided below:

Age	Recommended unit per week
Adults (≥ 19 and < 65 years)	Male: 8 units/week Female: 4 units/week
Elderly (≥ 65 years)	Male: 4 units/week Female: 2 units/week

6. Subjects of usual smoker (exceeding 10 cigarettes per day)
7. Subjects who have a past history of autoimmune diseases (e.g.: rheumatoid arthritis, etc.) or active immune diseases that may affect the immune system [e.g.: flu, cancer, Human immunodeficiency virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV) etc.], diabetes mellitus, heart diseases, asthma, sinusitis, chronic urticaria, dermographism, or any skin conditions that may affect post-IP administration assessments (e.g.: Dermatitis, dermatomycosis and other skin diseases or tattoo)
8. Subjects with known allergic reactions or hypersensitivity to bee sting or other common allergens or known contraindications to hyaluronidase, thimerosal (disinfectants) and EDTA
9. Subjects with hypersensitivity to the IP or its ingredients or a history of anaphylaxis
10. Past history of drug abuse
11. Subjects who have participated in other clinical trials within 6 months prior to the expected IP administration
12. Others determined ineligible for the study participation in the opinion of investigator

Part II-A

13. Subjects who have been treated with a drug-metabolizing enzyme inducers and inhibitors* within 30 days prior to Visit 4

    * Example: Phenytoin, Carbamazepine, Barbiturates, Rifampicin, Griseofulvin, Cimetidine, Disulfiram, Erythromycin, Ketoconazole, Fluconazole, Itraconazole, Valproic acid, Isoniazid, Ciprofloxacin, Omeprazole, Clarithromycin, Quinidine, Sulfonamides, etc.
14. Subjects who have significant bleeding or blood loss within 60 days prior to Visit 4
15. Subjects who have donated whole blood within 60 days or donated blood by apheresis within 14 days or received transfusion within 14 days prior to Visit 4
16. Subjects who have consumed grapefruit juice within 7 days prior to Visit 4

Target Number of Subjects	1. Total number of subjects: At least 231 subjects (Maximum of 290 including subjects with allergic reactions, dropped out and excluded from analysis) 2. Number of subjects for each Part

<table>
<tr><td></td><td>
• Part I (Allergy assessment): At least 231 subjects
• Part II-A (PK assessment): Total 23 subjects (including 10% drop-out rate)
• Part II-B (Safety assessment): At least 208 subjects
(Study group- at least 139 subjects, Control group - at least 69 subjects; randomized in a 2:1 ratio)
</td></tr>
<tr><td>Study Period</td><td>
1. Entire Study Period
Approximately 18 months from approval of the Institutional Review Board (IRB) (however, it may be changed depending on the subject enrollment rate.)

2. Study Period for Individual Subjects
• Part I (Allergy assessment):
- Screening period: Up to 2 weeks
- Treatment with IP: 1 day (ID single dose)
- Follow-up period: 2 days (48 hours)

• Part II-A (Pharmacokinetic (PK) assessment):
- Treatment with IP: 1 day (SC single dose)
- PK Assessment: Total 15 times
■ Pre-dose: -2h, -1h, and 0h
■ Post-dose: 10 min (0.17h), 20 min (0.3h), 30 min (0.5h), 40 min (0.7h), 50 min (0.8h), 1h, 1h 15 min (1.25h), 1h 30 min (1.5h), 2h, 2 h 30 min (2.5h), 3h and 24h
- Follow-up period: 4 weeks

• Part II-B (Safety assessment):
- Treatment with IP: 1 day (SC single dose)
- Follow-up period: 4 weeks
</td></tr>
<tr><td>Investigational Product (IP)</td><td>
1. Study Drug: ALT-BB4 (Recombinant Hyaluronidase)
• Active ingredient and Content: ALT-BB4 (recombinant hyaluronidase) 1,500 IU/mL
• Formulation and Appearance: Colorless, clear liquid injection
• Manufacturer: Alteogen, Inc.
2. Comparator: Normal saline (0.9% NaCl)
• Active ingredient and Content: 0.9% NaCl
• Formulation and Appearance: Colorless, clear liquid injection
• Manufacturer: JW Pharmaceutical Corp.
<table>
<tr><th>Administration Method (Assessment)</th><th>Study drug (ALT-BB4, 1,500 IU/mL)</th><th>Comparator (0.9% NaCl)</th></tr>
<tr><td>ID injection (Allergy assessment)</td><td>30 IU (0.02 mL)</td><td>0.02 mL</td></tr>
<tr><td>SC injection (PK assessment)</td><td>1,500 IU (1 mL)</td><td>N/A</td></tr>
<tr><td>SC injection (Safety assessment)</td><td>1,500 IU (1 mL)</td><td>1 mL</td></tr>
</table>
</td></tr>
<tr><td>Dosage and Administration</td><td>
1. Part I (Allergy assessment)
Subjects will be randomized into the study group or control group at Baseline visit (Visit 2), and receive single dose of the study drug or
</td></tr>
</table>

comparator intradermally into the right or left forearm.

Treatment group	Study group	Control group
IP	Study drug (ALT-BB4, 1,500 IU/mL)	Comparator (0.9% NaCl)
ID dose	30 IU (0.02 mL)	0.9% NaCl (0.02 mL)
Administration site*	Right or left forearm	Forearm in the opposite side of the previous dose

* For example, when the study drug is intradermally injected into the left forearm, the comparator will be intradermally injected into the right forearm.

2. Part II-A (PK assessment)

Part II-A PK assessment will be performed only in subjects with drug allergy test negative at Visit 4, 2 days (48 hours) after the ID injection in Part I. All subjects participating in Part II-A will receive single dose of the study drug subcutaneously into the right or left upper arm.

Treatment group	Study group
IP	Study drug (ALT-BB4, 1,500 IU/mL)
SC dose	1,500 IU (1 mL)
Administration site	Right or left upper arm

3. Part II-B (Safety assessment)

Part II-B safety assessment will be performed only in subjects with drug allergy test negative at Visit 4, 2 days (48 hours) after the ID injection in Part I, randomized into the study group and control group in a 2:1 ratio. Subjects assigned in each group of Part II-B will receive single dose of the study drug or comparator subcutaneously into the right or left upper arm.

Treatment group	Study group	Control group
IP	Study drug (ALT-BB4, 1,500 IU/mL)	Comparator (0.9% NaCl)
SC dose	1,500 IU (1 mL)	1 mL
Administration site	Right or left upper arm	Right or left upper arm

Methods

This is a Phase 1 study to perform allergy assessment and PK profiling as well as to evaluate the safety and tolerability following administration of ALT-BB4 (recombinant hyaluronidase) in healthy volunteers. This study consists of Part I (Allergy assessment), Part II-A (PK assessment) and Part II-B (Safety assessment). In Part I, drug allergy assessment will be performed first. Then only in eligible subjects, PK of ALT-BB4 will be assessed in Part II-A, and the safety and tolerability assessment will be performed in Part II-B.

Part I (Allergy assessment)

For subjects who have agreed to participate by giving a written informed consent, Screening tests will be performed. By confirming the Screening test results and the inclusion/exclusion criteria, eligible subjects for participating in Part I of the study for the drug allergy assessment will be enrolled. Enrolled subjects will visit the institution and receive the IP intradermally. For up to 30 minutes after the IP administration, subjects will be observed for cutaneous hypersensitivity of immediate allergic reaction and subjects without any adverse event will be discharged. Later at Visit 3, subjects will be contacted via phone to monitor concomitant medications

	and adverse events. At Visit 4, subjects will visit the institution for monitoring of cutaneous hypersensitivity of delayed allergic reaction occurring within 48 hours after the ID injection, blood collection, change of concomitant medications and adverse events, performing scheduled tests. ALT-BB4 allergy assessment will be conducted through scheduled tests and evaluations for each visit. **Part II-A (PK assessment)** Eligible subjects for Part II-A of the study will be enrolled only from subjects with drug allergy test negative in Part I. Enrolled subjects will receive the IP subcutaneously at the institution and perform scheduled tests and blood collection at designated time points. At 3 hours after the IP administration, scheduled tests will be completed and subjects without any adverse event will be discharged. In a case of adverse events, the subjects will be treated by investigator as required before discharge. Later at Visit 5, subjects will visit the institution for blood collection, review of any change in concomitant medications and adverse events, and scheduled tests, and at Visit 6 (End of Study visit), subjects will visit the institution for review of any change in concomitant medications and adverse events and scheduled tests. ALT-BB4 PK assessment will be conducted through scheduled tests and blood collection for PK assessment for each visit. **Part II-B (Safety assessment)** Eligible subjects for Part II-B of the study will be enrolled only from subjects with drug allergy test negative in Part I. Subjects enrolled in Part II-B study will be randomized into the study group or control group at the institution and receive single dose of the IP subcutaneously into the right or left upper arm. For up to 30 minutes after the IP administration, subjects will be discharged if there is no adverse event when monitoring systemic and administration site adverse events. In a case of adverse events, the subjects will be treated by investigator as required before discharge. Later at Visit 5, subjects will visit the institution for blood collection, review of any change in concomitant medications and adverse events, and scheduled tests. Visit 6 is a phone visit, where concomitant medications and adverse events including systemic and administration site events will be monitored. At Visit 7, the End of Study visit, scheduled tests and safety assessment will be performed to evaluate the safety and tolerability of ALT-BB4.
Endpoints and Assessment Method	**Efficacy Endpoints** The study aims to evaluate allergy, PK, safety and tolerability and efficacy analysis will not be separately performed. **Safety Endpoints** Part I study will evaluate the drug allergy and adverse events occurring following ID injection of the IP. Part II-A study will evaluate adverse events occurring following SC injection of the IP. Part II-B study will evaluate the safety and tolerability following SC injection of the IP. **1. Primary Endpoints** **1) Incidence rate of drug allergy following ID injection of the IP* in Part I** *Subjects developing either immediate or delayed allergic reaction are considered to have drug allergy. The number and percentage (%) of subjects showing any change of drug allergy from the IP administration to the end of study or early

termination in the control group and study group will be provided. In addition, as the primary analysis, the upper limit of binomial exact 95% two-sided confidence interval (97.5% one-sided confidence interval) < 10% is considered statistically significant. In addition, the analysis will be performed using the McNemar's test to compare the incidence rate of drug allergy between the study group and the control group.

2) Safety and tolerability assessment following SC injection of the IP in Part II**

** Incidence rate of adverse events, including systemic and administration site-related adverse events

To evaluate the safety and tolerability, systemic and administration site-related adverse events will be assessed following SC injection of the IP.
In addition to the routine safety tests of physical examination, vital signs, ECG and laboratory tests, comprehensive data about clinical adverse events will be evaluated to determine relationship of the adverse drug reactions, including the nature, signs and type of adverse events, severity, frequency and duration, occurrence site and types of disease, and symptoms of AEs by system organ class (SOC).
The number and percentage (%) of subjects developing adverse events will be provided and the analysis will be performed using the Pearson's chi-square test or Fisher's exact test to compare the incidence rate of AEs between the study group and the control group..

2. Secondary Endpoints

1) Incidence rate of immediate allergic reactions† occurring within 30 minutes after the ID injection of the IP

†defined as drug allergy associated with wheal ≥ 8 mm with or without pseudopod (elevation considered due to an injection is not considered wheal), erythema or localized itching occurring in the administration site within 30 minutes after IP administration

The number and percentage (%) of subjects showing any change of immediate allergic reaction occurring within 30 minutes after the IP administration in the control group and study group will be provided and analyzed using the McNemar's test to compare the incidence of immediate allergic reaction between the study group and the control group.

2) Incidence rate of delayed allergic reactions‡ occurring within 48 hours after the ID injection of the IP

‡drug allergy occurring from 30 minutes to 48 hours after the IP administration, including but not limited to redness, rash, urticaria, edema, and erythema in the administration site as well as acute generalized exanthematous pustulosis and erythema multiforme. Investigator will decide whether an adverse event is a delayed allergic reaction.

The number and percentage (%) of subjects showing any change of delayed allergic reaction occurring from 30 minutes to 48 hours after the IP administration in the control group and study group will be provided and analyzed using the McNemar's test to compare the incidence of delayed allergic reaction between the study group and the control group.

3) Incidence rate of administration site events* at 30 min. and 48 hours after the IP administration

* classified into wheal, erythema, localized itching and other adverse events, occurring after the IP administration, at each time point

For wheal, erythema, localized itching and other adverse events at 30 minutes and 48 hours after the IP administration in the control group and study group, the number and percentage (%) of subjects showing any change of each event will be provided and analyzed using the McNermar's test in Part 1 and Pearson's chi-square test or Fisher's exact test in Part 2 to compare the incidence of allergic reaction between the study group and control group by administration site-related AE.

4) Size of wheal and erythema occurring at 30 min. and 48 hours after the IP administration

For the size of wheal and erythema occurring at 30 minutes and 48 hours and change in the size of wheal and erythema occurring at 48 hours after the IP administration compared to 30 minutes after the IP administration in the control group and study group, descriptive statistics (mean, standard deviations, median, min and max) will be provided. The analysis will be performed using the Paired t-test or Wilcoxon signed-rank sum test to compare the change in the size of wheal and erythema occurring at 48 hours after the IP administration compared to 30 minutes, and for the comparison between the study group and control group, an analysis of covariance (ANCOVA) with the baseline as a covariate will be performed. If the assumption of normality is not satisfied, a rank ANCOVA will be performed.

3. Other Safety Endpoints

1) Adverse Events

All adverse events will be coded by using the most recent version of Medical dictionary for regulatory activities (MedDRA). The number of subjects with adverse events and adverse drug reactions, incidence rate (%) and frequency by treatment group will be provided, along with its 95% confidence interval.

In addition, the number of subjects with adverse events and adverse drug reactions, incidence rate and frequency by SOC and PT will be provided.

2) Physical Examination, Vital Signs, ECG and Laboratory Tests

For continuous data, descriptive statistics (mean, standard deviation, median, min and max) for absolute values and changes from baseline at each time point of visits and Paired t-test or Wilcoxon signed-rank test will be performed. For laboratory tests and ECG values, the frequency and proportion of subjects shifting from normal/clinically insignificant abnormal value before the IP administration (Screening) to clinically significant abnormal value after the IP administration will be provided and McNemar's test will be performed.

PK Endpoints

	1. Primary endpoints: C_{max}, AUC_{last}, $t_{1/2,}$ and T_{max} of ALT-BB4 2. Secondary endpoints: Baseline-adjusted C_{max}, AUC_{last}, $t_{1/2}$, and T_{max} of ALT-BB4 For PK endpoints, statistical test is not separately performed. PK assessment results can be provided in a separate report.

2.2 Table of Study Schedule

After allergy assessment in Part I, Part II-A and Part II-B will be performed as provided in the schedule below.

2.2.1 Part I (Allergy assessment)

Period	Screening[1]	Baseline[1]	Follow up (Wash-out before Part II)	
Visit	Visit 1	Visit 2[2]	Visit 3 (Phone Visit)	Visit 4[3]
Day (D)	–14D ~ –1D	0D	1D	2D
Written informed consent[4]	X			
Assigning screening number	X			
Demographic data[5]	X[6]			
Medical history[7]	X	X		
Prior/Concomitant medications[8]	X	X	☎	X
Vital signs[9]	X	X		X
ECG	X			
Physical examination[10]	X			
Laboratory test[11]	X	X		X
Immunogenicity test	X			
Pregnancy test[12]	X			
Check of inclusion/exclusion criteria	X	X		
Randomization		X		
ID injection of the IP		X		
Observation of administration site following ID injection of the IP[13]		X	☎[9]	X
Photographing of the ID injection site of the IP [14]		X	(☎)	X
Monitoring of AEs[15]		X	☎	X

1. Screening visit and baseline visit can be performed on the same day.
2. Vital signs and laboratory tests will be performed before the ID injection of the IP.
3. Visit 4 will be the last visit of Part I for allergy assessment and will be 2 days after ID injection of IP (visit 2) (Visit 3 is a phone visit). Subjects with negative allergy test result on ID injection will proceed to Part II-A and Part II-B baseline visit. On baseline visit for Part II-A and Part II-B, SC injection will be conducted.
4. Written informed consent must be obtained from subjects prior to any study-related procedures.
5. Sex, date of birth, skin conditions (presence of tattoo, acne, dermatitis, pigmentation, lesion and skin damage on the administration site), smoking (non-smoker, former smoker or current smoker), alcohol intake (non-drinker, former drinker or current drinker), weekly alcohol intake, and daily smoking amount will be investigated.
6. Only for subjects considering participating in Part II-A, information about history of whole blood donation within 60 days, apheresis within 14 days, and transfusion within 14 days prior to Visit 4 will be investigated.
7. Medical history (surgical history within 3 years prior to screening visit, past medical history within 1 year prior to screening visit and current medical conditions) and past drug allergy will be checked.
8. At screening visit, medications administered within 3 months prior to screening will be investigated, and after screening visit, any change of medications investigated in the previous visit will be reviewed.
9. After taking a rest for at least 5 minutes, sitting systolic and diastolic blood pressures, respiratory rate,

pulse rate and temperature will be measured, before performing other tests.

