**Report on the Deliberation Results**

February 20, 2019
Medical Device Evaluation Division,
Pharmaceutical Safety and Environmental Health Bureau,
Ministry of Health, Labour and Welfare

| | |
|---|---|
| **Category** | Gene Therapy Products, 1 Plasmid vector products |
| **Non-proprietary Name** | Beperminogene Perplasmid |
| **Brand Name** | Collategene Intramuscular Injection 4 mg |
| **Applicant** | AnGes, Inc. |
| **Date of Application** | January 22, 2018 (marketing application) |

**Results of Deliberation**

In its meeting held on February 20, 2019, the Committee on Regenerative Medicine Products and Biotechnology made the following decision and concluded that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product may be approved. A conditional and time-limited approval is applicable to the product. The conditions of approval and the duration of approval are as follows.

**Conditions of Approval**

1. The applicant is required to ensure that the product is used under the supervision of physicians with adequate knowledge of and experience in treating severe chronic arterial occlusive disease at medical institutions where wound management is performed by collaboration among multiple departments.
2. The applicant is required to conduct an approval condition-based post-marketing evaluation in all patients treated with the product during the period between the conditional and time-limited approval and reapplication for marketing approval.

**Duration of Approval**

5 years

**Review Report**

February 4, 2019
Pharmaceuticals and Medical Devices Agency

The following are the results of the review of the following regenerative medical product submitted for marketing approval conducted by the Pharmaceuticals and Medical Devices Agency (PMDA).

| | |
|---|---|
| **Brand Name** | Collategene Intramuscular Injection 4 mg |
| **Category** | Gene Therapy Products, 1 Plasmid vector products |
| **Non-proprietary Name** | Beperminogene Perplasmid |
| **Applicant** | AnGes, Inc. |
| **Date of Application** | January 22, 2018 |
| **Dosage Form/Strength** | Solution for injection: Each vial contains 4 mg of Beperminogene Perplasmid. |

**Shape, Structure, Active Ingredient, Quantities, or Definition**

Beperminogene Perplasmid is a plasmid DNA encoding the gene of human hepatocyte growth factor. Beperminogene Perplasmid consists of 5181 base pairs, containing the human hepatocyte growth factor cDNA regulated by cytomegalovirus promoter/enhancer, pUC-derived sequence, kanamycin resistance gene, etc.

| | |
|---|---|
| **Application Classification** | (1-1) New regenerative medical products |
| **Items Warranting Special Mention** | None |
| **Reviewing Office** | Office of Cellular and Tissue-based Products |

**Results of Review**

On the basis of the data submitted, PMDA has concluded that the product is expected to have a certain level of efficacy in improving ulcers in patients with chronic arterial occlusive disease (arteriosclerosis obliterans and Buerger's disease) who do not respond adequately to standard drug therapy and are poor candidates for revascularization, and that the product has acceptable safety (see Attachment). However, since the currently available information is limited, the efficacy of the product should be evaluated and confirmed also after marketing approval.

As a result of its review, PMDA has concluded that the product may be approved for the indication or performance and dosage and administration or method of use shown below. The approval should be time-limited and conditional in accordance with Article 23-26 of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. The time limit according to the Article should be 5 years.

**Indication or Performance**

Improvement of ulcers in patients with chronic arterial occlusive disease (arteriosclerosis obliterans and Buerger's disease) who do not respond adequately to standard drug therapy and are poor candidates for revascularization

**Dosage and Administration or Method of Use**

Usually for adults, 0.5 mg per site of the product should be injected into the muscles of the ischemic lower limb in 8 sites every 4 weeks on 2 occasions (a total of 4 mg per occasion). If clinical symptoms remain, a third set of injections may be given 4 weeks after the second set of injections. The product should be diluted in Isotonic Sodium Chloride Solution (The Japanese Pharmacopoeia [JP]) before use, injections of 3 mL per site of diluted product should be delivered, and if the muscle to be injected is small, the injection volume may be reduced to 2 mL.

**Conditions of Approval**

1. The applicant is required to ensure that the product is used under the supervision of physicians with adequate knowledge of and experience in treating severe chronic arterial occlusive disease at medical institutions where wound management is performed by collaboration among multiple departments.
2. The applicant is required to conduct an approval condition-based post-marketing evaluation in all patients treated with the product during the period between the conditional and time-limited approval and reapplication for marketing approval.

**Attachment**

**Review Report (1)**

December 3, 2018

The following is an outline of the data submitted by the applicant and content of the review conducted by the Pharmaceuticals and Medical Devices Agency (PMDA).

**Product Submitted for Approval**

| | |
|---|---|
| **Brand Name** | Collategene Intramuscular Injection 4 mg |
| **Category** | Gene Therapy Products, 1 Plasmid vector products |
| **Non-proprietary Name** | Beperminogene Perplasmid |
| **Applicant** | AnGes, Inc. |
| **Date of Application** | January 22, 2018 |
| **Dosage Form/Strength** | Solution for injection: Each vial contains 4 mg of Beperminogene Perplasmid. |

**Shape, Structure, Active Ingredient, Quantities, or Definition**

Beperminogene Perplasmid is a plasmid DNA encoding the gene of human hepatocyte growth factor. Beperminogene Perplasmid consists of 5181 base pairs, containing the human hepatocyte growth factor cDNA regulated by cytomegalovirus promoter/enhancer, pUC-derived sequence, kanamycin resistance gene, etc.

**Proposed Indication or Performance**

Improvement of ulcers and rest pain in chronic arterial occlusive disease (arteriosclerosis obliterans/Buerger's disease)

**Proposed Dosage and Administration or Method of Use**

The product should be diluted in Isotonic Sodium Chloride Solution (JP), and 0.5 mg per site of the product should be injected into the muscles of the ischemic lower limb in 8 sites (a total of 4 mg). Injections of 3 mL per site of diluted product should be delivered, and if the muscle to be injected is small, the injection volume may be reduced to 2 mL. Injection locations should be selected based on the ischemic state of the limb. The product should be dosed every 4 weeks on 2 occasions. If clinical symptoms remain, a third set of injections may be given.

**Table of Contents**



**List of Abbreviations**

See Appendix.

## 1. Origin or History of Discovery, Use in Foreign Countries, and Other Information

### 1.1 Outline of the proposed product

Hepatocyte growth factor (HGF) was discovered as a protein involved in hepatocyte proliferation in 1984 with complementary deoxyribonucleic acid (cDNA) cloning in 1989 (*Biochem Biophys Res Commun*. 1989;163:967-73). HGF has been reported to be produced in the injured organ following liver or kidney injury or by mesenchymal cells of the lung etc. and acts on the epithelial cells of the injured organ to promote regeneration (*J Biochem*. 1996;119:591-600, *Proc Natl Acad Sci USA*. 1993;90:1937-41). HGF has also been reported to potently stimulate vascular endothelial cell growth and plays an important role in angiogenesis (*J Cell Biol.* 1992;119:629-41, etc.).

Collategene (HGF plasmid) is a regenerative medical product, and its main component is a plasmid DNA encoding the hHGF gene of 5181 base pairs, containing the cDNA for hHGF (Beperminogene Perplasmid). It is intended to treat critical limb ischemia of arteriosclerosis obliterans (ASO) or Buerger's disease. It was developed with an expectation of its pharmacological therapeutic effects, as with pharmaceuticals. Collategene administered by injection into muscles in close proximity to the ischemic focus is expected to induce angiogenesis through HGF production/release and improve the ischemic state of the limb by increasing the number of blood vessels and blood flow. It is considered to exert its therapeutic effects in the treatment of critical limb ischemia in patients with ASO or Buerger's disease.

### 1.2 Development history etc.

ASO is caused by chronic atherosclerotic changes in the trunk arteries in the upper and lower extremities, which often involves the iliac artery/femoral artery, and patients with ASO present with clinical symptoms of circulatory disturbance due to peripheral arterial stenosis and occlusion (rest pain, ulcers, gangrene, etc.). For the treatment of critical limb ischemia due to ASO, revascularization via endovascular techniques or surgical bypass is performed, which cannot achieve adequate hemodynamic improvement and fails to improve clinical symptoms in not a few patients. There are also patients in whom revascularization itself cannot be performed or it is difficult to perform revascularization. There are no useful treatment options for patients who have not responded adequately to revascularization or are not candidates for revascularization, and not a few patients require lower-extremity amputation in the end. The prognosis of patients who have undergone lower-extremity amputation is poor, and lower limb salvage as well as improvement of clinical symptoms are important issues also in terms of survival prognosis.

Buerger's disease is also called thromboangiitis obliterans (TAO). It is an inflammatory disease that affects the trunk arteries in the upper and lower extremities, characterized by occlusive inflammation involving all layers of the vessel wall. It is considered to be associated with vasospasm induced by smoking, but its precise etiology is unknown. Lesions can occur in any artery of the extremities, particularly frequently in the arteries of the lower limb, and patients with severe Buerger's disease present with clinical symptoms in the lower limbs (rest pain, ulcers, gangrene, etc.), which are similar to those of ASO. Since more distal arteries are occluded in Buerger's disease compared with ASO, the incidence of ulcerations on the end of the toes is especially high. The basic treatment for Buerger's disease is abstinence from smoking and drug therapy, and revascularization

is rarely possible for patients with Buerger's disease for an anatomic reason, i.e. the distal nature of the disease. Sympathectomy is considered for patients who are not candidates for revascularization, but its effectiveness is limited. As with ASO, there are no other useful treatment options, and not a few patients with Buerger's disease require lower-extremity amputation.

The Fontaine classification is used to grade the severity of the clinical symptoms of patients with ASO or Buerger's disease, and Stage I is defined as asymptomatic, Stage II as intermittent claudication, Stage III as rest pain, and Stage VI as ulcers or gangrene (Guidelines for the management of peripheral arterial occlusive diseases (JCS 2015) [Japanese clinical practice guidelines]).

A first-in-human study of HGF plasmid was initiated as "a clinical research study of gene therapy for peripheral vascular disease" at the Osaka University Hospital in May 2001. Then, in Japan, ASO phase III study in ASO patients with critical limb ischemia who had not responded adequately to standard drug therapy and were poor candidates for revascularization began in January 2004. TAO open-label clinical study in Buerger's disease patients with ischemic ulcers who had failed conventional medical treatment was undertaken in May 2004.

In the US, US phase II study in ASO patients with critical limb ischemia who were poor candidates for revascularization was initiated in April 2003. HGF plasmid was injected at predefined sites in US phase II study, unlike in the Japanese clinical studies. Thus, HGF plasmid was injected into the muscles of the ischemic lower limb in US second phase II study beginning in August 2005, as in the Japanese phase III study. However, US second phase II study was terminated in [redacted] 20[redacted] because many patients who were unsuitable for efficacy evaluation were enrolled in the study, which made it difficult to achieve the purpose of the study.

Based on the results from ASO phase III study, TAO open-label clinical study, etc., the applicant submitted a new drug marketing application for HGF plasmid, Collategene Intramuscular Injection 4 mg, for the indication of "arteriosclerosis obliterans/Buerger's disease with critical limb ischemia (rest pain, ischemic ulcers)" as of March 27, 2008. PMDA conducted a regulatory review and expressed the opinion that a definitive conclusion could not be made, based on the submitted clinical study data, concerning the efficacy and safety of HGF plasmid for the claimed indication. Accordingly, the applicant concluded that further clinical data would be needed for obtaining the marketing approval and withdrew the marketing application as of [redacted], 20[redacted].

Then, US phase IIb pilot study was initiated in March 2014 to evaluate the safety of a modified dosage regimen [see Section 7.2.3] of HGF plasmid in ASO patients with critical limb ischemia who were poor candidates for revascularization. Although global phase III study with the same dosage regimen as in US phase IIb pilot study began in November 2014, further enrollment was discontinued in [redacted] 20[redacted] due to slow subject enrollment.

On the other hand, in Japan, advanced medical care B clinical research study was undertaken in October 2014 in patients with chronic arterial occlusive disease (ASO and Buerger's disease) who had not responded adequately to standard drug therapy and were poor candidates for revascularization.

The applicant has now filed a marketing application for the regenerative medical product, HGF plasmid, based on the results from newly conducted advance medical care B clinical research study, in addition to the results from ASO phase III study, TAO open-label clinical study, etc. that were submitted with the previous new drug marketing application, as the main study data.

As of November 2018, HGF plasmid has not been approved in any country or region.

## 2. Manufacturing Process and Specifications and Outline of the Review Conducted by PMDA

### 2.1 Drug substance

A Drug Master File (DMF) for the drug substance Beperminogene Perplasmid (DMF Registration Number, [redacted]) has been registered by [redacted].

#### 2.1.1 Generation and control of cell substrate

See Supplement for the generation and control of master cell bank (MCB) and working cell bank (WCB). The drug substance is manufactured in an *Escherichia coli* (*E. coli*) cell substrate.

#### 2.1.2 Manufacturing process

See Supplement.

#### 2.1.3 Safety evaluation of adventitious agents

No raw materials of biological origin etc. are used in the drug substance manufacturing process.

#### 2.1.4 Manufacturing process development

See Supplement.

#### 2.1.5 Characterization

##### 2.1.5.1 Structure and properties

Characterization studies performed are shown in Table 1.

**Table 1. Characterization attributes**

| | |
|---|---|
| Primary structure | restriction map, nucleotide sequence |
| Higher-order structure | covalently closed circular structure, open circular structure, linear structure |
| Biological activity | HGF expression activity [see Section 4.1.1], [redacted], [redacted] |
| Immunochemical properties [see Section 6.2.4] | heterologous passive cutaneous anaphylaxis test (mouse), active systemic anaphylaxis test (guinea pig), homologous passive cutaneous anaphylaxis test (guinea pig) |

The culture supernatant from [redacted] cells transfected with HGF plasmid was added to genetically modified [redacted] cells established, and the activation of the c-Met receptor tyrosine kinase was assessed. The biological

functional activity of HGF was assessed by measuring VEGF in the culture supernatant obtained by adding the culture supernatant from [redacted] cells transfected with HGF plasmid to [redacted] cell line [redacted] cells.

#### 2.1.5.2 Product-related substances/Product-related impurities

The open circular (OC) form (a single-strand nick in the covalently closed circular DNA [the CCC form, the main structural form of HGF plasmid]), the linear (LN) form (double-stranded nicks in the DNA duplex [both DNA strands are cut at the same or nearly the same position in the DNA duplex]), and other impurities were considered product-related impurities. All product-related impurities are controlled by the drug substance and product specifications [see Section 3.R.1].

#### 2.1.5.3 Process-related impurities

Host cell protein (HCP), host cell DNA, host cell RNA, endotoxins, [1], [2], residual proteins, and [3] were considered process-related impurities. All of the process-related impurities have been demonstrated to be adequately removed during the manufacturing process.

### 2.1.6 Control of drug substance

The proposed specifications for the drug substance consist of content, description, identification ([redacted], [redacted]), pH, the content of the CCC form (HPLC method, [redacted]), purity (the OC form, the LN form, other impurities, host cell DNA, host cell RNA, residual proteins [[redacted]], HCP [[redacted]], [3]), endotoxins, microbial limits, and assay ([redacted]).

Tests for the OC form, the LN form, and other impurities (purity tests) were added in the course of regulatory review [see Section 3.R.1].

## 2.2 Product

### 2.2.1 Description and composition of product and product development

The product is a solution for injection. Each glass vial (3 mL) contains 4 mg of Beperminogene Perplasmid and the following excipients: sodium chloride, sodium hydroxide, and water for injection. A carton is used as secondary packaging.

### 2.2.2 Manufacturing process

The product is manufactured through a process comprised of blending of the drug substance, [redacted], [redacted], sterile filtration, filling, stoppering/screw capping, storage, thawing, packaging, testing, and storage.

[redacted], [redacted], sterile filtration, and filling have been defined as critical steps.

Process validation of the commercial-scale product manufacturing process has been performed.

### 2.2.3 Manufacturing process development

Major changes made to the product manufacturing process during development are shown below. The product manufactured by [redacted] process (Process B) or [redacted] process (Process C), using the drug substance produced by [redacted] process (Process A), is referred to as the A-B product or the A-C product, respectively. The product manufactured by [redacted] process (Process E) or [redacted] process (Process F), using the drug substance produced by [redacted] process (Process D), is referred to as the D-E product or the D-F product, respectively.

- Process B→Process C: change of [redacted], and [redacted], [redacted], [redacted], [redacted], [redacted], and [redacted] etc.
- Process C→Process E: change of [redacted], [redacted] and [redacted] etc.
- Process E→Process F: change of [redacted], [redacted] and [redacted] etc.
- Process F→The proposed commercial process: change of [redacted], [redacted] etc.

The A-B product was used in Osaka University clinical research study, the A-C product in ASO phase III study, TAO open-label clinical study, US phase II study, US second phase II study, and US IHD phase I study, and the D-F product in US phase IIb pilot study, a global phase III study, and advanced medical care B clinical research study.

When these changes were made to the manufacturing process, the quality attributes of the product were evaluated, which demonstrated comparability between pre-change and post-change product.

### 2.2.4 Control of product

The proposed specifications for the product consist of strength, description, identification ([redacted]), pH, osmotic pressure ratio, the content of the CCC form (HPLC method, [redacted]), purity (the OC form, the LN form, and other impurities), extractable volume, foreign insoluble matter, insoluble particulate matter, sterility, biological activity ([redacted]), and assay ([redacted]).

Tests for the OC form, the LN form, and other impurities (purity tests) were added in the course of regulatory review [see Section 3.R.1].

## 2.R Outline of the Review Conducted by PMDA

See Section 3.R.

# 3 Stability and Outline of the Review Conducted by PMDA

## 3.1 Stability of drug substance

The primary stability studies on the drug substance are shown in Table 2.

**Table 2. Overview of primary stability studies on drug substance**

| | No. of batches | Manufacturing process | Storage conditions | Storage package | Testing period |
|---|---|---|---|---|---|
| Long-term testing | 7 [a] | Process D1 [b] | −70 ± 10°C | 1.8 mL polypropylene cryopreservation tube | [redacted] months |
| | 1 | Process D2 [b] | | | |
| | 1 | Proposed commercial process | −70 ± 10°C | 1.8 mL polypropylene cryopreservation tube | [redacted] months [c] |
| | 3 | Proposed commercial process | −80 ± 10°C | 30 mL polypropylene bottle | [redacted] months [c] |
| Accelerated testing | 1 | Process D1 [b] | 25 ± 2°C 60%RH ± 5%RH | 1.8 mL polypropylene cryopreservation tube | [redacted] months |
| | 1 | Process D2 [b] | | | |
| | 4 | Proposed commercial process | | | |

a: One batch was subjected to testing up to 24 months and then discontinued from testing (not tested at ≥36 months).
b: The manufacturing processes before and after changes were made to Process D are referred to as Process D1 and Process D2, respectively.
c: Stability testing is ongoing.

The long-term testing showed no significant changes in any of the attributes tested throughout the testing period. Under the accelerated condition, the content of the CCC form, [redacted], and [redacted] markedly decreased over time.

Based on the above, a shelf-life of [redacted] months has been proposed for the drug substance when stored below −70°C in a polypropylene bottle with a cap, protected from light.

### 3.2 Stability of product

The primary stability studies on the product are shown in Table 3.

**Table 3. Overview of primary stability studies on product**

| | No. of batches | Product | Storage conditions | Testing period | Storage package |
|---|---|---|---|---|---|
| Long-term testing | 3 | D1[b]-E product | −20°C ± 5°C | [redacted] months | Chlorobutyl rubber stopper/glass vial |
| | 7 | D1[b]-F product | | | |
| | 3 [a] | Proposed commercial process | | | |
| Accelerated testing (1) | 3 | D1[b]-E product | 5°C ± 3°C | [redacted] months | |
| Accelerated testing (2) | 3 | D1[b]-E product | 25°C ± 2°C 60%RH ± 5%RH | [redacted] months | |
| | 3 | D1[b]-F product | | | |
| Stress testing | 1 | D1[b]-E product | 40 ± 2°C 75%RH ± 5%RH | [redacted] months | |
| Photostability testing | 1 | D1[b]-E product | An overall illumination of not less than 1.2 million lux·h + an integrated near ultraviolet energy of not less than 200 W·h/m$^2$ | | |

a: Testing is conducted on the first 3 batches of the commercial product to be released. Stability testing is ongoing.
b: The manufacturing processes before and after changes were made to Process D are referred to as Process D1 and Process D2, respectively.

The long-term testing showed no significant changes in any of the quality attributes tested throughout the testing period.

The accelerated testing (1) showed a trend towards a decrease in the content of the CCC form at ≥[redacted] months. The accelerated testing (2) showed a marked decrease in the content of the CCC form over time. There were large batch-to-batch differences in the degree of decrease in the content of the CCC form.

Under the stress condition, a marked decrease in the content of the CCC form was observed.

The results of the photostability testing indicated that the product is photosensitive.

Based on the above, taking account of [redacted], a shelf-life of 60 months has been proposed for the product when packaged in a glass vial with a chlorobutyl rubber stopper and stored in a carton to protect from light at −35°C to −15°C [see Section 3.R.2].

## 3.R Outline of the review conducted by PMDA

### 3.R.1 Control of product-related impurities

For the control of product-related impurities, content uniformity testing was included in the proposed drug substance and product specifications, in order to control the percentage of the CCC form and the percentage of known isomers (the sum of the amounts of the CCC form, the OC form, and the LN form), compared with the total amount of the CCC form, the OC form, the LN form, and other impurities.

PMDA considered as follows and requested the applicant to take action:
Since the OC form, the LN form, and other impurities have lower biological activity than the CCC form and are positioned as product-related impurities, it is necessary to control their limits. However, as to the proposed content uniformity testing by HPLC, its capability to control [redacted] OC form and the LN form [redacted] has not been evaluated. Thus, it is necessary to include suitable purity tests for the control of the content of individual isomers in the specifications.

The applicant responded that suitable purity tests for the control of the content of individual product-related impurities will be included.

PMDA accepted the applicant's response. The analytical procedure for impurities is currently being tested, including the determination of conditions, which will be reported in the Review Report (2).

### 3.R.2 Effects on stability

PMDA asked the applicant to explain the reason for large batch-to-batch variations in the stability profile, i.e. large batch-to-batch differences in the content of the CCC form, in the accelerated and stress testing of the product.

The applicant's response:
Under the accelerated and stress conditions, the content of the CCC form in the product decreased over time, and there was a batch-to-batch variability in the degree of decrease. In the accelerated testing at 25°C, the degree of decrease in the content of the CCC form over time tended to differ according to the pH of the product, and a marked decrease was seen at pH[redacted]. Thus, the pH of the product was suspected to be the main factor affecting the stability of the CCC form. On the other hand, there was no pH-dependent decrease in the content of the CCC form under the long-term condition.

PMDA asked the applicant to explain the measures to reduce effects on the stability profile, i.e. the content of the CCC form.

The applicant's response:
In the accelerated testing of the product, no decrease in the content of the CCC form was seen at 1 month. Within the product pH range of [redacted] to [redacted], no marked decrease in the content of the CCC form was observed for [redacted] months even under the accelerated condition of 25°C. Thus, the stability of the content of the CCC form can be ensured by controlling the pH of the product through an acceptance criterion of pH 5.0 to 7.5. In the manufacturing process, the vialed product after thawing is exposed to room temperature for a certain period of time during packaging. In order to ensure the stability, the operation time at room temperature in this step will be limited to [redacted] hours. The package insert will advise that at the medical institution, the product should be used within [redacted] hours after thawing.

PMDA concluded that although the content of the CCC form in the product decreased at 25°C, as there is no problem with stability under the proposed product storage conditions, pH will be controlled properly, and the operation time at room temperature during production will be managed, the measures against a decrease in the content of the CCC form as observed in stability studies have been taken, and there are no problems. Thus, PMDA accepted the proposed shelf-life of 60 months for the product stored at −35°C to −15°C, protected from light.

## 4 Indication or Performance and Outline of the Review Conducted by PMDA

### 4.1 *In vitro* studies

#### 4.1.1 Analysis of protein expressed from HGF plasmid in cell line (Attached document 4.2.1.1-1)

When Chinese hamster ovary (CHO) cells were transfected with HGF plasmid or negative control, p[redacted], by lipofection and incubated for 24 hours, the hHGF concentrations in the culture supernatants were determined by an enzyme-linked immunosorbent assay (ELISA). The hHGF concentrations in the culture supernatants were 73.23 ± 7.47 ng/mL (mean ± standard deviation [SD], n = 3) with HGF plasmid and below the lower limit of quantification (LLOQ) ([redacted] ng/mL, n = 3) with p[redacted].

*In vivo*, HGF is produced as prepro-HGF and then secreted into the extracellular matrix as pro-HGF. Pro-HGF undergoes extracellular proteolytic cleavage, resulting in a mature, heterodimeric, active form consisting of an α- and β-chain.

CHO cells were transfected with HGF plasmid, and the molecular structure of hHGF released in the culture supernatant was analyzed by Western blotting. According to an analysis with monoclonal antibody against the α-chain of hHGF, signals were detected at around 92 kDa, likely corresponding to pro-HGF, and at around 69 kDa, likely corresponding to mature HGF α-chain. According to an analysis with monoclonal antibody against the β-chain of hHGF, signals were detected at around 92 kDa, likely corresponding to pro-HGF and at around 32 kDa and 34 kDa, likely corresponding to mature HGF β-chain (the 2 signals represented mature HGF β-chain with or without a sugar chain).

### 4.1.2 Induction of HGF expression in human skeletal muscle cells by transfection of HGF plasmid (Attached document 4.2.1.1-2)

When normal human skeletal muscle cells were transfected with HGF plasmid or p[redacted] by the method as described in Section 4.1.1 and incubated for 24 hours, the hHGF concentrations in the culture supernatants were 3.45 ± 0.46 ng/mL with HGF plasmid (n = 3) and below the LLOQ ([redacted] ng/mL) with p[redacted] (n = 3).

### 4.1.3 Human vascular endothelial cell proliferation assay with HGF expressed from HGF plasmid (Attached document 4.2.1.1-3)

CHO cells were transfected with HGF plasmid by the method as described in Section 4.1.1, and human umbilical vein endothelial cell (HUVEC) proliferation induced by HGF produced/secreted by the transfected CHO cells was assessed by WST-1 assay based on the amount of formazan dye produced by the metabolism of the tetrazolium salt by viable cells. Cell proliferation was dependent on the concentration of HGF expressed from HGF plasmid.

## 4.2 Studies in rats

### 4.2.1 Study to evaluate the therapeutic efficacy of HGF plasmid in a rat model of hindlimb ischemia (Attached document 4.2.1.1-6, Reference data 4.2.1.1-7 and 4.2.1.1-8)

A single dose of 0.5 mL/body of HGF plasmid 0.2 or 2 mg/mL or p[redacted] 2.5 mg/mL was injected into the muscles of the left medial thigh (the ischemic hindlimb) in a male SD rat model of left hindlimb ischemia. Blood flow in the hindlimb was measured using a laser Doppler perfusion imager at Days 14 and 26, and improvement of blood flow was assessed by the Doppler ratio (the ratio of blood flow in the left hindlimb to the right hindlimb) (Table 4). A significant increase in blood flow was observed in the HGF plasmid groups compared with the p[redacted] group at Day 26 ($P < 0.01$, Dunnett's test).

**Table 4. Doppler ratio (%) after injection of HGF plasmid in rat model of hindlimb ischemia**

| Sample | HGF plasmid | | p[redacted] |
|---|---|---|---|
| | 0.1 mg/body | 1.0 mg/body | |
| Predose | 40.71 ± 6.85 | 40.31 ± 6.87 | 40.28 ± 7.08 |
| Day 14 | 50.74 ± 13.10 | 50.72 ± 14.09 | 42.49 ± 14.23 |
| Day 26 | 64.76 ± 10.56 | 64.96 ± 8.42 | 50.97 ± 8.72 |

Mean ± SD (n = 16/group)

The angiogenic effect of HGF plasmid was assessed by counting the number of capillary vessels in alkaline phosphatase (ALP)-stained sections from the injection-site muscles at Day 28, using an image analyzer (n = 15 or 16). The numbers of capillary vessels (/body) were 300 ± 99 in the p[redacted] group, and 324 ± 49 and 383 ± 63 in the HGF plasmid 0.1 and 1.0 mg/body groups, respectively. Injection of HGF plasmid tended to increase the number of capillary vessels in a dose-dependent manner, and there was a significant difference between the HGF plasmid 1 mg/body and p[redacted] groups ($P < 0.01$, Dunnett's test).

#### 4.2.2 Comparison of HGF expression in rats between A-C product and D-E product (Attached document 4.2.1.1-9)

A single dose of HGF plasmid (the A-C product or the D-E product) 0.75 mg/0.3 mL was injected into the left or right tibialis anterior muscles of male SD rats (9 weeks of age), and the hHGF concentrations in the injection-site muscles at Day 7 were determined by ELISA. The expression levels of hHGF following injection of HGF plasmid (the A-C product and the D-E product) were 1.500 ± 0.954 and 1.899 ± 1.195 ng/g tissue, respectively (n = 10/group).

### 4.3 Studies in rabbits

A single dose of HGF plasmid was injected into the left or right [redacted] muscles of normal male NZW rabbits (14 weeks of age). The effects of the injection solution concentration and the injection volume on hHGF expression were assessed by measuring hHGF expression in the injection-site muscles over time by ELISA.

#### 4.3.1 Effect of HGF plasmid concentration in injection solution on HGF expression in rabbit skeletal muscles (Attached document 4.2.1.1-10)

A single dose of HGF plasmid 0.01 or 0.5 mg was injected (injection volume, 2.0 mL), and hHGF expression in the injection-site muscle at Day 7 was measured (n = 20/group). In the 0.01 mg group, hHGF expression was measurable in 8 of 20 rabbits, and the expression level of hHGF was 0.345 ± 0.492 ng/g tissue (calculated by letting levels below the LLOQ equal zero). In the 0.5 mg group, hHGF expression was measurable in all 20 rabbits, and the expression level of hHGF was 14.824 ± 12.366 ng/g tissue. hHGF expression was injection solution concentration-dependent.

#### 4.3.2 Effect of injection volume on HGF expression in rabbit skeletal muscles (Attached document 4.2.1.1-11)

A single dose of HGF plasmid 0.5 mg was injected (0.5 mL [1.0 mg/mL] or 2.0 mL [0.25 mg/mL]), and hHGF expression in the injection-site muscle at Day 7 was measured (n = 20/group). The expression level was 5.174 ± 4.662 ng/g tissue (detected in 19 of 20 rabbits) in the 0.5 mL group and 15.628 ± 17.047 ng/g tissue (detected in 19 of 20 rabbits) in the 2.0 mL group. hHGF expression was injection volume-dependent.

### 4.3.3 HGF expression in rabbit skeletal muscles over time (Attached document 4.2.1.1-12)

Following a single injection of HGF plasmid 0.5 mg (2.0 mL [0.25 mg/mL]), hHGF expression in the injection-site muscle was measured at Days 1, 7, 14, and 28 (Table 5). Both the number of animals with hHGF expression and its expression level peaked at Day 7.

**Table 5. hHGF expression following a single intramuscular injection in rabbits**

| Sample | Injection-site muscle | | Serum |
|---|---|---|---|
| | No. of animals with HGF expression (n) | hHGF concentration [a] (ng/g tissue) | hHGF concentration (ng/mL) |
| Day 1 | 10/20 | 0.737 ± 0.914 | Below the LLOQ [b] |
| Day 7 | 18/19 | 3.754 ± 3.079 | Below the LLOQ [b] |
| Day 14 | 16/20 | 2.331 ± 2.954 | Below the LLOQ [b] |
| Day 28 | 5/20 | 0.362 ± 0.764 | Below the LLOQ [b] |

a: Mean ± SD (n = 20/group), Calculated by letting levels below the LLOQ ([redacted] ng/mL) equal zero.
b: [redacted] ng/mL

## 4.4 Safety pharmacology

### 4.4.1 Effects on central nervous system (Attached document 4.2.1.3-1 and 4.2.1.3-2)

When a single dose of HGF plasmid 0.5 or 5.0 mg/kg or vehicle (saline) was injected into the right thigh muscles (at 2 sites) of male SD rats (6 weeks of age) (total injection volume, 2 mL/kg), the effects on the central nervous system were assessed by functional observation battery (FOB) up to 168 hours post-dose. There were no effects on clinical signs, behavior, or body temperature. Although defecation was significantly increased in the 5.0 mg/kg group (n = 8) than in the vehicle group (n = 8) at 1 hour post-dose, as there were no changes in associated measures of emotionality, the applicant discussed that this finding was incidental.

When a single dose of HGF plasmid 0.25 or 2.5 mg/kg or vehicle was injected into the left or right thigh muscles (at multiple sites) (approximately 0.6 mL per site) of male cynomolgus monkeys (2-3 years of age, n = 4/group) (total injection volume, 1 mL/kg), clinical signs and behavior were observed. There were no evident changes.

### 4.4.2 Effects on respiratory/cardiovascular system (Attached document 4.2.1.3-2)

When a single dose of HGF plasmid 0.25 or 2.5 mg/kg or vehicle (saline) was injected into the left or right thigh muscles (at multiple sites) (approximately 0.6 mL per site) of male cynomolgus monkeys (2-3 years of age, n = 4/group) (total injection volume, 1 mL/kg), the effects on the respiratory/cardiovascular system were assessed using telemetry and Holter ECG. There were no effects on blood pressure, respiratory rate, heart rate, or ECG up to 24 hours post-dose and also at 168 hours post-dose. Although arterial blood gas analysis at 1 hour post-dose showed significantly higher $O_2$ partial pressure in the 2.5 mg/kg group than in the vehicle group, there were no effects on arterial blood pH, $CO_2$ partial pressure, or $O_2$ saturation.

## 4.R Outline of the review conducted by PMDA

### 4.R.1 Mechanism of action of HGF plasmid

The applicant's explanation about the mechanism of action of intramuscular HGF plasmid in the treatment of critical limb ischemia:

Mature hHGF is produced/secreted by cells transfected with HGF plasmid, and hHGF expressed from HGF plasmid specifically promotes proliferation of vascular endothelial cells, suggesting the possibility that

injection of HGF plasmid induces angiogenesis. Taking also into account that measurable hHGF expression in the muscles was observed after injection of HGF plasmid in rabbit skeletal muscles and rat tibialis anterior muscles, and that injection of HGF plasmid in the thigh muscles increased blood flow and the number of blood vessels in a rat model of hindlimb ischemia, hHGF expressed from gene transfer via HGF plasmid in skeletal muscles is considered to induce angiogenesis via proliferation of vascular endothelial cells.

PMDA considers that the mechanism of action of HGF plasmid has been discussed to a certain degree, based on the currently available findings.

### 4.R.2 Safety pharmacology

The applicant explained that it is inferred from the results of safety pharmacology studies of HGF plasmid that there are no safety concerns about the CNS, respiratory, or cardiovascular effects of HGF plasmid.

PMDA concluded that safety evaluation for humans based on the results of safety pharmacology studies is acceptable.

## 5. Biodistribution of the Product and Outline of the Review Conducted by PMDA

### 5.1 Non-clinical biodistribution

The time course of blood concentrations, distribution, metabolism, and urinary excretion following intramuscular or intravenous injection of HGF plasmid in male and female rats, and the time course of blood concentrations following intramuscular injection of HGF plasmid in male cynomolgus monkeys were determined.

HGF plasmid concentrations in rat blood and urine and in monkey blood were determined by quantitative polymerase chain reaction (Q-PCR), and primers that specifically detect a [redacted] bp sequence containing the cytomegalovirus (CMV) promoter and the hHGF cDNA out of the entire sequence comprising HGF plasmid (5181 bp) were used (Q-PCR assay A). Furthermore, for determination of HGF plasmid concentrations in rat tissues, primers for amplifying a [redacted] bp sequence containing this [redacted] bp were also used in order to distinguish functioning plasmid (capable of inducing hHGF expression) from its metabolites, inactive DNA fragments (Q-PCR assay B). The LLOQ of Q-PCR assay was 10 copies/μL in blood (Q-PCR assays A and B), 50 copies/μg DNA in tissues (Q-PCR assays A and B), and 50 copies/μL in urine (Q-PCR assay A only). For the pharmacokinetic parameters of HGF plasmid, blood concentrations are expressed as equivalents of HGF plasmid, based on $5.68\times10^{-6}$ pg per copy. Southern blotting was used for HGF plasmid metabolite analysis.

### 5.1.1 Absorption

#### 5.1.1.1 Time course of blood concentrations following a single intramuscular or intravenous injection in rats (Attached document 4.2.2.3-1, 4.2.2.3-3, 4.2.2.3-4, and 4.2.2.3-6)

Male and female SD rats (9 weeks of age) received a single injection of HGF plasmid 0.06, 0.3, or 1.5 mg/kg (injection volume, 1 mL/kg) in the right thigh muscles or a single intravenous injection of HGF plasmid 1.5 mg/kg, and blood concentrations of HGF plasmid were determined by Q-PCR assay A (Table 6). When the AUC following intramuscular injection of HGF plasmid 1.5 mg/kg was compared with the AUC following intravenous injection, the absolute bioavailability was 0.7% in female rats and 0.2% in male rats, which indicated that when HGF plasmid is administered by intramuscular injection, very little enters the systemic circulation.

**Table 6. Pharmacokinetic parameters of HGF plasmid in blood following single intramuscular or intravenous injection in male and female rats**

| Route of administration | Dose (mg/kg) | Sex | $C_{max}$ (pg eq./mL) | $AUC_{last}$ (pg eq.·h/mL) | $AUC_{inf}$ (pg eq.·h/mL) | $t_{max}$ (h) | $t_{1/2}$ (h) |
|---|---|---|---|---|---|---|---|
| IM | 0.06 | F | 3.07 ± 2.05 | 9.97 ± 6.50 | Nc | 6.5 ± 3.0 | Nc |
| | 0.3 | | 1010 ± 1780 | 616 ± 782 | 1130 [a] | 0.44 ± 0.13 | 0.99 [a] |
| | 1.5 | | 5120 ± 5710 | 20,600 ± 18,800 | 20,600 ± 18,800 | 4.3 ± 4.3 | 3.7 ± 4.2 |
| IV | 1.5 | | | 3,120,000 ± 910,000 | 3,120,000 ± 910,000 | | 7.0 ± 3.0 |
| IM | 0.06 | M | 20.4 ± 33.4 | 73.9 ± 107.4 | 28.2 [a] | 2.1 ± 1.5 | 4.5 [a] |
| | 0.3 | | 623 ± 653 | 562 ± 400 | Nc | 1.3 ± 1.8 | Nc |
| | 1.5 | | 6440 ± 5050 | 8420 ± 6680 | 10,200 ± 6900 [b] | 0.31 ± 0.13 | 4.0 ± 3.8 [b] |
| IV | 1.5 | | | 4,250,000 ± 1,020,000 | 4,250,000 ± 1,020,000 | | 12 ± 3 |

Mean ± SD (n = 4/group); Nc, not calculable; a, Mean (n = 2); b, Mean ± SD (n = 3)

### 5.1.1.2 Time course of blood concentrations following 2 intramuscular injections at monthly intervals in rats (Attached document 4.2.2.3-2 and 4.2.2.3-6)

HGF plasmid 1.5 mg/kg (injection volume, 1 mL/kg) was injected into the right thigh muscles of male and female SD rats (9 weeks of age) on 2 occasions at monthly intervals, and blood concentrations of HGF plasmid were determined by Q-PCR assay A (Table 7). There were no significant differences in the AUC between the first dose [see Section 5.1.1.1] and the second dose.

**Table 7. Pharmacokinetic parameters of HGF plasmid in blood following 2 intramuscular injections at monthly intervals in male and female rats**

| Route of administration | Sex | $C_{max}$ (pg eq./mL) | $AUC_{last}$ (pg eq.·h/mL) | $t_{max}$ (h) | $t_{1/2}$ (h) |
|---|---|---|---|---|---|
| IM | F | 5880 ± 6510 | 7010 ± 7290 | 2.1 ± 2.2 | 2.1 ± 0.4 |
| | M | 9700 ± 15,890 | 20,800 ± 28,000 | 2.6 ± 3.6 | 1.8 [a] |

Mean ± SD (n = 4); a, Mean (n = 2)

### 5.1.1.3 Time course of blood concentrations following a single intramuscular injection in monkeys (Attached document 4.2.2.3-5 and 4.2.2.3-6)

Male cynomolgus monkeys (3-5 years of age) received a single intramuscular injection of HGF plasmid 0.5 or 2.5 mg/kg, and blood concentrations of HGF plasmid were determined by Q-PCR assay A (Table 8). While higher values were observed in monkeys, the $t_{max}$ was similar between monkeys and rats.

**Table 8. Pharmacokinetic parameters of HGF plasmid in blood following a single intramuscular injection in male cynomolgus monkeys**

| Route of administration | Dose (mg/kg) | Sex | $C_{max}$ (ng eq./mL) | $AUC_{last}$ (ng eq.·h/mL) | $t_{max}$ (h) | $t_{1/2}$ (h) |
|---|---|---|---|---|---|---|
| IM | 0.5 | M | 123 ± 155 | 640 ± 748 | 3.0 ± 4.3 | 6.2 ± 1.6 |
| | 2.5 | | 483 ± 640 | 10,500 ± 17,600 | 0.50 ± 0.43 | 11 ± 4 |

Mean ± SD (n = 3/group)

### 5.1.2 Distribution

#### 5.1.2.1 Tissue distribution following a single intramuscular injection in rats (Attached document 4.2.2.3-1 and 4.2.2.3-6)

Following a single intramuscular injection of HGF plasmid 1.5 mg/kg (injection volume, 1 mL/kg) in male and female SD rats (9 weeks of age, n = 4), tissue concentrations of HGF plasmid were determined at 1, 4, and 7 days post-dose. In addition, following a single intramuscular injection of the same dose of HGF plasmid in male rats, HGF plasmid concentrations in the injection-site muscles were determined at 14, 28, 60, and 90 days post-dose.

Based on quantification with Q-PCR assay A, at 1 day post-dose, 0.014% to 0.015% of the administered dose of HGF plasmid was present in the injection-site muscles of male and female rats, and HGF plasmid was detected also in the blood, lung, heart, spleen, kidney, pancreas, small intestine, cerebrum, adrenal gland, and non-injection site muscles. At ≥4 days post-dose, HGF plasmid was below the LLOQ in tissues other than the injection-site muscles. On the other hand, based on quantification with Q-PCR assay B, HGF plasmid was undetectable in all tissues other than the injection-site muscles of male and female rats at all time points, and the HGF plasmid concentrations in the injection-site muscles of male rats were below the LLOQ at 60 days post-dose. Based on the above results, HGF plasmid detected in tissues were considered its metabolites, DNA fragments, and it was inferred that functioning plasmid was not distributed in tissues.

#### 5.1.2.2 Tissue distribution following a single intravenous injection in rats (Attached document 4.2.2.3-4 and 4.2.2.3-6)

Following a single intravenous injection of HGF plasmid 1.5 mg/kg in male and female SD rats (9 weeks of age, n = 4), tissue concentrations of HGF plasmid were determined at 1, 4, and 7 days post-dose.

Based on quantification with Q-PCR assay A, HGF plasmid was detected in extensive tissues at 1 and 4 days post-dose and in the bone marrow, lung, heart, kidney, testis, ovary, spleen, and muscles at 7 days post-dose. Based on quantification with Q-PCR assay B, HGF plasmid was detected in the bone marrow, lung, heart, kidney, spleen, liver, pancreas, adrenal gland, muscles, and cerebrum at 1 day post-dose and in the spleen of males only at 4 days post-dose, and was below the LLOQ in all tissues at 7 days post-dose. The above results showed that HGF plasmid was metabolized by 7 days post-dose, with no functioning plasmid in tissues.

### 5.1.2.3 Placental transfer following a single intramuscular injection in pregnant rats (Attached document 4.2.2.3-7 to 4.2.2.3-9)

Following a single intramuscular injection of HGF plasmid 0.15 or 1.5 mg/kg in pregnant SD rats on gestation day 13 or a single intramuscular injection of HGF plasmid 1.5 mg/kg in pregnant rats on gestation days 6, 8, 10, and 16, tissue concentrations of HGF plasmid were determined (Table 9). Since functioning plasmid was not detected except for some animals, it was considered that functioning plasmid is unlikely to cross the placenta and distribute into the fetus.

**Table 9. Evaluation of placental transfer following a single intramuscular injection of HGF plasmid in pregnant rats**

| | Dose (mg/kg) | Tissues analyzed | Day (after administration) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
| | | | A | B | A | B | A | B | A | B | A | B | A | B |
| Gestation day 6 (n = 5) | 1.5 | Injection-site muscle | + | + | + | + | + | + | + | + | + | + | + | + |
| | | Maternal blood | − | − | − | − | − | − | − | − | − | − | − | − |
| | | Uterus | + | − | − | − | − | − | − | − | − | − | − | − |
| | | Conceptus [a] | + | − | − | − | − | − | − | − | − | − | − | − |
| Gestation day 8 (n = 5) | 1.5 | Injection-site muscle | + | + | + | + | + | + | + | + | | | | |
| | | Maternal blood | − | − | △ | − | − | − | − | − | | | | |
| | | Uterus | + | − | − | − | − | − | − | − | | | | |
| | | Conceptus [a] | + | − | △ | − | − | − | − | − | | | | |
| Gestation day 10 (n = 5) | 1.5 | Injection-site muscle | + | + | + | + | | | | | | | | |
| | | Maternal blood | − | − | − | − | | | | | | | | |
| | | Uterus | + | − | − | − | | | | | | | | |
| | | Conceptus [a] | + | − | − | − | | | | | | | | |
| Gestation day 13 (n = 3) | 0.15 | Injection-site muscle | + | | + | | | | + | | | | | |
| | | Maternal blood | − | | − | | | | − | | | | | |
| | | Uterus | − | | − | | | | − | | | | | |
| | | Placenta | − | | − | | | | − | | | | | |
| | | Embryo/Fetus | − | | − | | | | − | | | | | |
| | | Amniotic fluid | − | | − | | | | − | | | | | |
| | 1.5 | Injection-site muscle | + | | + | | | | + | | | | | |
| | | Maternal blood | △ | | △ | | | | − | | | | | |
| | | Uterus | △ | | − | | | | − | | | | | |
| | | Placenta | △ | | − | | | | − | | | | | |
| | | Embryo/Fetus | − | | − | | | | − | | | | | |
| | | Amniotic fluid | − | | − | | | | − | | | | | |
| Gestation day 16 (n = 5) | 1.5 | Injection-site muscle | + | + | + | + | | | | | | | | |
| | | Maternal blood | + | △ | △ | − | | | | | | | | |
| | | Uterus | + | − | + | − | | | | | | | | |
| | | Placenta | + | △ | + | − | | | | | | | | |
| | | Fetus | − | − | − | − | | | | | | | | |
| | | Amniotic fluid | − | − | − | − | | | | | | | | |

A, Q-PCR assay A; B, Q-PCR assay B; a, Products of conception, including the placenta, embryo/fetus, and amniotic fluid
−, below LLOQ; △, Detected in some animals.

## 5.1.3 Metabolism

### 5.1.3.1 *In vitro* metabolism (Attached document 4.2.2.4-1)

In order to evaluate species differences in the metabolism of HGF plasmid in serum, HGF plasmid 1.0 μg/mL was added to human or rat serum, incubated, and analyzed by Southern blotting using a probe that can specifically detect a [redacted] bp hHGF cDNA out of the entire sequence comprising HGF plasmid (5181 bp).

When added to human or rat serum, the CCC form, the main structural form of HGF plasmid, was rapidly metabolized to the OC form and the LN form, and then to small DNA fragments. In human serum, the CCC form was detected up to 1 to 5 minutes after addition, and the OC form and the LN form were detected up to 15 to 30 minutes after addition. In rat serum, the CCC form was detected up to 1 minute after addition, and the OC form and the LN form were detected up to 5 minutes after addition. In this test system, the $t_{1/2}$ of the CCC form was calculated to be 37 seconds in human serum and 28 seconds in rat serum, showing that HGF plasmid is rapidly metabolized in both human and rat serum.

#### 5.1.3.2 *In vivo* metabolism (Attached document 4.2.2.3-1 and 4.2.2.3-3)

Following a single intramuscular injection of HGF plasmid 1.5 mg/kg (injection volume, 1 mL/kg) in male and female rats, metabolites in blood up to 8 hours post-dose were analyzed by Southern blotting. The CCC form was not present in any sample, and the OC form and the LN form were detected in samples at 15 minutes, 30 minutes, and 1 hour post-dose. In samples at ≥2 hours post-dose, neither the OC form nor the LN form was detected, and smears, which were considered small DNA fragments, were found.

Following a single intravenous injection of HGF plasmid 1.5 mg/kg (injection volume, 1 mL/kg) in male and female rats, metabolites in blood up to 4 hours post-dose were analyzed in the same manner. The CCC form was not present in any sample. Although the OC form and the LN form only were detected in samples up to 5 minutes post-dose, neither the OC form nor the LN form was detected, and smears, which were considered small DNA fragments, were found, in samples at ≥15 minutes post-dose.

The above results suggested that in blood, HGF plasmid is converted from the CCC form to the OC form or the LN form, and is metabolized to inactive DNA fragments in the end.

### 5.1.4 Excretion (Attached document 4.2.2.3-3)

Following a single intravenous injection of HGF plasmid 1.5 mg/kg in male and female rats, HGF plasmid concentrations in the urine were determined by Q-PCR assay A. Urinary concentrations of HGF plasmid were below the LLOQ at all time points, i.e. 8, 24, 48, 72, 96, 120, 144, and 168 hours post-dose.

## 5.2 Clinical pharmacology

### 5.2.1 Blood concentrations of HGF plasmid after intramuscular injection

The time course of blood concentrations of HGF plasmid was determined in US phase II study, US second phase II study, and Stage 2 of Osaka University clinical research study [see Section 7]. HGF plasmid in human blood was quantified by Q-PCR assay A (LLOQ, 50 copies/20 μL in the US clinical studies, 50 copies/10 μL in Osaka University clinical research study).

In these studies, HGF plasmid was detected in the blood of almost all subjects at 4 hours or 1 day after injection of HGF plasmid and only in some subjects at Day 7. Also when the second or third dose was given 2 weeks apart, similar results were obtained. HGF plasmid was detectable in the blood of a limited number of

subjects at Week 2 or 4 and was below the detection limit (10 copies/20 μL in the US clinical studies, 10 copies/10 μL in Osaka University clinical research study) in all subjects at Month 3 or 6.

### 5.2.2 Serum hHGF concentrations after intramuscular injection

The time course of serum hHGF concentrations was determined in ASO phase III study, TAO open-label clinical study, advanced medical care B clinical research study, US phase II study, and US second phase II study [see Section 7]. Serum hHGF was quantified by ELISA (LLOQ, [redacted] ng/mL).

In all studies, there were no clear changes in serum hHGF concentrations after administration of HGF plasmid.

### 5.R Outline of the review conducted by PMDA

The applicant's explanation about the biodistribution of HGF plasmid:

As the time course of HGF plasmid concentrations in the injection-site muscles following a single intramuscular injection in rats was similar to that following 2 intramuscular injections at monthly intervals, there seemed to be no effect of multiple dosing. When the time course of blood HGF plasmid concentrations following a single intramuscular injection was compared with that following 2 intramuscular injections at monthly intervals, there were no significant differences in the AUC, and multiple dosing did not alter exposure levels.

The AUC of blood HGF plasmid following a single intramuscular injection of HGF plasmid in rats was <1% of the AUC following a single intravenous injection of the same dose of HGF plasmid, which indicated that when HGF plasmid is administered by intramuscular injection, very little enters the systemic circulation.

Following a single intravenous injection of HGF plasmid in rats, urinary HGF plasmid concentrations were below the LLOQ at all time points up to 168 hours post-dose. Analysis of HGF plasmid metabolites in blood by Southern blotting indicated that as HGF plasmid is rapidly metabolized in blood, it may be excreted as short DNA fragments that cannot be detected in the urine. A study of placental transfer of a single intramuscular dose of HGF plasmid in rats indicated that HGF plasmid does not cross the placenta into the embryo/fetus or amniotic fluid.

Since clinical pharmacology studies showed no clear changes in blood HGF plasmid concentrations or serum hHGF concentrations after administration of HGF plasmid, when HGF plasmid is administered by intramuscular injection, very little enters the systemic circulation, also in humans. Based on the results of analysis of metabolites in blood, even if HGF plasmid enters the systemic circulation, it will be rapidly metabolized. Thus, it is inferred that administration of HGF plasmid has little systemic effect.

PMDA accepted the applicant's explanation.

## 6. Nonclinical Safety Data and Outline of the Review Conducted by PMDA

The applicant submitted the results from non-clinical safety studies of HGF plasmid: single-dose toxicity, repeated-dose toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, local tolerance, and other toxicity studies (antigenicity studies). The A-B product, the A-C product, and the D-E product were used in these studies [see Section 2.2.3].

Unless otherwise specified, saline was used as vehicle in *in vivo* studies.

### 6.1 General toxicity

Intramuscular toxicity studies in rats and monkeys and intravenous toxicity studies in rats were conducted (Table 10, Table 11). The principal finding following administration of HGF plasmid was mononuclear cell infiltration at the injection site.

**Table 10. Single-dose toxicity studies**

| Test system | Route of administration | Dose (mg/kg) | Principal findings | Attached document |
|---|---|---|---|---|
| Male and female rats (SD) | IM | 0, 10 (A-B product) | 10: Mononuclear cell infiltration at the injection site | 4.2.3.1-1 |
| Male and female rats (SD) | IV | 0, 10 (A-B product) | 10: Venous endothelial hyperplasia at the injection site | 4.2.3.1-2 |
| Male and female rats (SD) | IV | 0, 10 (D-E product)<br>10 (A-C product) | No noteworthy findings | 4.2.3.1-3 |
| Male cynomolgus monkey | IM | 2.5 | 2.5: Increased basophil count and percentage | 4.2.3.1-4 |

**Table 11. Repeated-dose toxicity studies**

| Test system | Route of administration | Duration of dosing | Dose (A-B product) | Principal findings | NOAEL | Attached document |
|---|---|---|---|---|---|---|
| Male and female rats (SD) | IM | Every month for 4 months (once monthly) + 1-month recovery period | 0, 0.5, 1.5, 5.0 (mg/kg/month) (A-B product) | ≥1.5: A rise in anti-hHGF antibody titer<br>Reversible | 5.0 (mg/kg/month) | 4.2.3.2-1 |
| Male and female rats (SD) | IM | Every week for 5 weeks (once weekly) + 4-week recovery period | 0, 5.0 (mg/kg/week) (A-B product) | 5.0: The following findings at the injection site: degeneration/necrosis/regeneration of myofibers, interstitial fibrosis, hemorrhage, and mononuclear cell infiltration<br>Reversible | 5.0 (mg/kg/week) | 4.2.3.2-2 |
| Male and female rats (SD) | IV | 14 days (once daily) + 14-day recovery period | 0, 0.6, 2.5, 10 (mg/kg/day) (A-B product) | ≥0.6: Mononuclear cell infiltration at the injection site<br>Reversible | 10.0 (mg/kg/day) | 4.2.3.2-3 |

| Test system | Route of administration | Duration of dosing | Dose (A-B product) | Principal findings | NOAEL | Attached document |
|---|---|---|---|---|---|---|
| Male and female cynomolgus monkeys | IM | Every month for 4 months (once monthly) + 1-month recovery period | 0, 0.25, 0.8, 2.5 (mg/kg/month) (A-B product) | 2.5: Diarrhoea (Female) | 2.5 (mg/kg/month) | 4.2.3.2-4 (Reference data) |

## 6.2. Other safety data

### 6.2.1 Comet assay in multiple organs of rat

hHGF is expressed from HGF plasmid administered, and it would not cross the membrane of cells and therefore would not interact directly with DNA or other chromosomal material. Thus, no genotoxicity studies taking account of hHGF were conducted. Since HGF plasmid was distributed primarily in the lung and kidney at 1 day after intravenous injection in rats [see Section 5.1.2.2], a comet assay in multiple organs of the rat was performed to determine HGF plasmid-induced early DNA damage in the lung and kidney. HGF plasmid did not induce early DNA damage (Table 12).

**Table 12. Comet assay in multiple organs of rat (*in vivo*)**

| Test system | Route of administration | Dose (mg/kg/day) (D-E product) | Cells assessed | Test result | Attached document |
|---|---|---|---|---|---|
| Male rat (SD) | IV | 10 (2 injections at intervals of 24 hours/IV) | Cells derived from the lung and kidney | Negative | 4.2.3.3.2-1 |

### 6.2.2 Study in tumor-bearing mice to assess the effect of HGF plasmid on tumor growth

The receptor for hHGF expressed from HGF plasmid administered is a proto-oncogene product, c-MET. Activation of the HGF/c-MET signaling pathway is known to be involved in the proliferation, invasion, and metastasis of cancer cells, and if a tumor is in the proximity of the injection site, the possibility that injection of HGF plasmid affects tumor growth cannot be ruled out. A human tumor cell line (Mewo cells or HT-29 cells) was inoculated subcutaneously into the thigh of mice, and using these tumor-bearing mice, the effect of HGF plasmid on tumor growth was assessed. HGF plasmid did not promote tumor growth or metastasis of either tumor cell line (Table 13).

**Table 13. Study in tumor-bearing mice to assess the effect of HGF plasmid on tumor growth**

| Test system | Route of administration | Duration of dosing | Tumor cells | Dose (mg/kg/day) (D-E product) | Results of observation | Attached document |
|---|---|---|---|---|---|---|
| Female nude mouse (Balb/cA Jcl-nu) | IM (in the proximity of tumor) | Single dose | Mewo cell line [a] | 0<br>0.05<br>0.25 | There were no significant differences in tumor volume or weight between the HGF plasmid and control groups, and metastasis of tumor cells did not occur, up to 21 days after injection of HGF plasmid in mice inoculated with either tumor cell line. | 4.2.3.4.3-1 |
| | | | HT-29 cell line [b] | 0<br>0.05<br>0.25 | | |

a, Human malignant melanoma cell line; b, Human colon adenocarcinoma cell line

### 6.2.3 Local tolerance

In rabbits, the local tolerance of HGF plasmid was considered almost similar to that of negative control, saline (Table 14).

**Table 14. Intramuscular local tolerance studies in rabbits**

| Test system | Site of administration | Test method [a] | Principal findings | Attached document |
|---|---|---|---|---|
| Male rabbit (NZW) | IM | Single dose of 1 mL of 0.17 mg/mL (D-E product) | At 2 days after injection, degeneration or necrosis of myofibers, infiltration of histiocytes and pseudoeosinophils, and hemorrhage<br>At 7 days after injection, histiocytic infiltration<br>The above findings resolved by 14 days after injection. | 4.2.3.6-1 |
| | | Single dose of 1 mL of 0.17 mg/mL (A-B product) | At 2 days after injection, degeneration or necrosis of myofibers, infiltration of histiocytes and pseudoeosinophils, and hemorrhage<br>At 7 days after injection, histiocytic infiltration, hemorrhage, and fibrosis<br>The above findings improved by 14 days after injection. | 4.2.3.6-2 |

a: Saline was used as negative control, and 0.425% and 1.7% acetic acid were used as positive control.

### 6.2.4 Antigenicity

A heterologous passive cutaneous anaphylaxis test in male mice (Hetero-PCA test), and an active systemic anaphylaxis test (ASA test) and a homologous passive cutaneous anaphylaxis test (Homo-PCA test) in male guinea pigs were performed. The antigenicity of the D-E product and the A-B product were evaluated, and anti-HGF plasmid antibodies in sera from sensitized mice and guinea pigs were measured by ELISA. All test results were negative, and neither product was considered to be antigenic (Table 15).

**Table 15. Antigenicity studies in mice and antigenicity studies in guinea pigs**

| Type of study | Test system | Test method | Test results | Attached document |
|---|---|---|---|---|
| Hetero-PCA test | Male mouse (Balb/c and C3H/He) and male rat (SD) | Sera from mice sensitized by intraperitoneal injections of 25 mg/kg (D-E product) (3 doses, 2 weeks apart) were injected intradermally to rats (SD), and 24 hours later, a single dose of 2.5 mg/kg (D-E product) was injected intravenously. | HGF plasmid did not cause PCA reactions.<br>No anti-HGF plasmid antibodies were detected in sera from sensitized mice. | 4.2.3.7.1-1<br>4.2.3.7.1-3<br>(Reference data) |
| ASA test and Homo-PCA test | Male guinea pig (Hartley) | Guinea pigs were sensitized by subcutaneous injections of 2.5 mg/kg (D-E product) (3 doses, 2 weeks apart), and 28 days after the final sensitization, 2.5 mg/kg (D-E product) was injected intravenously (ASA test).<br>Sera from sensitized guinea pigs were injected intradermally to untreated guinea pigs, and 4 hours later, a single dose of 2.5 mg/kg (D-E product) was injected intravenously (Homo-PCA test). | HGF plasmid did not cause ASA reactions or PCA reactions.<br>No anti-HGF plasmid antibodies were detected in sera from sensitized guinea pigs. | 4.2.3.7.1-1 |
| Hetero-PCA test | Male mouse (Balb/c and C3H/He) and male rat (SD) | Sera from mice sensitized by intraperitoneal injections of 25 mg/kg (A-B product) (3 doses, 2 weeks apart) were injected intradermally to rats (SD), and 24 hours later, a single dose of 2.5 mg/kg (A-B product) was injected intravenously. | HGF plasmid did not cause PCA reactions.<br>No anti-HGF plasmid antibodies were detected in sera from sensitized mice. | 4.2.3.7.1-2 |
| ASA test and Homo-PCA test | Male guinea pig (Hartley) | Guinea pigs were sensitized by subcutaneous injections of 2.5 mg/kg (A-B product) (3 doses, 2 weeks apart), and 28 days after the final sensitization, 2.5 mg/kg (A-B product) was injected intravenously (ASA test).<br>Sera from sensitized guinea pigs were injected intradermally to untreated guinea pigs, and 4 hours later, a single dose of 2.5 mg/kg (A-B product) was injected intravenously (Homo-PCA test). | HGF plasmid did not cause ASA reactions or PCA reactions.<br>No anti-HGF plasmid antibodies were detected in sera from sensitized guinea pigs. | 4.2.3.7.1-2 |

## 6.R Outline of the review conducted by PMDA

### 6.R.1 Carcinogenic risk of HGF plasmid

The applicant explained that although HGF is known to promote tumor cell proliferation, the carcinogenic risk of HGF plasmid is considered low for the following reasons.