10. Height, weight, and BMI measurements will be performed, and allergy, cardiovascular system, respiratory system, gastrointestinal system/hepatobiliary system, metabolism/endocrine system, renal/urinary system, reproductive system, musculoskeletal system, skin and connective tissues, nervous system, psychiatric system and other body organs will be examined.
11. Tests results for baseline (Visit 2) may be replaced with the results obtained within 7 days prior to baseline visit (Visit 2), if available.
12. Pregnancy test (serum β hCG or urine hCG) will be performed in women of child-bearing potential who have not received a sterilization procedure, or women who have the last menstrual period within 12 months or are not diagnosed with menopause only.
13. After the ID injection, injection site reaction and skin in the administration site will be observed for any abnormality. In a phone visit (Visit 3), subjects will report findings after observation on the ID injection site to the investigator via telephone, message or social media, etc. together with adverse events.
14. Photograph of administration site following ID injection of the IP will be taken at Visit 2 and Visit 4. For details about photographing method, follow a separate manual. Through a phone visit (Visit 3), photograph of the administration site taken by the subject will be sent to the investigator, if necessary.
15. AEs occurring following ID injection of the IP will be collected. Later, presence of AEs will be monitored for specified visits.

2.2.2 Part II-A (PK assessment)

Period	Baseline				Follow up		USV
Visit	Visit 4				Visit 5	Visit 6	
Day (D)	0D				1D	28D	
Visit Window: day	-				-	±2days	
PK Sampling Timepoint[1]	Pre-dose[2]	Administration	30 min post-dose	3h post-dose	24h post-dose	-	
Concomitant medications[3]	X				X	X	(X)
Vital signs[4]	X			X	X	X	(X)
ECG						X	(X)
Physical examination[5]						X	(X)
Laboratory test	X			X	X	X	(X)
Immunogenicity test						X	
Pregnancy test[6]						X	(X)
Blood collection for PK (total 15 times)	①~③		④~⑥	⑦~⑭	⑮		
Assigning allocation number		X					
SC injection of the IP		X					
Observation of administration site following SC IP injection[7]		X	X	X	X	X	(X)
Photographing of the SC injection site of the IP[8]		(X)	(X)	(X)	(X)	(X)	(X)
Monitoring of AEs[9]		X	X	X	X	X	(X)

USV: Unscheduled visit

1. Blood collection time points for PK assessment (total 15 times)

Pre-dose (3 times)	Pre-dose	①2 h (-2h±10mins), ②1 h (-1h±10mins), ③0 h (0h-10mins, blood collection before SC injection)
Post-dose (12 times)	30min post-dose	④10 min (0.17h±3mins), ⑤20 min (0.3h±3mins), ⑥30 min (0.5h±3mins)
	3h post-dose	⑦40 min (0.7h±3mins), ⑧50 min (0.8h±3mins), ⑨1 h (1h±5mins), ⑩1 h 15 min (1.25h±5mins), ⑪1 h 30 min (1.5h±5mins), ⑫2 h (2h±5mins), ⑬2 h 30 min (2.5h±10mins), ⑭3 h (3h±10mins)
	24h post-dose	⑮24 h (24h±60mins)

2. Vital signs and laboratory tests will be performed before the SC injection of the IP and may be replaced with the results obtained at Visit 4 of Part I.
3. Any change of medications investigated in the previous visit will be reviewed.
4. After taking a rest for at least 5 minutes, sitting systolic and diastolic blood pressures, respiratory rate, pulse rate and temperature will be measured, before performing other tests.
5. Cardiovascular system, respiratory system, gastrointestinal system/hepatobiliary system, metabolism/endocrine system, renal/urinary system, reproductive system, musculoskeletal system, skin and connective tissues, nervous system, psychiatric system and other body organs will be

examined.

6. Pregnancy test (serum β hCG or urine hCG) will be performed in women of child-bearing potential who have not received a sterilization procedure, or women who have the last menstrual period within 12 months or are not diagnosed with menopause only.
7. After the SC injection, injection site reaction and skin in the administration site will be observed for any abnormality.
8. Photographing will be performed when there is any visible abnormality in the administration site (if necessary). For details about photographing method, follow a separate manual. Through a phone visit, photograph of the administration site taken by the subject will be sent to the investigator.
9. AEs occurring before IP injection will be collected. Later, presence of AEs will be monitored for specified visits.

2.2.3 Part II-B (Safety assessment)

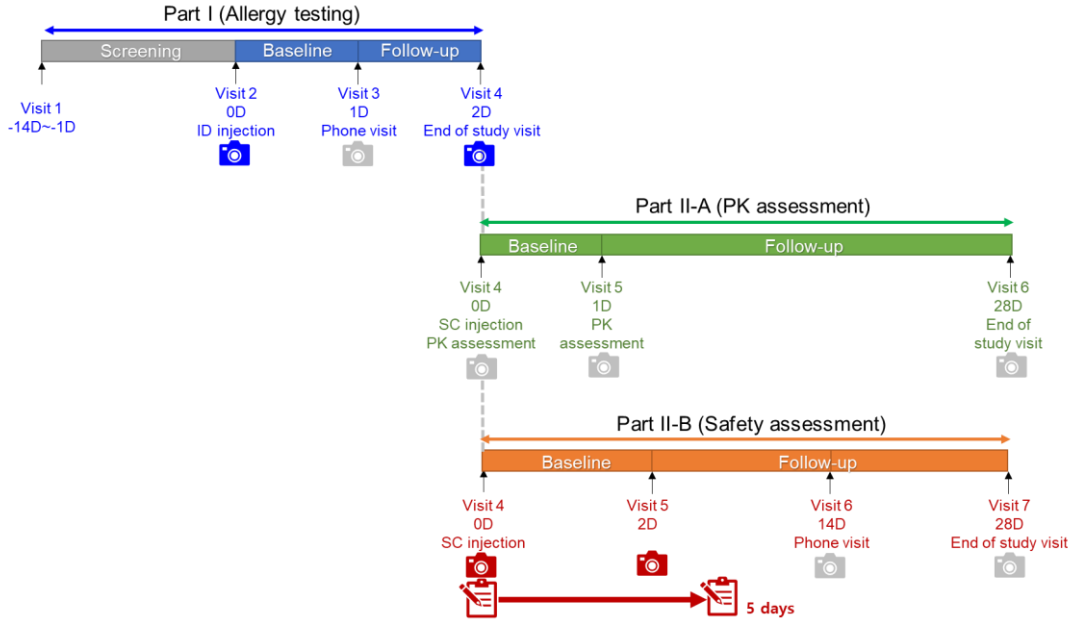
Period	Baseline	Follow up			USV
Visit	Visit 4[1]	Visit 5	Visit 6 (Phone Visit)	Visit 7	
Day (D)	0D	2D	14D	28D	
Visit Window: day	-	±1day	±2days	±2days	
Concomitant medications[2]	X	X	☎	X	(X)
Vital signs[3]	X	X		X	(X)
ECG				X	(X)
Physical examination[4]				X	(X)
Laboratory test	X	X		X	(X)
Immunogenicity test				X	
Pregnancy test[5]				X	(X)
Randomization[6]	X				
SC injection of the IP[7]	X				
Observation of administration site following SC injection of the IP[8]	X	X	☎[7]	X	(X)
Photographing of the SC injection site of the IP[9]	X	X	(☎)	(X)	(X)
Monitoring of AEs[10]	X	X	☎	X	(X)
Intensive monitoring of AEs[10]		☎: 1D~5D			

USV: Unscheduled visit

1. Vital signs and laboratory tests will be performed before the SC injection of the IP and may be replaced with the results obtained at Visit 4 of Part I.
2. Any change of concomitant medications investigated in the previous visit will be reviewed.
3. After taking a rest for at least 5 minutes, sitting systolic and diastolic blood pressures, respiratory rate, pulse rate and temperature will be measured, before performing other tests.
4. Cardiovascular system, respiratory system, gastrointestinal system/hepatobiliary system, metabolism/endocrine system, renal/urinary system, reproductive system, musculoskeletal system, skin and connective tissues, nervous system, psychiatric system and other body organs will be examined.
5. Pregnancy test (serum β hCG or urine hCG) will be performed in women of child-bearing potential who have not received a sterilization procedure, or women who have the last menstrual period within 12 months or are not diagnosed with menopause only.
6. Randomization into the study group or control group for SC injection in Part II-B.
7. SC injection site and presence of injection reaction will be observed for 30 minutes after SC injection of the IP and subjects without any symptom will be discharged. More information about adverse events following SC injection will be given by the principal investigator.
8. After the SC injection, injection site reaction and skin in the administration site will be observed for any abnormality. In a phone visit (Visit 6), subjects will report findings after observation on the SC injection site to the investigator via telephone, message or social media, etc. together with adverse events.
9. Photograph of administration site following SC injection of the IP will be taken at Visit 4 and Visit 5. For details about photographing method, follow a separate manual. In other visits, photographing will be performed when there is any visible abnormality in the administration site (if necessary), and subjects will photograph the administration site and send it to the investigator.
10. Adverse events before and after SC injection will be monitored. In particular, other adverse events following SC injection will be monitored, other than those found during observation of injection site reactions and skin in the SC injection site. Later, presence of AEs will be monitored for specified visits. However, investigator will contact subjects by telephone every day up to Day 5 after SC injection to check presence of AEs.

2.3 Diagram

Part I (Allergy assessment), Part II-A (PK assessment)/ Part II-B (Safety assessment)



3. Introduction

3.1. Study Background

Hyaluronic acid (HA) is composed of disaccharide repeats of N-acetylglucosamine and glucuronic acid joined alternately in a form of chain. HA is a natural high-molecular weight substance in the extracellular matrix (ECM) and exists in various parts, such as animal vitreous humor, cartilage, synovia, and placenta[1]. HA fills any empty space in the cells or fiber tissues, stimulating collagen regeneration and inhibiting germ invasion[2]. Regarding drug administration, HA binds to water, forming jelly-like form and thus inhibiting dispersion and absorption of a drug subcutaneously injected[3], which limits a dose per subcutaneous (SC) injection[4]. SC injection may improve patient's quality of life compared to intravenous (IV) injection, but it is associated with many problems due to limited dosage per injection and relatively lower bioavailability, increasing required amount of a drug, which increases viscosity of the drug during administration. Increased SC injection frequency with low dose causes a concern of immunogenicity. To overcome these problems, collagen, one composition of ECM, was degraded and used to enhance penetration of a drug subcutaneously injected. However, with known half-life of approx. 15 years, collagen is associated with concerns of permanent skin tissue changes. On the contrary, HA has relatively short half-life of 15-20 hours, compared to collagen. Thus, hyaluronidase, an enzyme that can degrade HA, has drawn attention.

Hyaluronidase is an endoglycosidase that degrades the glycosidic bond of HA which is a disaccharide, between N-acetylglucosamine and glucuronic acid into monosaccharide[5], which is found in various species, such as bacteria, fish and mammal[6]. Human hyaluronidase is classified into 6 subtypes, Hyaluronidase 1-4, PH20 and Hyaluronoglucosaminidase pseudogene 1 (HYALP1), among which Hyaluronidase 1 is known to play the most important role[5]. A subtype of hyaluronidase, Sperm adhesion molecule 1 (SPAM 1, PH20), is located on the surface of sperm, degrading HA on the surface of egg at the time of conception, and thus increasing penetration into the egg[3]. Focusing on such functions, bovine or chicken-derived hyaluronidase has been used to enhance the absorption and dispersion of a drug since 1940s[7]. However, animal-derived hyaluronidase causes concerns of allergic reactions, such as anaphylaxis, and spread of zoonosis, such as variant Creutzfeldt-Jakob disease[4]. To solve the problems, Halozyme based in the US developed an allogenic human-derived hyaluronidase, called HYLENEX, which was approved in 2005 by the US Food and Drug Administration (FDA) and has been sold until now. Active ingredient of HYLENEX is the 5th subtype of human hyaluronidase, PH20 and the most frequently reported adverse events were mild local adverse events, such as erythema or pain, with incidence rate of allergic reaction less than 0.1%.[8] HYLENEX has been approved and used as a spreading factor for anticancer agents or immunodeficiency treatment, for example HyQvia and Herceptin SC.

Human-derived hyaluronidase allows change of drug administration route from IV to SC, which can enhance patient's quality of life and improve problems, such as limited dosage or increased dosing frequency with the existing SC injection. However, no human-derived hyaluronidase has been developed until now since Halozyme's HYLENEX. ALT-B4 is the second human-derived hyaluronidase developed after HYLENEX and provides additional benefits, increasing heat stability for increased protein stability, while maintaining the same mechanism of action and enzyme activity as the human-derived hyaluronidase of Halozyme.

Adverse drug reaction (ADR) refers to any undesirable and untoward events occurring at any dose of an IP, where causality to the IP cannot be denied. Based on expectedness, ADRs are classified into Type A reactions, which are predictable, and Type B reactions, which are not predictable.[9), 10)] In Type B reaction, an ADR caused by immune mechanism is called drug allergy.[11), 12)] Drug allergy can be classified into immediate allergic reaction, mediated by immunoglobulin E (IgE) and appearing within 1 hour after drug administration, and non-immediate (delayed) allergic reaction, mediated by T cells and appearing within 1-24 hours or at the latest 48 hours after drug administration[13), 14)]. Medications known to have high prevalence of drug allergy include penicillins antibiotics, cephalosporins antibiotics, non-steroidal anti-inflammatory drugs, narcotic analgesics and anticonvulsants[15)], and frequent drug use, high single dose, and administration route of injection, instead of oral, and patients with asthma, sinusitis and chronic urticaria are known factors of allergic reaction[15)]. Estimated prevalence of drug allergy is about 5-

10%, and drug allergy may increase mortality and economic burden[10].

3.2. Study Rationale

The investigational product (IP), ALT-BB4, is a drug product containing ALT-B4 as an active ingredient. The active ingredient, ALT-B4, is a hyaluronidase that facilitates dispersion of other medications and reconstructs the dermal layer, which has the same enzyme activity as the commercial hyaluronidase available in the market. Enzyme activities of ALT-B4 are measured by turbidimetric assay and/or viscometric assay. Turbidimetric assay uses ultrasound-visible light spectrophotometer to measure turbidity due to HA-albumin complex, which uses a principle where degradation of HA into hyaluronidase reduces the amount of HA-albumin complex, decreasing absorbance at 600 nm wavelength (A600 nm). Enzyme activities of the sample are calculated by using A600 nm and external calibration curve of the standard hyaluronidase. When the turbidimetric assay established by Alteogen was applied to commercially available drug products containing recombinant human hyaluronidase (rHuPH20) (Herceptin SC, and Mabthera SC), it was found that they were very similar to activities specified in each product insert. The assay is in vitro assessment of the same mechanism as found in the human body, and thus can be applied to existing indications. Viscometric assay measures a change of viscosity caused by degradation of HA with hyaluronidase, which is applied to animal testis-derived hyaluronidase commercially available. Like turbidimetric assay, it was proven to provide very similar activity results to those specified in each product insert.

Mechanism related to clearance of hyaluronidase administered is not well known. However, it is known that hyaluronidase is inactivated in blood of many mammals. Known half-life of bovine testis-derived hyaluronidase in human serum is 3.2 minutes. Reconstruction level of damaged skin barrier due to intradermal (ID) injection of hyaluronidase at 0.02-~20 Units/mL was checked for at 24 hours, which showed that the recovery of barrier was incomplete and was in inverse proportion to the amount of enzyme administered. Complete recovery was found at 48 hours.

Hyaluronidase has been administered as a mixture with anticancer agents to enhance absorption of anticancer agents subcutaneously injected.

3.3. Risk and Benefit Assessment

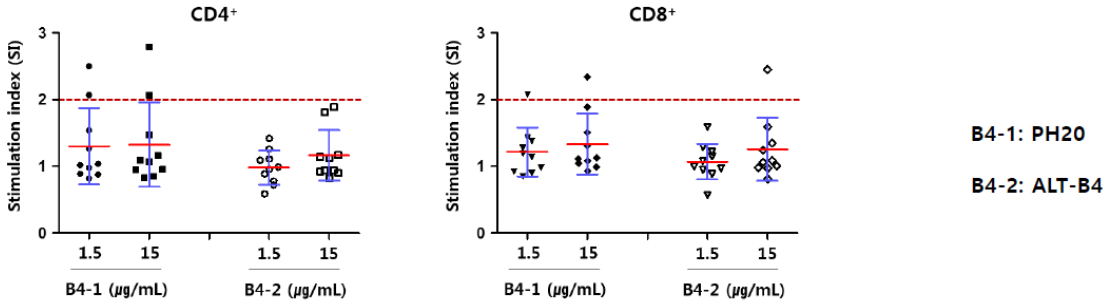
Summary of results from non-clinical trials is provided. For details, refer to the Investigator's Brochure (IB).