- Biodistribution studies showed that administered HGF plasmid is rapidly metabolized and inactivated in blood [see Section 5.1].
- hHGF at the injection site was below the LLOQ in most rabbits at 28 days after intramuscular injection of HGF plasmid, and HGF plasmid is dosed every 4 weeks on 2 or 3 occasions in a clinical setting. Given these points, hHGF concentrations in the injection-site tissue will return to its physiological levels by 4 weeks after the last dose of HGF plasmid, and long-term local exposure of hHGF to the injection site is unlikely [see Section 4.3.3].
- A comet assay in multiple organs of the rat showed that HGF plasmid is not an initiator of carcinogenesis [see Section 6.2.1].

- There was no evidence of preneoplastic lesions in repeated-dose toxicity studies of HGF plasmid [see Section 6.1].
- In a study in tumor-bearing mice to assess the effect of HGF plasmid on tumor growth, HGF plasmid did not promote tumor growth or metastasis [see Section 6.2.2].

Although the currently available toxicity data suggested no carcinogenic risk, and no clinical studies indicated that HGF plasmid causes tumor progression, hHGF expressed from HGF plasmid may promote the growth of malignant tumors through its angiogenic effect. Thus, the package insert will state that HGF plasmid is contraindicated in patients with current or previous malignant tumors in close proximity to its injection sites (muscles and surrounding tissue) and will advise that the use of HGF plasmid should be determined carefully for patients with malignant tumors at other locations.

PMDA accepts the applicant's explanation, but considers that it is necessary to carefully collect post-marketing safety information regarding the carcinogenic risk of HGF plasmid.

**6.R.2 Reproductive and developmental toxicity**

PMDA asked the applicant to explain the reason for not conducting a reproductive and developmental toxicity study of HGF plasmid, the assessment of reproductive and developmental toxicity, and the potential for HGF plasmid to affect the offspring via milk excretion.

The applicant's explanation:

Although no reproductive and developmental toxicity studies of HGF plasmid have been conducted, HGF plasmid at the proposed dosage regimen is unlikely to cause reproductive and developmental toxicity for the following reasons. However, as the occurrence of developmental abnormalities in transgenic mice overexpressing HGF has been reported (*Proc Natl Acad Sci*. 1996;93:5866-71), excessive exposure to HGF during the embryo-fetal development should be avoided.

- Since a plasmid vector is used for the proposed product, the risk of integration into germ cells should be low.
- In a study of placental transfer of a single intramuscular dose of HGF plasmid in pregnant rats [see Section 5.1.2.3], trace amounts of nucleic acids derived from HGF plasmid were detected in the conceptus, but only inactive DNA fragments not capable of inducing hHGF expression were considered to be distributed. Thus, HGF plasmid is unlikely to affect the embryo/fetus.
- Given that serum hHGF concentrations following intramuscular injection of HGF plasmid in rabbits were below the LLOQ and that serum hHGF concentrations following administration of HGF plasmid did not rise above its physiological levels in clinical studies of HGF plasmid, HGF plasmid and hHGF expressed from HGF plasmid are unlikely to have systemic effects, including reproductive effects.

Although HGF plasmid is unlikely to affect suckling infants via milk excretion for the following reasons, the package insert will advise against use in nursing mothers.

- Although DNA fragments derived from HGF plasmid were detected in blood after administration of HGF plasmid in clinical studies, the results of biodistribution studies etc. indicated that functioning plasmid is unlikely to be excreted in milk.
- Since HGF plasmid is DNA, even if suckling infants ingest HGF plasmid excreted in milk, HGF plasmid will be rapidly degraded by gastric acid and digestive enzymes.

PMDA accepted the applicant's explanation.

## 7 Clinical Data and Outline of the Review Conducted by PMDA

The applicant submitted efficacy and safety evaluation data, in the form of the results from 7 studies presented in Table 16. The applicant also submitted the results from 2 studies presented in Table 16 as reference data. During the period between the withdrawal of a new drug marketing application and the filing of the present application, advanced medical care B clinical research study [see Section 7.1.3], US phase IIb pilot study [see Section 7.2.3], and a global phase III study [see Section 7.2.4], and an additional long-term follow-up survey for ASO phase III study, TAO open-label clinical study, US phase II study, and US second phase II study [see Sections 7.1.1, 7.1.2, 7.2.1, and 7.2.2] were conducted.

**Table 16. Listing of clinical studies**

| | Geographical location | Study title | Phase | Study population | No. of patients enrolled | Dosage regimen [a] | Main endpoints |
|---|---|---|---|---|---|---|---|
| Evaluation data | Japan | ASO phase III | III | ASO patients | (1) 29<br>(2) 15<br>(3) 10 | Stage 1<br>(1) 2 sets of 8 injections of HGF plasmid 0.5 mg (a total of 4 mg) Q4W<br>(2) 2 sets of 8 injections of placebo Q4W<br>Stage 2<br>(3) 2 sets of 8 injections of HGF plasmid 0.5 mg (a total of 4 mg) Q4W | Efficacy<br>Safety |
| | | TAO open-label | — | Patients with Buerger's disease | 10 | 2 or 3 sets of 8 injections of HGF plasmid 0.5 mg (a total of 4 mg) Q4W | Efficacy<br>Safety |
| | | Advanced medical care B clinical research study | — | Patients with ASO or Buerger's disease | 6 | 2 or 3 sets of 8 injections of HGF plasmid 0.5 mg (a total of 4 mg) Q4W | Efficacy<br>Safety |
| | Global | Global phase III | III | ASO patients | (1) 23<br>(2) 23 | (1) 4 sets of 8 injections of placebo Q2W<br>(2) 4 sets of 8 injections of HGF plasmid 0.5 mg (a total of 4 mg) Q2W<br>(1)(2) The same dose regimen was repeated at Months 3, 9, and 12. | Efficacy<br>Safety |
| | Non-Japanese | US phase II | II | ASO patients | (1) 26<br>(2) 26<br>(3) 25<br>(4) 27 | (1) 3 sets of 8 injections of placebo Q2W<br>(2) 3 sets of 8 injections of HGF plasmid 0.05 mg (a total of 0.4 mg) Q2W<br>(3) 2 sets of 8 injections of HGF plasmid 0.5 mg (a total of 4 mg) Q4W (placebo at Week 2)<br>(4) 3 sets of 8 injections of HGF plasmid 0.5 mg (a total of 4 mg) Q2W | Efficacy<br>Safety |
| | | US second phase II | II | ASO patients | (1) 6<br>(2) 21 | (1) 3 sets of 8 injections of placebo Q2W<br>(2) 3 sets of 8 injections of HGF plasmid 0.5 mg (a total of 4 mg) Q2W | Efficacy<br>Safety |
| | | US phase IIb pilot | II | ASO patients | 10 | 4 sets of 8 injections of HGF plasmid 0.5 mg (a total of 4 mg) Q2W<br>The same dose regimen was repeated at Months 3, 9, and 12. | Efficacy<br>Safety |
| Reference data | Japan | Osaka University clinical research study | I | Patients with ASO or Buerger's disease | (1) 6<br>(2) 8<br>(3) 8 | Stage 1<br>(1) HGF plasmid 0.4 mg followed by 2 sets of 4 injections of HGF plasmid 0.5 mg (a total of 2 mg) Q4W<br>Stage 2<br>(2) 2 sets of 4 injections of HGF plasmid 0.5 mg (a total of 2 mg) Q4W<br>(3) 2 sets of 8 injections of HGF plasmid 0.5 mg (a total of 4 mg) Q4W | Efficacy<br>Safety |
| | Non-Japanese | US IHD phase I | I | Ischemic heart disease | (1) 3<br>(2) 6 | (1) HGF plasmid [redacted] mg was injected in [redacted] sites (a total of 0.4 mg).<br>(2) HGF plasmid [redacted] mg was injected in [redacted] sites (a total of 4 mg). | Efficacy<br>Safety |

a: HGF plasmid was injected into the muscles of the lower limb to be treated above and below the knee in the US phase II study, into the ischemic myocardium in the US IHD phase I study, and into the muscles in the ischemic region of the lower limb to be treated in other studies.

The clinical studies are summarized below.

## 7.1 Japanese clinical studies

### 7.1.1 ASO phase III study (Attached document 5.3.5.1-1.1 to 5.3.5.1-1.5, 5.3.5.1-2.1, and 5.3.5.1-2.2; Study period (trial period, Stage 1, January 2004 to [redacted] 20[redacted]/Stage 2, [redacted] 20[redacted] to [redacted] 20[redacted]; follow-up period, Stage 1, until [redacted] 20[redacted]/Stage 2, until [redacted] 20[redacted])

A placebo-controlled, randomized, double-blind[1)] study was conducted at 57 sites in Japan to evaluate the efficacy and safety of HGF plasmid in ASO patients (Fontaine classification III or IV) with critical limb ischemia who had not responded adequately to standard drug therapy and were poor candidates for revascularization.

The study included a 4-week screening phase, and patients who met the key inclusion and exclusion criteria presented in Table 17 were enrolled in the study at the end of the screening phase. The enrolled patients entered Stage 1 and were randomized using a dynamic allocation procedure stratifying for severity (Fontaine classification) at enrollment, sex, study site, and inpatient/outpatient to receive HGF plasmid or placebo in a double-blind manner. Patients randomized to the placebo group in Stage 1 could choose to enter Stage 2 and receive HGF plasmid.

**Table 17. Key inclusion and exclusion criteria**

| | |
|---|---|
| Key inclusion criteria | • Patients with occluded or stenotic superficial femoral artery, popliteal artery, or infrapopliteal artery in the lower limb to be treated, confirmed by angiography, computed tomography angiography (CTA), or magnetic resonance angiography (MRA), and rest pain (VAS ≥20 mm) or ulcer(s) associated with the occlusion or stenosis<br>• Patients had both of the following hemodynamic indicators in the lower limb to be treated.<br>· Resting ankle brachial pressure index (ABPI) ≤0.6 at all time points during screening phase<br>· Mean ankle pressure <70 mmHg during screening phase<br>• Poor candidates for revascularization (including endovascular intervention) of the lower limb to be treated<br>• No improvement in the symptoms of the lower limb to be treated despite conventional medical treatment or intervention during 4-week screening phase[2)] |
| Key exclusion criteria | • Patients with necrotic ulcers and/or ulcers with tendon or bone exposure in the lower limb to be treated or contralateral lower limb<br>• Patients with evidence or history of malignant neoplasm, or patients had the following test results and the possibility of malignant neoplasm could not be ruled out.<br>· Tumor marker, PSA-ACT ≥5.5 ng/mL<br>· Cervical cytology, Class IV or V<br>· Additional testing other than the above could not exclude the presence of malignant neoplasm.<br>• Patients with proliferative diabetic retinopathy (untreated proliferative retinopathy, mid-/late-stage proliferative retinopathy) or exudative age-related macular degeneration |

The dosage regimen

[Stage 1]

HGF plasmid 0.5 mg per site or placebo (saline) was to be injected into the muscles in the ischemic region of the lower limb to be treated in 8 sites (a total of HGF plasmid 4 mg) Q4W on 2 occasions. HGF plasmid was

1) Treatment effect was evaluated at 12 weeks after the first set of injections of HGF plasmid or placebo, and subjects' treatment allocations were unblinded one-by-one.

2) No improvement was defined as follows: "a reduction in rest pain VAS of <20 mm" and "rises in ABPI and TBPI of <0.1 if measurable" for Fontaine III patients; and "a reduction in the size of the ulcer to be assessed for the primary endpoint √(major axis × minor axis) of <25%" and "rises in ABPI and TBPI of <0.1 if measurable" for Fontaine IV patients.

to be diluted in saline. Injections of 3 mL per site of diluted HGF plasmid were to be delivered, and if the muscle to be injected was small, the injection volume was allowed to be reduced to 2 mL. Injection locations were to be selected after angiographically locating arterial occlusion, and intramuscular injections were to be performed under ultrasound guidance.

[Stage 2]
See the dosage regimen for the HGF plasmid group in Stage 1.

For the treatment of ASO and symptoms associated with ASO, revascularization of the lower limb to be treated or contralateral limb, sympathetic block, sympathectomy, epidural anesthesia, and the use of trafermin were all prohibited. Moreover, from the start of screening phase until the final assessments at 12 weeks after the first set of injections, a drug change or a dose increase [(1) a >1.5-fold and (2) a >1.3-fold increase in the mean daily dose] was not permitted for (1) concomitant medications for ASO and ulcers associated with ASO and (2) analgesics.

The primary endpoint was the improvement rate for rest pain measured by Visual Analogue Scale (VAS) or ulcer size at 12 weeks after the first set of injections of HGF plasmid or placebo (or at withdrawal). Improvement was defined by Fontaine's stage:

- For Fontaine III patients, a reduction from baseline in rest pain (VAS) of ≥20 mm. Note that subjects who required a >1.3-fold increase from screening phase in analgesic use were to be handled as "no improvement."
- For Fontaine IV patients, a reduction in the size of the ulcer to be assessed for the primary endpoint[3] (√[major axis × minor axis]),[4] which was designated at enrollment, to ≤75% of the baseline value.

The applicant determined a target sample size of 120 subjects (80 in the HGF plasmid group, 40 in the placebo group) at the time of initiation of the study, and did not plan an interim analysis before the initiation of the study. However, a very limited number of patients met the eligibility criteria for the study, and when about [redacted] years had passed since the initiation of the study, accrual of the target sample size seemed difficult. Furthermore, based on the results from 11 subjects who had been unblinded at this time point (as of [redacted], 20[redacted]), the applicant considered that the efficacy of HGF plasmid was likely to be much higher than initially predicted, and amended the protocol to perform one interim analysis when 40 subjects in the Full Analysis Set (FAS) were unblinded (version [redacted], as of [redacted], 20[redacted]). The Lan-DeMets method was used to control the type I error rate from the interim analysis. Based on the Lan-DeMets method, a two-sided significance level of 2.00% at the interim analysis (3.49% at final analysis) was chosen.

[3] The largest ulcer that was measurable at Week 4 of screening phase

[4] Ischemic ulcer size was assessed using √(major axis × minor axis), and the major axis of the tissue loss and the orthogonal minor axis were measured using a scale supplied by the sponsor. Even when the shape of the ulcer changed during the trial period, what were originally the major and minor axes measured at the start of screening phase were measured. Ischemic ulcer size was assessed in the same manner also in other Japanese studies.

In this study, 46 subjects (30 in the HGF plasmid group, 16 in the placebo group) were randomized, of whom 44 subjects (29 in the HGF plasmid group, 15 in the placebo group) received study drug. The date the 41st subject (28 in the HGF plasmid group, 13 in the placebo group) completed the final assessments ([redacted], 20[redacted]) was defined as the data cutoff date, and an interim analysis was performed. Three subjects (42nd to 44th subjects) receiving HGF plasmid as of the data cutoff date were excluded from efficacy and safety analyses.

Forty-one subjects who had completed the final assessments at interim analysis (28 in the HGF plasmid group, 13 in the placebo group) were included in the safety analysis population. After excluding 1 subject in the HGF plasmid group who was diagnosed with vasculitis and found not to have the target disease after receiving HGF plasmid, 40 subjects (27 in the HGF plasmid group, 13 in the placebo group) were included in the FAS, and the FAS was used as the efficacy analysis population. Thirty-nine of the 41 subjects completed the 12-week treatment phase. The 3 subjects who were not included in the interim analysis population were unblinded before database lock following the independent data monitoring committee for the interim analysis.

**1) Stage 1**

An interim analysis was performed using the data cutoff date of [redacted], 20[redacted]. The primary efficacy endpoint of the improvement rate for rest pain (VAS) or ulcer size is shown in Table 18. The independent data monitoring committee recommended early termination of the study, and the applicant terminated the study.

**Table 18. Improvement rate for ulcer size or rest pain (VAS) at 12 weeks after the first dose in Stage 1 (FAS, data cutoff date of [redacted], 20[redacted])**

| Severity | Fontaine classification | | | | | |
|---|---|---|---|---|---|---|
| | III + IV | | III | | IV | |
| Endpoint | Ulcer or rest pain | | Rest pain (VAS) | | Ulcer size | |
| Group | HGF plasmid N = 27 | Placebo N = 13 | HGF plasmid N = 16 | Placebo N = 8 | HGF plasmid N = 11 | Placebo N = 5 |
| No. of subjects with improvement (Proportion [%]) | 19 (70.4) | 4 (30.8) | 8 (50.0) | 2 (25.0) | 11 (100) | 2 (40.0) |
| *P*-value | 0.014 [a] | | — | | — | |
| Difference in improvement rate between the placebo and HGF plasmid groups [95% CI] | 39.6 [9.2, 70.0] | | 25.0 [−13.7, 63.7] | | 60.0 [17.1, 100.0] | |

N: Number of subjects analyzed; Improvement was determined based on the results at withdrawal for withdrawal cases.
a: Mantel-Haenszel test stratified by Fontaine classification; level of significance (two-sided) of 2%; The Lan-DeMets method was used to adjust for multiplicity in hypothesis testing.

The results of secondary efficacy endpoints of the change in rest pain (VAS) over time in Fontaine III patients (Figure 1), the changes in rest pain (VAS) in individual Fontaine III patient (Table 19), the changes in the size of the ulcer to be assessed for the primary endpoint (the largest ulcer) and total ulcer size in Fontaine IV patients (Table 20, Figure 2), ABPI (Table 21), toe-brachial pressure index (TBPI), the change in Fontaine's stage, angiogenesis, the incidence of requiring no analgesics, and the incidence of amputation of the treated limb are shown below.

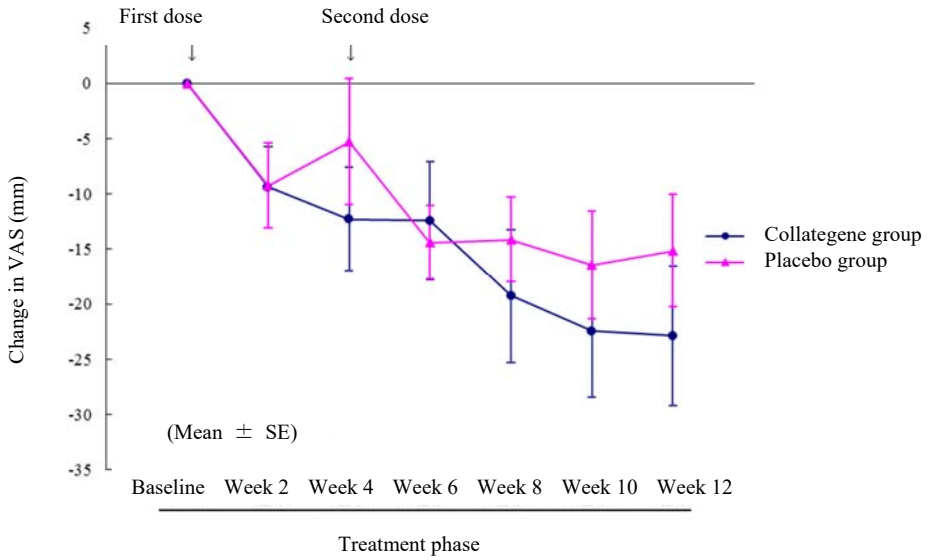


**Figure 1. Change in rest pain (VAS) over time in Fontaine III patients in Stage 1 (FAS)**

**Table 19. Changes in rest pain (VAS, mm) in individual Fontaine III patient in Stage 1 (FAS)**

| | | HGF plasmid N = 16 | | Placebo N = 8 |
|---|---|---|---|---|
| Values of individual subject (Baseline→Last time point) | | 34 → 30 | 72 → 30 | 75 → 66 |
| | | 43 → 0 | 74 [a] → 85 [a] | 77 → 65 |
| | | 48 → 22 | 74 → 70 | 25 → 1 |
| | | 54 → 55 | 77 → 70 | 44 → 5 |
| | | 56 → 4 | 78 → 49 | 64 → 45 |
| | | 63 → 26 | 78 → 61 | 50 → 44 |
| | | 66 → 70 | 82 → 94 | 91 → 93 |
| | | 70 → 10 | 88 → 43 | 85 → 77 |
| Upper row: Mean ± SD<br>Lower row: Median (Min.-Max.) | Baseline | 66.1 ± 15.2<br>71.0 (34-88) | | 63.9 ± 22.6<br>69.5 (25-91) |
| | Last time point | 44.9 ± 28.9<br>46.0 (0-94) | | 49.5 ± 32.8<br>55.0 (1-93) |
| | Change | −21.1 ± 23.6<br>−21.5 (−60 to 12) | | −14.4 ± 12.7<br>−10.5 (−39 to 2) |

a: The subject who underwent a major amputation by Month 15 [see (3) Follow-up survey].

**Table 20. Ulcer size (√[major axis × minor axis]) in Fontaine IV patients in Stage 1 (FAS)**

| Endpoint | | Change in largest ulcer size (mm) | | Change in total ulcer size (mm) | |
|---|---|---|---|---|---|
| Group | | HGF plasmid N = 11 | Placebo N = 5 | HGF plasmid N = 11 | Placebo N = 5 |
| Mean ± SD | Baseline | 9.68 ± 6.36 | 9.30 ± 4.51 | 13.33 ± 10.32 | 9.68 ± 4.12 |
| | 12 weeks after the first dose | 3.33 ± 3.75 | 11.77 ± 11.39 | 4.76 ± 5.88 | 11.77 ± 11.39 |
| | Percent change (%) | −69.53 ± 27.60 | 6.97 ± 77.46 | −71.82 ± 24.58 | −0.78 ± 75.13 |

N: Number of subjects analyzed

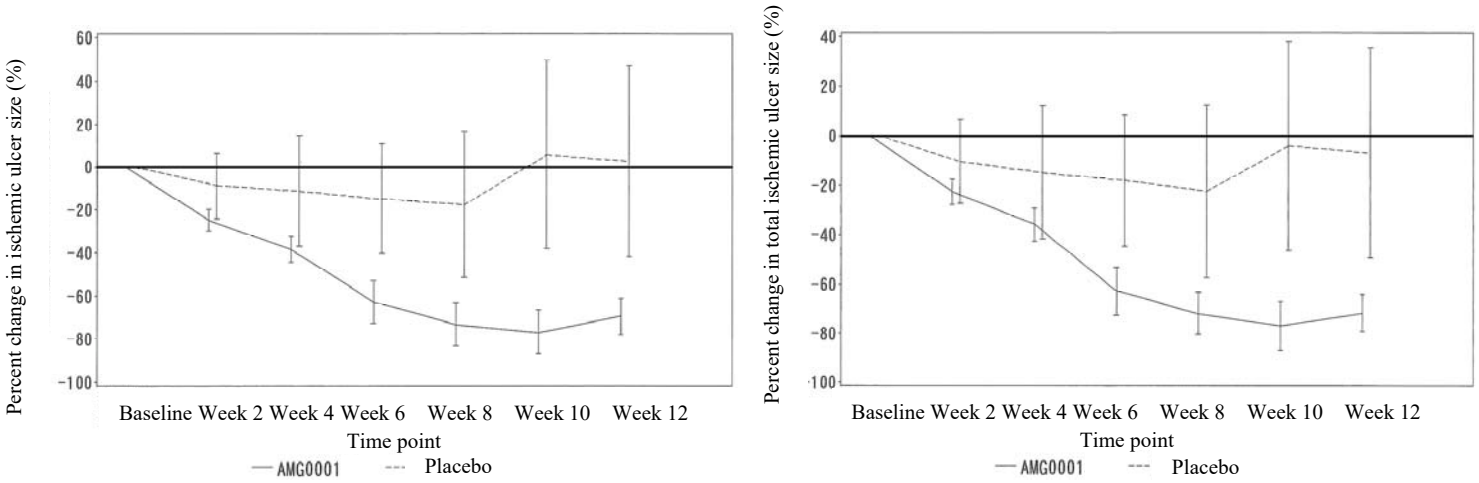


**Figure 2. Percent change in ulcer size over time in Fontaine IV patients in Stage 1**
**(FAS; mean ± standard error [SE]; left figure, largest ulcer; right figure, all ulcers; AMG0001 group = HGF plasmid group)**

**Table 21. Improvement rate for ABPI at 12 weeks after the first set of injections in Stage 1 (FAS)**

| Severity | Fontaine classification | | | | | |
|---|---|---|---|---|---|---|
| | III + IV | | III | | IV | |
| Group | HGF plasmid N = 25 | Placebo N = 12 | HGF plasmid N = 16 | Placebo N = 8 | HGF plasmid N = 9 | Placebo N = 4 |
| No. of subjects with improvement [a] (Proportion [%]) | 11 (44.0) | 6 (50.0) | 7 (43.8) | 3 (37.5) | 4 (44.4) | 3 (75.0) |
| 95% CI for improvement rate | 24.4, 65.1 | 21.1, 78.9 | 19.8, 70.1 | 8.5, 75.5 | 13.7, 78.8 | 19.4, 99.4 |

N: Number of subjects analyzed
a: "Improvement" was defined as a change of ≥0.1, referring to "Classification for limb ischemia (Revised version)"[5)]

5) Takehisa Iwai. Supervisor of translation. Classification for limb ischemia (Revised version) 1998

As to the changes in ulcer size in individual patient, the ulcer size was reduced in all of the 11 subjects in the HGF plasmid group, and complete ulcer closure was observed in 4 of the 11 subjects. In the placebo group, complete ulcer closure was observed in 1 of the 5 subjects, and the ulcer size was reduced in another subject (Table 22, Figure 3). Four subjects in the HGF plasmid group had multiple ulcers, and 6 ulcers of these subjects were not included in the analysis. Among these 6 ulcers, 2 ulcers were almost unchanged, and 4 ulcers were completely closed.

**Table 22. Ulcer sizes (√[major axis × minor axis]) in individual Fontaine IV patients in Stage 1 (FAS)**

| Group | Subject # | Largest ulcer size | | | | Sizes of individual ulcers other than the largest ulcer | | | Total ulcer size | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Baseline (mm) | After treatment (mm) | Percent change (%) | Improvement | Baseline (mm) | After treatment (mm) | Percent change (%) | Baseline (mm) | After treatment (mm) | Percent change (%) |
| HGF plasmid | 1 | 13.1 | 8.9 | −31.6 | Yes | — | — | — | 13.1 | 8.9 | −31.6 |
| | 2 | 12.7 | 9.2 | −27.2 | Yes | 9.8 | 0.0 | −100.0 | 22.5 | 9.2 | −58.9 |
| | 3 [a] | 3.9 | 5.8 | 50.6 | No | — | — | — | 3.9 | 5.8 | 50.6 |
| | 4 | 12.0 | 6.0 | −49.8 | Yes | 8.0 | 0.0 | −100.0 | 20.0 | 6.0 | −69.9 |
| | 5 | 7.5 | 0.0 | −100.0 | Yes | — | — | — | 7.5 | 0.0 | −100.0 |
| | 6 | 3.6 | 1.7 | −53.7 | Yes | — | — | — | 3.6 | 1.7 | −53.7 |
| | 7 | 15.7 | 0.0 | −100.0 | Yes | — | — | — | 15.7 | 0.0 | −100.0 |
| | 8 | 1.4 | 0.0 | −100.0 | Yes | — | — | — | 1.4 | 0.0 | −100.0 |
| | 9 | 5.2 | 1.6 | −70.0 | Yes | — | — | — | 5.2 | 1.6 | −70.0 |
| | 10 [b] | 23.2 | 7.2 | −69.0 | Yes | 9.5 | 11.5 | 21.1 | 36.7 | 18.7 | −49.1 |
| | | | | | | 4.0 | 0.0 | −100.0 | | | |
| | 11 | 5.5 | 2.0 | −63.5 | Yes | 5.5 | 4.2 | −24.4 | 14.4 | 6.2 | −56.7 |
| | | | | | | 3.5 | 0.0 | −100.0 | | | |
| | 12 | 6.6 | 0.0 | −100.0 | Yes | — | — | — | 6.6 | 0.0 | −100.0 |
| Placebo | 1 | 5.0 | 7.0 | 40.9 | No | 1.9 | 0.0 | −100.0 | 6.9 | 7.0 | 2.1 |
| | 2 | 4.4 | 0.0 | −100.0 | Yes | — | — | — | 4.4 | 0.0 | −100.0 |
| | 3 | 14.4 | 29.5 | 104.3 | No | — | — | — | 14.4 | 29.5 | 104.3 |
| | 4 | 12.8 | 15.9 | 23.6 | No | — | — | — | 12.8 | 15.9 | 23.6 |
| | 5 | 9.8 | 6.5 | −33.9 | Yes | — | — | — | 9.8 | 6.5 | −33.9 |

a: The subject who was excluded from the FAS (The subject was diagnosed with vasculitis and was found not to have the target disease after receiving HGF plasmid).
b: The sujbect who underwent a major amputation by Month 15 [see (3) Follow-up survey].

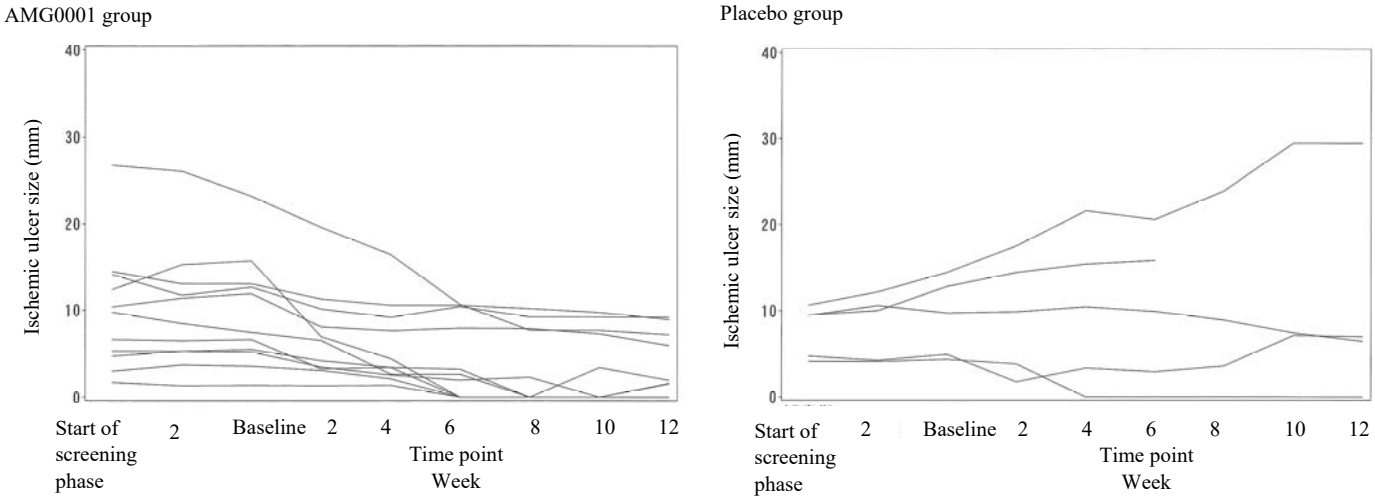


**Figure 3. Changes in ulcer size in individual Fontaine IV patients in Stage 1 (FAS; AMG0001 group = HGF plasmid group)**

TBPI was measured in 8 of the 40 subjects (4/group), and the changes from baseline at 12 weeks after the first dose (mean ± SD) were 0.048 ± 0.123 in the HGF plasmid group and 0.000 ± 0.000 in the placebo group. Only 1 subject in the HGF plasmid group had improvement in TBPI (an increase of ≥0.1 from baseline).

The changes in Fontaine's stage were as follows: HGF plasmid group, 16 stage III subjects and 11 stage IV subjects at baseline and 3 stage IIb subjects, 17 stage III subjects, and 7 stage IV subjects at 12 weeks after the first set of injections (or at withdrawal); placebo group, 8 stage III subjects and 5 stage IV subjects at baseline and 1 stage IIa subject, 1 stage IIb subject, 7 stage III subjects, and 4 stage IV subjects at 12 weeks after the first set of injections (or at withdrawal). No subjects worsened from stage III to stage IV in either group.

Patients who underwent angiography at 8 to 12 weeks after the first set of injections were assessed by their physician for the presence or absence of angiogenesis. Angiogenesis was present in 50.0% (11 of 22 subjects) in the HGF plasmid group and 33.3% (3 of 9 subjects) in the placebo group.

The incidence of requiring no analgesics at 12 weeks after the first set of injections (or at withdrawal) in subjects who were receiving analgesics at the start of treatment phase was 14.3% (2 of 14 subjects) in the HGF plasmid group and 0.0% (0 of 8 subjects) in the placebo group.

As to the incidence of amputation of the treated limb, 1 subject in the placebo group underwent a minor amputation[6)] by 12 weeks after the first set of injections.

6) In the Case Report Form (CRF), amputation was divided into 3 categories: major amputation (above the knee), major amputation (below the knee), and minor amputation.

Regarding safety, the incidences of adverse events during the treatment phase (form the first dose through Week 12) were 96.4% (27 of 28 subjects, 139 events) in the HGF plasmid group and 92.3% (12 of 13 subjects, 57 events) in the placebo group. Adverse events reported by ≥10% of subjects in either group are shown in Table 23.

**Table 23. Adverse events reported by ≥10% of subjects in either group in Stage 1 (Safety analysis population)**

| | n (Incidence [%]) | |
|---|---|---|
| | HGF plasmid<br>N = 28 | Placebo<br>N = 13 |
| Nasopharyngitis | 6 (21.4) | 3 (23.1) |
| CRP increased | 5 (17.9) | 2 (15.4) |
| Gastritis erosive | 4 (14.3) | 1 ( 7.7) |
| Colonic polyp | 4 (14.3) | 0 ( 0.0) |
| Blood pressure increased | 3 (10.7) | 1 ( 7.7) |
| Injection site pain | 3 (10.7) | 1 ( 7.7) |
| Gastritis | 3 (10.7) | 0 ( 0.0) |
| Oedema peripheral | 3 (10.7) | 0 ( 0.0) |
| Contusion | 3 (10.7) | 0 ( 0.0) |

N: Number of subjects analyzed MedDRA/J ver.18.0

Serious adverse events occurred in 8 subjects in the HGF plasmid group (peritonitis; acute renal failure; bladder perforation; bacterial pneumonia; post procedural haematoma; prostate cancer; cerebellar infarction; and peripheral ischaemia, 1 subject each) and 3 subjects in the placebo group (embolism; gangrene; and pain in extremity, 1 subject each). Among the serious adverse events reported in the HGF plasmid group, a causal relationship to HGF plasmid could not be ruled out for 2 cases (bladder perforation; and prostate cancer). The subject with bladder perforation (an 84-year-old woman) also had haemorrhagic cystitis, neurogenic bladder, and chronic cystitis, who presented with chills etc. at 36 days after treatment with HGF plasmid and received the diagnosis of panperitonitis due to bladder wall perforation after work-up. No deaths were reported.

Among the 3 subjects who were not included in the interim analysis population, 1 subject in the HGF plasmid group experienced 3 adverse events, and 1 of the 2 subjects in the placebo group experienced 8 adverse events, but no serious adverse events were reported.

**2) Stage 2**

Among the patients randomized to the placebo group in Stage 1 (15 patients), 10 patients who wished to receive HGF plasmid were treated with the same dosage regimen of HGF plasmid as in Stage 1. All of the 10 subjects who received HGF plasmid were included in efficacy and safety analyses. The same efficacy endpoints as in Stage 1 were selected, and the starting point for calculating changes was at baseline in Stage 2 (within 14 days prior to administration of HGF plasmid). Fontaine's stage at enrollment was IIa (1 subject), IIb (1 subject), III (5 subjects), and IV (3 subjects), and 1 Fontaine IIa (mild intermittent claudication) subject and 1 Fontaine IIb (moderate to severe intermittent claudication) subject were excluded from the efficacy analysis population. The 1 Fontaine IIa subject was discontinued from the study due to an adverse event (congestive cardiac failure) before the second dose of HGF plasmid.

The primary endpoint of the improvement rate for rest pain or ulcer size at 12 weeks after the first dose (or at withdrawal) [95% CI] is shown in Table 24. The results of key secondary efficacy endpoints are shown in Table 25.

**Table 24. Improvement rate for ulcer size or rest pain (VAS) at 12 weeks after the first dose in Stage 2 (Efficacy analysis population)**

| Severity | Fontaine classification | | |
|---|---|---|---|
| | III + IV<br>N = 8 | III<br>N = 5 | IV<br>N = 3 |
| Endpoint | Ulcer or rest pain | Rest pain (VAS) | Ulcer |
| No. of subjects with improvement (Proportion [%]) | 4 (50.0) | 1 (20.0) | 3 (100.0) |
| 95% CI for improvement rate | 15.7, 84.3 | 0.5, 71.6 | 29.2, 100.0 |

N: Number of subjects analyzed

**Table 25. Ulcer size, rest pain, and ABPI in Stage 2 (Efficacy analysis population)**

| Severity | | Fontaine classification | | | |
|---|---|---|---|---|---|
| | | IV | | III | II + III + IV |
| Endpoint | | Ulcer √(major axis × minor axis) (mm)<br>N = 3 | | Rest pain (VAS, mm)<br>N = 5 | ABPI<br>N = 9 |
| | | Largest ulcer size | Total ulcer size | | |
| Mean ± SD | Baseline | 16.76 ± 11.01 | 21.57 ± 13.64 | 59.0 ± 25.2 | 0.372 ± 0.206 |
| | 12 weeks after the first dose | 0.00 ± 0.00 | 4.22 ± 7.30 | 48.2 ± 27.7 | 0.439 ± 0.154 |
| | Percent change (%) | −100.00 ± 0.00 | −86.35 ± 23.65 | — | — |
| | Change | — | — | 10.8 ± 11.7 | 0.067 ± 0.109 |

N: Number of subjects analyzed

As to the changes in ulcer size in individual patients, complete ulcer closure was observed in all 3 subjects (Table 26). In Subject 3 with multiple ulcers, 2 ulcers other than the ulcer to be assessed for the primary endpoint (the largest ulcer) almost unchanged.

**Table 26. Ulcer sizes (√[major axis × minor axis]) in individual Fontaine IV patient in Stage 2 (Efficacy analysis population)**

| Subject # | Largest ulcer size | | | | Sizes of individual ulcers other than the largest ulcer | | | Total ulcer size | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline (mm) | After treatment (mm) | Percent change (%) | Improvement | Baseline (mm) | After treatment (mm) | Percent change (%) | Baseline (mm) | After treatment (mm) | Percent change (%) |
| 1 | 27.9 | 0.0 | −100.0 | Yes | — | — | — | 27.9 | 0.0 | −100.0 |
| 2 | 5.9 | 0.0 | −100.0 | Yes | — | — | — | 5.9 | 0.0 | −100.0 |
| 3 | 16.4 | 0.0 | −100.0 | Yes | 6.3<br>8.1 | 6.3<br>6.3 | 0.0<br>−22.2 | 30.8 | 12.6 | −59.0 |

In 4 subjects with TBPI measurements, the change from baseline to after treatment (mean ± SD) was 0.088 ± 0.176.

As to the change in Fontaine's stage, there were no changes in Fontaine's stage for 2 Fontaine II and 5 Fontaine III subjects, and 2 of 3 Fontaine IV subjects improved to stage III. Angiogenesis was present as assessed by their physician in 1 of 4 subjects who underwent angiography.

Regarding safety, the incidence of adverse events during the treatment phase (from the first dose through Week 12) was 80.0% (8 of 10 subjects, 30 events), and no events were reported by ≥2 subjects. Serious adverse events occurred in 4 subjects (congestive cardiac failure; colonic polyp; cholelithiasis; and gastric cancer, 1 subject each), and a causal relationship to HGF plasmid could not be ruled out for 2 cases (gastric cancer; and colonic polyp). In the subject with gastric cancer (a 76-year-old man), upper gastrointestinal tract endoscopy at Week 12 in Stage 2 revealed a lesion suspected of gastric adenoma, and a biopsy specimen of the lesion was histologically diagnosed as gastric cancer. Although endoscopic photograph at Week 12 in Stage 1 also showed minimal elevation as seen in Stage 2, a biopsy was not performed, and the time of onset of gastric cancer was unknown. In the subject with colonic polyp (a 66-year-old man), lower gastrointestinal tract endoscopy and resection of large intestine polyps were performed at the start of screening phase in Stage 1, at Week 12 in Stage 1, and at Week 12 in Stage 2, and histological examinations of all polyps excluded malignant tumors. The subject who was discontinued from the study due to congestive cardiac failure died 121 days after the first dose.

**3) Follow-up survey**

Twenty-eight subjects in the HGF plasmid group included in the safety analysis population for Stage 1, and 9 of 10 subjects treated with HGF plasmid in Stage 2 (excluding 1 subject who died from congestive cardiac failure during the treatment phase) were followed-up for 15 months after the first dose of HGF plasmid to evaluate the efficacy and safety of HGF plasmid.

Regarding efficacy, among the 37 subjects, 2 subjects in the HGF plasmid group in Stage 1 had amputation, both of whom had a major amputation proximal to the knee joint. One of them had Fontaine III at enrollment and showed no improvement in rest pain, leading to a major amputation 280 days after the first dose of HGF plasmid. The other subject had Fontaine IV at enrollment and showed ulcer reduction during the treatment phase, but was contused in the ulcer site about 4 months after the second dose of HGF plasmid, leading to a major amputation 278 days after the first dose. Ulcer remained resolved at Month 15 in 1 of 2 subjects with Month 15 assessment, among 7 subjects with complete ulcer closure (4 subjects in Stage 1 and 3 subjects in Stage 2).

Regarding safety, serious adverse events occurring during the follow-up period were acute myocardial infarction, angina pectoris, congestive cardiac failure, myocardial infarction, gastric ulcer, acute pancreatitis, gangrene, abscess limb, burns second degree, joint sprain, anorexia, dehydration, gout, malnutrition, loss of consciousness, asthenia, and anaphylactic reaction (1 subject each). During the follow-up period in Stage 1, 1 death occurred (acute myocardial infarction), and its causal relationship to HGF plasmid was denied. Serious adverse events occurring during the follow-up period in Stage 2 were chest pain, multi-organ failure, pancreatic carcinoma, femoral neck fracture, cerebral infarction, pleurisy, pneumothorax, and deep vein thrombosis (1 subject each). During the follow-up period in Stage 2, 2 deaths occurred (multi-organ failure; and pancreatic carcinoma), and a causal relationship to HGF plasmid was denied for 1 case of multi-organ failure. In the 1 subject with pancreatic carcinoma for

which a causal relationship to HGF plasmid could not be ruled out (a 74 year-old woman), CT scans before and after administration of HGF plasmid showed the dilatation of the main pancreatic duct, but not a tumor. About 1 year later, CT scan was performed for scrutiny for thoracic aortic aneurysm, which revealed pancreatic carcinoma, and about 2 months later, the subject died from pancreatic carcinoma.

**4) Long-term follow-up survey**

A total of 35 patients, after excluding 3 patients who died during the follow-up period from 28 patients in the HGF plasmid group included in the safety analysis population for Stage 1 and 10 patients treated with HGF plasmid in Stage 2, were followed-up from Month 15 through Month 36 (a long-term follow-up survey). Among the 35 patients, 26 were alive, 7 died, and 2 were lost to follow-up. Of whom, 1 patient underwent a major amputation and died 8 months later.

**5) Additional long-term follow-up survey**

An additional long-term follow-up survey following the long-term follow-up survey was conducted up to 13 years after the first dose. Among the 26 patients, 6 were alive, 11 died, and 9 were lost to follow-up. Of whom 1 patient underwent a major amputation and died 2 months later.

### 7.1.2 TAO open-label clinical study (Attached document 5.3.5.2-2.1 to 5.3.5.2-2.4; Study period (trial period, May 2004 to [redacted] 20[redacted]; follow-up period, until [redacted] 20[redacted])

An open-label, uncontrolled study was conducted at 8 sites in Japan to evaluate the efficacy and safety of HGF plasmid in Buerger's disease patients with ulcers who had not responded adequately to standard drug therapy.

This study included a 4-week screening phase. At the end of the screening phase, patients who met the key inclusion and exclusion criteria presented in Table 27 were to enter the treatment phase and receive HGF plasmid.

**Table 27. Key inclusion and exclusion criteria**

| | |
|---|---|
| Key inclusion criteria | • Patients with occluded or stenotic superficial femoral artery, popliteal artery, or infrapopliteal artery in the lower limb to be treated, confirmed by angiography, CTA, or MRA, and ulcer(s) associated with the occlusion or stenosis<br>• No improvement in the symptoms of the lower limb to be treated (defined as "a reduction in the size of the ulcer to be assessed for the primary endpoint (√[major axis × minor axis]) of <25%" and "rises in ABPI and TBPI of <0.1 if measurable") despite conventional medical treatment or intervention during screening phase (≥4 weeks) |
| Key exclusion criteria | • Patients with necrotic ulcers and/or ulcers with tendon or bone exposure in the lower limb to be treated or contralateral lower limb<br>• Patients with evidence or history of malignant neoplasm, or patients had the following test results and the possibility of malignant neoplasm could not be ruled out.<br>· Tumor marker, PSA-ACT ≥5.5 ng/mL<br>· Cervical cytology, Class IV or V<br>· Additional testing other than the above could not exclude the presence of malignant neoplasm.<br>• Patients with proliferative diabetic retinopathy (untreated proliferative retinopathy, mid-/late-stage proliferative retinopathy) or exudative age-related macular degeneration |

Dosage regimen

HGF plasmid 0.5 mg per site was to be injected into the muscles in the ischemic region of the lower limb to be treated in 8 sites (a total of 4 mg) Q4W on 2 occasions. At 4 weeks after the second dose of HGF plasmid, patients with a reduction in the size of the ulcer to be assessed for the primary endpoint (√[major axis × minor axis]) of <15% were to receive a third dose of HGF plasmid. HGF plasmid was to be diluted in saline. Injections of 3 mL per site of diluted HGF plasmid were to be delivered, and if the muscle to be injected was small, the injection volume was allowed to be reduced to 2 mL. Injection locations were to be selected after angiographically locating arterial occlusion, and intramuscular injections were to be performed under ultrasound guidance.

For the treatment of Buerger's disease and symptoms associated with Buerger's disease, revascularization of the lower limb to be treated or contralateral limb, sympathetic block, sympathectomy, epidural anesthesia, and the use of trafermin were all prohibited. Subjects did not receive smoking cessation instruction. Moreover, from the start of screening phase until the final assessments at 12 weeks after the first set of injections, a drug change or a dose increase [(1) a >1.5-fold and (2) a >1.3-fold increase in the mean daily dose] was not permitted for (1) concomitant medications for Buerger's disease and ulcers associated with Buerger's disease and (2) analgesics.

The primary endpoint was the improvement rate for ulcer size at Week 12 (or at withdrawal). Improvement was defined as a reduction in ulcer[7)] size (√[major axis × minor axis]) to ≤75% of the baseline value.

At the initiation of the study, the target sample size was 15 subjects. Of 21 patients tentatively enrolled, 10 did not meet the eligibility criteria. Of the 11 enrolled patients, 10 patients excluding 1 patient whose ulcer had almost healed at baseline, received HGF plasmid. Of whom 2 patients received a third dose of HGF plasmid. Ten patients treated with HGF plasmid were included in the safety analysis population, and after excluding 1 patient who was diagnosed with systemic scleroderma and found not to have the target disease at Month 9 during the follow-up period (the patient who received a third dose of HGF plasmid), 9 patients were included in the FAS, which was used as the efficacy analysis population.

The primary endpoint of the improvement rate for the largest ulcer size [95% CI] was 66.7% (6 of 9 subjects) [29.9, 92.5].

The results of secondary endpoints of ulcer size, rest pain, and ABPI are shown in Table 28 and Figure 4.

7) The largest ulcer that was measurable at Week 4 of screening phase

**Table 28. Ulcer size, rest pain, and ABPI (FAS)**

| Endpoint | | Ulcer √(major axis × minor axis) (mm) N = 9 | | Rest pain (VAS, mm) N = 7 [a] | ABPI N = 8 [b] |
|---|---|---|---|---|---|
| | | Largest ulcer size | Total ulcer size | | |
| Mean ± SD | Baseline | 16.31 ± 8.14 | 18.74 ± 7.75 | 59.6 ± 15.0 | 0.581 ± 0.238 |
| | 12 weeks after the first dose | 11.73 ± 18.82 | 12.54 ± 18.40 | 23.3 ± 22.6 | 0.655 ± 0.216 |
| | Percent change (%) | −53.23 ± 65.39 | −51.60 ± 63.79 | — | — |
| | Change | — | — | −36.3 ± 19.8 | 0.074 ± 0.103 |

N: Number of subjects analyzed

a: Two subjects with a baseline value of ≤20 mm were excluded from the analysis.

b: One subject with a history of amputation of the lower limb to be treated at the ankle joint before participation in the study, which made it impossible to measure ABPI, was excluded.

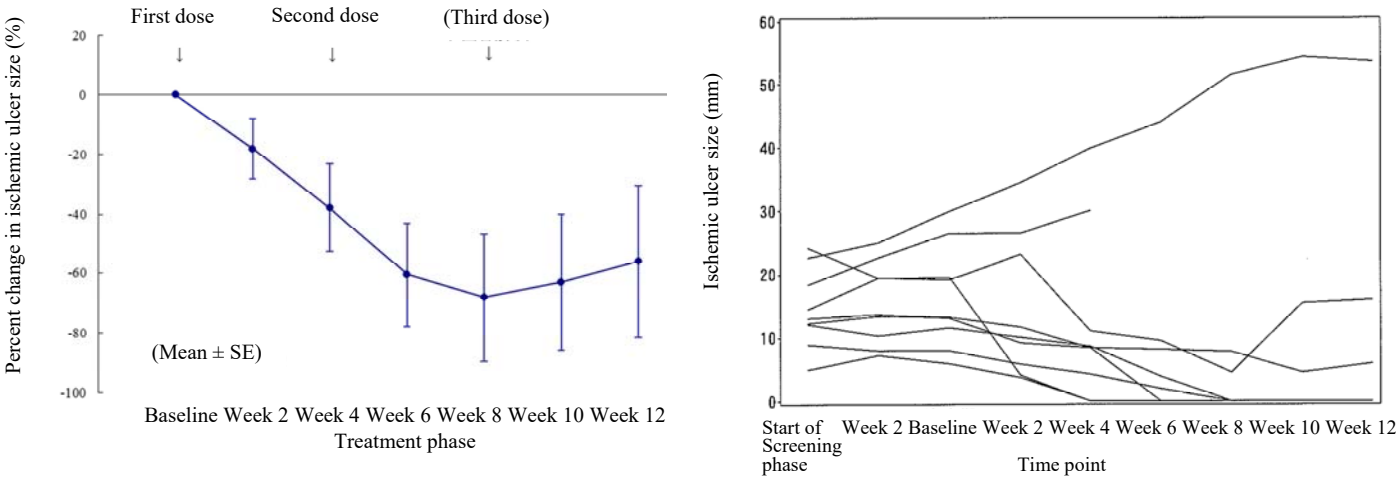


**Figure 4. Percent change in largest ulcer size (left figure) and largest ulcer size over time in individual patients (right figure) (FAS)**

As to the changes in ulcer size in individual patient, 6 of the 9 patients had a reduction in ulcer size to ≤75% of the baseline value and 5 of the 6 patients had complete ulcer closure (Table 29). In 3 subjects with multiple ulcers (Subjects 3, 4, and 10), complete closure or reduction of their ulcers other than those analyzed was observed.

**Table 29. Ulcer sizes (√[major axis × minor axis]) in individual patient (FAS)**

| Subject # | Largest ulcer size | | | | Sizes of individual ulcers other than the largest ulcer | | | Total ulcer size | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline (mm) | After treatment (mm) | Percent change (%) | Improvement | Baseline (mm) | After treatment (mm) | Percent change (%) | Baseline (mm) | After treatment (mm) | Percent change (%) |
| 1 | 19.5 | 0.0 | −100.0 | Yes | — | — | — | 19.5 | 0.0 | −100.0 |
| 2 | 29.9 | 53.7 | 79.2 | No | — | — | — | 29.9 | 53.7 | 79.2 |
| 3 | 13.0 | 5.9 | −54.6 | Yes | 6.9 | 0.0 | −100.0 | 20.0 | 5.9 | −70.4 |
| 4 | 13.2 | 0.0 | −100.0 | Yes | 5.9<br>5.0 | 3.9<br>3.4 | −33.9<br>−32.0 | 24.1 | 7.3 | −69.6 |
| 5 [a] | 28.8 | 32.2 | 11.6 | No | 4.6 | 2.0 | −56.5 | 33.4 | 34.2 | 2.3 |
| 6 | 19.2 | 16.0 | −16.7 | No | — | — | — | 19.2 | 16.0 | −16.7 |
| 7 | 26.5 | 30.0 | 13.1 | No | — | — | — | 26.5 | 30.0 | 13.1 |
| 8 | 5.9 | 0.0 | −100.0 | Yes | — | — | — | 5.9 | 0.0 | −100.0 |
| 9 | 11.5 | 0.0 | −100.0 | Yes | — | — | — | 11.5 | 0.0 | −100.0 |
| 10 | 7.9 | 0.0 | −100.0 | Yes | 4.0 | 0.0 | −100.0 | 11.9 | 0.0 | −100.0 |

a: The subject who was found to have the primary disease of systemic scleroderma and excluded from the FAS

The results of other key secondary endpoints are shown below:
TBPI was measured in 3 subjects (Subjects # 8, 9, and 10), and the changes from baseline at Week 12 were 0.08→0.13, 0.00→0.00, and 0.00→0.00, respectively.

Based on the angiographic findings, 6 subjects were assessed by their physician for the presence or absence of angiogenesis, and angiogenesis was present in none of the subjects.

$TcPO_2$ was measured in 6 subjects, and no significant changes from baseline were seen after treatment.

The incidence of requiring no analgesics at Week 12 (or at withdrawal) in subjects who were receiving analgesics at the start of treatment phase was 12.5% (1 of 8 subjects).

As to the incidence of amputation of the treated limb, 1 subject underwent a minor amputation[8)] by Week 12.

Regarding safety, the incidence of adverse events during the treatment phase (from the first dose through Week 12) was 100% (10 of 10 subjects, 53 events), and adverse events reported by ≥2 subjects were CRP increased (6 subjects), blood triglycerides increased (3 subjects), blood phosphorus decreased (3 subjects), alanine aminotransferase increased (2 subjects), pruritus (2 subjects), and hepatic steatosis (2 subjects). Serious adverse events occurred in 3 subjects (3 events) (infected skin ulcer; osteomyelitis; and oesophageal squamous cell carcinoma, 1 subject each), and a causal relationship to HGF plasmid could not be ruled out for 1 case of oesophageal squamous cell carcinoma (a 49-year-old man). In this subject, upper gastrointestinal tract endoscopy during the screening phase showed oesophagitis, and upper gastrointestinal tract endoscopy after administration of HGF plasmid revealed oesophageal squamous cell carcinoma. No deaths were reported.

8) In the CRF, amputation was divided into 3 categories: major amputation (above the knee), major amputation (below the knee), and minor amputation.

Salivary cotinine testing was conducted to assess smoking status. Salivary cotinine was negative in 4 subjects, positive in 2 subjects, and unknown in 3 subjects at baseline, and negative in 3 subjects, positive in 5 subjects, and unknown in 1 subject at Week 12 (or at withdrawal).

In this study, 8 of the 9 subjects, after excluding 1 subject who was lost to follow-up during the treatment phase, were followed-up from the end of treatment phase until Month 15 to evaluate the efficacy and safety of HGF plasmid.

As to efficacy, 3 of the 8 subjects underwent amputation of the treated lower limb, all of whom had a minor amputation.[7)] Ulcer remained resolved in all 3 subjects with Month 15 assessment, among 5 subjects with complete ulcer closure. Regarding safety, serious adverse events occurring during the follow-up period were peripheral ischaemia (2 subjects); and infected skin ulcer; osteomyelitis; macular pseudohole; and oesophageal squamous cell carcinoma (1 subject each), and no deaths were reported.

Furthermore, a long-term follow-up survey was conducted from Month 15 through Month 36. None of 7 patients surveyed had lower-extremity amputation or died. According to an additional long-term follow-up survey conducted up to 13 years after the first dose following the long-term follow-up survey, among the 7 patients, 4 were alive without major amputation, 1 had a major amputation, and 3 were lost to follow-up, and no deaths were reported.

### 7.1.3 Advanced medical care B clinical research study (Attached document 5.3.5.2-3.1; Study period, September 2014 to August 2017; follow-up period, until [redacted] 20[redacted])

An open-label, uncontrolled clinical research study was conducted as advanced medical care B at 5 sites in Japan to obtain consistent results with those from ASO phase III study and TAO open-label clinical study, with respect to the efficacy and safety of HGF plasmid in Fontaine III or IV ASO or Buerger's disease patients with critical limb ischemia who had not responded adequately to standard drug therapy and were poor candidates for revascularization.

This study included a 2-week screening phase. At the end of the screening phase, patients who met the key inclusion and exclusion criteria presented in Table 30 were to enter the treatment phase and receive HGF plasmid. Patients received smoking cessation instruction before the screening phase and were monitored for smoking status until the end of the screening phase.

**Table 30. Key inclusion and exclusion criteria**

| | |
|---|---|
| Key inclusion criteria | • Patients with occluded or stenotic superficial femoral artery, popliteal artery, or infrapopliteal artery in the lower limb to be treated, confirmed by CTA or MRA<br>• In ASO patients, the mean ankle pressure in the lower limb to be treated is ≤70 mmHg or ankle-branchial index (ABPI) ≤0.6 during screening phase.<br>• Patients with rest pain (VAS during screening phase ≥20 mm) (Fontaine III) or ulcer(s) (Fontaine IV)<br>• Poor candidates for revascularization (including endovascular intervention) of the lower limb to be treated; or revascularization was a possible option, but poor operative risk<br>• No improvement in the symptoms of the lower limb to be treated (improvement was defined as "a reduction in VAS of ≥20 mm" for Fontaine III patients, and "a reduction in the size of the ulcer to be assessed for the primary endpoint [√{major axis × minor axis}] to ≤75%" for Fontaine IV patients) despite conventional medical treatment or intervention during screening phase |
| Key exclusion criteria | • Patients with necrotic ulcers and/or ulcers with tendon or bone exposure in the lower limb to be treated or contralateral lower limb<br>• Patients with evidence or history of malignant tumor, except for fully resolved basal cell carcinoma of the skin.<br>• Patients with untreated or refractory proliferative diabetic retinopathy, or exudative age-related macular degeneration |

Dosage regimen

HGF plasmid 0.5 mg per site was to be injected into the muscles in the ischemic region of the lower limb to be treated in 8 sites (a total of 4 mg) Q4W on 2 occasions. HGF plasmid was to be diluted in saline. Injections of 3 mL per site of diluted HGF plasmid were to be delivered, and if the muscle to be injected was small, the injection volume was allowed to be reduced to 2 mL. Injection locations were to be individualized for each subject and selected for each occlusion site (the region to which the anterior tibial artery, posterior tibial artery, or peroneal artery supplies blood) by locating arterial occlusion or reduced collateral blood flow using CTA or MRA and ultrasonography. If (1) the criteria for improvement in the primary endpoint (improvement was defined as "a reduction in VAS of ≥20 mm" for Fontaine III patients and "a reduction in the size of the ulcer to be assessed for the primary endpoint (√[major axis × minor axis]) to ≤75%" for Fontaine IV patients) were not met at 4 weeks after the second dose of HGF plasmid, (2) the disease condition at baseline was severe and the criteria for improvement were met, but certain symptoms remained, or (3) the criteria for improvement were met, but the symptoms over time were unstable, a third dose of HGF plasmid was to be given at 4 weeks after the second dose.

For the treatment of ASO and Buerger's disease and symptoms associated with either disease, sympathetic block, epidural anesthesia, the use of trafermin, revascularization (including endovascular intervention) of the lower limb to be treated or contralateral limb, amputation, and sympathectomy were all prohibited. Moreover, from the start of screening phase until the final assessments, a drug change or a dose increase [(1) a >1.5-fold and (2) a >1.3-fold increase in the mean daily dose] was not permitted for (1) concomitant medications for ASO and Buerger's disease or symptoms associated with either disease, and (2) analgesics.

The primary endpoint was the improvement rate for rest pain (VAS) or ulcer size at Week 12 or at treatment discontinuation. Improvement was defined as follows:

- For Fontaine III patients, a reduction from baseline in rest pain (VAS) of ≥20 mm. Note that subjects who required a >1.3-fold increase from screening phase in analgesic use were to be handled as "no improvement."