3.3.1. In Vitro Pharmacology

1. Immunogenicity Assessment

By checking for its capability of inducing stimulus reaction in CD4 and CD8+ cells, potential immunogenicity of ALT-B4 was confirmed and ALT-BB4 and PH20 were compared at the concentration of 1.5 and 15 μg/mL (n=10 Per condition). As shown in Figure 3.3.1, majority of the ALT-BB4 and PH20 results had stimulation index (SI) no more than 2 (positive threshold). Especially for ALT-BB4, all results did not exceed the positive threshold, except one result in CD8+ cells at the concentration of 15 μg/mL.

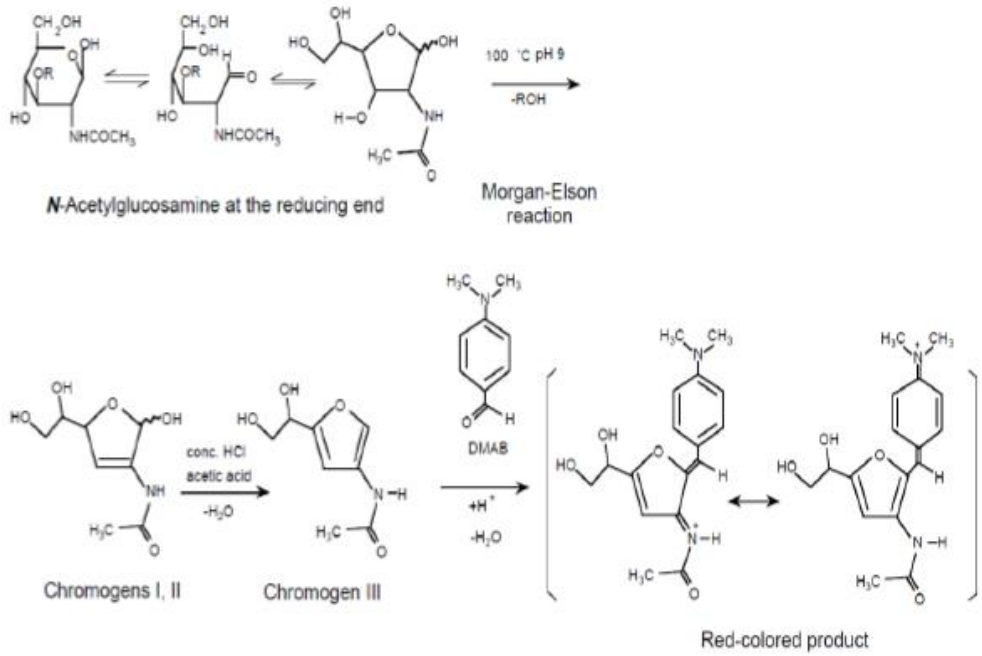
Figure 3.3.1 Stimulatory Potential of ALT-B4



2. Enzyme Activity

Alteogen, Inc. performed enzymatic kinetics assay (Morgan-elson assay) to determine enzyme kinetic parameters of ALT-B4, compared to PH20. Colorimetric morgan-elson assay is based upon a reducing N-acetyl-D-glucosamine terminus generated by cleavage reaction of enzyme (Figure 3.3.2).

Figure 3.3.2 Morgan-Elson Reaction



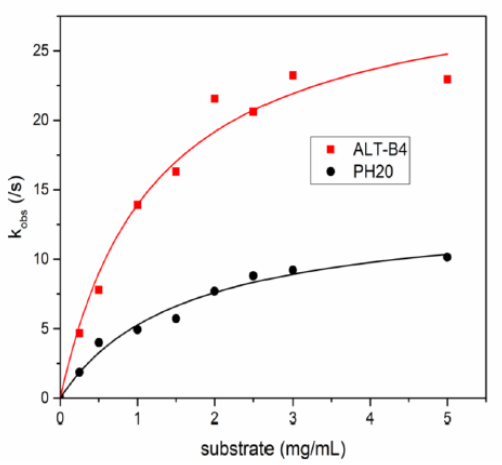
Morgan-Elson assay results are provided in Table 3.3.1 and Figure 3.3.3. The results showed that ALT-B4 has approximately 3-fold higher catalytic efficiency, compared to PH20.

Table 3.3.1 Enzyme Kinetic Parameters at 37°C, pH 5.7

Parameter	PH20	ALT-B4
K_M (mg/mL)	1.59	1.21
k_{cat} (/sec)	13.65	30.77
k_{cat}/K_M (mL/mg sec)	8.58	25.40

k_{cat}=Catalytic rate constant; K_M=Substrate concentration at which the reaction rate is half of V_{max}; V_{max}=Maximum rate achieved.

Figure 3.3.3 Michaelis-Menten Plot of ALT-B4 and PH20



3.3.2. In Vivo Pharmacology

1. Study Y01934

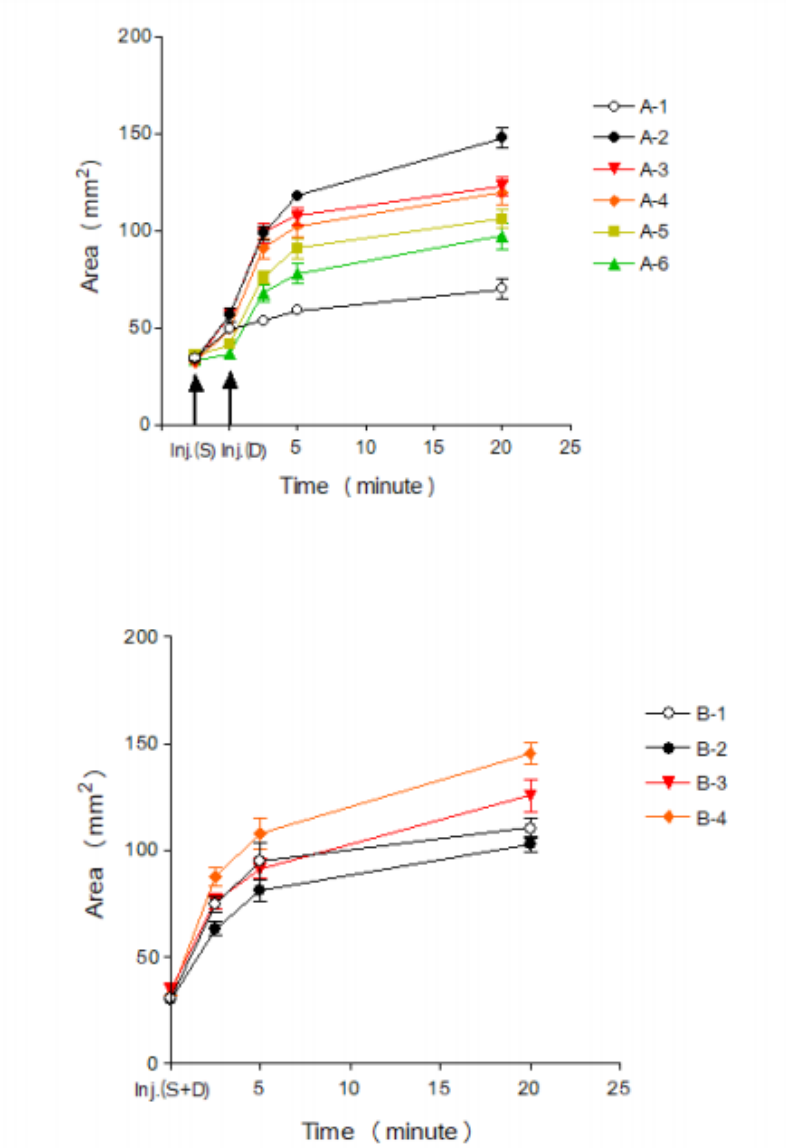
To evaluate efficacy of enzymes that help dispersion and absorption of other medications, dye dispersion study was performed, and sequential administration of ALT-B4 followed by Trypan blue was compared to concurrent administration of ALT-B4 and Trypan blue by mixing. The study results are provided in Table 3.3.2 and Figure 3.3.4. Dispersion of Trypan blue was proportional to ALT-B4 concentration, and shorter injection interval between ALT-B4 and Trypan blue was associated with larger area of dispersion. Mortality and adverse events were not observed following ID injection of ALT-BB4.

Table 3.3.2 Diffusion Test

Cohort No.	Admin.	ALT-B4 Conc. (U/mL)	Time Between ALT-B4 and Trypan blue	0 min		2.5 min		5 min		20 min	
				AVG (mm^2)	SEM	AVG (mm^2)	SEM	AVG (mm^2)	SEM	AVG (mm^2)	SEM
1	Co-mix	0	0 min	30.7	2.1	74.5	3.8	94.9	8.5	110.4	4.9
2	Seq.		1 min	49.3	1.3	53.7	1.9	59.0	2.7	70.1	5.4
3	Co-mix	100	0 min	29.6	2.0	63.2	3.1	81.3	4.9	102.7	3.9
4		500		35.0	0.9	16.3	3.7	91.4	4.6	125.5	7.3
5		5,000		31.9	1.2	87.7	4.2	107.8	6.9	145.6	5.1
6	Seq.	100	1 min	56.6	3.3	98.5	3.5	118.0	2.5	147.8	5.3
7		100	20 min	56.6	2.4	98.8	5.0	107.7	4.5	123.1	4.9
8		100	4 hrs	49.3	2.7	90.8	4.8	102.4	6.1	119.3	5.8
9		100	24 hrs	41.3	1.6	76.0	3.0	90.9	5.1	106.1	5.0
10		100	48 hrs	36.6	1.9	67.9	4.2	78.0	5.1	97.2	7.0

AVG=Average; Co-mix=Concomitant; SEM=Standard error of the mean; Seq=sequential.

Figure 3.3.4 Diffusion Test



Inj.: Injection, S: Sample, D: Dye

A-1: Vehicle, A-2: ALT-B4 (1m), A-3: ALT-B4 (20m), A-4: ALT-B4 (4h), A-5: ALT-B4 (24h), A-6: ALT-B4 (48h), B-1: Vehicle, B-2: ALT-B4 (100 U/mL), B-3: ALT-B4 (500 U/mL), B-4: ALT-B4 (5000 U/mL)

SEM: standard error of the mean

3.3.3. Nonclinical Pharmacokinetics

1. Study 2519133

Single dose pharmacokinetic (PK) study of ALT-B4 was performed in Sprague dawley rats. Single dose of ALT-B4 at 1 mg/kg was injected intravenously in 4 male rats, and blood collection was performed pre-dose, and 1, 2, 3, 4, 8, 16, 30, and 60 minutes post-dose. Half-life in plasma was

6.9 (±3.8) min. Mortality and adverse events were not observed.

2. Study 2089-034 (ANC028)

The study evaluated PK of ALT-B4 following single IV and SC injections of ALT-B4 in Cynomolgus monkeys. In Phases 1 and 2, single dose of ALT-B4 at 0.3, 3, 15 or 30 mg/kg was injected intravenously at 3 mL per kg to each animal, while in Phases 3 and 4, a single dose of ALT-B4 at 1, 3, 10 or 30 mg/kg was injected subcutaneously at 3 mL per kg. Blood samples for PK analysis were collected at 0 (pre-dose), 1, 3, 5, 10, 15, 30, and 60 minutes post-dose for IV injection, or 0.25, 0.5, 1, 4, 8, and 24 hours post-dose for SC injection.

Until Phase 3, no clinical adverse event was found after injections.

Table 3.3.3 Study design of monkey PK

Group	Test Article	No. of Males	Dose Route	Vehicle	Dose Level (mg/kg)	Dose Volume (mL/kg)	Collection Intervals
Phase 1							
1	ALT-B4	3	IV	Histidine buffer	0.3	3	Blood[a]
2	ALT-B4	3	IV	Histidine buffer	3	3	Blood[a]
Phase 2							
1	ALT-B4	3	IV	Histidine buffer	15	3	Blood[a]
2	ALT-B4	3	IV	Histidine buffer	30	3	Blood[a]
Phase 3							
1	ALT-B4	3	SC	Histidine buffer	1	3	Blood[b]
2	ALT-B4	3	SC	Histidine buffer	3	3	Blood[b]
Phase 4							
1	ALT-B4	3	SC	Histidine buffer	10	3	Blood[c]
2	ALT-B4	3	SC	Histidine buffer	30	3	Blood[c]

[a]Phase 1 and 2 (IV): Blood samples were collected at 0(pre-dose), 1, 3, 5, 10, 15, 30, and 60 minutes postdose

[b]Phase 3 (SC): Blood samples were collected at 0.25, 0.5, 1, 4, 8, and 24 hours postdose

[c]Phase 4 (SC): Blood samples were collected at 0.25, 0.5, 1, 4, 8, 24, and 48 hours postdose

Following IV injection at 3-30 mg/kg, blood ALT-B4 concentration was considered not affected by baseline value. C_{max} and AUC values showed dose-dependent results and half-life of ALT-B4 was found 0.3-0.87 hours, confirming rapid elimination from the body (Figure 3.3.5, Table 3.3.4). However, all PK parameters did not show significant difference between groups in the dose range of 0.3-30 mg/kg (one-way ANOVA, p-value>0.05).

Figure 3.3.5 Plasma concentration vs. time profiles of ALT-B4 after IV injections (upper panel: rectilinear scale, lower panel: semi-logarithmic scale)