- For Fontaine IV patients, a reduction in the size of the ulcer to be assessed for the primary endpoint[9] (√[major axis × minor axis]), which was designated at enrollment, to ≤75% of the baseline value. Note that subjects with a new ulcer in the ipsilateral lower limb were to be handled as "no improvement."

The target sample size was 6 subjects, and a total of 6 patients (2 ASO patients and 4 Buerger's disease patients) were enrolled. The 4 subjects with Buerger's disease were confirmed to have abstained from smoking. All of 6 subjects treated with HGF plasmid (among the 6 subjects, 2 received 2 sets of injections and 4 received 3 sets of injections) were included in the efficacy analysis population. However, 2 of them (1 subject in whom it was considered difficult to distinguish pain due to an adverse event of ankle sprain from rest pain, 1 subject in whom it was extremely difficult to measure the ulcer size due to crust formation in the ulcer-like skin lesion) were considered unassessable and excluded from the primary endpoint analysis.

The primary efficacy endpoint of the improvement rate for rest pain (VAS) or ulcer size (√[major axis × minor axis]) at Week 12 (or at withdrawal) is shown in Table 31.

**Table 31. Ulcer size, rest pain, and ABPI in the primary endpoint analysis population (Efficacy analysis population)**

| Endpoint | | Primary endpoint | | Secondary endpoint |
|---|---|---|---|---|
| | | Fontaine IV<br>Largest ulcer size<br>√(major axis × minor axis)<br>N = 1 | Fontaine III<br>Rest pain<br>(VAS, mm)<br>N = 3 | ABPI<br>N = 2 [a] |
| No. of subjects with improvement (n) | | 1 | 2 | 1 [b] |
| Improvement rate [95% CI] (%) | | 75.0 [19.4, 99.4] | | 50.0 [1.3, 98.7] |
| Mean ± SD | Baseline | 12.51 | 64.0 ± 21.3 | 0.858 ± 0.338 |
| | 12 weeks after the first dose | 0.00 | 41.4 ± 25.0 | 0.852 ± 0.299 |
| | Change | −12.51 | −25.4 ± 17.7 | −0.006 ± 0.100 |

N: Number of subjects analyzed
a: Only ASO patients were included in the analysis.
b: Improvement was defined as a rise of ≥0.1.

Table 32 shows the changes in the secondary efficacy endpoints of rest pain, ulcer size, ABPI, and Fontaine's stage.

**Table 32. Ulcer size, rest pain, and ABPI in patients treated with HGF plasmid (Efficacy analysis population)**

| Subject # | Target disease | Fontaine classification | Total dose administered (No. of doses) | Baseline→Week 12 | | |
|---|---|---|---|---|---|---|
| | | | | Largest ulcer size √(major axis × minor axis) | Rest pain (VAS, mm) | ABPI |
| 01 | Buerger's disease | III | 8 mg (2) | — | 43→26 | 0.91→0.76 |
| 02 | Buerger's disease | IV | 12 mg (3) | 12.51→0.00 | 77→75 | 1.14→1.08 |
| 03 | Buerger's disease | IV | 12 mg (3) | —[a] | 42→12 | 1.21→1.23 |
| 04 | ASO | III | 12 mg (3) | — | 50→No assessment [b] | 0.6→0.7 |
| 05 | ASO | III | 8 mg (2) | — | 87→37 | 0.43→0.49 |
| 06 | Buerger's disease | III | 12 mg (3) | — | 85→57 | No assessment [c] |

a: The subject was excluded from the efficacy analysis due to crust formation in the ulcer.
b: The subject was excluded from the efficacy analysis due to right ankle sprain at this time point.
c: Unmeasurable due to severe arterial calcification

9) The largest ulcer that was measurable at Week 2 of screening phase.

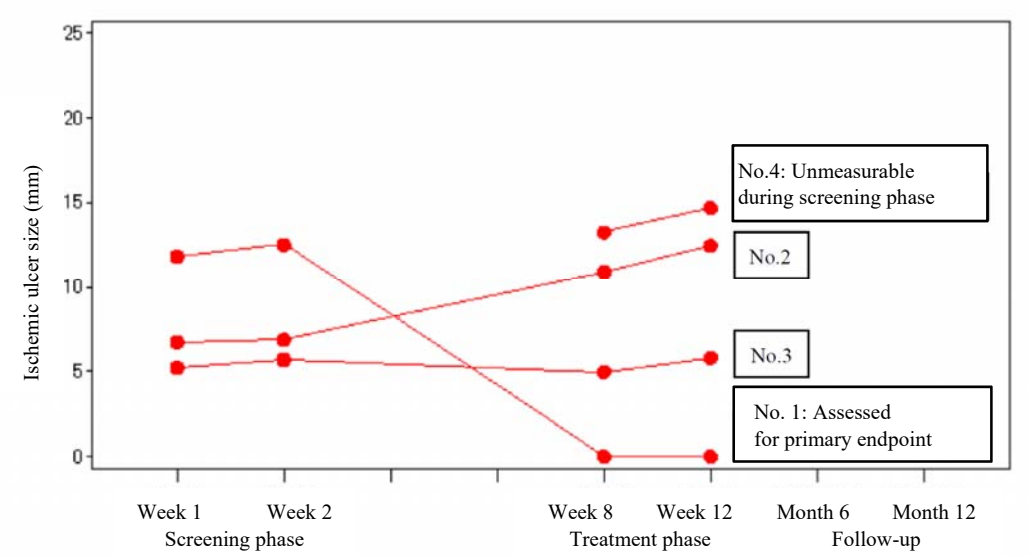


**Figure 5. Ulcer size over time in 1 subject with ulcers**

No.1: The root of the big toe (to be assessed for the primary endpoint)
No.2: The big toe side of the second toe
No.3: The second toe side of the big toe
No.4: The root of the second toe (observed as an unmeasurable ulcer at baseline and observable at Week 8.)

Four subjects were included in the primary endpoint analysis. The improvement rate for rest pain (VAS) at Week 12 (or at withdrawal) in Fontaine III patients was 66.7% (2 of 3 subjects): 100% (1 of 1 subject with ASO) and 50.0% (1 of 2 subjects with Buerger's disease). Only 1 subject with Fontaine IV Buerger's disease was included in the analysis for the improvement rate for ulcer size, and the improvement rate for ulcer size was 100% (1 of 1 subject). In this patient, the ulcer on the root of the big toe (assessed for the primary endpoint) resolved by Week 12, whereas no improvement was observed with other ulcers that were not assesed (the second toe side of the big toe, the big toe side of the second toe, the root of the second toe) (Figure 5).

Only ASO patients were included in the ABPI analysis, and the improvement rate for ABPI was 50.0% (1 of 2 subjects). There were no changes in Fontaine's stage after treatment for all patients.

The incidence of requiring no analgesics at Week 12 (or at withdrawal) in subjects who were receiving analgesics at the start of treatment phase was 50.0% (1 of 2 subjects with Fontaine IV Buerger's disease).

Regarding safety, the incidence of adverse events from the first dose through Week 12 was 83.3% (5 of 6 subjects, 9 events), and a causal relationship to HGF plasmid could not be ruled out for gastric cancer, anaemia, rash, and pain (2 of 6 subjects, 4 events). Gastric cancer reported by 1 subject was classified as a serious adverse event. No deaths were reported.

According to a follow-up survey up to [redacted] 20[redacted], 1 of the 6 patients underwent minor amputation of the right big toe/the tip of the third toe of the treated lower limb at 352 days after the first dose. This patient was the one

who was excluded from the efficacy analysis due to crust formation in the ulcer on the right big toe (assessed for the primary endpoint). The remaining 5 patients including 1 patient who died for causes other than the target disease, had no lower-extremity amputation.

## 7.2. Foreign clinical studies

### 7.2.1 US phase II study (Attached document 5.3.5.1-3.1 to 5.3.5.1-3.5; Study period, April 2003 to [redacted] 20[redacted])

A placebo-controlled, randomized, double-blind study was conducted to assess the safety and angiogenic effects of HGF plasmid and their correlation with the relief of critical limb ischemia symptoms in ASO patients with critical limb ischemia who were poor candidates for revascularization. Patients who met the key inclusion/exclusion criteria presented in Table 33 were enrolled in the study.

**Table 33. Key inclusion and exclusion criteria**

| | |
|---|---|
| Key inclusion criteria | • Patients had either of the following.<br>· Distal lower-extremity pain at rest that required analgesics for >2 weeks<br>· Lower extremity ulceration or gangrene<br>• $TcPO_2$ ≤40 mmHg (At 1 of the 3 predefined sites, the mean of $TcPO_2$ measured on 2 separate occasions during a 30-day screening period before enrollment ≤40 mmHg)<br>• Patients had one or both of the following hemodynamic indicators. If ankle pressure or toe pressure could not be measured appropriately, metatarsal pulse volume recording (PVR) or Doppler waveform was allowed to be used. In this case, PVR or Doppler waveform had to be flat or barely pulsatile.<br>· Ankle systolic pressure ≤70 mmHg<br>· Toe systolic pressure ≤50 mmHg<br>• Poor candidates for standard revascularization treatment options for peripheral arterial disease |
| Key exclusion criteria | • Patients with deep ulcerations with bone or tendon exposure, or clinical evidence of invasive infection (e.g., cellulitis, osteomyelitis) uncontrollable by antibiotics<br>• Patients with evidence or history of malignant neoplasm, except for fully resolved basal cell carcinoma of the skin. Patients who had successful tumor resection or radiochemotherapy of breast cancer more than 10 years before inclusion in the study, with no recurrence, were permitted in the study. Patients who had successful tumor resection or radiochemotherapy of all other tumor types more than 5 years before inclusion in the study, with no recurrence, could be enrolled in the study.<br>• Patients who had proliferative diabetic retinopathy or nonproliferative retinopathy, recent (within 6 months) retinal vein occlusion, macular degeneration with choroidal neovascularization, macular edema, or intraocular surgery within 3 months |

While the clinical study was ongoing, the protocol version [redacted] (as of [redacted], 20[redacted]) was amended to revise some of the inclusion criteria (ankle pressure, ≤60 mmHg → ≤70 mmHg; toe pressure, ≤40 mmHg → ≤50 mmHg, etc.).

The following 3 dose regimens of HGF plasmid were used. In all subjects, HGF plasmid was to be administered via 8 intramuscular injections into the affected limb, 4 above and 4 below the knee joint (predefined injection sites). HGF plasmid was to be diluted in saline, and injections of 2 mL per site of diluted HGF plasmid were to be delivered.

- 0.4 mg × 3 group: HGF plasmid 0.4 mg (0.05 mg × 8 sites) Q2W on 3 occasions
- 4 mg × 2 group: HGF plasmid 4 mg (0.5 mg × 8 sites) Q4W on 2 occasions
- 4 mg × 3 group: HGF plasmid 4 mg (0.5 mg × 8 sites) Q2W on 3 occasions

Hyperbaric oxygen therapy for the treatment of ASO and symptoms associated with ASO was prohibited. In addition, analgesics were not to be changed from the start of screening phase, wherever possible, and any change was to be documented in the CRF. Concomitant use of approved drugs for the treatment of ulcers was permitted.

The primary endpoint was the change in $TcPO_2$ at Month 3 at the initiation of the study, which was changed [redacted] years [redacted] months later to the assessments of all efficacy endpoints ($TcPO_2$, ulcer size, ankle systolic pressure, toe systolic pressure, ABPI, TBPI, revascularization, lower-extremity amputation, rest pain [VAS], and Rutherford classification[10]) at baseline (immediately before the first set of injections), Days 14, 28, and 49, and Months 3 and 6 (Analysis plan Ver.[redacted] as of [redacted], 20[redacted]). A central reader was to calculate the ulcer size by tracing the wound onto a sheet.

The target sample size was 25 subjects per group, a total of 100 subjects. Among 106 randomized subjects, 104 subjects (26 in the placebo group, 26 in the 0.4 mg × 3 group, 25 in the 4 mg × 2 group, 27 in the 4 mg × 3 group) excluding 2 subjects who did not receive study drug were included in the safety analysis population. After excluding 11 subjects (7 subjects who received <3 sets of injections, 2 subjects without critical limb ischemia, 1 subject who had a revascularization procedure 4 days before the second dose, 1 subject who had had below knee amputation before participation in the clinical study) from the safety analysis population, 93 subjects (24 in the placebo group, 25 in the 0.4 mg × 3 group, 21 in the 4 mg × 2 group, 23 in the 4 mg × 3 group) were included in efficacy analyses. There were 13 withdrawals, and the reasons for withdrawals were death (6 subjects), withdrawal of consent (6 subjects), and adverse event (1 subject).

Regarding efficacy, the changes in $TcPO_2$, ulcer size, and rest pain at Month 3 are shown in Table 34.

**Table 34. Changes in key endpoints (value at Month 3 minus baseline value) (Efficacy analysis population)**

| Group | | Placebo<br>N = 24 | 0.4 mg × 3<br>N = 25 | 4 mg × 2<br>N = 21 | 4 mg × 3<br>N = 23 |
|---|---|---|---|---|---|
| Mean ± SD | $TcPO_2$ (mmHg) | 6.1 ± 2.9 | 8.7 ± 4.3 | 5.8 ± 3.2 | 5.7 ± 4.0 |
| | Total ulcer size ($cm^2$) | 0.2 ± 1.1<br>(N = 19) | −1.0 ± 1.4<br>(N = 13) | 10.2 ± 10.8<br>(N = 9) | 0.3 ± 0.7<br>(N = 16) |
| | Rest pain (VAS, cm) | −1.78 ± 0.70 | −1.56 ± 0.63 | −1.00 ± 0.75 | −1.79 ± 0.64 |

N: Number of subjects analyzed
Total ulcer size was measured for subjects with ulcers.

Also as to the changes in ankle pressure, ABPI, toe pressure, and TBPI at Months 3 and 6, there were no significant differences among the treatment groups.

Major lower-extremity amputations occurred in 1 of 24 subjects in the placebo group, 2 of 25 subjects in the 0.4 mg × 3 group, 3 of 21 subjects in the 4 mg × 2 group, and 1 of 23 subjects in the 4 mg × 3 group by Month 6.

[10] Clinical classification of severity of chronic lower-extremity ischemia (resembles Fontaine classification). A 7-point scale from 0, which denotes asymptomatic disease to 6, which denotes major tissue loss.

Seven of 24 subjects in the placebo group, 12 of 25 subjects in the 0.4 mg × 3 group, 10 of 21 subjects in the 4 mg × 2 group, and 6 of 23 subjects in the 4 mg × 3 group experienced an improvement in Rutherford class by Month 6.

Regarding safety, adverse events through Month 12 occurred in 24 of 26 subjects in the placebo group (182 events), 24 of 26 subjects in the 0.4 mg × 3 group (169 events), 24 of 25 subjects in the 4 mg × 2 group (215 events), and 25 of 27 subjects in the 4 mg × 3 group (213 events). Adverse events reported by ≥20% of subjects in any group are shown in Table 35.

**Table 35. Adverse events reported by ≥20% of subjects in any group (Safety analysis population)**

| | n (Incidence [%]) | | | |
|---|---|---|---|---|
| | Placebo N = 26 | 0.4 mg × 3 N = 26 | 4 mg × 2 N = 25 | 4 mg × 3 N = 27 |
| Skin ulcer | 3 (11.5) | 6 (23.1) | 6 (24.0) | 7 (25.9) |
| Oedema peripheral | 6 (23.1) | 5 (19.2) | 5 (20.0) | 5 (18.5) |
| Pain in extremity | 0 ( 0.0) | 4 (15.4) | 6 (24.0) | 7 (25.9) |
| Peripheral ischaemia | 4 (15.4) | 2 ( 7.7) | 7 (28.0) | 6 (22.2) |
| Nausea | 3 (11.5) | 1 ( 3.8) | 6 (24.0) | 0 ( 0.0) |
| Diarrhoea | 2 ( 7.7) | 1 ( 3.8) | 5 (20.0) | 1 ( 3.7) |
| Anaemia | 1 ( 3.8) | 5 (19.2) | 3 (12.0) | 7 (25.9) |

N: Number of subjects analyzed; MedDRA/J ver.18.0

Serious adverse events occurred in 62 subjects (130 events) (15 of 26 subjects in the placebo group [32 events], 16 of 26 subjects in the 0.4 mg × 3 group [33 events], 12 of 25 subjects in the 4 mg × 2 group [26 events], 19 of 27 subjects in the 4 mg × 3 group [39 events]), and a causal relationship to HGF plasmid could not be ruled out for 2 cases of colon cancer (1 subject in the placebo group, 1 subject in the 4 mg × 2 group). Two subjects in the placebo group (adenocarcinoma; and cardiac disorder), 2 subjects in the 0.4 mg × 3 group (myocardial infarction; and small intestinal obstruction), 1 subject in the 4 mg × 2 group (respiratory failure), and 2 subjects in the 4 mg × 3 group (cerebrovascular accident; and gun shot wound) died, and a causal relationship to HGF plasmid was denied for all those cases.

According to an additional follow-up survey conducted up to 13 years after the first dose, among 78 patients surveyed, 3 were alive without a major amputation, 15 had a major amputation, 19 died, and 56 were lost to follow up.

### 7.2.2 US second phase II study (Attached document 5.3.5.1-4.1 to 5.3.5.1-4.3; Study period, August 2005 to [redacted] 20[redacted])

A placebo-controlled, randomized, double-blind study was conducted to assess the efficacy and safety of HGF plasmid in ASO patients with critical limb ischemia who were poor candidates for revascularization. Patients who met the key inclusion/exclusion criteria presented in Table 36 were enrolled in the study.

**Table 36. Key inclusion and exclusion criteria**

| | |
|---|---|
| Key inclusion criteria | • Patients had appropriately sized ulcer(s) on the lower limb to be treated.<br>• Patients had one or both of the following hemodynamic indicators.<br>· Ankle systolic pressure ≤70 mmHg<br>· Toe systolic pressure ≤50 mmHg<br>• Poor candidates for standard revascularization treatment options for peripheral arterial disease |
| Key exclusion criteria | • Patients with deep ulcerations with bone or tendon exposure, or clinical evidence of invasive infection (e.g., cellulitis, osteomyelitis) uncontrollable by antibiotics<br>• Patients with evidence or history of malignant neoplasm, except for fully resolved basal cell carcinoma of the skin. Patients who underwent successful tumor resection or radio-chemotherapy of breast cancer more than 10 years prior to inclusion in the study, with no recurrence, could be enrolled in the study. Patients who had successful tumor resection or radio-chemotherapy of all other tumor types more than 5 years prior to inclusion in the study, with no recurrence, could be enrolled in the study.<br>• Patients who had proliferative diabetic retinopathy or nonproliferative retinopathy, recent (within 6 months) retinal vein occlusion, macular degeneration with choroidal neovascularization, macular edema, or intraocular surgery within 3 months |

The dosage regimen

HGF plasmid 0.5 mg per site or placebo was to be injected into the muscles in the ischemic region of the lower limb to be treated in 8 sites (a total of HGF plasmid 4 mg) Q2W on 3 occasions. HGF plasmid was to be diluted in saline. Injections of 3 mL per site of diluted HGF plasmid or placebo were to be delivered. Injection locations were individualized for each subject based on the ischemic state of the limb.

Hyperbaric oxygen therapy for the treatment of ASO and symptoms associated with ASO was prohibited. In addition, analgesics were not to be changed from the start of screening phase, wherever possible, and any change was to be documented in the CRF. Concomitant use of approved drugs for the treatment of ulcers was permitted.

In this study, a blinded interim analysis was performed after the 21st patient completed Month 3 assessments, which revealed that many patients unsuitable for efficacy evaluation of HGF plasmid, e.g. patients who had ulcers with necrotic tissue, had been enrolled in the study. Thus, potential difficulty in achieving the purpose of the study was predicted, and a decision to terminate the clinical study was made as of [redacted], 20[redacted].

In this study, all of 27 randomized subjects (21 in the HGF plasmid group, 6 in the placebo group) received HGF plasmid or placebo and were included in safety analyses. After excluding 3 subjects who did not receive all 3 doses of HGF plasmid (1 death, 2 subjects who had amputation of the treated limb), 24 subjects (18 in the HGF plasmid group, 6 in the placebo group) were included in the modified intent to treat (MITT) population, which was used for efficacy analyses. There were 8 withdrawals (7 in the HGF plasmid group, 1 in the placebo group), and the reasons for withdrawals were death (4 subjects), major amputation of the treated limb (1 subject), major contralateral amputation (1 subject), multi-organ failure (1 subject), and the deterioration of the primary disease (1 subject).

The changes in the efficacy endpoints are shown in Table 37. There were no significant differences between the HGF plasmid and placebo groups for any of the endpoints at Month 3 or 6.

**Table 37. Changes in key endpoints (value at each time point minus baseline value) (MITT population)**

| Group | | Month 3 | | Month 6 (Month 12 for ischemic ulcers only) | |
|---|---|---|---|---|---|
| | | HGF plasmid N = 18 | Placebo N = 6 | HGF plasmid N = 18 | Placebo N = 6 |
| Mean ± SE | Largest ulcer size ($cm^2$) | 0.456 ± 0.6974 | −0.875 ± 0.5543 | −0.074 ± 0.7957 | −3.667 ± 3.0705 |
| | Total ulcer size ($cm^2$) | 9.956 ± 6.2594 | −0.458 ± 1.2997 | 10.559 ± 6.4438 | −3.458 ± 3.3705 |
| | Rest pain (cm) | −0.85 ± 0.770 | −0.38 ± 0.902 | −1.04 ± 1.214 | −0.18 ± 0.816 |
| | ABPI | −0.008 ± 0.0402 | 0.010 ± 0.0643 | −0.036 ± 0.0570 | −0.116 ± 0.1429 |
| | TBPI | 0.035 ± 0.0361 | −0.114 ± 0.0273 | 0.046 ± 0.0435 | −0.142 ± 0.0398 |

N: Number of subjects analyzed

Lower-extremity amputations occurred in 4 of 18 subjects in the HGF plasmid group, but not in the placebo group.

Among the 27 HGF plasmid- or placebo-treated subjects, 33% (2 of 6) of subjects in the placebo group and 4.8% (1 of 21) of subjects in the HGF plasmid group underwent a revascularization procedure by Month 12.

Regarding safety, adverse events through Month 12 occurred in 20 of 21 subjects in the HGF plasmid group (249 events) and 5 of 6 subjects in the placebo group (63 events). Adverse events reported by ≥20% of subjects in either group are shown in Table 38.

**Table 38. Adverse events reported by ≥20% of subjects in either group (Safety analysis population)**

| | n (Incidence [%]) | |
|---|---|---|
| | HGF plasmid N = 21 | Placebo N = 6 |
| Peripheral arterial occlusive disease | 11 (52.4) | 2 (33.3) |
| Ischaemic ulcer | 8 (38.1) | 1 (16.7) |
| Anaemia | 7 (33.3) | 3 (50.0) |
| Constipation | 5 (23.8) | 1 (16.7) |
| Osteomyelitis | 5 (23.8) | 0 ( 0.0) |
| Pyrexia | 5 (23.8) | 1 (16.7) |
| Confusional state | 3 (14.3) | 2 (33.3) |
| Cellulitis | 2 ( 9.5) | 3 (50.0) |
| Renal impairment | 0 ( 0.0) | 2 (33.3) |

N: Number of subjects analyzed; MedDRA/J ver.18.0

Serious adverse events occurred in 17 of 21 subjects in the HGF plasmid group (37 events) and 3 of 6 subjects in the placebo group (13 events), but a causal relationship to HGF plasmid was denied for all those events. Four subjects in the HGF plasmid group (death from natural causes; multi-organ failure; sepsis; aortic thromboembolism) and 1 subject in the placebo group (cerebrovascular accident) died.

According to an additional follow-up survey conducted up to 13 years after the first dose, among 21 patients surveyed, no patients were confirmed to be alive, 7 had a major amputation, 9 died, and 12 were lost to follow up.

### 7.2.3 US phase IIb pilot study (Attached document 5.3.5.2-4.1; Study period, February 2014 to [redacted] 20[redacted])

An open-label, uncontrolled study was conducted to evaluate the safety and feasibility of a modified dosage regimen of HGF plasmid, which was chosen through discussion with FDA, in ASO patients with critical limb ischemia who were poor candidates for revascularization. Patients who met the key inclusion/exclusion criteria presented in Table 39 were enrolled in the study.

**Table 39. Key inclusion and exclusion criteria**

| | |
|---|---|
| Key inclusion criteria | • Patients were required to meet either of the following:<br>· Rutherford 4 patients with distal lower-extremity pain at rest that required analgesics<br>· Rutherford 5 patients with lower-extremity ulcer ≤20 $cm^2$ (≤10 $cm^2$ if on the heel) or gangrene<br>• Patients had the following hemodynamic indicators. If ankle pressure or toe pressure could not be measured appropriately, metatarsal PVR or Doppler waveform was allowed to be used. In this case, PVR or Doppler waveform had to be flat or barely pulsatile.<br>· Rutherford 4 patients: ankle pressure ≤50 mmHg or toe pressure ≤30 mmHg<br>· Rutherford 5 patients: ankle pressure ≤70 mmHg or toe pressure ≤50 mmHg<br>• Poor candidates for standard revascularization treatment options for peripheral arterial disease |
| key exclusion criteria | • Patients with deep ulcerations with bone or tendon exposure, an ulcer >20 $cm^2$ (>10 $cm^2$ if on the heel), or clinical evidence of invasive infection (e.g., cellulitis, osteomyelitis) uncontrollable by antibiotics<br>• Patients with proliferative diabetic retinopathy or non-proliferative retinopathy, macular oedema, or previous panretinal photocoagulation therapy |

<u>The dosage regimen</u>

HGF plasmid 0.5 mg per site was to be injected into the muscles in the ischemic region of the lower limb to be treated in 8 sites (a total of 4 mg) Q2W on 4 occasions. This dose regimen was to be repeated at Months 3, 9, and 12 (up to 16 sets of injections). HGF plasmid was to be diluted in saline. Injections of 3 mL per site of diluted HGF plasmid were to be delivered.

Hyperbaric oxygen therapy for the treatment of ASO and symptoms associated with ASO was prohibited. In addition, analgesics were not to be changed from the start of screening phase, wherever possible, and any change was to be documented in the CRF. Concomitant use of approved drugs for the treatment of ulcers was permitted.

In light of the nature of this study, no primary efficacy endpoint was defined, and ulcer size, rest pain (VAS), VascuQOL questionnaire score, lower-extremity amputation, and revascularization were to be assessed. Assessments occurred at Months 3, 6, 9, 12, and 18. The largest ulcer was to be assessed. For the accurate measurement of ulcer size, necrotic tissue, crusts, and calluses were to be removed before assessment.

The target sample size was 10 subjects. Ten subjects (5 Rutherford 4 subjects, 5 Rutherford 5 subjects) were enrolled within [redacted] years after the initiation of the study, all of whom were treated with HGF plasmid and included in the efficacy and safety analysis populations. Of the 10 subjects, 3 completed all 16 sets of injections, and 5 completed 18-month visit.

Regarding efficacy, the number of subjects with improvement of the largest ulcer or rest pain is shown in Table 40. The ulcer size was reduced by ≥20% in 1 of 4 Rutherford 5 subjects who completed Month 6 assessments, and none had complete ulcer closure. VAS was reduced from baseline by ≥20 mm in 1 of 8 Rutherford 4 or 5 subjects who completed Month 6 assessments. The change from baseline in VascuQOL questionnaire score was not significant at any time point.

**Table 40. Number of subjects with improvement of the largest ulcer or rest pain of the treated limb (Efficacy analysis population)**

| Time point (after the first set of injections) | Largest ulcer | | | Rest pain (VAS) | |
|---|---|---|---|---|---|
| | N | No. of subjects with complete ulcer closure | No. of subjects with a reduction from baseline [a] in ulcer size of ≥20% [b] | N | No. of subjects with a reduction from baseline [a] in VAS of ≥20 mm |
| Months 0-6 | 4 | 0 | 1 | 8 | 1 |
| Months 6-12 | 1 | 0 | 0 | 6 | 2 |
| Months 12-18 | 0 | 0 | 0 | 4 | 2 |

N: Number of subjects analyzed
a: Baseline value was defined as the data closest to and before the first set of injections of HGF plasmid.
b: If data were available, the area of reduction was calculated based on the ulcer area estimated from the diameter of the largest ulcer.

By Month 18, 20% (2 of 10) of subjects had a major lower-extremity amputation, and 30% (3 of 10) of subjects had a revascularization procedure.

Regarding safety, adverse events through Month 18 occurred in all subjects (10 of 10 subjects), and those reported by ≥3 subjects were administration site reaction (9 subjects), abrasion (4 subjects), pneumonia (4 subjects), and contusion (3 subjects). Adverse events for which a causal relationship to HGF plasmid could not be ruled out occurred in 5 of 10 subjects. Among those events, peripheral ischaemia (1 subject) and uterine leiomyosarcoma (1 subject) were classified as serious adverse events, and 1 subject with cardiac arrest, 1 subject with intestinal ischaemia, and 1 subject with cardiac failure and pneumonia had a fatal outcome.

As to long-term lower-extremity amputation and survival outcome, the lower-extremity amputation-free survival at 18 months was estimated at 0.57 [95% CI, 0.22, 0.81].

### 7.2.4 Global phase III study (Attached document 5.3.5.1-5.1 and 5.3.5.1-5.2; Study period, November 2014 to [redacted] 20[redacted])

A global, placebo-controlled, randomized, double-blind study was conducted to evaluate the efficacy and safety of the same dosage regimen of HGF plasmid as in the US phase IIb pilot study in ASO patients with critical limb ischemia who were poor candidates for revascularization. Patients who met the same key inclusion/exclusion criteria as in the US phase IIb pilot study (Table 39) were enrolled in the study.

The dosage regimen

HGF plasmid 0.5 mg per site or placebo was to be injected into the muscles in the ischemic region of the lower limb to be treated in 8 sites (a total of HGF plasmid 4 mg) Q2W on 4 occasions. This dose regimen was to be repeated at Months 3, 9, and 12 (up to 16 sets of injections). HGF plasmid was to be diluted in saline. Injections of 3 mL per site of diluted HGF plasmid were to be delivered.

Hyperbaric oxygen therapy for the treatment of ASO and symptoms associated with ASO was prohibited. In addition, analgesics were not to be changed from the start of screening phase, wherever possible, and any change was to be documented in the CRF. Concomitant use of approved drugs for the treatment of ulcers was permitted.

The primary endpoint was the time to major amputation of the lower limb to be treated or all-cause death.

Although the target sample size for this study was 500 subjects (250 in the HGF plasmid group, 250 in the placebo group), patient enrollment was slow, and when [redacted] had passed since the initiation of the study, it was considered difficult to accrue the target sample size. Thus, the study was terminated in [redacted] 20[redacted]. A total of 46 subjects (23 HGF plasmid-treated subjects in the HGF plasmid group [12 Rutherford 4 subjects and 11 Rutherford 5 subjects] and 23 subjects in the placebo group [10 Rutherford 4 subjects, 13 Rutherford 5 subjects]) were included in safety and efficacy analyses.

Since the number of patients enrolled was well below the target sample size, the efficacy endpoints were assessed in an exploratory manner. The percentages of subjects who had undergone a major amputation of the lower limb to be treated or had died at Month 6 were 21.7% (5 of 23 subjects) in the HGF plasmid group and 34.8% (8 of 23 subjects) in the placebo group. The median times to lower-extremity amputation or all-cause death were 505 days in the HGF plasmid group and 381 days in the placebo group.

The percentage of subjects who had undergone revascularization of the lower limb to be treated, and the incidences of myocardial infarction and stroke at Month 6 were 13.0% (3 of 23 subjects), 4.3% (1 of 23 subjects), and 0% (0 of 23 subjects), respectively, in the HGF plasmid group and 8.7% (2 of 23 subjects), 0% (0 of 23 subjects), and 4.3% (1 of 23 subjects), respectively, in the placebo group.

Improvement of the largest ulcer or rest pain is shown in Table 41.

**Table 41. Number of subjects with improvement of the largest ulcer or rest pain of the lower limb to be treated (Efficacy analysis population)**

| Time point (after the first set of injections) | HGF plasmid | | | | Placebo | | | |
|---|---|---|---|---|---|---|---|---|
| | Largest ulcer | | Rest pain (VAS) | | Largest ulcer | | Rest pain (VAS) | |
| | N | No. of subjects with complete ulcer closure | N | No. of subjects with a reduction from baseline of ≥20 mm | N | No. of subjects with complete ulcer closure | N | No. of subjects with a reduction from baseline of ≥20 mm |
| Months 0-6 | 11 | 2 | 23 | 4 | 13 | 1 | 23 | 6 |
| Months 6-12 | | 3 | | 9 | | 1 | | 6 |
| Months 12-18 | | 3 | | 9 | | 1 | | 6 |

N: Number of subjects analyzed

Regarding safety, the incidences of adverse events through Month 18 were 73.9% (17 of 23 subjects) in the HGF plasmid group and 78.3% (18 of 23 subjects) in the placebo group. Adverse events reported by ≥10% of subjects in either group are shown in Table 42.

**Table 42. Adverse events reported by ≥10% of subjects in either group**

| | n (Incidence [%]) | |
|---|---|---|
| | HGF plasmid N = 23 | Placebo N = 23 |
| Urinary tract infection | 3 (13.0) | 1 ( 4.3) |
| Nausea | 0 ( 0.0) | 3 (13.0) |

N: Number of subjects analyzed; MedDRA/J ver.19.0

The incidences of serious adverse events were 30.4% (7 of 23 subjects) in the HGF plasmid group and 9% (9 of 23 subjects) in the placebo group. There were no serious adverse events causally related to HGF plasmid. A total of 6 subjects (3 in the HGF plasmid group and 3 in the placebo group) died, and the causes of death were respiratory failure, multi-organ failure, and non-small cell lung cancer in the HGF plasmid group and cardiac arrest, ventricular tachycardia, and stroke in the placebo group, and a causal relationship to study product was denied for all those cases.

## 7.3 Reference data

### 7.3.1 Osaka University clinical research study (Attached document 5.3.5.2-1.1 to 5.3.5.2-1.3; Study period, May 2001 to [redacted] 20[redacted]; follow-up period, until [redacted] 20[redacted])

An open-label, uncontrolled clinical research study was conducted at a single center to evaluate the safety, efficacy, and effective dose of HGF plasmid in ASO or Buerger's disease patients with critical limb ischemia who had not responded adequately to standard drug therapy and were poor candidates for revascularization. Patients who met the key inclusion/exclusion criteria presented in Table 43 were enrolled in the study.

**Table 43. Key inclusion and exclusion criteria**

| | |
|---|---|
| Key inclusion criteria | • ASO or Buerger's disease patients with markedly impaired QOL who were not candidates for revascularization, had been resistant to conventional medical treatments, and might require an amputation in future<br>• Patients with the following clinical symptoms:<br>Stage 1: Patients with rest pain or ulcers/necrosis<br>Stage 2: Patients with severe claudication and patients with rest pain or ulcers/necrosis |
| Key exclusion criteria | • Patients with severe diabetic retinopathy (untreated proliferative retinopathy, mid-/late-stage proliferative retinopathy) |

<u>The dosage regimen</u>

<u>Stage 1</u>

Two weeks after a preliminary intramuscular injection of 0.4 mg of HGF plasmid, HGF plasmid 0.5 mg per site was to be injected into the muscles in the ischemic region of the lower limb to be treated in 4 sites (a total of 2 mg) Q4W on 2 occasions. A total of 2.0 mg of HGF plasmid was to be administered via 4 intramuscular injections, 2 above and 2 below the knee, in patients with poor limb circulation caused by a distal occlusion of the superficial femoral artery, etc., and a total of 2.0 mg of HGF plasmid was to be injected into the muscles of the affected limb in 4 sites below the knee, in patients with a more distal occlusion. In the case of atrophy

of the muscle to be injected or stenotic vessels at different sites, injection locations were to be selected according to the patient's disease condition. Safety in all patients in Stage 1 was to be evaluated by the indication/evaluation subcommittee, and if the subcommittee concluded that there were no problems, the study would proceed to Stage 2 after the approval by the gene therapy clinical research review board.

Stage 2

HGF plasmid 0.5 mg per site was to be injected into the muscles in the ischemic region of the lower limb to be treated in 4 sites (a total of 2 mg) or in 8 sites (a total of 4 mg) Q4W on 2 occasions.

A total of 6 patients (3 patients with ASO and 3 patients with Buerger's disease) were enrolled in Stage 1. In Stage 2, a total of 8 patients (5 patients with ASO and 3 patients with Buerger's disease) were enrolled in the 2 mg group and a total of 8 patients (6 patients with ASO and 2 patients with Buerger's disease) were enrolled in the 4 mg group. Both lower limbs were treated in a total of 5 patients in stage 1 and stage 2 of this study, and each limb was counted as 1 case. In either stage, the treatment phase was from the first dose of HGF plasmid (excluding a preliminary injection) until Week 12, and a follow-up survey was conducted up to Month 27. No primary efficacy endpoint was defined.

The improvement rates for ulcer size or rest pain in Stage 1 and Stage 2 are shown in Table 44.

**Table 44. Improvement rates for ulcer size (the major axis of the largest ulcer) or rest pain (VAS)**

| Endpoint | Ulcer (the major axis of the largest ulcer) [a] | | | Rest pain (VAS) [b] | | |
|---|---|---|---|---|---|---|
| Group | Stage 1 | Stage 2 | | Stage 1 | Stage 2 | |
| | N = 4 [c] | 2 mg N = 4 [d] | 4 mg N = 3 [d] | N = 6 | 2 mg N = 4 [e] | 4 mg N = 3 [e] |
| No. of subjects with improvement (%) | 3 (75.0) | 2 (50.0) | 2 (66.7) | 4 (66.7) | 3 (75.0) | 1 (33.3) |
| 95% CI for improvement rate | 19.4, 99.4 | 6.8, 93.2 | 9.4, 99.2 | 22.3, 95.7 | 19.4, 99.4 | 0.8, 90.6 |

N: Number of subjects analyzed
Both limbs were treated in 5 patients in Stage 2, and each limb was counted as 1 case.
a: "Improvement" was defined as a reduction in the size of the largest ulcer to ≤75% of the baseline value.
b: "Improvement" was defined as a reduction from baseline in VAS of ≥2 cm.
c: Among 6 patients, 4 patients with ulcers were included in the analysis.
d: Four of 8 patients in the 2 mg group and 3 of 8 patients in the 4 mg group had ulcers.
e: Rest pain (VAS) could be measured in 4 of 8 patients in the 2 mg group and 3 of 8 patients in the 4 mg group.

Regarding safety, during the treatment phase in Stage 1, the incidence of adverse events was 100% (6 of 6 patients, 90 events). As a serious adverse event, cerebral infarction occurred in 1 patient 3 weeks after the second dose, and its causal relationship to HGF plasmid was denied. During the treatment phase in Stage 2, the incidences of adverse events were 100% (8 of 8 limbs, 125 events) in the 2 mg group and 100% (8 of 8 limbs, 144 events) in the 4 mg group. Two serious adverse events (pneumonia and cardiac failure; these events were counted twice, but occurred in the same patient who was treated bilaterally at the same time) were reported, and a causal relationship to HGF plasmid was denied for both events. No deaths were reported in Stage 1 or Stage 2.

During a follow-up survey through Month 25 following the treatment phase, among the 22 patients, 1 patient in Stage 1 and 1 patient in Stage 2 died from hyperkalaemia-related arrhythmia and pneumonia, respectively.

According to an additional follow-up survey conducted from 2 years through ≥10 years after the initiation of treatment, among 20 patients surveyed, 1 had a major amputation, 2 died, and 9 were lost to follow-up.

### 7.3.2 US IHD phase I study (Attached document 5.3.5.4-1.1 to 5.3.5.4-1.3; Study period, November 2004 to [redacted])

An open-label, uncontrolled study was conducted to assess the safety and tolerability of HGF plasmid in patients with ischemic heart disease not amenable to coronary artery bypass grafting or percutaneous coronary intervention.

The dosage regimen

*Stage 1*

HGF plasmid [redacted] mg per site was to be administered via intramyocardial injection catheter into the ischemic region of [redacted] in [redacted] sites (a total of 0.4 mg). The study was to proceed to Stage 2 after the safety evaluation committee concluded that no patients in Stage 1 had a safety problem.

*Stage 2*

HGF plasmid [redacted] mg per site in [redacted] sites (a total of 4 mg) was to be administered via intramyocardial injection catheter into the ischemic region of [redacted].

Three patients were enrolled in Stage 1, and 6 patients were enrolled in Stage 2. The study included a 12-month follow-up phase. Regarding safety, the incidences of adverse events were 100% (3 of 3 subjects, 32 events) in the 0.4 mg group and 100% (6 of 6 subjects, 58 events) in the 4 mg group. Serious adverse events occurred in 2 subjects in the 0.4 mg group (3 events, myocardial infarction, myocardial ischaemia, gastrointestinal haemorrhage) and 2 subjects in the 4 mg group (3 events, angina pectoris, myocardial infarction, chronic obstructive pulmonary disease), and a causal relationship to HGF plasmid was denied for all those events. No deaths were reported in either stage.

## 7.4 Antibody production following administration of HGF plasmid

Anti-hHGF antibody titer, anti-DNA antibody titer, and anti-*E. coli* protein antibody titer in human sera were measured by an ELISA method (The LLOQs were [redacted] ng/mL, [redacted] IU/mL, and [redacted] ng/mL, respectively).

(1) ASO phase III study

Serum anti-hHGF antibody titer, serum anti-DNA antibody titer, and serum anti-*E. coli* protein antibody titer were measured at baseline (before administration of HGF plasmid or placebo) and Weeks 4, 8, and 12 in 28 subjects in the HGF plasmid group and 13 subjects in the placebo group.

No serum anti-hHGF antibodies were detected in either group.

Following administration of HGF plasmid, no patients tested positive for serum anti-DNA antibodies or serum anti-*E. coli* protein antibodies.

(2) TAO open-label clinical study
Serum anti-hHGF antibody titer, serum anti-DNA antibody titer, and serum anti-*E. coli* protein antibody titer were measured at baseline (before administration of HGF plasmid) and Weeks 4, 8, and 12, but none of these antibodies were detected.

(3) US phase II study
Serum anti-hHGF antibodies were measured at baseline (before administration of HGF plasmid), Days 7, 14, 21, 28, 35, and 49, and Months 3 and 6, and no antibodies were detected in any group.

(4) US second phase II study
Serum anti-hHGF antibodies were measured at baseline (before administration of HGF plasmid), Days 14, 28, and 49, and Months 3 and 6, and no antibodies were detected in either group.

(5) US phase IIb pilot study
Serum anti-hHGF antibodies were measured at baseline (before administration of HGF plasmid) and Months 3, 6, 9, 12, 15, and 18, and no antibodies were detected.

(6) Global phase III study
Serum anti-hHGF antibodies were measured at baseline (before administration of HGF plasmid), Months 3, 6, 9, 12, 15, and 18, and no antibodies were detected.

## 7.R Outline of the review conducted by PMDA

### 7.R.1 Review strategy

PMDA considers that among the evaluation data submitted, the pivotal studies to evaluate the efficacy and safety of HGF plasmid in Japan are ASO phase III study and TAO open-label clinical study, which were submitted with a new drug marketing application for HGF plasmid as of March 27, 2008. PMDA re-evaluates the efficacy and safety of HGF plasmid, after reviewing the results of advanced medical care B clinical research study, which was conducted by the applicant after withdrawing the marketing application as of [redacted], 20[redacted], in addition to the above 2 studies, and taking also account of the current environment in the treatment of ASO and Buerger's disease, etc.

### 7.R.2 Clinical positioning

The applicant's explanation about the clinical positioning of HGF plasmid in the treatment of ASO and Buerger's disease:
No treatment has been established for critical limb ischemia in ASO patients who do not respond adequately to standard drug therapy and are poor candidates for revascularization or Buerger's disease patients who do not respond to conventional medical treatment, and the 1-year limb amputation rate in such ASO patients has been

reported to be 10% to 14% (*JACC Cardiovasc Interv*. 2017;10:1147-57). It has been reported that 11.5% of Buerger's disease patients with or without critical limb ischemia underwent lower-extremity amputation within 9 years (*Journal of Japanese College of Angiology*. 1997;37:883-6). Lower-extremity amputation is associated with a poor prognosis in ASO patients, and limb salvage as well as improvement of symptoms are important issues also in terms of survival prognosis. However, given that the decision regarding lower-extremity amputation is made comprehensively based on the state of ulcers (size, depth, with or without infection or necrosis), the patient's condition (underlying disease, operability), etc., there are limitations to evaluating the efficacy of HGF plasmid based on lower-extremity amputation. On the other hand, relief of rest pain and ulcer healing in patients with critical limb ischemia improve quality of life and are considered clinically meaningful.

When HGF plasmid is injected into muscles in close proximity to the ischemic focus, the muscle cells take up injected HGF plasmid, which is expected to improve blood flow in the limbs by inducing angiogenesis through HGF production/release. HGF plasmid is positioned as a new treatment option for improvement of rest pain and ulcers in patients with critical limb ischemia who do not respond adequately to standard drug therapy and are poor candidates for revascularization.

PMDA's view:
Although the true endpoint for the treatment of critical limb ischemia in patients with ASO or Buerger's disease who do not respond adequately to standard drug therapy and are poor candidates for revascularization is limb salvage, pain relief and healing of tissue loss are also listed as the treatment goals for critical limb ischemia in the Japanese clinical practice guidelines. ASO phase III study, TAO open-label clinical study, etc. were not designed to assess lower-extremity amputation and assessed the relief of rest pain, ulcer size reduction, etc. Taking account of this point, it is possible to assess the appropriateness of positioning HGF plasmid as a product expected to relieve rest pain or heal ulcers in patients with critical limb ischemia who show no improvement in symptoms despite conventional medical treatment and do not respond adequately to revascularization or are poor candidates for revascularization.

### 7.R.3 Efficacy

#### 7.R.3.1 Patient populations included in clinical studies

PMDA's view:
Patients with ASO or Buerger's disease who were not candidates for revascularization or medical treatment were included and treated with HGF plasmid in ASO phase III study, TAO open-label clinical study, advanced medical care B clinical research study, etc., which is appropriate in light of the pharmacological effects and clinical positioning expected of HGF plasmid.

### 7.R.3.2 Endpoints

The applicant's explanation about the reasons for the choice of the primary endpoint for ASO phase III study and TAO open-label clinical study:

For the following reasons, the composite endpoint of "rest pain VAS" in Fontaine III patients (with rest pain) and "ulcer size" in Fontaine IV patients (with ulcers) was chosen as the primary endpoint for ASO phase III study, and "the improvement rate for ulcer size" in patients with Fontaine IV Buerger's disease was chosen as the primary endpoint for TAO open-label clinical study.

- The Japanese clinical practice guidelines state that pain relief and ulcer healing are both treatment goals for critical limb ischemia of ASO and Buerger's disease, and these were considered clinically meaningful endpoints.
- Although rest pain and ulcers are clinically different findings, both are symptoms caused by chronic obstruction of the arterial circulation, and it was considered possible to use these as a composite endpoint of efficacy.

Furthermore, "improvement" was defined as a reduction in VAS of ≥20 mm or a reduction in ulcer size (√[major axis × minor axis]) to ≤75% of the baseline value, for the following reasons.

- For assessment of rest pain associated with ASO or Buerger's disease, the VAS was selected as it is commonly used for ratings of pain intensity. Improvement of rest pain was defined as a reduction of ≥ 20 mm, referring to a report that the minimal clinically important difference of VAS back pain was 18 to 19 mm in a clinical trial in patients with chronic low back pain (*Eur Spine J*. 2003;12:12-20), etc.
- There is no established definition of improvement in ulcers. Thus, based on the opinion of the medical expert familiar with the treatment of ASO and Buerger's disease, and the European Wound Management Association (EWMA)'s recommendations (wound healing for leg ulceration etc. is defined as a 50% reduction in wound size [*J Wound Care*. 2010;19:237-68]), "improvement" was defined as a reduction in ulcer size (geometric mean of major and minor axis lengths: √[major axis × minor axis]) to ≤75%: Assuming that the ulcer to be assessed in a clinical study of HGF plasmid is an ellipse, if the lengths of both major and minor axis are reduced to 75%, the reduced ulcer size will be 56.3% of its original size, which is a value close to 50%.

PMDA's view:

Although "a reduction in rest pain VAS of 20 mm" and "a reduction in ulcer size √(major axis × minor axis) to ≤75%" were used as the improvement criteria for the primary endpoint for ASO phase III study and TAO open-label clinical study, these are not established criteria, and there are limitations to assessing the efficacy of HGF plasmid based only on these criteria.

However, given the clinical positioning of HGF plasmid [see Section 7.R.2] and the following points, the efficacy of HGF plasmid can be evaluated based on the degree of improvement of rest pain (VAS) and complete ulcer closure after assessing the improvement rates for "rest pain VAS" and "ulcer size" separately. Since limb salvage is clinically important, the efficacy of HGF plasmid can be evaluated by assessing also lower-extremity amputation as supportive information.

- For the analysis of the primary endpoint for ASO phase III study, Fontaine III patients were analyzed for

"rest pain VAS" and Fontaine IV patients were analyzed for "ulcer size," and the definition of "improvement" was different according to Fontaine's stage. Taking account of these points, there are limitations to assessing "rest pain VAS" and "ulcer size" together, and these need to be assessed separately in efficacy evaluation of HGF plasmid.

- Although there is no established measure of rest pain associated with critical limb ischemia, the VAS is commonly used for ratings of pain intensity, and pain relief is expected to improve quality of life (QOL) and activity of daily life (ADL). Given these points, rest pain measured by VAS is clinically meaningful to a certain extent, depending on the degree of improvement in VAS.
- Complete ulcer closure is expected to restore the skin barrier, e.g. infection control, which is clinically meaningful.

**7.R.3.3 Efficacy**

The applicant's explanation about the efficacy of HGF plasmid in the improvement of ulcers and rest pain:

Ulcers

The improvement rates for ulcer size in clinical studies and a clinical research study are shown below:

- In Stage 1 of ASO phase III study, improvement of ulcer size was observed in 11 of 11 subjects (100%) in the HGF plasmid group and 2 of 5 subjects (40.0%) in the placebo group [see Section 7.1.1, Table 18].
- In TAO open-label clinical study, improvement of ulcer size was observed in 6 of 9 subjects (66.7%) [see Section 7.1.2, Table 28].
- In advanced medical care B clinical research study, improvement of ulcer size was observed in 1 of 1 subject (100%) [see Section 7.1.3, Table 31].

Furthermore, achievement of complete ulcer (the ulcer to be assessed) closure in Fontaine IV patients is shown below:

- In Stage 1 of ASO phase III study, complete ulcer closure was observed in 4 of 11 subjects (36.4%) in the HGF plasmid group and 1 of 5 subjects (20.0%) in the placebo group [see Section 7.1.1, Table 22].
- In TAO open-label clinical study, complete ulcer closure was observed in 5 of 9 subjects (55.6%) [see Section 7.1.2, Table 29], and ulcer remained resolved in all 3 subjects with Month 15 assessment, among the 5 subjects with complete ulcer closure.
- In advanced medical care B clinical research study, complete ulcer closure was observed in 1 of 1 subject (100.0%) [see Section 7.1.3, Table 32].

In the 1 patient with complete ulcer closure in advance medical care B clinical research study, among 4 ulcers on the ipsilateral limb, only 1 ulcer assessed for the primary endpoint resolved, and no improvement of the other 3 ulcers was observed, etc. PMDA asked the applicant to explain the efficacy of HGF plasmid in this patient.

The applicant's response:

In advanced medical care B clinical research study, "the ulcer with the largest diameter that existed at the start of the study" was to be treated to evaluate the efficacy of HGF plasmid. This patient had 4 ulcers on the big and

second toes of the ipsilateral limb, of which the ulcer on the root of the big toe had the largest diameter, and intramuscular injections of HGF plasmid were given in the area, where HGF plasmid seemed most effective for the ulcer with the largest diameter (a group of muscles through which the arteries to the big toe pass: tibialis anterior muscle, soleus muscle, extensor hallucis longus muscle, flexor hallucis longus muscle, and flexor hallucis brevis muscle). As a result, the ulcer with the largest diameter only closed completely. Injections could not improve blood flow to the remaining 3 ulcers, possibly because these 3 ulcers were not located in the area of blood supply. Based on the above, no improvement of the remaining 3 ulcers does not deny the efficacy of HGF plasmid.

The above results etc. indicated that the efficacy of HGF plasmid in the improvement of ulcers is expected.

Rest pain

The improvement rates for rest pain in Fontaine III patients in clinical studies and a clinical research study are shown below:

- In Stage 1 of ASO phase III study, improvement of rest pain was observed in 8 of 16 subjects (50.0%) in the HGF plasmid group and 2 of 8 subjects (25.0%) in the placebo group [see Section 7.1.1, Table 18]. The median changes in VAS [95% CI] were −21.5 mm [−33.7, −8.6] in the HGF plasmid group and −10.5 mm [−25.0, −3.7] in the placebo group [see Section 7.1.1, Table 19].
- In TAO open-label clinical study, improvement of rest pain was observed in 3 of 7 subjects (42.9%). The median change in VAS [95% CI] was −41.0 mm [−54.6, −18.0].
- In advanced medical care B clinical research study, improvement of rest pain was observed in 2 of 3 subjects (66.7%) [see Section 7.1.3, Table 31]. The median change in VAS [95% CI] was −28 mm [−50.7, −12.7].

In ASO phase III study, the effect of HGF plasmid, compared with placebo, in improving rest pain, was small. It is difficult to assess the efficacy of HGF plasmid in the improvement of rest pain based on the results from uncontrolled studies, i.e. TAO open-label clinical study and advanced medical care B clinical research study. PMDA asked the applicant to further explain the efficacy of HGF plasmid in the improvement of rest pain.

The applicant's response:

In ASO phase III study, improvement of rest pain was observed in 8 of 16 subjects (50.0%) in the HGF plasmid group and 2 of 8 subjects (25.0%) in the placebo group.

Using the pooled data from Fontaine III patients assessed for rest pain in a Japanese clinical study and Japanese clinical research studies (26 patients in the HGF plasmid group [22 in ASO phase III study, 3 in advanced medical care B clinical research study, and 1 in Osaka University clinical research study], 9 patients in the placebo group [9 in ASO phase III study[11)]]), the efficacy of HGF plasmid in the improvement of rest pain was examined. Based on the pooled data, improvement of rest pain was observed in 11 of

11) Analysis was performed, also including 1 patient without rest pain assessment as of the data cutoff date of June 29, 2007 in ASO phase III study.

26 patients (42.3%) in the HGF plasmid group and 2 of 9 patients (22.2%) in the placebo group. Moreover, as it has been reported that placebo is likely to affect pain ratings (*Curr Pain Headache Rep*. 2014;18:419), and that there is a large placebo effect, especially in patients with less pain (*Am J Cardiol*. 2002;90:1314-9), 9 patients in the placebo group were divided into subgroups of [redacted] before administration of placebo (saline) ≥[redacted] (7 patients) or <[redacted] (2 patients) to analyze the change in [redacted]. The mean ± SE values for the change in [redacted] after placebo administration in the subgroups of [redacted] ≥[redacted] or <[redacted] were [redacted]±[redacted] and [redacted]±[redacted], respectively, suggesting a larger placebo effect in the subgroup of [redacted] <[redacted] than in the subgroup of [redacted] ≥[redacted]. Based on the above, it was considered that the efficacy of HGF plasmid in the improvement of rest pain can be evaluated with higher sensitivity in the subgroup of ≥[redacted], and improvement of [redacted] in a subgroup of Fontaine III patients with ≥[redacted] (20 patients in the HGF plasmid group, 7 patients in the placebo group) was analyzed. As a result, improvement of [redacted] was observed in 9 of 20 patients (45.0%) in the HGF plasmid group and 0 of 7 patients in the placebo group.

Although the above results suggested the efficacy of HGF plasmid in the improvement of rest pain, given that the number of patients analyzed was not sufficient to confirm the efficacy of HGF plasmid in the improvement of rest pain, etc., it cannot be concluded at present that a certain level of efficacy of HGF plasmid has been demonstrated.

[Other efficacy assessment]

The applicant's explanation about lower-extremity amputation:

The incidences of lower-extremity amputation in clinical studies and a clinical research study are as follows.

- Among 38 patients treated with HGF plasmid in Stages 1 and 2 of ASO phase III study, 2 patients (5.3%) underwent a major amputation between the start of treatment phase and the end of follow-up survey (15 months). Additional 1 patient underwent a major amputation by Month 36. Moreover, an additional long-term follow-up survey beyond Month 36 (up to 13 years from the first dose) was conducted, which showed that among 26 patients surveyed, 1 patient (3.8%) underwent a major amputation.
- Among 9 patients treated with HGF plasmid in TAO open-label clinical study, 1 patient (11.1%) underwent a major amputation between the start of treatment phase and the end of follow-up survey (15 months). No additional major amputation occurred up to Month 36. Moreover, an additional long-term follow-up survey beyond Month 36 (up to 13 years from the first dose) was conducted, which showed that among 7 patients surveyed, 1 patient (14.3%) underwent a major amputation.
- Among 22 patients treated with HGF plasmid in Osaka University clinical research study, no major amputation occurred between the start of treatment phase and the end of follow-up survey (25 months). Moreover, an additional follow-up survey beyond Month 25 (up to 16 years from the first dose) was conducted, which showed that among 20 patients surveyed, 1 patient (5.0%) underwent a major amputation.

In Japan, ASO patients with critical limb ischemia were observed for up to 3 years, 24.7% of the patients required lower-limb amputation, and two-thirds of them had a major amputation above or below the knee

(*Therapeutic Research*. 1992;13:4099-109). Taking account of this report, the efficacy of HGF plasmid in limb salvage is expected.

PMDA asked the applicant to explain the reasons for differences in efficacy results between ASO phase III study and US phase II/second phase II studies in ASO patients.

The applicant's response:
For the following reasons, the applicant considers that differences in the injection locations, the dose per site, the total dose per occasion, dosing interval, and the disease condition among the studies may have affected the efficacy results of these studies.

- Patients with necrotic ulcers on the lower limb to be treated were enrolled in US phase II and second phase II studies, while such patients were excluded from ASO phase III study.
- The ulcers of enrolled patients were larger in US phase II and second phase II studies than in ASO phase III study.
- Injection locations were individualized for each patient after assessing the ischemic state of the limb using angiography and ultrasonography in ASO phase III study, whereas study drug was injected at predefined sites, 4 above and 4 below the knee joint, regardless of the ischemic state of the limb, in US phase II study.
- Non-clinical studies in rabbits showed that the level of hHGF expressed from HGF plasmid is injection solution concentration- and injection volume-dependent [see Sections 4.3.1 and 4.3.2], and hHGF production is considered to be increased by increases in both the dose and injection volume. The dose per site was 0.5 mg/3 mL (the injection volume was allowed to be reduced to 2 mL if the muscle was small) in ASO phase III study and 0.5 mg/2 mL in US phase II study. The level of hHGF expression may have been higher in ASO phase III study that employed a higher volume of injection.

PMDA's view:
In ASO phase III study, subjects' treatment allocations were planned to be unblinded one-by-one, the results from subjects who were unblinded were reviewed during the study, and moreover, an interim analysis for early termination for efficacy, which had not been pre-specified, was planned based on the data from the ongoing study. These were all inappropriate and would significantly compromise the integrity of the study and the reliability of the results. Thus, interpretation of the interim analysis results has limitations. However, it is possible to assess the efficacy of HGF plasmid, taking account of these limitations.

A certain level of efficacy of HGF plasmid in the improvement of ulcers is expected, given that the proportion of patients with complete ulcer closure tended to be higher in the HGF plasmid group than in the placebo group in ASO phase III study. In advanced medical care B clinical research study, no improvement of other ulcers was observed in 1 patient with a complete ulcer closure. The applicant's explanation about this finding as described above is understandable, but it is necessary to continue to investigate the association between the injection locations and efficacy of HGF plasmid [see Section 8.1].

On the other hand, the improvement rates for rest pain in the placebo and HGF plasmid groups in the only placebo-controlled clinical study, ASO phase III study, are shown in Table 18 in Section 7.1.1. A subjective measure of pain, VAS, is likely to lead to bias in the results of an uncontrolled study, and there are limitations to using the pooled data from controlled and uncontrolled studies for evaluation. Given that a subgroup analysis of patients with ≥[redacted] is a post-hoc exploratory analysis, etc., it is difficult to explain that the efficacy of HGF plasmid is expected based on the results of this subgroup analysis. Thus, it cannot be concluded at present that the efficacy of HGF plasmid in the improvement of rest pain is expected.

As the above clinical studies and clinical research study were not designed to assess lower-extremity amputation, and the information on amputations other than major amputations is also limited, the efficacy of HGF plasmid in limb salvage is unknown at present. However, taking account of a report on lower-extremity amputations in patients with critical limb ischemia, at least, the use of HGF plasmid had no clear adverse effects on limb salvage.

Based on the above, PMDA concluded that HGF plasmid is expected to have a certain level of efficacy in improving ulcers in ASO or Buerger's disease patients with critical limb ischemia who do not respond adequately to standard drug therapy and are poor candidates for revascularization. Although the definitive reason for the failure to show the efficacy of HGF plasmid in US phase II study is unknown, HGF plasmid may not be effective in patients with necrotic ulcers on the limb to be treated or those with ulcers with a large diameter.

The above conclusion by PMDA will be discussed at the Expert Discussion. The intended population for HGF plasmid is further discussed in the indication or performance section [see Section 7.R.4].