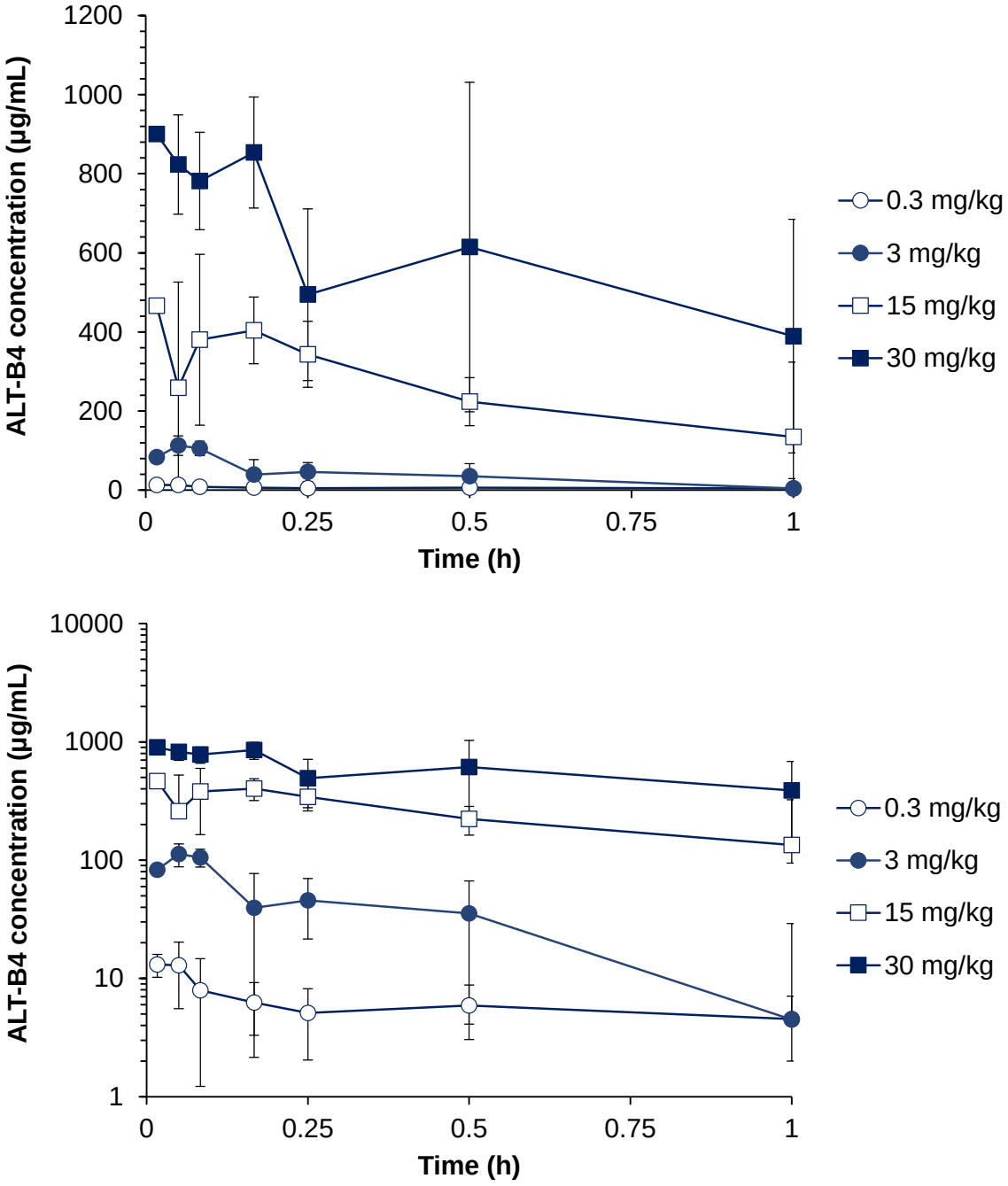


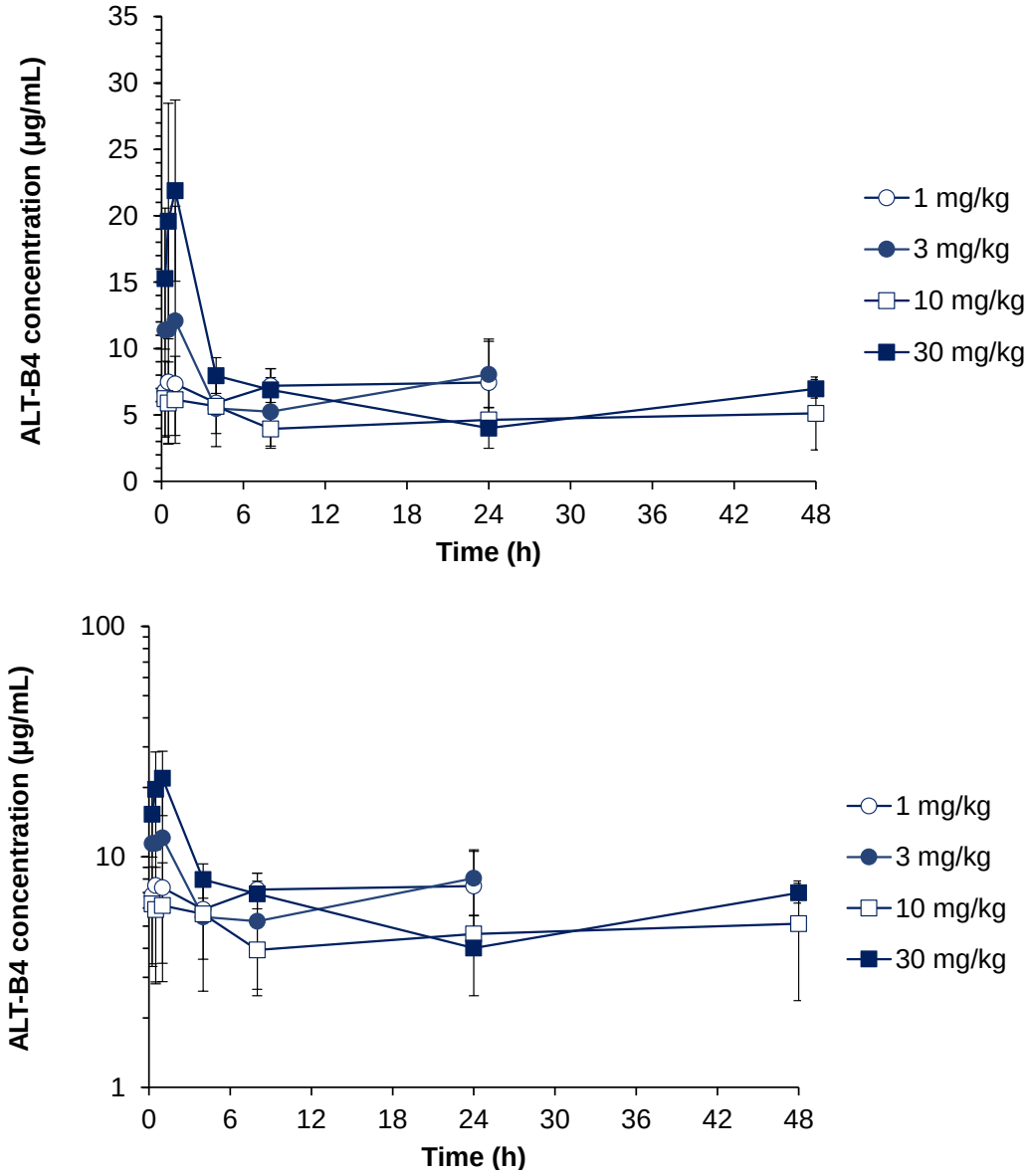
Table 3.3.4 Average PK parameters of ALT-B4 after IV injections

Parameter	ALT-B4 dose			
	0.3 mg/kg	**3 mg/kg**	**15 mg/kg**	**30 mg/kg**
$t_{1/2}$ (h)	1.13 ± 0.31	0.3 ± 0.19	0.75 ± 0.66	0.87 ± 0.46
C_0 (µg/mL)	4.27 ± 2.87	7.52 ± 4.22	6.02 ± 5.39	13.9 ± 9.01
AUC_{last} (µg·h/mL)	5.14 ± 3.07	37.38 ± 10.71	250.88 ± 97.82	576.95 ± 223.42
AUC_{inf} (µg·h/mL)	11.24 ± 7.87	39.23 ± 10.3	460.83 ± 323.39	1155.31 ± 570.04
Cl (mL/h/kg)	42.87 ± 37.57	80.22 ± 21.69	52.99 ± 47.25	32.98 ± 21.6
V_{ss} (mL/kg)	53.29 ± 27.33	27.57 ± 6.69	31.16 ± 7.43	33.87 ± 10.89

$t_{1/2}$: Elimination half-life, C_0: Plasma concentration at time zero, AUC_{last} and AUC_{inf}: Area under the concentration, Cl: Clearance, V_{ss}: Steady state volume

For ALT-B4 subcutaneously injected at 1-30 mg/kg, PK parameters, such as C_{max} and AUC were not dose dependent and in all animals, they couldn't be calculated due to effects of baseline values and absence of observation in the elimination phase. There was no meaningful difference in absorption kinetics among 1, 3, and 10 mg/kg groups. Considering increased ALT-B4 concentration in blood at the highest dose (30 mg/kg), compared to lower doses, it is assumed that ALT-B4 has been absorbed into the body circulatory system. Although the finding that the highest concentration of ALT-B4 was reached within 0.25-1 hour after SC injection showed rapid absorption into body circulatory system (Figure 3.3.6), exposure to body circulatory system following SC injection of ALT-B4 was very low when comparing to IV injection group of the same dose.

Figure 3.3.6 Plasma concentration vs. time profiles of ALT-B4 after SC injections (upper panel: rectilinear scale, lower panel: semi-logarithmic scale)



3.3.4. Toxicology

1. Study 2089-031 (ANC021)

Non-GLP dose-finding and tolerability studies were performed following single dose. Vehicle, and 0.04, 0.2, 2 mg/kg of ALT-B4 were administered at a dose of 0.5 mL/kg to all directions of the trunk of 4 male minipigs aged 5 months.

There was no clinical or veterinary symptom related to local toxicity of ALT-B4, and skin evaluation did not show any ALT-B4-related symptoms.

The study results showed good tolerability of single SC injection of ALT-B4 up to 2 mg/kg.

2. Study 891-0003-TX (ANC026)

A single dose rat study was performed for 7 days, where toxicity of ALT-B4 IV and SC injection was evaluated, and reversibility, persistence or delayed toxicity that can occur during 7-day

recovery period were evaluated.

Mortality was not observed, and there was no change related to ALT-B4 in aspects of clinical sign, weight, food intake, hematology, serum chemistry, coagulation, urinalysis, organ weight, gross pathology, and histopathology.

Single IV and SC injection of ALT-B4 to rats at 0, 0.04, 0.2, and 2 mg/kg were found well tolerable and no observed adverse effect level (NOAEL) was determined 2 mg/kg.

3. Study 891-0004-TX (ANC027)

A GLP repeat dose toxicity rat study was performed for 29 days, where toxicity and toxicokinetics of repeat weekly SC injection of ALT-B4 were evaluated for 5 weeks (Days 1, 8, 15, 22, and 29).

During the study, there was no mortality and no ALT-B4-related change was observed in aspects of clinical sign, weight, food intake, ophthalmic examinations, functional observational battery, clinical pathology (hematology, serum chemistry, coagulation, urinalysis), gross (necropsy) evaluation, organ weight, and histopathology.

Exposure to body circulatory system following weekly SC injection of ALT-B4 to male and female rats at 0.04, 0.2, and 2 mg/kg for 5 weeks was clear in 2 mg/kg group only. In both male and female rats, T_{max} was 0.5 hours after injection, and time for recovery to below the limit of quantification (BLQ) of mean blood ALT-B4 concentration was 8 hours after injection. 2 mg/kg group did not show noticeable difference in ALT-B4 exposure to body circulatory system by sex and there was no difference in exposure between Day 1 and Day 22.

In conclusion, weekly repeat dose SC injection of ALT-B4 at 0, 0.04, 0.2, and 2 mg/kg/dose for 5 weeks (Days 1, 8, 15, 22 and 29) to Sprague dawley rats showed good tolerability and there was no mortality and adverse events related to ALT-B4 in aspects of clinical sign, weight, food intake, ophthalmology, functional observational battery, clinical pathology, and pathology. NOAEL was determined 2 mg/kg/dose.

4. Study 20219-234 (ANC020)

A repeat dose toxicity study of ALT-B4 in Cynomolgus monkeys (ALT-B4-19001, weekly/total 5 SC injections) was performed (Table 3.3.5).

Table 3.3.5 Experimental design of monkey repeat dose toxicity study

Group No.	Test Material	Dose Level (mg/kg/dose)	Dose Volume (mL/kg)[a]	Dose Concentration (mg/mL)	No. of Animals[b]			
					Main Study		Recovery Study	
					Males	Females	Males	Females
1	Control	0	1.0	0	3	3	2	2
2	ALT-B4	0.04	1.0	0.04	3	3	-	-
3		0.2	1.0	0.2	3	3	-	-
4		2	1.0	2	3	3	2	2

- = Not applicable.

[a]Based on the most recent body weight measurement.

[b]Main Study animals were euthanized on Day 30. Recovery animals were euthanized on Day 43.

SC injection in Cynomolgus monkeys showed good tolerability at all doses. Mortality was not observed and there were no clinical signs, ophthalmic effect, abnormal electrocardiogram (ECG) or any change in body weight and food intake related to ALT-B4.

Despite difference in ALT-B4 injection concentrations by multiple folds among groups, there was no detectable additive effect in all blood samples collected, confirming absence of ALT-B4-related change in hyaluronidase activity in plasma.

Final euthanasia found microscopic findings including mixed cell inflammation (minimal-mild), hemorrhage, regionally extensive myocyte degeneration in the SC injection site at all doses of

ALT-B4, which were not observed in recovery group.

In the conclusion based on meaningful study findings, weekly SC injection of ALT-B4 at 0, 0.04, 0.2, and 2 mg/kg/dose for total 5 weeks (total 5 injections) was well tolerable and NOAEL was determined 2 mg/kg/dose.

3.4. Rationale for Dose Selection

Allergy reactivity is assumed caused by animal-derived proteins mostly included in animal-derived hyaluronidase. Therefore, skin allergy reaction test should be performed by using a small amount of hyaluronidase before administration of hyaluronidase to identify patients who may develop hypersensitivity to hyaluronidase.

Traditionally, skin allergy reaction test for hyaluronidase has been performed by using 4-8 units of hyaluronidase[16]. For skin allergy reaction test, a high dose of hyaluronidase is diluted and appropriate dosing volume is used. Recent study results showed that low doses of 10, 20, and 30 units have a chance of false negative, and allergy reactivity was found dose-dependent[17]. Therefore, use of a higher dose of hyaluronidase is recently recommened[19], for example directly using 1,500 IU/mL dose of hyaluronidase without dilution in the skin allergy reaction test[18].

A representative skin allergy reaction test for hyaluronidase is ID test[20, 21], and following precautions are required.

- Bubbles must be removed carefully before injection to make sure that there will be no splash reaction.
- Caution should be taken not to burst capillaries under the epidermis while inserting the needle.
- A syringe should be inserted at 45 degrees. The sloping side of the needle should face the skin and the entire sloping side should be fully inserted into the skin before administering the reagent.
- **0.01-0.05 mL will be injected to make a small blister with a diameter of 2-3 mm.**
- **Do not inject 0.05 mL or more.**
- If injected subcutaneously, false negative develops.

For an allergy test through ID test, it is mandatory to form appropriate size of blister by injecting appropriate volume of the study drug before observation of the reactions. Therefore, generally no more than 0.05 mL is injected and in other hyaluronidase clinical trials, this was reflected and 0.02-0.05 mL of the study drug was injected for testing.

Dosing volumes of study drugs in other hyaluronidase clinical trials are provided below:

Study drug	Dosing volume
Amphdase	0.02 mL
Vitrase	0.02 mL
Hydase	0.02 mL
Hylenex	0.10 mL

As per the provided grounds,

- 1,500 IU/mL of concentration is used to minimize a chance of false negative result, and
- 0.02 mL is injected to form an appropriate blister for the ID test.

This means that 30 IU/20 μL of ALT-BB4 will be used as an ID injection dose for allergy test.

For hyaluronidase products approved in Korea, dosage regimen of SC and intramuscular (IM) injections are provided below. Based on the clinical literatures reported in Korea,[22),23)] general clinical dose of hyaluronidase would be 1,500 IU or less. In addition, most animal-derived hyaluronidase products available in Korean market are lyophilized products with potency of 1,500

IU. These products are used after dissolving in 1 mL water for injection. Therefore, the safety of ALT-BB4 at 1,500 IU contained in a 1mL vial will be secured through a clinical study.

<Dosage regimen of animal-derived hyaluronidase products approved in Korea>

- SC infusion (mass SC injection)

  1,500 IU as hyaluronidase will be dissolved in 1 mL of water for injection or normal saline injection and administered into applicable site before starting SC infusion, or administered into a tube about 2 cm above the needle for infusion when starting the infusion. At administration of 500-1,000 mL of fluid, approximately 1,500 IU of this drug is appropriate.

- SC injection or IM injection

  1,500 IU of this drug will be directly dissolved in the injection solution to be administered.

- Extravasation

  In the event of extravasation, especially when there is diffusion, compared to regional case, 1,500 IU of this drug will be dissolved in 1 mL of water for injection or normal saline injection and infiltrated into the lesion area as soon as possible.

- Hematoma

  1,500 IU of this drug will be dissolved in 1 mL of water for injection or normal saline injection and infiltrated into the applicable site. This drug and about 1 mL of water for injection will be dissolved in solution for injection immediately before use.

Therefore, 1,500 IU was decided as a clinical dose.

To check for toxicity of ALT-B4, it was injected IV in rats, and IV and SC in monkeys. In Cynomolgus monkeys, 0.3-30 mg/kg was injected after 4-5 times of washout period. Through PK studies, very low bioavailability and very rapid exposure to body circulatory system of ALT-B4 were confirmed. In Minipigs, a dose-finding study was performed following single SC injection and no adverse event was found at the highest dose (2 mg/kg). In addition, single dose and repeat dose toxicity studies in rats were performed. In the single dose toxicity study, ALT-B4 was injected IV or SC at 0.04, 0.2, and 2 mg/kg, while it was injected SC in the repeat dose toxicity study. At the same dose, a repeat dose toxicity study was performed in Cynomolgus monkeys. In both animal species, no toxicity was found at all doses, and NOAEL was determined 2 mg/kg.

As ALT-B4 was inactive at approx. 70,000 IU/mg, the highest dose in the non-clinical trials, 2 mg/kg/dosage corresponds to approx. 140,000 IU/kg/dosage, which is 9,800,000 IU/dosage for a 70-kg adult. Considering that the actual treatment dose is ≤1,500 IU (single fixed dose), the dose in the non-clinical trials is much higher than the expected clinical dose.

4. Study Objectives

4.1. Primary Objective

To evaluate the allergic reactivity, safety and tolerability of ALT-BB4 (recombinant hyaluronidase) by checking for drug allergy following single ID injection of ALT-BB4 and monitoring systemic reactions and adverse events following its single SC injection

4.2. Secondary Objectives

To evaluate the PK profile of single dose of ALT-BB4

4.3. Exploratory Objectives

To evaluate the immunogenicity of ALT-BB4 (Visit 1 and End of Study visit)

5. Study Population (Sample Size and Subject Selection)

5.1. Sample Size

1. Total number of subjects: At least 231 subjects (Maximum of 290 including subjects with allergic reactions, dropped out and excluded from analysis)
2. Number of subjects for each Part
   - Part I (Allergy assessment): At least 231 subjects
   - Part II-A (PK assessment): Total 23 subjects (including 10% drop-out rate)
   - Part II-B (Safety assessment): At least 208 subjects (Study group- at least 139 subjects, Control group - at least 69 subjects; randomized in a 2:1 ratio)

5.2. Rationale for Sample Size Determination

The minimum target number of subjects 231 was based on the empirical cases of previous clinical trials and statistical hypothesis. Based on the existing similar clinical trials of allergy hypersensitivity with clinically significant effect size of 10% and existing literature, the number of subjects was determined for the sample size.

The number of subjects who participated in a previous similar clinical trial with an animal-derived/human gene recombinant hyaluronidase was 100 for Hylenex, 162 for Amphadase and 65 for Vitrase. In the study to check for allergenicity of hyaluronidase products approved by the FDA, incidence of positive skin reaction was 0% (0/100 subjects) with a recombinant hyaluronidase, called Hylenex, and 5% (8/162 subjects) with an animal-derived hyaluronidase, called Amphadase. In the recent Study NCT01689363, "Evaluation of the Allergenicity of AMPHADASE INJECTION (Hyaluronidase Injection USP) (H001-A2)", it was 0% (0/183 subjects) in PP group, 2.7% (7/253 subjects) in ITT group, and 0% (0/65 subjects) in Vitrase group.

This study aims to evaluate the safety, tolerability and PK following administration of ALT-BB4 (recombinant hyaluronidase) in healthy volunteers. The study consists of Part I for allergy assessment, Part II-A for PK assessment, and Part II-B for safety assessment.

- Part I

Allergy incidence rate (%) is defined as proportion of subjects with positive allergy test result, which will be used to determine the target number of subjects. Statistical hypothesis is provided below:

$$H_0: p_T \geq p_0 \qquad \text{vs.} \qquad H_A: p_T < p_0$$

p_T: Allergy incidence rate in the study group (ALT-BB4)

p_0 : Allergy incidence rate recommended by the FDA

Main purpose of Part I is to identify the allergy incidence rate in the study group (ALT-BB4) and to determine whether it is appropriate. Therefore, it is deemed inappropriate to determine the sample size under hypothesis where allergy incidence rates between the placebo-control group (saline) and the study group (ALT-BB4) would be statistically compared. Thus, a hypothesis to test the incidence rate of the study group by comparing with the target incidence rate was used to determine the target number of subjects.

As suggested in the FDA medical review for Amphadase, the FDA-recommended allergy incidence rate is 10%. This was used as the test threshold (p_0 =0.1) in the statistical hypothesis. In addition, allergy incidence rate in Study API-H001-CLN-A of Amphadase was 5% (7/142 subjects). As the study group using a similar agent (ALT-BB4) is expected to show the same level of allergy incidence rate, p_T was assumed 0.05.

The target number of subjects using “Numeric Results for Testing One Proportion using the Exact Test” of PASS 15 with one-sided significance level of 2.5% and 80% power is 231 subjects. However, when the target number of subjects (231) required for analysis has not been met due to severe protocol deviation, additional subjects can be recruited to maintain statistical power, and up to 290 subjects may be enrolled.

- Part II-A

Among the 231 subjects who have completed Part I, 23 subjects, which accounts for 10%, will be enrolled in Part II-A. Assuming 10% of drop-out rate, we can expect that at least 20 subjects will

finally complete Part II-A.

- Part II-B

Among 231 subjects who will complete Part I, 208 subjects will be enrolled in Part II-B, except 23 subjects who will be enrolled in Part II-A. The sample size is considered sufficient to assess the safety in the Phase 1 study.

5.3. Subject Selection

5.3.1. Inclusion Criteria

Common

1. Healthy volunteers aged ≥19 years at the time of Screening (Visit 1)
2. Female subjects or male subjects' female partner must be menopausal or should have received a sterilization procedure or have agreed to use contraceptive methods during the study period, as defined below:
   - Post-menopausal female subjects or male subjects' female partners (non-drug induced amenorrhea for at least 12 months or confirmed diagnosis with menopause)
   - Female subjects or male subjects' female partners who have received a sterilization procedure (removal of ovary and/or uterus)
   - Subjects who have agreed to practice total abstinence during the study period [For female subjects, periodic abstinence (e.g., ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception.]
   - When female subjects or male subjects' female partners are women of childbearing potential (WOCBP) who have not received a sterilization procedure, they must agree to use of following contraceptive methods:
     - Hormones (implantable, patch, and oral)
     - Intrauterine device (IUD)
     - Double barrier methods (concomitant use of two of following contraceptive methods: male condom, female condom, cervical cap, diaphragm, sponge, spermicide) (However, concomitant use of male condom and female condom is excluded from double barrier methods.)
3. WOCBP or females who have the last menstrual period within 12 months must have a negative serum or urine pregnancy test at Screening (Visit 1).
4. Subjects who have voluntarily decided to take part in the study and able to comply with the study protocol
5. Subjects who have no tattoo, acne, dermatitis, pigmentation or lesion on the administration site and who have no damage in the skin, so that they can receive the IP and allergy test
6. Subjects determined eligible for the study through Screening tests (vital signs, physical examination, medical history and surgery history, ECG, and laboratory tests)

Part II-A

7. Subjects with BMI no less than 18.5 kg/m^2, no greater than 24.9 kg/m^2

5.3.2. Exclusion Criteria

Below are the study exclusion criteria:

Common

1. Subjects who have received or treated with following medications within the specified timeframe prior to Baseline (Visit 2) or who are expected to receive them during the study period:
   - Within 1 month: Hyaluronidase, Chemotherapeutic agent, penicillins antibiotics (e.g.: Amoxicillin, Ampicillin, etc.), cephalosporins antibiotics (e.g.: Cefaclor, Cefadroxil, Cefixime, etc.), sulfonamides antibiotics (e.g.: Sulfadiazine, Sulfamethoxazole, etc.), quinolones antibiotics (e.g.: Ciprofloxacin, Levofloxacin, etc.), Glucocorticosteroid, Immunosuppressive agent
   - Within 14 days: Antihistamine (e.g.: Chlorpheniramine, Hydroxyzine, Ketotifen, etc.), non-steroidal anti-inflammatory drugs (NSAIDs; e.g.: Aspirin, Aceclofenac, etc.)[24-6)]

2. Subjects who have current or prior history of clinically significant liver, kidney, gastrointestinal, cardiovascular, respiratory, endocrine, immune system, psychiatric/nerve system, and blood/oncological disorders
3. Subjects with acute fever > 37.5℃ within 7 days from the expected IP administration or showing symptoms suspecting acute diseases within 14 days from the expected IP administration
4. Subjects who are diagnosed with hypertension/hypotension or subjects who are with clinically significant blood pressure*

   *Hypertension=systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg[27)]

   *Hypotension=systolic blood pressure ≤ 90 mmHg and/or diastolic blood pressure ≤ 60 mmHg[28)-30)]
5. Subjects who persistently drink more than the weekly recommended alcohol units*

   14 g of alcohol content per unit is applied, which corresponds to 1 can (small bottle) of beer (5%), 350 mL of draft beer (5%), 1 cup (300 mL) of makgeoli, 1 glass (150 mL) of wine (12%), 1/4 bottle (90 mL) of soju (20%), and 1 shot (45 mL) of liquor (40%). Moderate amount of alcohol by age and sex is provided below:[31)]

Age	Recommended unit per week
Adults (≥ 19 and < 65 years)	Male: 8 units/week Female: 4 units/week
Elderly (≥ 65 years)	Male: 4 units/week Female: 2 units/week

6. Subjects of usual smoker (exceeding 10 cigarettes per day)
7. Subjects who have a past history of autoimmune diseases (e.g.: rheumatoid arthritis, etc.) or active immune diseases that may affect the immune system [e.g.: flu, cancer, Human immunodeficiency virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV) etc.], diabetes mellitus, heart diseases, asthma, sinusitis, chronic urticaria, dermographism, or any skin conditions that may affect post-IP administration assessments (e.g.: Dermatitis, dermatomycosis and other skin diseases or tattoo)
8. Subjects with known allergic reactions or hypersensitivity to bee sting or other common allergens or known contraindications to hyaluronidase, thimerosal (disinfectants) and EDTA
9. Subjects with hypersensitivity to the IP or its ingredients or a history of anaphylaxis
10. Past history of drug abuse
11. Subjects who have participated in other clinical trials within 6 months prior to the expected IP administration
12. Others determined ineligible for the study participation in the opinion of investigator

Part II-A

13. Subjects who have been treated with a drug-metabolizing enzyme inducers and inhibitors*[32), 33)] within 30 days prior to Visit 4

    * Example: Phenytoin, Carbamazepine, Barbiturates, Rifampicin, Griseofulvin, Cimetidine, Disulfiram, Erythromycin, Ketoconazole, Fluconazole, Itraconazole, Valproic acid, Isoniazid, Ciprofloxacin, Omeprazole, Clarithromycin, Quinidine, Sulfonamides, etc.
14. Subjects who have significant bleeding or blood loss within 60 days prior to Visit 4
15. Subjects who have donated whole blood within 60 days or donated blood by apheresis within 14 days or received transfusion within 14 days prior to Visit 4
16. Subjects who have consumed grapefruit juice[34)] within 7 days prior to Visit 4

5.3.3. Withdrawal Criteria

Subjects can withdraw their consent without providing any reason at any time during the study. In addition, subjects who are considered unable to continue the study due to their clinical conditions will be discontinued from IP administration by investigator. Subjects will discontinue the study or be withdrawn/dropped out from the study by investigator for following reasons:

1. Voluntary consent withdrawal of subject (or subject's legally acceptable representative)
2. Subjects who cannot perform the study due to adverse events in the investigator's opinion
3. Subjects who cannot receive SC injection due to drug allergy following ID injection in the investigator's opinion
4. Major protocol deviation/violation (inclusion/exclusion criteria violation, use of forbidden concomitant medications, etc.)
5. Subjects who have received a drug that may affect the IP assessments (allergy assessment, safety and tolerability assessment, and PK assessment) in the investigator's opinion
6. Subjects lost to follow-up (except those considered not affecting the PK and safety assessments)
7. Females with confirmed pregnancy during the study period
8. In the opinion of the investigator, a subject should discontinue the study participation

5.3.4. Protocol Deviation/Violation

Principal investigator and subinvestigator of the study must be fully aware of the protocol and strictly follow it to prevent any protocol deviation/violation. Principal investigator (or its designee) will take appropriate measures to ensure subjects can return the institution for their scheduled visits for IP administration and tests, for example providing written notice or phone call as a reminder of the next visit. In addition, investigator must inform the sponsor of any protocol deviation/violation occurring in a subject as soon as possible and determine whether the subject can continue the study participation. Drop-out due to protocol deviation/violation must be documented in the electronic case report form (eCRF). Following cases are considered as major protocol deviation/violations:

1. Violations against the inclusion or exclusion criteria
2. Use of forbidden concomitant medications that may affect the PK, safety and tolerability assessments of the IP
3. Other cases considered as major protocol deviation/violations

For minor deviation/violations that are considered not affecting interpretation of the study results, the level of deviation/violation or delay and reasons must be accurately documented in the eCRF and it will be comprehensively discussed in a blind meeting whether it has affected the study.

6. Study Design

6.1. Study Period

1. Entire Study Period

Approximately 18 months from approval of the Institutional Review Board (IRB) (however, it may be changed depending on the subject enrollment rate.)

2. Study Period for Individual Subjects

- Part I (Allergy assessment):
  - Screening period: Up to 2 weeks
  - Treatment with IP: 1 day (ID single dose)
  - Follow-up period: 2 days (48 hours)
- Part II-A (PK assessment):
  - Treatment with IP: 1 day (SC single dose)
  - PK Assessment: Total 15 times
    - Pre-dose: -2h, -1h, and 0h
    - Post-dose: 10 min (0.17h), 20 min (0.3h), 30 min (0.5h), 40 min (0.7h), 50 min (0.8h), 1h, 1h 15 min (1.25h), 1h 30 min (1.5h), 2h, 2 h 30 min (2.5h), 3h and 24h
  - Follow-up period: 4 weeks

- Part II-B (Safety assessment)
  - Treatment with IP: 1 day (SC single dose)
  - Follow-up period: 4 weeks

6.2. Study Group/Control Group

1. Part I (Allergy assessment)

Treatment group	Study group	Control group
IP	Study drug (ALT-BB4, 1,500 IU/mL)	Comparator (0.9% NaCl)
ID dose	30 IU (0.02 mL)	0.9% NaCl(0.02 mL)
Administration site*	Right or left forearm	Forearm in the opposite side of the study drug administration

* For example, when the study drug is intradermally injected into the left forearm, the comparator will be intradermally injected into the right forearm.

2. Part II-A (PK assessment)

Treatment group	Study group
IP	Study drug (ALT-BB4, 1,500 IU/mL)
SC Dose	1,500 IU (1 mL)
Administration site	Right or left upper arm

3. Part II-B (Safety assessment)

Treatment group	Study group	Control group
IP	Study drug (ALT-BB4, 1,500 IU/mL)	Comparator (0.9% NaCl)
SC Dose	1,500 IU (1 mL)	1 mL
Administration site	Right or left upper arm	Right or left upper arm

6.3. Randomization

1. Part I (Allergy assessment) and Part II-B (Safety assessment)

Randomization will be used to ensure the scientific validity of the study and to avoid subjectivity of the investigator in the subject assignment to each treatment group. Biostatistician independent from the study will send the randomization list to an IP packaging staff and prepare and send a blinded envelop to the principal investigator of the institution. The list will be kept blinded.

1. Allocation number will be generated by using SAS® 9.4 or higher Proc plan procedure.
2. Subjects enrolled in Part I (allergy assessment) will be randomized to receive the study drug or comparator into the both arms in a ratio of 1:1 based on their allocation number. Allocation number will be assigned in a form of R00-000. For example, the allocation number of the first subject enrolled in Institution 01 will be R01-001.

   Part I allocation number: R@@-### (@@: Institution No., ###: Serial number, Example: R01-001)

3. Subjects enrolled in Part II-B (Safety assessment) will be randomized into the study group and control group in a ratio of 2:1. Allocation number will be assigned in a form of B00-000. For example, the allocation number of the first subject assigned in Part II-B in Institution 01 will be B01-001.

   Part II-B allocation number: B@@-### (@@: Institution No., ###: Serial number, Example: B01-001)

The investigator will review the subject inclusion/exclusion criteria and assign a unique number to subjects determined eligible for the study; and will use this number to prescribe the IP.

Until the end of the study, investigators, subjects, clinical pharmacists and people conducting the study should be kept blinded and when unblinding is required during the study, it will be performed based on the procedure as specified in Section 6.4.2 of the protocol.

2. Part II-A (PK assessment)

Part II-A of the study has a single arm and an open-labeled design, and randomization will not be separately performed. However, subject number (allocation number) will be sequentially assigned to subjects in Part II-A. For example, the subject number of the first subject assigned in Part II-A in Institution 01 will be A01-001.

Part II-A allocation number: A@@-### (@@: Institution No., ###: Serial number, Example: A01-001)

6.4. Blinding

Part I and Part II-B will be performed based on a double-blinded design. In this study, double-blinding will be performed in accordance with a separate blinding procedure to ensure that subjects in Part I and Part II-B cannot know whether they are receiving the study drug or comparator in the administration site. For Part II-A performed with a single arm, a separate blinding process will not be performed.

6.4.1. Double-blinding

This is a Phase 1 study. To exclude subjective judgment of investigator and subjects, Part I and Part II-B will be performed based on a double-blinded design. This means that both investigator and subject will not know which IP is administered (a person who conducts administration will be unblinded).

In Part I allergy assessment, subjects will be blinded to the IP administered and administration site, while unblinded staff administers the IP as randomized.

In Part II-B safety assessment, subjects will be blinded to the type of IP administered, while unblinded staff administers the IP as randomized.

To maintain double-blinding in Part I and Part II-B, unblinded administration staff will prepare the IP in an isolated place from subjects. At that time, subjects cannot see the IP preparation process in a syringe by administration staff, and will be asked to turn their head during administration not to see the administration process and administration site. Maintaining double-blinding for investigator and subjects will prevent bias in the safety and tolerability assessment by the investigator or subjects. To maintain double-blinding, only allocation number will be used to identify subjects and the list of allocation number assignment by group will not be disclosed until the end of the study.