### 7.R.4 Safety

PMDA asked the applicant to explain angiogenesis-related adverse events (the risk of benign or malignant neoplasms etc., the new onset and exacerbation of diabetic retinopathy/age-related macular degeneration), which are expected from the mechanism of action etc. of HGF plasmid, and anaphylactic reaction.

#### 7.R.4.1 The risk of benign or malignant neoplasms etc.

The applicant's explanation:

According to 7 sets of evaluation data and Osaka University clinical research study submitted with the present application, the occurrence of benign or malignant neoplasms etc. is shown in Table 45, and benign or malignant neoplasms etc. occurred in 41 of 209 patients (19.6%) in the HGF plasmid group and 3 of 70 patients (4.3%) in the placebo group. No benign or malignant neoplasms were reported in US second phase II study.

**Table 45. Occurrence of benign or malignant neoplasms etc.**

| | n (%) | | | | | |
|---|---|---|---|---|---|---|
| | ASO phase III study | | TAO open-label clinical study | Advanced medical care B clinical research study | US phase II study | |
| | HGF plasmid N = 39 | Placebo N = 15 | HGF plasmid N = 10 | HGF plasmid N = 6 | HGF plasmid N = 78 | Placebo N = 26 |
| Any event | 12 (28.2) | 1 (6.7) | 3 (30.0) | 1 (16.7) | 10 (12.8) | 2 (7.7) |
| Large intestine polyp | 5 (12.8) | 0 | 1 (10.0) | 0 | 0 | 0 |
| Benign duodenal neoplasm | 2 (5.1) | 0 | 0 | 0 | 0 | 0 |
| Gastric adenocarcinoma | 1 (2.6) | 0 | 0 | 0 | 0 | 0 |
| Pancreatic carcinoma | 1 (2.6) | 0 | 0 | 0 | 0 | 0 |
| Prostate cancer | 2 (5.1) | 0 | 0 | 0 | 0 | 0 |
| Gastric polyps | 1 (2.6) | 1 (6.7) | 0 | 0 | 0 | 0 |
| Duodenal polyp | 1 (2.6) | 0 | 0 | 0 | 1 (1.3) | 0 |
| Oesophageal squamous cell carcinoma | 0 | 0 | 1 (10.0) | 0 | 0 | 0 |
| Squamous cell carcinoma of pharynx | 0 | 0 | 1 (10.0) | 0 | 0 | 0 |
| Gastric cancer | 0 | 0 | 0 | 1 (16.7) | 0 | 0 |
| Basal cell carcinoma | 0 | 0 | 0 | 0 | 4 (5.1) | 0 |
| Adrenal adenoma | 0 | 0 | 0 | 0 | 1 (1.3) | 0 |
| Colon cancer | 0 | 0 | 0 | 0 | 1 (1.3) | 1 (3.8) |
| Lipoma | 0 | 0 | 0 | 0 | 1 (1.3) | 0 |
| Squamous cell carcinoma | 0 | 0 | 0 | 0 | 1 (1.3) | 0 |
| Hilar lymphadenopathy | 0 | 0 | 0 | 0 | 1 (1.3) | 0 |
| Adenocarcinoma | 0 | 0 | 0 | 0 | 0 | 1 (3.8) |

| | n (%) | | | |
|---|---|---|---|---|
| | US phase IIb pilot study | Global phase III study | | Osaka University clinical research study |
| | HGF plasmid N = 10 | HGF plasmid N = 23 | Placebo N = 23 | HGF plasmid N = 22 |
| Any event | 1 (10.0) | 1 (4.3) | 0 | 13 (59.1) |
| Uterine leiomyosarcoma | 1 (10.0) | 0 | 0 | 0 |
| Non-small cell lung cancer | 0 | 1 (4.3) | 0 | 0 |
| Benign gastric neoplasm | 0 | 0 | 0 | 2 (9.1) |
| Lipoma | 0 | 0 | 0 | 2 (9.1) |
| Oesophageal polyp | 0 | 0 | 0 | 2 (9.1) |
| Skin neoplasm | 0 | 0 | 0 | 1 (4.5) |
| Skin papilloma | 0 | 0 | 0 | 1 (4.5) |
| Benign pancreatic neoplasm | 0 | 0 | 0 | 1 (4.5) |
| Gastric polyps | 0 | 0 | 0 | 1 (4.5) |
| AFP increased | 0 | 0 | 0 | 1 (4.5) |
| CEA increased | 0 | 0 | 0 | 4 (18.2) |
| Tumour marker increased | 0 | 0 | 0 | 1 (4.5) |
| CA19-9 increased | 0 | 0 | 0 | 3 (13.6) |

**Table 46. Listing of patients with benign or malignant neoplasms etc.**

| Study | Treatment group | Age | Sex | Event | Time to onset [a] (days) | Causality |
|---|---|---|---|---|---|---|
| ASO phase III study | HGF plasmid | 84 | M | Duodenal polyp | 78 | No |
| | | 73 | M | Gastric polyps | 81 | No |
| | | | | Oesophageal polyp | 81 | No |
| | | 75 | M | Gastric adenocarcinoma | 83 | Yes |
| | | 81 | F | Benign duodenal neoplasm | 90 | No |
| | | 73 | F | Pancreatic carcinoma | 432 | Yes |
| | | 72 | M | Prostate cancer | 80 | Yes |
| | | 77 | M | Large intestine polyp | 90 | No |
| | | 79 | M | Large intestine polyp | 88 | No |
| | | 67 | M | Large intestine polyp | 89 | No |
| | | 66 | M | Large intestine polyp | 78 | No |
| | | | | Benign duodenal neoplasm | 79 | No |
| | | 70 | M | Large intestine polyp | 79 | No |
| | | 73 | M | Prostate cancer | 973 | Yes |
| | Placebo | 84 | M | Gastric polyps | 83 | No |
| TAO open-label clinical study | HGF plasmid | 48 | M | Oesophageal squamous cell carcinoma | 91 | Yes |
| | | 57 | M | Large intestine polyp | 117 | No |
| | | 49 | M | Laryngeal squamous cell carcinoma | 603 | Yes |
| Advanced medical care B clinical research study | HGF plasmid | 70 | M | Gastric cancer | 33 | Yes |
| US phase II study | HGF plasmid | 84 | M | Basal cell carcinoma | 171 | No |
| | | 70 | F | Basal cell carcinoma | 44 | No |
| | | | | Basal cell carcinoma | 122 | No |
| | | | | Basal cell carcinoma | 358 | No |
| | | 78 | M | Basal cell carcinoma | 91 | No |
| | | | | Basal cell carcinoma | 140 | No |
| | | 80 | M | Squamous cell carcinoma | 169 | No |
| | | 73 | M | Duodenal polyp | 92 | No |
| | | 78 | F | Hilar lymphadenopathy | 112 | No |
| | | 75 | F | Lipoma | 26 | No |
| | | 68 | M | Basal cell carcinoma | 48 | No |
| | | 65 | F | Adrenal adenoma | 16 | No |
| | | 77 | F | Colon cancer | 365 | Yes |
| | Placebo | 64 | M | Colon cancer | 224 | No |
| | | 74 | F | Adenocarcinoma | 114 | No |
| US phase IIb pilot study | HGF plasmid | 62 | F | Uterine leiomyosarcoma | 349 | Yes |
| Global phase III study | HGF plasmid | 70 | M | Non-small cell lung cancer | 493 | No |
| Osaka University clinical research study | HGF plasmid | 63 | F | AFP increased | 97 | No |
| | | 66 | M | CA19-9 increased | 194 | No |
| | | | | Benign gastric neoplasm | 595 | No |
| | | 69 | M | CA19-9 increased | 216 | No |
| | | | | CEA increased | 216 | No |
| | | | | Skin papilloma | 258 | No |
| | | | | Skin neoplasm | 267 | No |
| | | | | CA19-9 increased | 378 | No |
| | | | | CEA increased | 714 | No |
| | | | | Skin neoplasm | 718 | No |
| | | 67 | M | CA19-9 increased | 175 | No |
| | | | | Benign gastric neoplasm | 367 | No |
| | | 66 | M | Lipoma | 694 | No |
| | | 66 | M | Oesophageal polyp | 680 | No |
| | | 69 | F | Benign pancreatic neoplasm | 704 | No |
| | | 58 | M | Gastric polyps | 355 | No |

| Study | Treatment group | Age | Sex | Event | Time to onset [a] (days) | Causality |
|---|---|---|---|---|---|---|
| | | 66 | M | Lipoma | 574 | No |
| | | 27 | F | Tumour marker increased | 209 | No |
| | | | | CEA increased | 553 | No |
| | | 66 | M | Oesophageal polyp | 589 | No |
| | | 66 | M | CEA increased | 122 | No |
| | | | | CEA increased | 357 | No |
| | | 66 | M | CEA increased | 73 | No |
| | | | | CEA increased | 308 | No |

a: No. of days from the first dose

The applicant's explanation about the risk of benign or malignant neoplasms etc. associated with HGF plasmid:
The incidence of benign or malignant neoplasms etc. was higher in the HGF plasmid group than in the placebo group. However, a causal relationship to HGF plasmid could not be ruled out for 7 cases only, patients were followed-up for a longer period of time in the HGF plasmid group than in the placebo group in ASO phase III study (up to 13 years in the HGF plasmid group, 12 weeks in the placebo group), and furthermore, patients who are candidates for treatment with HGF plasmid are in age groups with an increased risk of developing cancer. Given these points etc., the relationship between HGF plasmid and benign or malignant neoplasms etc. is unclear at present.

PMDA's view:
The applicant's explanation about the risk of benign or malignant neoplasms etc. associated with HGF plasmid is understandable, and the relationship between HGF plasmid and benign or malignant neoplasms etc. is unclear at present. However, given that HGF plasmid induces angiogenesis through hHGF production/release [see Section 4.2.1], it is necessary to collect post-marketing information on the risk of benign or malignant neoplasms etc. following administration of HGF plasmid.

**7.R.4.2 New onset and exacerbation of diabetic retinopathy/age-related macular degeneration**

The applicant's explanation about the new onset and exacerbation of diabetic retinopathy and age-related macular degeneration associated with HGF plasmid-induced angiogenesis:
According to 7 sets of evaluation data and Osaka University clinical research study submitted with the present application, the occurrence of diabetic retinopathy or age-related macular degeneration and potentially associated adverse events is shown in Table 47, and these events occurred in 12 of 209 patients (5.7%) in the HGF plasmid group and 1 of 70 patients (1.4%) in the placebo group. In TAO open-label clinical study, advance medical care B clinical research study, US second phase II study, and global phase III study, no diabetic retinopathy or age-related macular degeneration was reported.

**Table 47. Occurrence of diabetic retinopathy or age-related macular degeneration**

| | n (%) | | | | | |
|---|---|---|---|---|---|---|
| | ASO phase III study | | US phase II study | | US phase IIb pilot study | Osaka University clinical research study |
| | HGF plasmid N = 39 | Placebo N = 15 | HGF plasmid N = 78 | Placebo N = 26 | HGF plasmid N = 10 | HGF plasmid N = 22 |
| Any event | 2 (5.1) | 0 | 3 (3.8) | 1 (3.8) | 2 (20.0) | 5 (22.7) |
| Diabetic retinopathy | 1 (2.6) | 0 | 0 | 0 | 1 (10.0) | 0 |
| Retinal exudates | 1 (2.6) | 0 | 0 | 0 | 0 | 0 |
| Retinal haemorrhage | 0 | 0 | 2 (2.6) | 0 | 0 | 5 (22.7) |
| Macular degeneration | 0 | 0 | 1 (1.3) | 0 | 0 | 0 |
| Retinopathy | 0 | 0 | 0 | 1 (3.8) | 0 | 0 |
| Macular oedema | 0 | 0 | 0 | 0 | 1 (10.0) | 0 |

Among the cases of diabetic retinopathy or age-related macular degeneration in the HGF plasmid group, a causal relationship to HGF plasmid could not be ruled out for only 2 cases of retinal haemorrhage in Osaka University clinical research study (the patient was treated bilaterally and counted as 2 cases). The event occurred at Day 368 and was classified as non-serious.

The applicant's explanation about the risk of the new onset and exacerbation of diabetic retinopathy and age-related macular degeneration:
According to 7 sets of evaluation data and Osaka University clinical research study submitted with the present application, 37 patients had pre-existing non-proliferative diabetic retinopathy and 3 patients had pre-existing non-exudative age-related macular degeneration at baseline, but none of these patients progressed to proliferative diabetic retinopathy or neovascular (exudative) age-related macular degeneration after administration of HGF plasmid.

Based on the above, administration of HGF plasmid is unlikely to cause the new onset or exacerbation of diabetic retinopathy or age-related macular degeneration.

However, since patients with proliferative diabetic retinopathy or exudative age-related macular degeneration based on prior fundus or retinal examination findings were excluded and were not to receive HGF plasmid or placebo in the 7 studies (evaluation data) or Osaka University clinical research study submitted with the present application, there is no clinical experience with HGF plasmid in patients with proliferative diabetic retinopathy or exudative age-related macular degeneration, and the possibility that administration of HGF plasmid causes the exacerbation of the disease cannot be ruled out. Thus, the package insert will advise that the use of HGF plasmid in patients with proliferative diabetic retinopathy or exudative age-related macular degeneration should be determined carefully.

PMDA's view:
At present, there is limited clinical experience with HGF plasmid, and the relationship between administration of HGF plasmid and the new onset/exacerbation of diabetic retinopathy/age-related macular degeneration is unclear. However, given the mechanism of action of HGF plasmid, attention should be paid to the new onset

or exacerbation of diabetic retinopathy and age-related macular degeneration following administration of HGF plasmid, and it is necessary to collect the relevant post-marketing information as well.

The applicant's explanation (there is no clinical experience with HGF plasmid in patients with proliferative diabetic retinopathy or exudative age-related macular degeneration, and the package insert will advise that the use of HGF plasmid should be determined carefully) is acceptable.

**7.R.4.3 Anaphylactic reaction**

The applicant's explanation about anaphylactic reaction:
In ASO phase III study, 1 of 39 patients (2.6%) in the HGF plasmid group had an anaphylactic reaction. This was considered to be an anaphylactic reaction to gabexate mesilate, which was administered for acute exacerbation of chronic pancreatitis, and its causal relationship to HGF plasmid was denied.

PMDA's view:
In clinical studies, an anaphylactic reaction occurred following administration of HGF plasmid in the above 1 patient only, and no events for which a causal relationship to HGF plasmid could not be ruled out have been reported at present. Although the relationship between HGF plasmid and anaphylactic reaction is unknown, as the currently available information is limited, it is necessary to collect post-marketing information.

The above conclusion by PMDA will be discussed at the Expert Discussion.

**7.R.5 Indication or performance**

The applicant's explanation about "INDICATION OR PERFORMANCE" and "PRECAUTIONS FOR INDICATION OR PERFORMANCE":
On the basis of the results from ASO phase III study, TAO open-label clinical study, advanced medical care B clinical research study, etc., HGF plasmid is expected to have a certain level of efficacy in patients with ulcers from critical limb ischemia due to chronic arterial occlusive disease (ASO and Buerger's disease) who do not respond adequately to standard drug therapy and are poor candidates for revascularization.

The applicant's view on the use of HGF plasmid in (1) patients with necrotic tissue extending over a wide area of the lower limb and patients with life-threatening infection of the lower limb uncontrollable by antibiotics, (2) patients with ulcerations with necrosis or bone or tendon exposure, and (3) tumor-bearing patients, who were excluded from these clinical studies and clinical research study:

(1) In these patients, restoration of blood flow cannot be expected with the use of HGF plasmid, and infection control should take precedence. Thus, the use of HGF plasmid cannot be recommended for these patients.
(2) In patients with ulcerations with necrotic tissue or bone or tendon exposure, the use of HGF plasmid can be recommended if necrotic tissue or bone/tendon is excised, resulting in ulcers without necrotic tissue or bone/tendon exposure.
(3) Although there are no clinical study data showing that HGF plasmid exacerbates tumors, taking account of the mechanism of action of HGF plasmid, the possibility that it exacerbates tumors cannot be ruled out.

Especially, HGF plasmid will be contraindicated in patients with current or previous malignant tumors in close proximity to its injection sites (muscles and surrounding tissue), and the package insert will advise that the use of HGF plasmid should be determined carefully for patients with malignant tumors at other locations.

Based on the applicant's explanation about the reasons for differences in efficacy results between ASO phase III study and US phase II/second phase II studies [see Section 7.R.3.3], the patient's ulcer size may affect efficacy. PMDA asked the applicant to explain the need for defining the maximum size of ulcer that can be treated effectively with HGF plasmid.

The applicant's response:
In 24 Japanese patients with ulcers treated with HGF plasmid (14 in ASO phase III study, 9 in TAO open-label clinical study, 1 in advanced medical care B clinical research study), the association between ulcer size and efficacy was investigated. The ulcer size at baseline (√[major axis × minor axis]) widely ranged from 1.4 to 27.9 mm in 13 patients with complete ulcer closure at Week 12, was 3.6 to 23.2 mm in 8 patients with a reduction in ulcer size to ≤75% at Week 12, and was 19.2 to 29.9 mm in 3 patients with unchanged or increased ulcer size at Week 12. Based on the above, the association between ulcer size and the efficacy of HGF plasmid is unknown, and there is no need to restrict the use of HGF plasmid based on the patient's ulcer size.

In addition to the above, it is necessary to fully understand the content of the CLINICAL STUDIES section of the package insert for the use of HGF plasmid. Thus, the "INDICATION OR PERFORMANCE" and "PRECAUTIONS FOR INDICATION OR PERFORMANCE" sections have been proposed as follows.

**Indication or Performance**
Improvement of ulcers in patients with chronic arterial occlusive disease (arteriosclerosis obliterans/Buerger's disease) who do not respond adequately to standard drug therapy and are poor candidates for revascularization

**Precautions for Indication or Performance**
- Patients for use of Collategene should be selected with a full knowledge of the content of the CLINICAL STUDIES section and an adequate understanding of the efficacy and safety of Collategene.
- In patients with ulcerations with bone or tendon exposure or necrosis, necrotic tissue should be removed or bone tissue should be resected, etc. Then, the use of Collategene should be considered.
- The use of Collategene is not recommended for the treatment of the wounds of ulcers with bone or tendon exposure or necrosis only.
- The use of Collategene is not recommended for patients with necrotic tissue extending over a wide area of the lower limb or patients with life-threatening infection of the lower limb uncontrollable by antibiotics.

PMDA's view:
Based on the considerations in Section "7.R.2 Clinical positioning," Section "7.R.3 Efficacy," and Section "7.R.4 Safety," HGF plasmid is positioned as a treatment option for patients with ulcers caused by

chronic arterial occlusive disease (ASO and Buerger's disease) who do not respond adequately to standard drug therapy and are poor candidates for revascularization. Thus, the "INDICATION OR PERFORMANCE" section proposed by the applicant is acceptable.

Next, the applicant's explanation that the association between ulcer size and the efficacy of HGF plasmid is unknown at present, is understandable, and there is no need to specify the ulcer size in the INDICATION OR PERFORMANCE section to restrict the use of HGF plasmid. However, the efficacy of HGF plasmid in the improvement of ulcers that are larger than those for which HGF plasmid has been effective in clinical studies is unknown, and administration of HGF plasmid for such ulcers should not be continued without careful consideration. Thus, the sizes of ulcers for which HGF plasmid has been effective should be specified in the CLINICAL STUDIES section of the package insert, and then the "PRECAUTIONS FOR INDICATION OR PERFORMANCE" section should advise that patients for use of HGF plasmid should be selected with a full knowledge of the content of the CLINICAL STUDIES section concerning the sizes of ulcers for which HGF plasmid has been effective, and an adequate understanding of the efficacy and safety of HGF plasmid.

Furthermore, the "PRECAUTIONS FOR INDICATION OR PERFORMANCE" section should also advise that the efficacy of HGF plasmid in the improvement of rest pain and limb salvage has not been established.

Given the following points, the applicant's explanation (the use of HGF plasmid can be recommended for patients with ulcerations with necrosis or bone or tendon exposure if the necrotic tissue etc. have been removed) is not acceptable, and the use of HGF plasmid in these patients cannot be recommended at present. Thus, the "PRECAUTIONS FOR INDICATION OR PERFORMANCE" section should advise against using HGF plasmid in these patients.

- The efficacy of HGF plasmid has not been demonstrated in US phase II study involving patients with ulcerations with necrosis or bone or tendon exposure.
- Patients with ulcerations with necrosis or bone or tendon exposure were excluded from Japanese clinical studies and clinical research study, there is no clinical experience with HGF plasmid after removing necrotic tissue etc., and there are no clinical study data demonstrating the efficacy and safety of HGF plasmid in these patients.

Based on the above, the appropriate statements in the INDICATION OR PERFORMANCE and PRECAUTIONS FOR INDICATION OR PERFORMANCE sections should be as follows.

**Indication or Performance**

Improvement of ulcers in patients with chronic arterial occlusive disease (arteriosclerosis obliterans and Buerger's disease) who do not respond adequately to standard drug therapy and are poor candidates for revascularization

**Precautions for Indication or Performance**

- The efficacy of Collategene in the improvement of rest pain and limb salvage has not been established.

- Patients for use of Collategene should be selected with a full knowledge of the content of the CLINICAL STUDIES section concerning the sizes of ulcers for which Collategene has been effective, etc., after carefully considering the use of other treatments as well.
- Dot not use Collategene in patients with ulcerations with bone or tendon exposure or necrosis.
- Do not use Collategene in patients with necrotic tissue extending over a wide area of the lower limb or patients with life-threatening infection of the lower limb uncontrollable by antibiotics.

The above conclusion by PMDA will be discussed at the Expert Discussion.

**7.R.6 Dosage and administration or method of use**

The applicant's explanation about "DOSAGE AND ADMINISTRATION OR METHOD OF USE" and "PRECAUTIONS FOR DOSAGE AND ADMINISTRATION OR METHOD OF USE":

**Dosage and Administration or Method of Use**

Collategene should be diluted in Isotonic Sodium Chloride Solution (JP), and 0.5 mg per site of Collategene should be injected into the muscles of the ischemic lower limb in 8 sites (a total of 4 mg). Injections of 3 mL per site of diluted Collategene should be delivered, and if the muscle to be injected is small, the injection volume may be reduced to 2 mL. Injection locations should be selected based on the ischemic state of the limb. Collategene should be dosed every 4 weeks on 2 occasions. If clinical symptoms remain, a third set of injections may be given.

**Precautions for Dosage and Administration or Method of Use**

- Prior to the use of Collategene, injection locations should be determined using angiography or ultrasonography. It is recommended that injections should be performed under ultrasound guidance to confirm intramuscular delivery of the drug solution.

The rationale for "DOSAGE AND ADMINISTRATION OR METHOD OF USE" is as follows:

As to the dose per administration, the number of injection sites, dosing interval, and route of administration of HGF plasmid, HGF plasmid 0.5 mg per site was to be injected into the muscles of the ischemic lower limb in 8 sites Q4W in ASO phase III study, TAO open-label clinical study, and advanced medical care B clinical research study, for the following reasons etc. The results of these studies demonstrated a certain level of efficacy of HGF plasmid.

- A non-clinical study using a rabbit hindlimb ischemia model showed that intramuscular injection of hHGF plasmid 0.5 mg per site (4 sites) resulted in an increase in blood flow (*Gene Ther*. 2001;8:181-9).
- Intramuscular injection of VEGF165 plasmid (0.5 mg per site was injected into the muscles of the ischemic limb in 4 sites Q4W) induced angiogenesis in a foreign clinical trial conducted to analyze the efficacy etc. of VEGF165 plasmid in patients with critical limb ischemia (*Circulation*. 1998;97:1114-23). Furthermore, the angiogenic effect of HGF has been reported to be more pronounced than that of VEGF165 in a rabbit model of hindlimb ischemia (*Circulation*. 1998;97:381-90).
- As to the number of injection sites, 0.5 mg per site of HGF plasmid was injected into the muscles of the

ischemic lower limb in 4 or 8 sites in Stage 2 of Osaka University clinical research study, and there were no clear differences in the efficacy and safety of HGF plasmid between 4 and 8 injections. However, hHGF expression induced by HGF plasmid is considered to be limited to an area in close proximity to the injection site, and higher efficacy of HGF plasmid is expected when injected in 8 sites than in 4 sites.

- As to the dosing interval, since a non-clinical study in rabbits showed that tissue hHGF concentration peaked at Day 7 and tapered off by Day 28 [see Section 4.3.3], it was considered useful to repeat dosing 4 weeks after the first dose to supplement hHGF in the muscle so as to sustain its angiogenic effect.
- As to the injection volume, as hHGF expression is considered to increase with increasing injection volume, 3 mL per site was selected as the maximum volume that can be clinically administered into the skeletal muscle of the lower limb of humans. However, considering the cases where the muscle to be injected is small, e.g. the foot and the distal muscles of the lower leg, the volume was allowed to be reduced to 2 mL.

While HGF plasmid was to be dosed on 2 occasions in ASO phase III study, if clinical symptoms remained etc., a third dose of HGF plasmid was permitted in TAO open-label clinical study and advanced medical care B clinical research study. A total of 6 patients received the third set of injections (2 in TAO open-label clinical study and 4 in advanced medical care B clinical research study), of whom 3 patients were included in efficacy analyses (1 in TAO open-label clinical study and 2 in advanced medical care B clinical research study). None of these 3 patients showed clear worsening of their symptoms after the 3rd set of injections. The 3rd set of injections is expected to contribute to stabilization of symptoms, and is considered clinically meaningful to a certain extent.

Moreover, in ASO phase III study, TAO open-label clinical study, and advanced medical care B clinical research study, angiography, CTA, MRA, or ultrasonography was used to locate arterial occlusion or reduced collateral blood flow, and injections were to be performed under ultrasound guidance to confirm that HGF plasmid was delivered into the muscle selected for each occlusion site (the region to which the anterior tibial artery, posterior tibial artery, or peroneal artery supplies blood). Thus, this information was included in the "PRECAUTIONS FOR DOSAGE AND ADMINISTRATION OR METHOD OF USE" section.

PMDA's view:
Whether 0.5 mg per site of HGF plasmid injected in a total of 8 sites (a total of 4 mg) is optimal is unknown because no other dosage regimens were explored. However, based on ASO phase III study etc., HGF plasmid is expected to have a certain level of efficacy in improving ulcers and has acceptable safety. Thus, 0.5 mg per site of HGF plasmid injected in a total of 8 sites (a total of 4 mg) is acceptable.

As to the third dose of HGF plasmid, only a limited number of patients received the 3rd set of injections, and there are no definitive study data showing the efficacy of 3 sets of injections. Thus, there is no solid evidence to recommend the 3rd set of injections, and HGF plasmid should be dosed on 2 occasions, as a rule.

However, given that no other effective treatments have been established for ulcers to be treated with HGF plasmid, leaving an option of the 3rd set of injections is understandable to a certain extent.

In addition, since selection of injection locations is important information for administration of HGF plasmid, the details of the procedure for selecting injection locations in ASO phase III study, TAO open-label clinical study, and advanced medical care B clinical research study should be described in the CLINICAL STUDIES section of the package insert, and then the PRECAUTIONS FOR DOSAGE AND ADMINISTRATION OR METHOD OF USE section should advise that HGF plasmid should be administered, referring to this procedure.

Based on the above, the statements in the DOSAGE AND ADMINISTRATION OR METHOD OF USE and PRECAUTIONS FOR DOSAGE AND ADMINISTRATION OR METHOD OF USE sections should be modified as shown below.

**Dosage and Administration or Method of Use**
Usually for adults, 0.5 mg per site of Collategene should be injected into the muscles of the ischemic lower limb in 8 sites every 4 weeks on 2 occasions (a total of 4 mg per occasion). If clinical symptoms remain, a third set of injections may be given 4 weeks after the second set of injections. Collategene should be diluted in Isotonic Sodium Chloride Solution (JP) before use, injections of 3 mL per site of diluted Collategene should be delivered, and if the muscle to be injected is small, the injection volume may be reduced to 2 mL.

**Precautions for Dosage and Administration or Method of Use**
Referring to the CLINICAL STUDIES section, injection locations should be selected after locating the ischemic region by diagnostic imaging such as angiography, CTA, MRA, and ultrasonography. Injections should be performed under ultrasound guidance to confirm intramuscular delivery of the drug solution.

The above conclusion by PMDA will be discussed at the Expert Discussion.

### 7.R.7 Qualifications of medical institutions and physicians to use HGF plasmid

HGF plasmid is administered by intramuscular injection, which does not require high technical skills. However, PMDA considers that it is necessary to define the qualifications of medical institutions and physicians to use HGF plasmid for the following reasons.

- When selecting injection locations, it is necessary to locate arterial occlusion or reduced collateral blood flow by diagnostic imaging such as angiography, CTA, MRA, and ultrasonography, and administer HGF plasmid by the method selected for each occlusion site (the region to which the anterior tibial artery, posterior tibial artery, or peroneal artery supplies blood) [see Section 7.R.6]. Injection locations of HGF plasmid need to be selected by physicians with adequate knowledge of and experience in the diagnostic imaging of limb ischemia.
- The wound management of ulcers is important in the treatment of critical limb ischemia. Since multiple departments need to take careful and appropriate actions to select patients who do not respond adequately

to standard drug therapy and are poor candidates for revascularization (the intended population), a system that enables collaboration among multiple departments for diagnosis and treatment is indispensable.

Based on the above, PMDA instructed the applicant to develop the requirements of medical institutions and physicians to use HGF plasmid, and the applicant presented the following requirements.

[Requirements of medical institutions and physicians to use HGF Plasmid]
Medical institutions are required to have physicians who fully understand the efficacy and safety of HGF plasmid and have adequate expertise/experience in the diagnosis/treatment of severe chronic arterial occlusive disease (ASO and Buerger's disease) (specialists of societies specializing in chronic arterial occlusive disease, e.g., the Japanese Circulation Society, the Japanese College of Angiology, the Japanese Society of Limb Salvage & Podiatric Medicine, the Japanese Society of Interventional Cardiology, the Japanese Society for Cardiovascular Surgery, and the Japanese Society for Vascular Surgery). Wound management must be performed with collaboration among multiple departments within the medical institution.

PMDA accepted the applicant's explanation.

The above conclusion by PMDA will be discussed at the Expert Discussion.

## 8 Risk Analysis and Outline of the Review Conducted by PMDA

### 8.1 Post-marketing investigations

The applicant's explanation about post-marketing surveillance of HGF plasmid:
Since the currently available information on the efficacy and safety of HGF plasmid is limited, a post-marketing comparative use-results survey of HGF plasmid-treated patients vs. untreated patients (Table 48) will be conducted to further evaluate the efficacy and safety of HGF plasmid. The data from the comparative use-results survey will be registered in the National Regenerative Medicine Database (NRMD) managed by the Japanese Society for Regenerative Medicine.

**Table 48. Outline of comparative use-results survey (draft)**

| | |
|---|---|
| Objective | To evaluate the efficacy and safety of HGF plasmid |
| Survey method | All-case surveillance |
| Population | Fontaine IV patients who meet the following criteria:<br>HGF plasmid group: All HGF plasmid-treated patients with chronic arterial occlusive disease who do not respond adequately to standard drug therapy and are poor candidates for revascularization<br>Control group: HGF plasmid-untreated patients with chronic arterial occlusive disease (arteriosclerosis obliterans/Buerger's disease) on conservative treatment who are considered poor candidates for revascularization |
| Observation period | 24 months from the start of treatment with HGF plasmid (until treatment discontinuation/dropout if applicable) |
| Efficacy survey items | <Primary endpoint><br>• Rate of complete closure of the ulcer to be assessed (the largest ulcer without necrosis at baseline or enrollment) at Week 12 in the HGF plasmid group or at 12 weeks after enrollment in the control group<br><Secondary endpoints><br>• Change in ulcer diameter (change and percent change) at Week 12 in the HGF plasmid group or at 12 weeks after enrollment in the control group<br>• Total number of ulcers/total ulcer diameter at Week 12 in the HGF plasmid group or at 12 weeks after enrollment in the control group<br>• Lower-extremity amputation and revascularization procedure (Yes/No), and survival outcome |
| Efficacy evaluation method | <Primary analysis><br>• Logistic regression analysis of rate of complete ulcer closure adjusted for the propensity score in the ITT population<br><Secondary analyses><br>• Analysis of covariance of changes in ulcer diameter with the propensity score as a covariate<br>• Comparison of rate of complete ulcer closure using inverse probability weighting of the propensity score or propensity score matching |
| Safety survey items | Presence or absence of adverse events (including administration related reaction [anaphylactoid reaction], haemangioma at administration site, increased progression of malignant tumors, and exacerbation of proliferative diabetic retinopathy or exudative age-related macular degeneration), and their incidences |
| Analysis population | As the ITT population, all patients enrolled in the survey will be included in the efficacy analysis population. The FAS consists of the ITT population, excluding the following patients.<br>HGF plasmid group: Patients who have received any intervention that would affect evaluation by Week 12<br>Control group: Patients other than those with arteriosclerosis obliterans/Buerger's disease |
| Target sample size | HGF plasmid group, 120 patients; control group, 120 patients (a planned sample size with an enrollment period of 3 years)<br><br>According to a pooled analysis of ASO phase III study, TAO open-label clinical study, advanced medical care B clinical research study, and Osaka University clinical research study, complete ulcer closure was observed in 13 of 27 patients (48.1%) in the HGF plasmid group and 1 of 6 patients (16.7%) in the placebo group at Week 12.<br><br>For example, 41 patients per group are required for a 1:1 matched comparison between the HGF plasmid and control groups with a two-sided type I error rate of 5% and 80% power. Even after allowing for dropouts, the above target sample size is sufficient to compare the efficacy and safety of HGF plasmid with those of the comparator. |

## 8.R Outline of the review conducted by PMDA

PMDA's view:

At present, the efficacy of HGF plasmid has not been confirmed, there is limited clinical experience with HGF plasmid, and the information on the safety of HGF plasmid is also limited. Thus, it is necessary to conduct a post-marketing comparative use-results survey, covering all patients treated with HGF plasmid, in order to confirm the efficacy of HGF plasmid and collect safety information.

Since the applicant's plan for a comparative use-results survey has not clearly specified the target number of patients analyzed or the efficacy evaluation method, these need to be discussed on another occasion. As to

the observation period of individual patients, since HGF plasmid is a gene therapy product, and the information on the long-term outcome in the HGF plasmid group is limited, safety information on each patient, and information on the survival rate and the rate of limb salvage should be collected also beyond 2 years, until the end of the survey.

Moreover, since there are no data comparing with the control group among patients with Buerger's disease, evaluation by disease (ASO and Buerger's disease) is also necessary.

The details of a post-marketing use-results survey will be finalized, taking also account of discussion on the efficacy and safety evaluation of HGF plasmid at the Expert Discussion.

## 9. Results of Compliance Assessment Concerning the Application Data and Conclusion Reached by PMDA

### 9.1 PMDA's conclusion concerning the results of document-based GLP/GCP inspections and data integrity assessment

The application data were subjected to a document-based compliance inspection and a data integrity assessment in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. On the basis of the inspection and assessment, PMDA concluded that there were no obstacles to conducting its review based on the application documents submitted.

### 9.2 PMDA's conclusion concerning the results of the on-site GCP inspection

The application data (CTD 5.3.5.1-1.1,[12)] CTD 5.3.5.1-1.2,[12)] CTD 5.3.5.1-2.1,[12)] CTD 5.3.5.2-2.1,[12)] CTD 5.3.5.2-2.2,[12)] CTD 5.3.5.2-3.1 [13)]) were subjected to an on-site GCP inspection, in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. On the basis of the inspection, PMDA concluded that since the studies as a whole were performed in compliance with GCP, there were no obstacles to conducting its review based on the application documents submitted. Though the outcome of the overall assessment of the studies was not affected significantly, the inspection revealed the following findings at some of the study sites used by the applicant and at the sponsor, and the heads of the relevant medical institutions and the sponsor were notified of these findings requiring corrective action.

*Findings requiring corrective action*

Study sites

- The head of the medical institution failed to seek the opinion of the institutional review board at least once a year, concerning the appropriateness of continuing the study at the site.

[12)] Although the present application was filed under the category of regenerative medical products, these clinical studies were initiated before the enforcement of the Ministerial Ordinance on GCP for Regenerative Medical Products. Therefore, the Ministerial Ordinance on GCP for Drugs was applicable to these clinical studies.

[13)] Data from advanced medical care B clinical research study, which was conducted in accordance with ICH-E6 (R1) guideline (ICH-GCP), etc.

- Flaws in the contract for the partial transfer of study-related duties
- Protocol deviations (noncompliance with the conditions of contrast agents for angiography)

Sponsor

- The sponsor failed to perform audits in accordance with the audit plan and the operating procedures.

## 10 Overall Evaluation during Preparation of the Review Report (1)

On the basis of the data submitted, PMDA has concluded that HGF plasmid is expected to have a certain level of efficacy in improving ulcers in patients with chronic arterial occlusive disease (arteriosclerosis obliterans/Buerger's disease) who do not respond adequately to standard drug therapy and are poor candidates for revascularization, and that HGF plasmid has acceptable safety in view of its benefits. Although the currently available information on the efficacy and safety of HGF plasmid is limited, making HGF plasmid available for clinical use as a new treatment option for patients with chronic arterial occlusive disease is of significance.

PMDA considers that HGF plasmid may be approved if HGF plasmid is not considered to have any particular problems based on comments from the Expert Discussion, with conditions and a time limit defined according to Article 23-26 of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. The conditions require the applicant to confirm the efficacy of HGF plasmid and further collect safety information until a certain time limit after market launch, etc. The time limit according to the Article will be finalized, taking account of the post-marketing surveillance plan (e.g., preparation time for marketing, patient enrollment period, observation period of individual patients, preparation time for reapplication for approval), based on comments from the Expert Discussion.

**Review Report (2)**

February 1, 2019

**Product Submitted for Approval**

| | |
|---|---|
| **Brand name** | Collategene Intramuscular Injection 4 mg |
| **Non-proprietary name** | Beperminogene Perplasmid |
| **Applicant** | AnGes, Inc. |
| **Date of Application** | January 22, 2018 |

**List of Abbreviations**

See Appendix.

## 1. Content of the Review

Comments made during the Expert Discussion and the subsequent review conducted by the Pharmaceuticals and Medical Devices Agency (PMDA) are summarized below. The expert advisors present during the Expert Discussion were nominated based on their declarations etc. concerning the product submitted for marketing approval, in accordance with the provisions of the Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

### 1.1 Efficacy

PMDA's conclusion:

Based on the results from ASO phase III study, TAO open-label clinical study, and advanced medical care B clinical research study, HGF plasmid is expected to have a certain level of efficacy in improving ulcers in ASO or Buerger's disease patients with critical limb ischemia who do not respond adequately to standard drug therapy and are poor candidates for revascularization (patients with ulcerations with bone or tendon exposure or necrosis were excluded). However, since the currently available information on the efficacy of HGF plasmid is extremely limited, it is necessary to evaluate the efficacy of HGF plasmid also after the market launch [Section "7.R.3 Efficacy" in the Review Report (1)].

At the Expert Discussion, the expert advisors supported the above conclusion by PMDA. The expert advisors made the following comment.

- Since the number of patients with complete ulcer closure was limited in the above clinical studies and clinical research study, and there were patients who were excluded from the studies, depending on their disease condition, the efficacy of HGF plasmid has been demonstrated in not all patients with critical limb ischemia, and patients for use of HGF plasmid should be selected, with an understanding of limitations of its efficacy. Thus, it is recommended that the information on arterial occlusion sites, the injection locations of HGF plasmid, and the change in ulcer size over time in individual patients, regardless of whether

HGF plasmid was effective or not, in the Japanese clinical studies and clinical research study, should be provided to healthcare professionals in clinical practice, using informative materials etc.

Though the true endpoint for the treatment of critical limb ischemia is limb salvage, the efficacy of HGF plasmid in limb salvage has not been demonstrated, and its effect in ulcer closure is also limited. Thus, PMDA instructed the applicant to develop the above-mentioned materials to be distributed to healthcare professionals in clinical practice. The applicant responded appropriately, and PMDA accepted it.

### 1.2 Safety

PMDA's conclusion:

PMDA reviewed the occurrence of angiogenesis-related adverse events (the risk of benign or malignant neoplasms etc., the new onset and exacerbation of diabetic retinopathy/age-related macular degeneration), which are expected from the mechanism of action etc. of HGF plasmid, and anaphylactic reaction, and concluded that the safety of HGF plasmid is tolerable in patients with critical limb ischemia. However, as the currently available safety information is limited, it is necessary to collect post-marketing information and appropriately provide the obtained information to healthcare professionals in clinical practice. The package insert should state that HGF plasmid is contraindicated in patients with current or previous malignant tumors in injection-site muscles and surrounding tissues, and advise that the use of HGF plasmid should be determined carefully for patients with proliferative diabetic retinopathy or exudative age-related macular degeneration [Section "7.R.4 Safety" in the Review Report (1)].

At the Expert Discussion, the expert advisors supported the above conclusion by PMDA.

### 1.3 Indication or performance

PMDA's conclusion:

In view of the considerations presented in Section "7.R.5 Indication or performance" in the Review Report (1), the appropriate statements in the INDICATION OR PERFORMANCE and PRECAUTIONS FOR INDICATION OR PERFORMANCE sections should be as follows.

**Indication or Performance**

Improvement of ulcers in patients with chronic arterial occlusive disease (arteriosclerosis obliterans and Buerger's disease) who do not respond adequately to standard drug therapy and are poor candidates for revascularization

**Precautions for Indication or Performance**

- The efficacy of Collategene in the improvement of rest pain and limb salvage has not been established.
- Patients for use of Collategene should be selected with a full knowledge of the content of the CLINICAL STUDIES section concerning the sizes of ulcers for which Collategene has been effective, etc., after carefully considering the use of other treatments as well.
- Dot not use Collategene in patients with ulcerations with bone or tendon exposure or necrosis.

- Do not use Collategene in patients with necrotic tissue extending over a wide area of the lower limb or patients with life-threatening infection of the lower limb uncontrollable by antibiotics.

At the Expert Discussion, the expert advisors supported the above conclusion by PMDA.

PMDA instructed the applicant to include the above statements in the INDICATION OR PERFORMANCE and PRECAUTIONS FOR INDICATION OR PERFORMANCE sections. The applicant responded appropriately, and PMDA accepted it.

**1.4 Dosage and administration or method of use**

PMDA's conclusion:

In view of the considerations presented in Section "7.R.6 Dosage and administration or method of use" in the Review Report (1), the statements in the DOSAGE AND ADMINISTRATION OR METHOD OF USE and PRECAUTIONS FOR DOSAGE AND ADMINISTRATION OR METHOD OF USE sections should be modified as shown below.

**Dosage and Administration or Method of Use**

Usually for adults, 0.5 mg per site of Collategene should be injected into the muscles of the ischemic lower limb in 8 sites every 4 weeks on 2 occasions (a total of 4 mg per occasion). If clinical symptoms remain, a third set of injections may be given 4 weeks after the second set of injections. Collategene should be diluted in Isotonic Sodium Chloride Solution (JP) before use, injections of 3 mL per site of diluted Collategene should be delivered, and if the muscle to be injected is small, the injection volume may be reduced to 2 mL.

**Precautions for Dosage and Administration or Method of Use**

Referring to the CLINICAL STUDIES section, injection locations should be selected after locating the ischemic region by diagnostic imaging such as angiography, CTA, MRA, and ultrasonography. Injections should be performed under ultrasound guidance to confirm intramuscular delivery of the drug solution.

At the Expert Discussion, the expert advisors supported the above conclusion by PMDA. The expert advisors made the following comment.

- Since the selection of injection locations and the method of injection are important information for administration of HGF plasmid, it is necessary to develop informative materials concerning the injection locations and the method of injection.

PMDA instructed the applicant to develop and distribute the above materials to healthcare professionals in clinical practice. The applicant responded appropriately, and PMDA accepted it.

### 1.5 Qualifications of medical institutions and physicians to use HGF plasmid

In view of the considerations presented in Section "7.R.7 Qualifications of medical institutions and physicians to use HGF plasmid" in the Review Report (1), PMDA accepted the following requirements of medical institutions and physicians to use HGF plasmid explained by the applicant.

[Requirements of medical institutions and physicians to use HGF Plasmid]
Medical institutions are required to have physicians who fully understand the efficacy and safety of HGF plasmid and have adequate expertise/experience in the diagnosis/treatment of severe chronic arterial occlusive disease (ASO and Buerger's disease) (specialists of societies specializing in chronic arterial occlusive disease, e.g., the Japanese Circulation Society, the Japanese College of Angiology, the Japanese Society of Limb Salvage & Podiatric Medicine, the Japanese Society of Interventional Cardiology, the Japanese Society for Cardiovascular Surgery, and the Japanese Society for Vascular Surgery). Wound management must be performed with collaboration among multiple departments within the medical institution.

At the Expert Discussion, the expert advisors made the following comments on the qualifications of medical institutions and physicians to use HGF plasmid proposed by the applicant.

- Physicians who have adequate expertise/experience in diagnosis/treatment have been defined as "specialists" of societies specializing in chronic arterial occlusive disease. Since some societies do not use the term "specialists," this should be modified.
- Physicians are required to attend workshop to learn the method of use, including the procedure for selecting injection locations, etc.

Taking account of the above comments from the Expert Discussion, PMDA instructed the applicant to reconsider the qualifications of medical institutions and physicians to use HGF plasmid and the prerequisite for using HGF plasmid (attendance at workshop on HGF plasmid to learn the method of use, etc.). The applicant appropriately addressed these issues and responded that the requirements of medical institutions and physicians to use HGF plasmid will be defined as follows.

[Requirements of medical institutions and physicians to use HGF plasmid]
Medical institutions are required to have physicians who fully understand the efficacy and safety of HGF plasmid, have adequate expertise/experience in the diagnosis/treatment of severe chronic arterial occlusive disease (ASO and Buerger's disease), and have been given necessary explanation about HGF plasmid at workshop provided by the manufacturer, etc. (board-certified physicians of societies specializing in chronic arterial occlusive disease, i.e., the Japanese Circulation Society, the Japanese College of Angiology, the Japanese Society of Limb Salvage & Podiatric Medicine, the Japanese Society of Footcare, the Japanese Society of Interventional Cardiology, the Japanese Society for Cardiovascular Surgery, and the Japanese Society for Vascular Surgery). Wound management must be performed with collaboration among multiple departments within the medical institution.

PMDA accepted the above.

### 1.6 Plan for approval condition-based post-marketing evaluation (draft)

In view of the considerations presented in Section "8.1 Post-marketing investigations" in the Review Report (1), PMDA concluded that the applicant should draft a post-marketing survey and the survey plan, taking account of the following points.

- At present, there is limited clinical experience with HGF plasmid, and the efficacy of HGF plasmid has not been fully confirmed. Thus, it is necessary to conduct an approval condition-based post-marketing evaluation, covering all patients treated with HGF plasmid, in order to confirm the efficacy of HGF plasmid and collect safety information.
- As to the observation period, since HGF plasmid is a gene therapy product, and the information on the long-term outcome in the HGF plasmid group is limited, safety information and information on the survival rate and the rate of limb salvage should be collected also beyond 2 years, until the end of the survey.
- The target number of patients analyzed has not been defined. It is necessary to discuss a clinically meaningful treatment difference in the rate of complete ulcer closure and then specify the target number of patients analyzed.
- As efficacy evaluation method, a covariate of the propensity score should be defined.

At the Expert Discussion, the expert advisors supported the above conclusion by PMDA. The expert advisors commented that hemodynamic parameters should be selected as a secondary endpoint, and that the survey items should include the details and use of foot care.

Taking account of the above comments from the Expert Discussion, PMDA instructed the applicant to reconsider the plan for the approval condition-based post-marketing evaluation.

The applicant submitted an outline of the plan for the approval condition-based post-marketing evaluation (draft) presented in Table 49 and explained that the comments from PMDA and the expert advisors will be addressed.

PMDA accepted the plan for the approval condition-based post-marketing evaluation (draft) and the applicant's explanation.

**Table 49. Plan for the approval condition-based post-marketing evaluation (draft)**

| | |
|---|---|
| Objective | To evaluate the efficacy and safety of HGF plasmid |
| Survey method | All-case surveillance |
| Population | Fontaine IV patients who meet the following criteria:<br>HGF plasmid group: All HGF plasmid-treated patients with chronic arterial occlusive disease (arteriosclerosis obliterans and Buerger's disease) who do not respond adequately to standard drug therapy and are poor candidates for revascularization<br>Control group: HGF plasmid-untreated patients with chronic arterial occlusive disease (arteriosclerosis obliterans and Buerger's disease) who do not respond adequately to standard drug therapy and are poor candidates for revascularization |
| Observation period | HGF plasmid group: up to 5 years<br>Control group: 12 weeks after enrollment |
| Efficacy survey items | <Primary endpoint><br>• Rate of complete closure of the ulcer to be assessed (the largest ulcer without necrosis at baseline or enrollment) at Week 12 in the HGF plasmid group or at 12 weeks after enrollment in the control group<br><Secondary endpoints><br>• Change in ulcer size (√[major axis × minor axis]) (change and percent change) at Week 12 in the HGF plasmid group or at 12 weeks after enrollment in the control group<br>• Total number of ulcers/total ulcer diameter at Week 12 in the HGF plasmid group or at 12 weeks after enrollment in the control group<br>• Hemodynamic parameters at Week 12 in the HGF plasmid group or at 12 weeks after enrollment in the control group<br>• Lower-extremity amputation and survival outcome in the HGF plasmid group |
| Efficacy evaluation method | <Primary analysis><br>• Logistic regression analysis of rate of complete ulcer closure using inverse probability weighting of the propensity score in the ITT population<br><Secondary analyses><br>• Logistic regression analysis of rate of complete ulcer closure with the propensity score as a covariate and analysis of covariance of ulcer size (√[major axis × minor axis])<br>• Comparison of rate of complete ulcer closure after propensity score matching using Fisher's exact test<br><br>The following items will be used to estimate the propensity score.<br>sex, age, disease type, size of the ulcer to be assessed before baseline or enrollment (√[major axis × minor axis]), the number of diseased arteries below the knee, previous revascularization before baseline or enrollment, BMI, diabetic neuropathy, dialysis, serum albumin level, local infection of the ulcer to be assessed, foot care, walking without assistance, smoking habit |
| Safety survey items | Presence or absence of adverse events (including administration related reaction [anaphylactoid reaction], haemangioma at administration site, increased progression of malignant tumors, and exacerbation of proliferative diabetic retinopathy or exudative age-related macular degeneration), and their incidences |
| Analysis population | As the ITT population, all patients enrolled in the survey will be included in the efficacy analysis population. The FAS consists of the ITT population, excluding patients who have received any intervention that would affect evaluation (revascularization, intensive wound care, lower-extremity amputation, etc.) by Week 12 in the HGF plasmid group or by 12 weeks after enrollment in the control group. |
| Target sample size | HGF plasmid group, 120 patients; control group, 80 patients<br><br>According to a pooled analysis of ASO phase III study, TAO open-label clinical study, advanced medical care B clinical research study, and Osaka University clinical research study, complete ulcer closure was observed in 13 of 27 patients (48.1%) in the HGF plasmid group and 1 of 6 patients (16.7%) in the placebo group at Week 12.<br><br>Assuming that the rate of complete ulcer closure is [redacted]% in the HGF plasmid group and [redacted]% in the control group, [redacted] patients in the HGF plasmid group and [redacted] patients in the control group are required to detect a treatment difference ([redacted]%) with a two-sided type I error rate of 5% and 80% power. Allowing for a dropout rate of 10% in each group, 108 patients in the HGF plasmid group and 72 patients in the control group are needed. This sample size is sufficient to detect a difference in the rate of complete ulcer closure between the HGF plasmid and control groups ([redacted]% in the HGF plasmid group and [redacted]% in the control group) with [redacted]% power. |

### 1.7 Others

#### 1.7.1 Quality

In response to PMDA's request during the preparation of the Review Report (1), the applicant explained purity tests for the control of product-related impurities [Section "3.R.1 Control of product-related impurities" in the Review Report (1)] as follows:

- Validation of the proposed HPLC method for content uniformity was performed, which demonstrated that this analytical procedure is suitable for the control of the content of the OC form and of the LN form in the product.
- This analytical procedure will be modified and included as purity tests for the drug substance and product.

The applicant also explained that endotoxins will be included in the product specifications.

PMDA accepted the applicant's explanation and concluded, based on the submitted data and the above considerations, that the quality of the drug substance and product is adequately controlled.

#### 1.7.2 Necessity of the designation of regenerative medical product

As regards the designation of biological products, specified biological products, and designated regenerative medical products (PFSB/ELD Notification No.1105-1 and No.1105-2, dated November 5, 2014), since no human- or animal-derived raw materials etc. are used in the drug substance or product manufacturing process, the product needs not be classified as a designated regenerative medical product.

## 2. Overall Evaluation

As a result of the above review, PMDA has concluded that the product may be approved after modifying the indication or performance and dosage and administration or method of use as shown below, provided that the package insert includes precautionary statements and healthcare professionals are informed of how to use the product properly after the market launch. The approval should be conditional and time-limited in accordance with Article 23-26 of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. The time limit according to the Article should be 5 years. The product needs not be classified as a designated regenerative medical product.

**Indication or Performance**

Improvement of ulcers in patients with chronic arterial occlusive disease (arteriosclerosis obliterans and Buerger's disease) who do not respond adequately to standard drug therapy and are poor candidates for revascularization

**Dosage and Administration or Method of Use**

Usually for adults, 0.5 mg per site of Collategene should be injected into the muscles of the ischemic lower limb in 8 sites every 4 weeks on 2 occasions (a total of 4 mg per occasion). If clinical symptoms remain, a third

set of injections may be given 4 weeks after the second set of injections. Collategene should be diluted in Isotonic Sodium Chloride Solution (JP) before use, injections of 3 mL per site of diluted Collategene should be delivered, and if the muscle to be injected is small, the injection volume may be reduced to 2 mL.

**Conditions of Approval**

1. The applicant is required to ensure that the product is used under the supervision of physicians with adequate knowledge of and experience in treating severe chronic arterial occlusive disease at medical institutions where wound management is performed by collaboration among multiple departments.
2. The applicant is required to conduct an approval condition-based post-marketing evaluation in all patients treated with the product during the period between the conditional and time-limited approval and reapplication for marketing approval.

**List of Abbreviations**

| | |
|---|---|
| ABPI | Ankle brachial pressure index |
| AEC | Anion exchange chromatography |
| AFP | Alpha-fetoprotein |
| AGE | Agarose gel electrophoresis |
| ALP | Alkaline phosphatase |
| ASO | Arteriosclerosis obliterans |
| AUC | Area under the drug concentration time curve |
| $AUC_{inf}$ | Area under the drug concentration time curve from 0 to infinity |
| $AUC_{last}$ | Area under the drug concentration time curve from 0 to the last quantifiable timepoint |
| BCA | Bicinchoninic acid |
| Beperminogene Perplasmid | Plasmid DNA encoding the gene of human HGF |
| B-C product | [redacted] product (the product produced [redacted] from the drug substance manufactured by [redacted]) |
| B-H product | [redacted] product (the product produced [redacted] from the drug substance manufactured by [redacted]) |
| bp | base pair |
| CA19-9 | Carbohydrate antigen 19-9 |
| CCC form | Covalently closed circular form |
| cDNA | Complementary deoxyribonucleic acid |
| CEA | Carcinoembryonic antigen |
| CHO cells | Chinese hamster ovary cells |
| CI | Confidence interval |
| Cmax | Maximum blood concentration |
| CMV | Cytomegalovirus |
| c-Met | HGF receptor tyrosine kinase |
| [redacted] cells | [redacted] cells |
| CRF | Case Report Form |
| CRP | C-reactive protein |
| CTA | Computed tomography angiography |
| DNA | Deoxyribonucleic acid |
| ELISA | Enzyme-linked immunosorbent assay |
| EWMA | European Wound Management Association |
| FAS | Full Analysis Set |
| FDA | Food and Drug Administration |
| FOB | Functional observation battery |
| HASMC | Human aortic smooth muscle cells |
| HCP | Host cell protein |
| HGF | Hepatocyte growth factor |
| hHGF | Human hepatocyte growth factor |
| HIC | Hydrophobic interaction chromatography |
| HPLC | High performance liquid chromatography |
| HUVEC | Human umbilical vein endothelial cells |
| Japanese clinical practice guidelines | Guidelines for the management of peripheral arterial occlusive diseases (JCS 2015) |
| LacZ | β−galactosidase |
| LN form | Linear form |
| MCB | Master cell bank |

| | |
|---|---|
| MedDRA/J | Medical Dictionary for regulatory activities Japanese version |
| MRA | Magnetic Resonance Angiography |
| NZW | New Zealand white |
| OC form | Open circular form |
| PMDA | Pharmaceuticals and Medical Devices Agency |
| Pro-HGF | Pro-Hepatocyte growth factor (an inactive precursor) |
| p[redacted] | [redacted] (a plasmid vector with no inserted hHGF gene) |
| PVR | Pulse volume recording |
| Q-PCR | Quantitative polymerase chain reaction |
| Q-PCR assay A | An assay using PCR amplification of 79 bp sequence |
| Q-PCR assay B | An assay using PCR amplification of 241 bp sequence |
| Q2W | quaque 2 weeks |
| Q4W | quaque 4 weeks |
| rhbFGF | Recombinant human basic fibroblast growth factor |
| rhHGF | Recombinant human hepatocyte growth factor |
| rhVEGF | Recombinant human vascular endothelial growth factor |
| RNA | Ribonucleic acid |
| SEC | Size exclusion chromatography |
| S-F product | [redacted] product (the product produced [redacted] from the drug substance manufactured by [redacted]) |
| S-P product | [redacted] product (the product produced [redacted] from the drug substance manufactured by [redacted]) |
| TAO | Thromboangiitis obliterans |
| TBPI | Toe-brachial Pressure Index |
| TcPO2 | Transcutaneous partial pressure of oxygen |
| The product | Collategene Intramuscular Injection 4 mg |
| $t_{max}$ | Time of maximum concentration |
| $t_{1/2}$ | Elimination half-life |
| [redacted] cells | [redacted] cells |
| VAS | Visual Analogue Scale |
| VEGF | Vascular endothelial growth factor |
| WCB | Working cell bank |

**Supplement**

**Summary of Referenced Data and Outline of PMDA Review Concerning Drug Master File for Collategen Intramuscular Injections 4 mg (DMF Registration Number, [redacted])**

| | |
|---|---|
| **Drug Master File** | |
| **Brand Name** | [redacted] |
| **Non-proprietary Name** | Beperminogene Perplasmid |
| **Name of Registrant** | [redacted] |
| **Registration Number** | [redacted] |

## 1. Summary of Referenced Data

### 1.1 Drug substance

#### 1.1.1 Generation and control of cell substrate

hHGF cDNA was selected from a human-leukocyte cDNA library by plaque hybridization. Gene fragments encoding the selected hHGF cDNA and the bovine growth hormone polyadenylation signal region were inserted into a p[redacted] vector having a minimally sized sequence homologous to human genome, to ensure that hHGF cDNA is expressed under the regulation of a cytomegalovirus (CMV) promoter/enhancer. This is how a plasmid vector with an expression construct (p[redacted]) was prepared. The plasmid vector was transfected into the *E. coli* strain ([redacted] strain) to select a clonal strain with a targeted phenotype from the transformed *E. coli*. The selected *E. coli* clonal strain was used to prepare the master cell bank (MCB) and the working cell bank (WCB).

MCB and WCB were subjected to characterization and in-process tests (viability assay, host identification, non-host contamination, plasmid DNA retention, [redacted], [redacted], and bacteriophage testing). Genetic stability during the production was confirmed by characterization (viability assay, plasmid DNA retention, [redacted], and [redacted]) of cells passaged more times than those in routine production. The MCB and WCB are stored at −70°C. A new MCB will not be prepared for the life of the product, but a new WCB will be prepared as necessary. When a new WCB is prepared, its qualification is confirmed by above-mentioned characterization testing.

#### 1.1.2 Manufacturing process

The manufacturing process for the drug substance consists of preculture and main culture, collection of cultured bacterial cells, bacteriolysis, ultrafiltration, ammonium sulfate precipitation and filtration, hydrophobic interaction chromatography (HIC), anion exchange chromatography (AEC), size exclusion chromatography (SEC), ultrafiltration, final filtration, testing, and storage. The prepared drug substance is stored in polypropylene container at −70°C, protected from light.

Of these processes, the critical steps are main culture, bacteriolysis, ammonium sulfate precipitation and filtration, HIC, and AEC.

Process validation of the manufacturing process for the drug substance was carried out at commercial scale.

### 1.1.3 Manufacturing process development

The major changes in the manufacturing process of the drug substance during its development are described below. The Osaka University clinical research study, ASO phase III study, TAO open-label clinical study, US phase II study, and US IHD phase I study used the drug product manufactured from the drug substance produced by [redacted] process (Process A). Advanced medical care B clinical research study used the drug product manufactured from the drug substance produced by [redacted] process (Process D1). In [redacted], it was later changed to Process D2 and Proposed Process.

- From Process A to Process D1: [redacted] and [redacted], [redacted], [redacted], etc.
- From Process D1 to Process D2: [redacted]
- From Process D2 to Proposed Process: [redacted]

The comparability between the pre-change and post-change drug substances was demonstrated by comparability assessment of their quality attributes performed at the time of process changes.