6.4.2. Unblinding

When unblinding is deemed necessary due to reasons such as emergency situation threatening the subject safety, investigator must inform Alteogen, Inc. or its designated CRO's monitor promptly. Monitor has responsibility for immediate contact to a representative of Alteogen, Inc. After receiving the information, the representative of Alteogen, Inc. will discuss with the investigator to determine whether to unblind and document the decision. After conferring with the sponsor, the investigator shall confirm the IP information of the relevant subject by opening the code breaking card box delivered at the kick-off meeting and sign it before keeping it in the investigator study file. When the sponsor, Alteogen, Inc. is not contactable immediately, investigator will conduct the unblinding process with the same procedure and must report the blind-breaking to Alteogen at the earliest time available and document the reason of conducting unblinding without discussion with the sponsor. When the investigator gets to know the subject's randomized IP during the study, the investigator must take effort to exclude bias in the safety and tolerability assessments.

After the end of the study, problems of all data will be solved out through query. Once integrity and accuracy of the database are confirmed, database is locked (DB lock) and the allocation number will be disclosed. Subsequent database change is only allowed with written consent of the sponsor and database manager.

7. Criteria for Study Termination and Early Termination

7.1. Scheduled Termination

Termination of entire study is defined as the study discontinuation of the last subject or discontinuation visit of the last subject.

7.2. Early Termination

Investigator may terminate the partial or entire study after discussion with the sponsor when continuation of the study is considered inappropriate based on the study findings.

The sponsor can terminate the entire study or the study at a particular institution early. Reasons of study termination by the sponsor include:

1. The institution fails in recruiting the target number of subjects
2. Any information that may significantly affect the study continuation becomes available
3. Deviation/violations of Good Clinical Practice (GCP), protocol or contract by the institution or investigator affecting continuation of the study
4. Other administrative reasons that may significantly affect the study continuation

In case of early termination or temporary termination of the study, investigator must inform the subjects immediately, make arrangement for appropriate actions and follow-up and report current study status and outcomes regarding subjects who had participated in the trial until the time of termination to the sponsor. In case of study termination, this must be reported to the IRB and the Ministry of Food and Drug Safety (MFDS). The entire study schedule may be suspended depending on the IRB or MFDS decision.

8. Investigational Product Information and Management

8.1. Investigational Product (IP)

8.1.1. Study Drug

1. Product name or Code name: ALT-BB4 (Recombinant Hyaluronidase)
2. Active ingredient and Content: ALT-BB4 (recombinant hyaluronidase) 1,500 IU/mL
3. Appearance and Formulation: Colorless, clear liquid injection
4. Manufacturer: Alteogen, Inc.

8.1.2. Comparator

1. Product name or Generic name: Normal saline (0.9% NaCl)
2. Active ingredient and Content: 0.9% NaCl
3. Appearance and Formulation: Colorless, clear liquid injection
4. Manufacturer: JW Pharmaceutical Corp.

8.2. Production/Packaging and Labeling

The sponsor will produce or purchase and then pack the IPs and provide them to the clinical pharmacist in an institution. IP labeling should be performed in accordance with Article 8.4 of [Attached Table 4-2] Good Manufacturing Practice for IPs of the Regulations on Safety of Drugs, Etc. and Article 7.7 of [Attached Table 11] IP Manufacturing of the Regulations on the Drug Manufacturing and Quality Control and should include the following:

1. Statement that it can be used for clinical trial only (e.g. “For clinical trial use only”)
2. Name or identification of the IP
3. Batch number or code number that can identify the contents and packaging process
4. Name, address and phone number of the sponsor (IND holder)
5. Shelf-life (expiry date)
6. Storage conditions
7. Reference code that can identify the clinical trial (Protocol No.)
8. Subject identification No., IP No., and Visit No. (can be omitted when documented)

8.3. Investigational Product Management

All IPs will be supplied to the institutions by the sponsor. IPs will be stored in a locked place with restricted access at refrigerated temperature (2-8℃).

After receipt of IPs, the clinical pharmacist at an institution will check for the quantity and conditions of them and ensure that they cannot be used without prescription of the principal investigator or designated study staff, and store and manage them so that they can be used for the study purpose only.

The clinical pharmacist must record accurate details, including quantity of IPs received from the sponsor and dispensed to subjects together with dispense date, in a form provided by the sponsor, while the monitor must examine the IP accountability by periodically monitoring the quantity kept by the investigator or clinical pharmacist and the storage condition.

The principal investigator or clinical pharmacist at each institution must keep unused IPs until monitored by the sponsor’s monitor. At the end of the study or upon the sponsor’s request, unused IPs will be collected or discarded by the sponsor. (Used empty containers will be discarded at the institution in accordance with the institution’s SOP, if feasible.)

The sponsor will check for the IP quantity and storage condition during the study period and make necessary measures to ensure smooth conduct of the study.

9. Methods and Administration Plan

9.1. Overall Methods

This is a Phase 1 study to perform allergy assessment and PK profiling as well as to evaluate the safety and tolerability following administration of ALT-BB4 (recombinant hyaluronidase) in healthy volunteers. This study consists of Part I (Allergy assessment), Part II-A (PK assessment) and Part II-B (Safety assessment). In Part I, ALT-BB4 allergy assessment will be performed first. Then only in eligible subjects, PK of ALT-BB4 will be assessed in Part II-A, and the safety and tolerability assessment will be performed in Part II-B.

Part I (Allergy assessment)

For subjects who have agreed to participate by giving a written informed consent, Screening tests will be performed. By confirming the Screening test results and the inclusion/exclusion criteria, eligible subjects for participating in Part I of the study for the drug allergy assessment will be enrolled. Enrolled subjects will visit the institution and receive the IP intradermally. For up to 30 minutes after the IP administration, subjects will be observed for cutaneous hypersensitivity of immediate allergic reaction and subjects without any adverse event will be discharged. Later at Visit 3, subjects will be contacted via phone to monitor concomitant medications and adverse events. At Visit 4, subjects will visit the institution for monitoring of cutaneous hypersensitivity of delayed allergic reaction occurring within 48 hours after the ID injection, blood collection, change of concomitant medications and adverse events, performing scheduled tests. ALT-BB4 allergy assessment will be conducted through scheduled tests and evaluations for each visit.

Part II-A (PK assessment)

Eligible subjects for Part II-A of the study will be enrolled only from subjects with drug allergy test negative in Part I. Enrolled subjects will receive the IP subcutaneously at the institution and perform scheduled tests and blood collection at designated time points. At 3 hours after the IP administration, scheduled tests will be completed and subjects without any adverse event will be discharged. In a case of adverse events, the subjects will be treated by investigator as required before discharge. Later at Visit 5, subjects will visit the institution for blood collection, review of any change in concomitant medications and adverse events, and scheduled tests, and at Visit 6 (End of Study visit), subjects will visit the institution for review of any change in concomitant medications and adverse events and scheduled tests. ALT-BB4 PK assessment will be conducted through scheduled tests and blood collection for PK assessment for each visit.

Part II-B (Safety assessment)

Eligible subjects for Part II-B of the study will be enrolled only from subjects with drug allergy test negative in Part I. Subjects enrolled in Part II-B study will be randomized into the study group or control group at the institution and receive single dose of the IP subcutaneously into the right or left upper arm. For up to 30 minutes after the IP administration, subjects will be discharged if there is no adverse event when monitoring systemic and administration site adverse events. In a case of adverse events, the subjects will be treated by investigator as required before discharge. Later at Visit 5, subjects will visit the institution for blood collection, review of any change in concomitant medications and adverse events, and scheduled tests. Visit 6 is a phone visit, where concomitant medications and adverse events including systemic and administration site events will be monitored. At Visit 7, the End of Study visit, scheduled tests and safety assessment will be performed to evaluate the safety and tolerability of ALT-BB4.

9.2. Investigational Product Dosage and Administration Method

1. Part I (Allergy assessment)

Subjects will be randomized into the study group or control group at Baseline visit (Visit 2), and receive single dose of the study drug or comparator intradermally into the right or left forearm.

Treatment group	Study group	Control group
IP	Study drug (ALT-BB4, 1,500 IU/mL)	Comparator (0.9% NaCl)
ID dose	30 IU (0.02 mL)	0.9% NaCl (0.02 mL)
Administration site*	Right or left forearm	Forearm in the opposite side of the study drug administration

* For example, when the study drug is intradermally injected into the left forearm, the comparator will be intradermally injected into the right forearm.

2. Part II-A (PK assessment)

Part II-A PK assessment will be performed only in subjects with drug allergy test negative at Visit 4, 2 days (48 hours) after the ID injection in Part I. All subjects participating in Part II-A will receive single dose of the study drug subcutaneously into the right or left upper arm.

Treatment group	Study group
IP	Study drug (ALT-BB4, 1,500 IU/mL)
SC Dose	1,500 IU (1 mL)
Administration site	Right or left upper arm

3. Part II-B (Safety assessment)

Part II-B safety assessment will be performed only in subjects with drug allergy test negative at Visit 4, 2 days (48 hours) after the ID injection in Part I, randomized into the study group and control group in a 2:1 ratio. Subjects assigned in each group of Part II-B will receive single dose of the study drug or comparator subcutaneously into the right or left upper arm.

Treatment group	Study group	Control group
IP	Study drug (ALT-BB4, 1,500 IU/mL)	Comparator (0.9% NaCl)
SC Dose	1,500 IU (1 mL)	1 mL
Administration site	Right or left upper arm	Right or left upper arm

9.3. Concomitant Medications and Therapies

9.3.1. Permitted Concomitant Medications and Therapies

In following cases, medications and therapies are allowed throughout the study period.

1. Non-forbidden concomitant medications and therapies can be administered during the study period, but only the concomitant medications and therapies considered not affecting the study result interpretation are allowed at the discretion of investigator.
2. Other medications and therapies for routine treatment of other diseases will be used concomitantly following discussion with the investigator.

Details (product name, purpose of administration, dosage, and treatment duration, etc.) of all concomitant medications and therapies (including treatment for other diseases or adverse events) will be documented in the electronic case report form (eCRF).

9.3.2. Forbidden Concomitant Medications and Therapies

Following medications are forbidden during the study period:

- Hyaluronidase, Chemotherapeutic agent, Glucocorticosteroid, immunosuppressive agent, Antihistamine, NSAIDs (e.g.: Aspirin, Aceclofenac, etc.), penicillins antibiotics (e.g.: Amoxicillin, Ampicillin, etc.), cephalosporins antibiotics (e.g.: Cefaclor, Cefadroxil, Cefixime, etc.), sulfonamides antibiotics (e.g.: Sulfadiazine, Sulfamethoxazole, etc.), quinolones antibiotics (e.g.: Ciprofloxacin, Levofloxacin, etc.), ethical-the-counter drugs*, herbal

medicines*, over-the-counter drug (OTC drug)*, health functional food*, Vitamins*, grapefruit juice**, drug-metabolizing enzyme-inducers and inhibitors**[32),33)] (e.g.: Phenytoin, Carbamazepine, Barbiturates, Rifampicin, Griseofulvin, Cimetidine, Disulfiram, Erythromycin, Ketoconazole, Fluconazole, Itraconazole, Valproic acid, Isoniazid, Ciprofloxacin, Omeprazole, Clarithromycin, Quinidine, Sulfonamides, etc.)

* Medications started before Screening visit and continuously used are allowed, while new medications after Screening are not allowed.

**For Part II-A only

In addition, whole blood donation, apheresis, and transfusion are prohibited for subjects in Part II-A.

9.4. Treatment Compliance

Single dose of IP will be administered for Part I (Allergy assessment), Part II-A (PK assessment) and Part II-B (Safety assessment). IP administration will be performed under supervision of the principal investigator (or its designee) at the institution and the time of administration to a subject will be documented in the eCRF.

10. Study Procedures and Assessment

10.1. Observations

10.1.1. Written Informed Consent Form and Assignment of Screening Number

Investigator must provide detailed explanation about the study purpose and what involved to subjects who want to take part in the study. Written informed consent must be obtained prior to any study procedures and a copy of signed informed consent form (ICF) will be provided to a subject by the investigator.

After obtaining written informed consent from a subject, the investigator will assign a screening number in the order of obtaining ICF. Screening number will be assigned in a form of S00-000. For example, the screening number of the first subject who provides the written informed consent in Institution 01 will be S01-001.

Screening number: S@@-### (@@: Institution No., ###: Serial number, Example: S01-001)

For a subject who failed screening, the subject's screening number will not be re-assigned to other subjects.

10.1.2. Demographic Data

Information about subject's sex, date of birth, skin conditions (presence of tattoo, acne, dermatitis, pigmentation, lesion and skin damage on the administration site), smoking (non-smoker, former smoker or current smoker), alcohol intake (non-drinker, former drinker or current drinker), weekly alcohol intake, and daily smoking amount will be investigated. In addition, only for subjects considering participating in Part II-A, information about history of whole blood donation within 60 days, apheresis within 14 days, and transfusion within 14 days prior to Visit 4 will be investigated.

10.1.3. Investigation of Medication History and Medical History

For medication history, information of medications will be recorded (product name or ingredient name, purpose of administration, dosage, and treatment duration, etc.).

For medical history, presence of past medical history and current medical conditions, time of drug allergy onset (dd/mmm/yyyy), ongoing status and investigator's opinion will be documented.

10.1.4. Vital Sign

Vital signs will be measured before other scheduled tests. After taking a rest for at least 5 minutes, sitting systolic and diastolic blood pressures, respiratory rate, pulse rate and temperature will be measured.

10.1.5. Electrocardiogram (ECG)

12-lead ECG will be performed after taking a rest for at least 3 minutes, and ventricular rate (beats/min), PR (msec), QRS (msec), QT (msec), and QTcF (msec) will be confirmed.

10.1.6. Physical Examination

Physical examination includes height (cm, whole number), weight (kg, one decimal place), and body mass index (BMI) (kg/m^2, round to one decimal place) measurements and comprehensive evaluation of allergy, cardiovascular system, respiratory system, gastrointestinal system/hepatobiliary system, metabolism/endocrine system, renal/urinary system, reproductive system, musculoskeletal system, skin and connective tissues, nervous system, psychiatric

system and other body organs.

Clinically meaningful symptoms will be recorded in the physical examination section in the eCRF. Any meaningful changes in physical examination findings that meet definitions of AEs after IP administration must be reported as AEs.

10.1.7. Laboratory Test

Tests results for baseline (Visit 2) may be replaced with the results obtained within 7 days prior to baseline visit (Visit 2), if available. Laboratory tests will be analyzed at the internal lab of the institution. Creatine phosphokinase in chemistry and serology will be performed at screening visit only. Subjects who do not meet inclusion/exclusion criteria based on the screening tests may undergo re-test once. Detailed tests are listed below:

Hematology	White blood cell (WBC), Red blood cell (RBC), Hemoglobin, Hematocrit, Platelet count, WBC diff count (Neutrophil, Lymphocyte, Monocyte, Eosinophil, Basophil)
Chemistry†	Na, K, Ca, Cl, Blood urea nitrogen (BUN), Creatinine, Uric acid, Total bilirubin, Albumin, Total protein, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), γ-GTP, Alkaline phosphatase (ALP), Glucose, Creatine phosphokinase (CPK)
Serology‡	HBsAg, Venereal disease research laboratory (VDRL), Anti-HBs, Anti-HIV, Anti-HCV
Urinalysis	pH, Specific gravity, Albumin, Bilirubin, Glucose, Urobilinogen, Ketone, Nitrite, Occult blood, Microscopy (Squamous cell, Urine RBC, urine WBC)

†Creatine phosphokinase test will be performed at screening visit (Visit 1) only.

‡For screening visit (Visit 1) only.

10.1.8. Immunogenicity Test

This test is for exploratory purpose and will be performed at screening visit (Visit 1 of Part 1) and the end of study visit (Visit 6 of Part II-A or Visit 7 of Part II-B)(total twice). Blood collection for immunogenicity test may be performed together with laboratory test. Details about blood collection, storage/disposition and analysis of the test will be described in a separate document.

10.1.9. Pregnancy Test

Pregnancy test will be performed in women of child-bearing potential who have not received a sterilization procedure and women who have the last menstrual period within 12 months or are not diagnosed with menopause only. Pregnancy test will be performed with blood or urine samples (serum β human Chorionic gonadotrophin (hCG) or Urine hCG).

10.1.10. Randomization

Only for subjects who are finally determined eligible, investigator will conduct randomization and assign allocation number for Part I (Allergy assessment) participation in the order of providing consent, as described in Section 6.3.

For subjects enrolled in Part I (Allergy assessment), both of their forearms will be randomized into the study group or control group in a ratio of 1:1 for ID injection.

Allocation number will be used as a subject forearm identification code during the study.

After evaluation of cutaneous hypersensitivity of delayed allergic reaction occurring from 30 minutes to 48 hours after IP administration at Visit 4 of Part I, only subjects with negative skin reaction of drug allergy can take part in Part II. Subjects will take part in Part II-A for PK assessment or Part II-B for safety assessment. Subjects enrolled in Part II-B (Safety assessment) will receive allocation numbers by randomization into the study group or control group in a ratio of 2:1 for SC injection.

10.1.11. PK Assessment

Subjects participating in Part II-A will be evaluated. Blood collection for PK assessment will be performed pre-dose (-2h, -1h, and 0h) and 10 min (0.17h), 20 min (0.3h), 30 min (0.5h), 40 min (0.7h), 50 min (0.8h), 1h, 1h 15 min (1.25h), 1h 30 min (1.5h), 2h, 2h 30 min (2.5h), 3h and 24 h post-dose. PK parameters (C_{max}, AUC_{last}, $t_{1/2}$, T_{max}) of plasma ALT-BB4 will be calculated and evaluated. In addition, PK parameters adjusted with baseline (pre-dose) will be calculated and evaluated.

C_{max}	Peak plasma drug concentration
AUC_{last}	Area under plasma concentration-time curve calculated until the time of measurable last blood collection
$t_{1/2}$	Drug elimination half-life
T_{max}	Time to peak plasma drug concentration

Below are acceptable windows.

Scheduled time	Window
Visit 4 pre-dose (-2h, -1h, pre-dose)	± 10 min
Visit 4 pre-dose (0h pre-dose)	- 10 min
Visit 4 0.17h, 0.3h, 0.5h, 0.7h, 0.8h post-dose	± 3 min
Visit 4 1h, 1.25h, 1.5h, 2h post-dose	± 5 min
Visit 4 2.5h, 3h post-dose	± 10 min
Visit 4 24h post-dose	± 60 min

10.1.12. Investigational Product Administration

At Baseline visit (Visit 2), cutaneous hypersensitivity due to drug allergy within 30 minutes after ID injection will be evaluated for Part I (drug allergy assessment). In the next visit, Visit 4, subjects with negative results evaluated from 30 minutes to 48 hours after IP administration can take part in Part II-A and Part II-B. Single dose of ALT-BB4 will be injected subcutaneously to the right or left upper arm of subjects participating in Part II-A and Part II-B, by principal investigator (or its designee).

Presence of temperature exceeding 37.5℃ within 7 days prior to IP administration and presence of acute disease within 14 days prior to IP administration will be checked before IP administration. Subjects with temperature exceeding 37.5℃ or history of acute diseases within past 14 days cannot receive the IP.

10.1.13. Monitoring of Adverse Event

Subjects will be monitored for adverse events after IP administration. In case of adverse events, the symptoms, start date/stop date, severity, causality, action taken to IP, action taken to other items except for IP, outcome and seriousness must be documented in detail.

For **presence of drug allergy**, immediate allergic reaction occurring within 30 (±5) minutes after ID injection of the IP at baseline visit (Visit 2) and delayed allergic reaction occurring from 30 minutes to 48 (±2) hours after ID injection of the IP will be identified. Administration site will be observed every minute for up to 5 minutes after IP administration and then every 5 minutes from 5 minutes to 30 minutes after IP administration. Subjects who show no immediate allergic reaction or whose symptoms disappear will be discharged, and then presence of delayed allergic reaction will be additionally monitored at 48 hours after IP administration.

Presence of any adverse events, including drug allergy, will be monitored from the time of IP administration at each visit. Investigator will advise the subjects to visit the institution to check presence of delayed allergic reaction and provide appropriate observation and treatment, if necessary, if he/she notices any adverse events occurring within 48 hours after IP administration.

10.1.14. Photographing of Investigational Product administration Site

Details about photographing method, collection/storage method of photographs taken, and quality assessment of collected photographs will be provided in a separate manual.

10.2. Efficacy Assessment

10.2.1. Efficacy Endpoints

The study aims to evaluate allergy, safety and tolerability and efficacy assessment will not be separately performed.

10.3. Safety Assessment

10.3.1. Safety Endpoints

Part I study will evaluate the drug allergy and adverse events occurring following ID injection of the IP. Part II-A study will evaluate adverse events occurring following SC injection of the IP. Part II-B study will evaluate the safety and tolerability following SC injection of the IP.

10.3.1.1. Primary Endpoints

1) **Incidence rate of drug allergy following ID injection of the IP* in Part I**

   *Subjects developing either immediate or delayed allergic reaction are considered to have drug allergy.

2) **Safety and tolerability assessment following SC injection of the IP** in Part II**

   ** Incidence rate of adverse events, including systemic and administration site-related adverse events

10.3.1.2. Secondary Endpoints

1) **Incidence rate of immediate allergic reactions† occurring within 30 minutes after the ID injection of the IP**

   †defined as drug allergy associated with wheal ≥ 8 mm with or without pseudopod (elevation considered due to an injection is not considered wheal), erythema or localized itching occurring in the administration site within 30 minutes after IP administration

2) **Incidence rate of delayed allergic reactions‡ occurring within 48 hours after the ID injection of the IP**

   ‡drug allergy occurring from 30 minutes to 48 hours after the IP administration, including but not limited to redness, rash, urticaria, edema, and erythema in the administration site as well as acute generalized exanthematous pustulosis and erythema multiforme. Investigator will decide whether an adverse event is a delayed allergic reaction.

3) **Incidence rate of administration site events* at 30 min. and 48 hours after the IP administration**

   * classified into wheal, erythema, localized itching and other adverse events, occurring after the IP administration, at each time point

4) **Size of wheal and erythema occurring at 30 min. and 48 hours after the IP administration**

10.3.1.3. Other Safety Endpoints

1) **Adverse Events**
2) **Physical Examination, Vital Signs, and ECG**
3) **Laboratory tests**

10.3.2. Safety Assessment Method

Part I study will evaluate the drug allergy and adverse events occurring following ID injection of the IP. Part II-A study will evaluate adverse events occurring following SC injection of the IP. Part II-B study will evaluate the safety and tolerability following SC injection of the IP.

10.3.2.1. Primary Endpoints

1) Incidence rate of drug allergy following ID injection of the IP* in Part I

*Subjects developing either immediate or delayed allergic reaction are considered to have drug allergy.

Incidence rate of drug allergy by treatment group following IP administration will be provided.

2) Safety and tolerability assessment following SC injection of the IP in Part II**

** Incidence rate of adverse events, including systemic and administration site-related adverse events

Systemic and administration site-related AEs following IP administration will be provided.

10.3.2.2. Secondary Endpoints

1) Incidence rate of immediate allergic reactions† occurring within 30 minutes after the ID injection of the IP

†defined as drug allergy associated with wheal ≥ 8 mm with or without pseudopod (elevation considered due to an injection is not considered wheal), erythema or localized itching occurring in the administration site within 30 minutes after IP administration

2) Incidence rate of delayed allergic reactions‡ occurring within 48 hours after the ID injection of the IP

‡drug allergy occurring from 30 minutes to 48 hours after the IP administration, including but not limited to redness, rash, urticaria, edema, and erythema in the administration site as well as acute generalized exanthematous pustulosis and erythema multiforme. Investigator will decide whether an adverse event is a delayed allergic reaction.

Incidence rate of immediate allergic reaction and delayed allergic reaction at 30 minutes and 48 hours after IP administration by treatment group will be provided.
In case of immediate allergic reaction assessment, localized itching will be assessed together. Localized itching will be rated with a numeric rating scale (NRS) between 0-10, and subjects will be asked to indicate the degree of itching in a scale from 0 (no itching) to 10 (unimaginably severe itching)[35]. Severity based on NRS score will be assessed by referring to the table below.

- Example of questions: Please tell us how serious itching have you experienced on a scale from 0 to 10.

0	1	2	3	4	5	6	7	8	9	10
No itching										Unimaginable itching

NRS Score	Severity
1-3	Mild
4-6	Moderate
7-10	Severe

3) Incidence rate of administration site events* at 30 min. and 48 hours after the IP administration

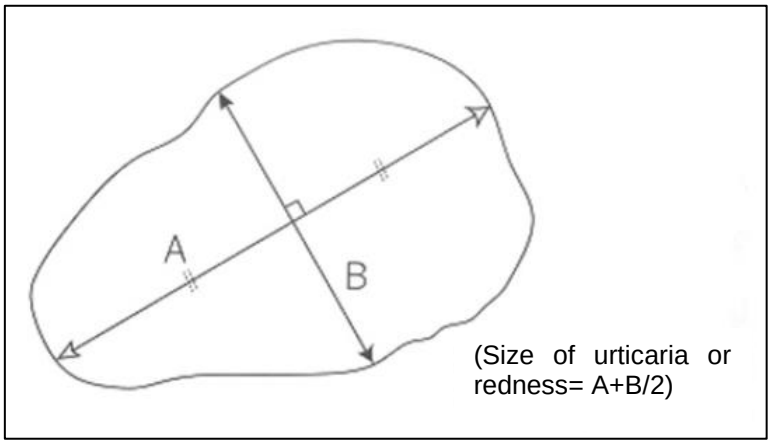
* classified into wheal, erythema, localized itching and other adverse events, occurring after the IP administration, at each time point. Incidence rate of adverse events (urticaria, erythema, localized itching and other adverse events) at 30 minutes and 48 hours after IP administration by administration will be provided.

4) Size of wheal and erythema occurring at 30 min. and 48 hours after the IP

administration

Size of wheal and erythema at each time point after IP administration will be provided. To measure size of urticaria or erythema, the length of the long axis and the length of the short axis that orthogonally crosses the middle of the long axis will be measured. The mean value of the length of both axes will be provided as the size of urticaria or erythema.

Figure 10.3.1 How to measure size of urticaria and erythema



10.3.2.3. Other Safety Endpoints

1) Adverse Events

AEs will be collected from baseline visit (Visit 2) to the end of the study, and in case of AEs, AE symptoms, start date/stop date, duration, severity and causality must be documented in eCRF without omission. All AEs will be classified by system organ class (SOC) and preferred term (PT) using the most recent version of Medical dictionary for regulatory activities (MedDRA).

2) Physical Examination, Vital Signs, and ECG

- **Physical examination**
  For physical examination, cardiovascular system, respiratory system, gastrointestinal system/hepatobiliary system, metabolism/endocrine system, renal/urinary system, reproductive system, musculoskeletal system, skin and connective tissues, nervous system, psychiatric system and other body organs will be comprehensively evaluated. Significant symptoms and meaningful symptoms detected during screening period will be recorded in the physical examination section in the eCRF. Any significant changes in physical examination findings that meet definitions of AEs after IP administration must be reported as AEs.

- **Vital sign**s
  It will be measured before other scheduled tests. After taking a rest for at least 5 minutes, sitting systolic and diastolic blood pressures, pulse rate and temperature will be measured.

- **ECG**
  Any significant changes in ECG findings that meet definitions of AEs after IP administration must be reported as AEs.

3) Laboratory tests

Laboratory test results will be classified into normal, not clinically significant (NCS) and clinically significant (CS). Clinically meaningful laboratory results based on comparison between IP pre-dose and post-dose will be reported as AEs.

10.4. Pharmacokinetic Assessment

10.4.1. PK Endpoints

1) Primary endpoints: C_{max}, AUC_{last}, $t_{1/2}$, and T_{max} of ALT-BB4
2) Secondary endpoints: Baseline-adjusted C_{max}, AUC_{last}, $t_{1/2}$, and T_{max} of ALT-BB4

10.4.2. PK Assessment Method

1) Blood collection method, pretreatment and storage method of isolated plasma
   - Blood collection will be performed in the forearm in the opposite side of IP administration and via a venipuncture or saline-locked angiocatheter inserted to the brachial vein. When saline-locked angiocatheter is used for blood collection, approximately 1 mL of blood will be drawn before collecting blood for PK assessment to fully remove any remaining normal saline in the blood collection set, and then 1 mL of saline will be injected to the catheter to prevent blood coagulation.
   - For the treatment and storage of collected blood samples, follow a separate document.

2) Sample Storage and Disposition

Samples collected for drug concentration analysis will be kept in the institution's sample storage below -70℃ and after the end of the study, they will be transferred after discussion with the analysis facility. Disposition of samples after drug concentration analysis will be performed in accordance with Standard Operating Procedures (SOPs) of the analysis facility. After submission of the Clinical Study Report (CSR) to the IRB, back-up samples under storage will be disposed or transferred to the sponsor after discussion with the sponsor and the storage period can be extended upon agreement. If it is disposed at the institution, the disposition will be performed in accordance with the SOPs of the institution.

3) Methods of analysis

Analysis methods will be described in a separate document.

10.5. Evaluation Criteria and Report of Adverse Events

10.5.1. Definition of Safety-related Terms

1) Adverse event (AE)

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

2) Adverse drug reaction (ADR)

All noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The phrase responses to a medicinal product means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

3) Serious AE/ADR (SAE/SADR)

Any untoward medical occurrence that at any dose:

① results in death, or is life-threatening,
② requires inpatient hospitalization or prolongation of existing hospitalization,
③ results in persistent or significant disability/incapacity, or

④ is a congenital anomaly/birth
⑤ results in any other medical event such as development of drug dependency or abuse or hematologic disease

4) Unexpected adverse drug reaction

It refers to any adverse drug reactions with different pattern or degree of harm, based on the related information available in the Investigator's Brochure or product insert.
In addition to the matters listed above, any medically significant event that may substantially affect the subject's well-being and health may be determined as a SAE based on the medical judgment of the study investigator (principal investigator or subinvestigator) and related experts and appropriate actions will be taken accordingly.

10.5.2. Evaluation Criteria of Adverse Events

10.5.2.1. Severity

Severity of AEs will be determined based on following criteria.

1) Mild: presence of conscious or objective symptoms, but not interfering with everyday activities
2) Moderate: discomfort interfering with everyday activities
3) Severe: preventing from performing normal everyday activities

10.5.2.2. Causality to the IP

Investigator will evaluate causality between the AE and the IP based on the following, and all AEs, except those determined as "Definitely not related, None" will be considered as adverse drug reactions.

1) Definitely related

① There is evidence of IP administration and plausible temporal relationship of AE onset
② The AE is most plausibly explained by IP administration than any other reasons
③ The AE disappears after the discontinuation of administration
④ Rechallenge (if feasible) result is positive
⑤ The AE shows consistent patterns with the information already known for the IP or one in the same class

2) Probably related

① There is evidence of IP administration and plausible temporal relationship of AE onset
② The AE is most plausibly explained by IP administration than any other reasons
③ The AE disappears after the discontinuation of administration

3) Possibly related

① There is evidence of IP administration and plausible temporal relationship of AE onset
② The AE is judged to be attributable to IP administration to the same extent as other possible causes
③ The AE disappears after the discontinuation of administration

4) Unlikely, Probably not related

① There is evidence of drug administration
② There are more possible causes of the AE
③ The result of the discontinuation of IP administration is negative or unclear
④ Rechallenge result is negative or unclear

5) Definitely not related, None

① Subject did not receive the IP

② Temporal relationship of the IP administration and AE onset is not reasonable
③ There is other clear cause of the AE

6) Unknown, Unassessable

① Lack of evidence to determine relationship
② Poor quality of source data or inconsistent data

10.5.2.3. Action taken and Outcome

During the study, principal investigator and subinvestigator must ensure the subject safety. In case of SADRs, prompt and appropriate actions must be taken to minimize the adverse events and the principal investigator may discontinue the study after discussion with the sponsor. For all AEs occurring during the study, even if they are not related to the IP, the symptoms and signs, start date/stop date, duration, severity, action taken and outcome, causality to the IP and seriousness must be documented in detail in the eCRF. In addition, the AE must be observed as far as possible until it recovers to pre-dose condition or baseline or is considered normalized by principal investigator or subinvestigator or no further observation is required.

Action taken to IPs for AEs, Action taken to other items except for IP and outcome will be completed as explained below.

1) Action taken to IPs

① Treatment stopped
② Dose reduced
③ Dose increased
④ Dose not change
⑤ Unknown
⑥ Not applicable

2) Action taken to other items except for IP

① No action taken
② Medication treatment
③ Non-medication treatment
④ Both medication and non-medication treatment

3) Outcome

① Recovered/resolved
② Recovering/resolving
③ Not recovered/not resolved
④ Recovered with sequela/resolved with sequela
⑤ Death
⑥ Unknown

10.5.3. Reporting Method for Adverse Events

10.5.3.1. Reporting of AE

Investigator will instruct the subjects (or their legally acceptable representative) to report all adverse events and symptoms that may occur after IP administration.
For systemic symptoms or various symptoms associated with laboratory tests after IP use, investigator will record the type, start date/stop date, severity, actions and progression, and causality to the IP in the eCRF. In addition, the AEs will be followed up until termination (resolution

of AE or loss of follow-up).

10.5.3.2. Expedited Reporting

Principal investigator and subinvestigator must report all SAEs occurring during the IP administration period, regardless of causality to the IP, to the sponsor or its designee, within 24 hours or by the next business day at the latest from the first recognition, via telephone, email or fax. Report to the IRB is also required in accordance with the institution regulations.
As far as possible, all information in the SAE Form should be included at the initial report. The completed form should be sent to the sponsor and AE page on the eCRF must be filled out.
Upon receipt of the initial report, the sponsor and the IRB must review the information and ask the subinvestigator for further information, if necessary. After review of the AE information, causality to the IP will be investigated.
If necessary, follow-up report containing any new information about a SAE must be submitted to the sponsor in the SAE Form.
The sponsor must report any suspected unexpected serious adverse reaction (SUSAR) to the investigator, Minister of Food and Drug Safety and if necessary, the IRB within the time frame based on the following classification:

- Death or life-threatening SUSARs: Within 7 days from the date the sponsor first gets the report or aware of it. However, when any information in Form 77, ADR Report is missed, such as ADR name, final observation outcome, and ADR summary, the sponsor must provide additional report containing details about the ADR within 15 days from the date he/she first gets the report or aware of it.
- Any other SUSARs must be reported within 15 days from the date he/she first gets the report or aware of it.

Contact Information for SAE Report:

- Contact: +82-70-4404-8793
- Phone: +82-10-8934-9157
- Email: shkim@alteogen.com
- Fax:+82-42-384-8770

10.5.3.3. Responsibilities of Staff in case of Serious Adverse Drug Reactions

Principal investigator and subinvestigator must make sure of subject safety and minimize AEs by taking prompt and appropriate actions during the study. When a SADR occurs during the study, responsibilities of each role are as below.

1) Principal Investigator
Principal investigator must report SADRs occurring during the study to the sponsor and the IRB within 24 hours or by the next business day at the latest from the first recognition to determine whether the study can continue or should discontinue.

2) Subinvestigator
Subinvestigator must report SADRs occurring during the study to the principal investigator and the sponsor immediately.

3) Institutional Review Board (IRB)
In case of SADRs, the IRB must require the principal investigator to take necessary actions, such as suspension of partial or entire study.

4) Sponsor
When a SUSAR is reported by principal investigator or subinvestigator, the sponsor must attach the ADR summary, such as Council for international organizations of medical sciences (CIOMS)-I Form to the ADR report and submit to the Minister of Food and Drug Safety and it must be immediately notified in accordance with the IRB regulations of the institution.

10.5.3.4. Pregnancy

Women of child-bearing potential who have not received a sterilization procedure and women who have the last menstrual period within 12 months must have negative pregnancy test to participate in the study and must maintain medically acceptable contraception during the study period. Medically acceptable contraception include hormones (implantable, patch, and oral), IUD, and double barrier methods (concomitant use of two of following contraceptive methods: male condom, female condom, cervical cap, diaphragm, sponge, spermicide) (However, concomitant use of male condom and female condom is excluded from double barrier methods.).

When a female subject or a male subject's spouse or sexual partner gets pregnant during the study, it is not considered as a SAE, but should be reported with the same reporting method for the SAE. Investigator must report pregnancy of a female subject or a male subject's spouse (or sexual partner) to the sponsor within 24 hours from the first recognition, and when the pregnancy is confirmed, follow-up will be performed for the female subject or male subject's spouse (or sexual partner) who have provided written informed consent with ICF for collection of pregnancy information (Annex 4. Informed Consent Form for Use of Pregnancy Information) until birth or termination of pregnancy.

Investigator will instruct the subjects to visit the investigator as soon as they become aware of the fact that they or their spouse (or sexual partner) got pregnant during the study for confirmation of the pregnancy. Female subjects with confirmed pregnancy will be dropped out from the study.

11. Data Analysis and Statistical Considerations

11.1. Analysis Sets

11.1.1. Safety Set

This includes subjects who have received IP.

Part I – Subjects receiving ID injection of the IP will be included.

Part II – Subjects who showed allergy test negative result in Part I and have received SC injection of the IP will be included.

11.1.2. Full Analysis Set

The study aims to evaluate allergy, safety and tolerability and efficacy set will not be separately defined.

11.1.3. PK Analysis Set

PK set includes all subjects who have at least 1 measurements of PK primary endpoints for ALT-BB4 after IP administration.

11.2. Statistical Analysis Method

11.2.1. General Principle of Result Analysis

For all statistical analysis, SAS® version 9.4 (or higher) will be used. Unless specified otherwise, two-sided test under significance level of 5% will be performed in principle. For continuous variables, descriptive statistics (sample size, mean, standard deviation, median, minimum, and maximum) will be presented, and for categorical variables, frequencies and percentages will be presented. For safety endpoints, missing data will not imputed and will be analyzed with OC (observed case) method. Analysis details are provided in the Statistical Analysis Plan.

11.2.2. Demographic Data and Medical History

For background and demographic data of subjects included in the safety set, descriptive statistics (sample size, mean, standard deviation, median, minimum, and maximum) for continuous variables (such as age) will be presented. Frequency and percentage (%) for categorical variables will be presented.

11.2.3. Efficacy Endpoints

The study aims to evaluate allergy, safety and tolerability and statistical test for efficacy analysis will not be separately performed.

11.2.4. Safety Endpoints

Part I study will evaluate the drug allergy and adverse events occurring following ID injection of the IP. Part II-A study will evaluate adverse events occurring following SC injection of the IP. Part II-B study will evaluate the safety and tolerability following SC injection of the IP.

11.2.4.1. Primary Endpoints

1) Incidence rate of drug allergy following ID IP administration* in Part I

*Subjects developing either immediate or delayed allergic reaction are considered to have drug allergy.

The number and percentage (%) of subjects showing any change of drug allergy from the IP administration to the end of study or early termination in the control group and study group will be

provided and as the primary analysis, the upper limit of binomial exact 95% two-sided confidence interval (97.5% one-sided confidence interval) < 10% is considered statistically significant.

In addition, the analysis will be performed using the McNemar's test to compare the incidence rate of drug allergy between the study group and the control group.

2) Safety and tolerability assessment following SC injection of the IP in Part II**

** Incidence rate of adverse events, including systemic and administration site-related adverse events

To evaluate the safety and tolerability, systemic and administration site-related adverse events will be assessed following SC injection of the IP.

In addition to the routine safety tests of physical examination, vital signs, ECG and laboratory tests, comprehensive data about clinical adverse events will be evaluated to determine relationship of the adverse drug reactions, including the nature, signs and type of adverse events, severity, frequency and duration, occurrence site and types of disease, and symptoms of AEs by system organ class (SOC).

The number and percentage (%) of subjects developing adverse events will be provided and the analysis will be performed using the Pearson's chi-square test or Fisher's exact test to compare the incidence rate of AEs between the study group and the control group.

11.2.4.2. Secondary Endpoints

1) Incidence rate of immediate allergic reactions† occurring within 30 minutes after the ID injection of the IP

†defined as drug allergy associated with wheal ≥ 8 mm with or without pseudopod (elevation considered due to an injection is not considered wheal), erythema or localized itching occurring in the administration site within 30 minutes after IP administration

The number and percentage (%) of subjects showing any change of immediate allergic reaction occurring within 30 minutes after the IP administration in the control group and study group will be provided and analyzed using the McNermar's test to compare the incidence of immediate allergic reaction between the study group and the control group.

2) Incidence rate of delayed allergic reactions‡ occurring within 48 hours after the ID injection of the IP

‡drug allergy occurring from 30 minutes to 48 hours after the IP administration, including but not limited to redness, rash, urticaria, edema, and erythema in the administration site as well as acute generalized exanthematous pustulosis and erythema multiforme. Investigator will decide whether an adverse event is a delayed allergic reaction.

The number and percentage (%) of subjects showing any change of delayed allergic reaction occurring from 30 minutes to 48 hours after the IP administration in the control group and study group will be provided and analyzed using the McNermar's test to compare the incidence of delayed allergic reaction between the study group and the control group.

3) Incidence rate of administration site events* at 30 min. and 48 hours after the IP administration

* classified into wheal, erythema, localized itching and other adverse events, occurring after the IP administration, at each time point

For wheal, erythema, localized itching and other adverse events at 30 minutes and 48 hours after the IP administration in the control group and study group, the number and percentage (%) of subjects showing any change of each event will be provided and analyzed using the McNermar's test in Part 1 and Pearson's chi-square test or Fisher's exact test in Part 2 to compare the incidence of allergic reaction between the study group and control group by administration site-

related AE.

4) Size of wheal and erythema occurring at 30 min. and 48 hours after the IP administration

For the size of wheal and erythema occurring at 30 minutes and 48 hours and change in the size of wheal and erythema occurring at 48 hours after the IP administration compared to 30 minutes after the IP administration in the control group and study group, descriptive statistics (mean, standard deviations, median, min and max) will be provided. The analysis will be performed using the Paired t-test or Wilcoxon signed-rank sum test to compare the change in the size of wheal and erythema occurring at 48 hours after the IP administration compared to 30 minutes, and for the comparison between the study group and control group, an analysis of covariance (ANCOVA) with the baseline as a covariate will be performed. If the assumption of normality is not satisfied, a rank ANCOVA will be performed.

11.2.4.3. Other Safety Endpoints

1) Adverse Events

All AEs will be coded by using the most recent version of MedDRA. The number of subjects with adverse events and adverse drug reactions, incidence rate (%) and frequency by treatment group will be provided, along with its 95% confidence interval.

In addition, the number of subjects with adverse events and adverse drug reactions, incidence rate and frequency by SOC, PT, and treatment group will be provided.

2) Physical Examination, Vital Signs, ECG and Laboratory Tests

For continuous data, descriptive statistics (mean, standard deviation, median, min and max) for absolute values and changes from baseline at each time point of visits and Paired t-test or Wilcoxon signed-rank test will be performed. For laboratory tests and ECG values, the frequency and proportion of subjects shifting from normal/clinically insignificant abnormal value before the IP administration (Screening) to clinically significant abnormal value after the IP administration will be provided and McNemar's test will be performed.

11.2.5. PK Endpoints

PK endpoints are PK parameters of ALT-BB4, which will be provided with descriptive statistics. For PK parameters, statistical test is not separately performed. PK assessment results can be provided in a separate report.

11.3. Planned Analysis Time

In this study, interim analysis is not planned and analysis will be performed after completing observation in all subjects enrolled.

12. Data Management

12.1. Record and Access

Source documents are defined as outcomes of study data collection activities and observation. Source documents include medical records, electronic data and measurements by instrument as well as other records. All source documents available in the study will be recorded and kept by the investigator at the institution and only authorized person can access it.

12.2. Data Collection

All electronic data capture (EDC) systems used in the study will comply with 21 CFR part 11 (Code of Federal Regulations). Only authorized person can access EDC system and all entries, corrections, saves and deletions in the eCRF will be tracked and recorded via EDC system. Investigator will ensure the eCRF data is accurate, complete and interpretable and timely through electronic signature.

After the end of the study, a copy of eCRFs prepared through EDC system will be transferred to the institution via electronic storage media and will be kept under the same standards as for other essential documents.

12.3. Record Protection and Storage

In accordance with relevant regulations on study record storage, principal investigator must store various study-related data (including electronic documents), such as protocol, and IP manufacturing and management records for 3 years from the date of study completion (or early termination) or product approval under appropriate storage conditions (The storage period can be extended, when determined necessary by the sponsor).

These documents will be subject to investigation in case of inspection of the sponsor or related regulatory authorities and investigator must not destroy any documents related to the study without written permission of the sponsor. Investigator must take appropriate measures to prevent any accidental damage or premature loss of these documents.

13. Ethical Considerations and Administrative Procedures

13.1. Compliance with Regulations, including Korean Good Clinical Practice, and Ethical Standards

This study will be conducted ethically and scientifically in accordance with the Korean Good Clinical Practice (KGCP) and all related regulations. In addition, based on the Helsinki Declaration, the study will respect human dignity and rights and will not cause any disadvantage to the subjects.

13.2. Institutional Review Board (IRB)

Investigator must obtain written approval for the protocol, ICFs, subject recruitment materials and procedures and subject information sheet (SIS) provided to the subjects from the IRB before the study starts. The IRB decision on the study implementation will be notified to the principal investigator and the sponsor in a written form before the study starts.

Principal investigator will report the study progression, SAEs and life-threatening problems or death to the IRB and must inform the IRB of study termination.

13.3. Informed Consent Process

Investigator must obtain written informed consent from subjects (or their legally acceptable representative) after fully explaining study details, procedures, and effects and possible adverse events of the study drug to conduct the study and the written informed consent process must be performed prior to any other study procedures (Annex 2 Subject Information Sheet and Informed Consent Form). Investigator must provide a copy of signed ICF and SIS to subjects (or their legally acceptable representative), and the original copy must be kept in the investigator file.

By signing the ICF, subjects will agree to study participation as well as collection and use of their personal information in relation to the study. The range of personal information to be collected in relation to the study includes personal information (date of birth, sex, etc.), demographic data, medical records (past medical history, treatment history, etc.) and study-related test results, and all data collected will be handled in accordance with Personal Information Protection Act, rules and regulations.

13.4. Confidentiality

All records that can identify subjects must be kept confidential. All study-related documents, including eCRFs, will be recorded and identified by using subject identification code, not name. When the study result is published, the subject identity will not be disclosed.

The sponsor or monitor and auditor as well as the MFDS and the IRB may access the subject's medical records to verify the information collected and during such activities, confidentiality of the subject information must be kept.

13.5. Subject Safeguards

13.5.1. Actions Taken for Adverse Events

When AEs occur due to the study, investigator must make arrangement for necessary tests and treatment without delay. In addition, AEs must be observed until the resolution of AE or loss of follow-up.

13.5.2. Post-Clinical-Study Treatment and Therapy for Subjects

Subjects dropped out from the study should be able to receive other appropriate treatment. After

the end of the study, subjects will be treated with the optimal treatment option, as determined by the investigator.

13.5.3. Patient Compensation Rule

Annex 3. Patient Compensation Rule

13.6. Quality Control and Reliability Assurance

13.6.1. Monitoring of Institutions

Monitoring will be performed to check for protection of subject rights and welfare and ensure that the study-related data reported is accurate, complete and verifiable, compared to the source documents and the study is conducted in accordance with the approved protocol, KGCP and related regulations.

Study monitoring will be performed via periodic institution visits and contacts of the sponsor (or its designee) or monitor. Their visit schedule will be established through discussion between the investigator and monitor.

Monitor must review the overall study process and basically check for source documents, IP management records and document storage status, etc. In addition, any issues found must be discussed with the investigator, and the investigator must cooperate with the process.

13.6.2. Audit

For reliability assurance during the study period, the sponsor may perform audit separately, in addition to routine monitoring. Audit will include confirmation that the study is conducted in accordance with the protocol, SOPs, KGCP and other related regulations and review of all source data, drug records and medical records, etc. The sponsor (or its designee) may ask access to the source documents and other essential documents for audit of the institution and the investigator must accept it and cooperate with the process.

13.6.3. Inspection

The MFDS may conduct inspection during or after the study and when an inspection is scheduled, the investigator must inform the sponsor immediately. The MFDS may ask access to the source documents and other essential documents for inspection of the institution and the investigator must accept it and cooperate with the process.

14. Sponsor's Information, and Principal Investigator's Name and Position

14.1. Sponsor Information

- Sponsor: Alteogen, Inc.
- CEO: Soon Jae Park
- Address: 62, Yuseong-daero 1628beon-gil, Yuseong-gu, Daejeon, Republic of Korea

14.2. Institution, and Principal Investigator's Name and Position

Annex 1 List of Institutions and Principal Investigators

15. Other Matters required for Safe and Scientific Conduct of Study

15.1. Protocol Amendment

To make a change in the approved protocol, the protocol amendment must be approved by the IRB and if necessary, by the Minister of Food and Drug Safety.

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17. Appendix

Appendix 1 Adverse Events of IP

This study is planned to monitor expected adverse events. However, based on human hyaluronidase, the active ingredient of Hylenex, a similar drug to ALT-BB4, and non-clinical trial data, expected adverse events are as below.

The most frequently reported adverse events with Hylenex were mild local adverse events, such as erythema or pain, and the reported incidence rate of allergic reaction was less than 0.1%.

At the highest dose in Minipig (2mg/kg) used for dose-finding, no adverse event was reported. In single dose and repeat dose toxicity studies of rats and repeat dose toxicity studies of cynomolgus monkeys, expected adverse event was not separately reported.

18. Annex

Annex 1 List of Institutions and Principal Investigators
Annex 2 Subject Information Sheet and Informed Consent Form
Annex 3 Patient Compensation Rule
Annex 4 Informed Consent Form for Use of Pregnancy Information