Report on the Deliberation Results

June 9, 2015
Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau
Ministry of Health, Labour and Welfare

[Brand name]	Xiaflex Inj.
[Non-proprietary name]	Collagenase (Clostridium Histolyticum) (JAN*)
[Applicant]	Asahi Kasei Pharma Corporation
[Date of application]	July 31, 2014

[Results of deliberation]
In the meeting held on June 5, 2015, the First Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The re-examination period is 8 years. The drug substance and the drug product are both classified as powerful drugs. The product is not classified as a biological product or as a specified biological product.

[Conditions for approval]
The applicant is required to:
• Develop and appropriately implement a risk management plan; and
• Take appropriate measures to ensure that the product is used only by physicians trained on the product and fully versed in its safety and efficacy, and therapeutic procedures.

**Japanese Accepted Name (modified INN)*

Review Report

May 18, 2015
Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

[Brand name]	Xiaflex Inj.
[Non-proprietary name]	Collagenase (Clostridium Histolyticum)
[Applicant]	Asahi Kasei Pharma Corporation
[Date of application]	July 31, 2014
[Dosage form/Strength]	Lyophilized powder for solution for injection: One vial contains 0.9 mg of Collagenase (Clostridium Histolyticum)[1] to be reconstituted prior to administration.
[Application classification]	Prescription drug, (1) Drug with a new active ingredient
[Definition]	Collagenase (Clostridium Histolyticum) is a mixture of class I and class II collagenases produced in *Clostridium histolyticum*. The class I and class II collagenases are consisting of 1008 and 991 amino acid residues each.
[Chemical Structure]	See Attachments 1 and 2
[Items warranting special mention]	None
[Reviewing office]	Office of New Drug I

[1] The vial, provided with diluent, is overfilled to ensure that a volume of the reconstituted solution containing the labeled dose (0.58 mg) can be withdrawn from it.

Amino acid sequence

Class I collagenase

IANTNSEKYD FEYLNGLSYT ELTNLIKNIK WNQINGLFNY STGSQKFFGD
KNRVQAIINA LQESGRTYTA NDMKGIETFT EVLRAGFYLG YYNDGLSYLN
DRNFQDKCIP AMIAIQKNPN FKLGTAVQDE VITSLGKLIG NASANAEVVN
NCVPVLKQFR ENLNQYAPDY VKGTAVNELI KGIEFDFSGA AYEKDVKTMP
WYGKIDPFIN ELKALGLYGN ITSATEWASD VGIYYLSKFG LYSTNRNDIV
QSLEKAVDMY KYGKIAFVAM ERITWDYDGI GSNGKKVDHD KFLDDAEKHY
LPKTYTFDNG TFIIRAGEKV SEEKIKRLYW ASREVKSQFH RVVGNDKALE
VGNADDVLTM KIFNSPEEYK FNTNINGVST DNGGLYIEPR GTFYTYERTP
QQSIFSLEEL FRHEYTHYLQ ARYLVDGLWG QGPFYEKNRL TWFDEGTAEF
FAGSTRTSGV LPRKSILGYL AKDKVDHRYS LKKTLNSGYD DSDWMFYNYG
FAVAHYLYEK DMPTFIKMNK AILNTDVKSY DEIIKKLSDD ANKNTEYQNH
IQELADKYQG AGIPLVSDDY LKDHGYKKAS EVYSEISKAA SLTNTSVTAE
KSQYFNTFTL RGTYTGETSK GEFKDWDEMS KKLDGTLESL AKNSWSGYKT
LTAYFTNYRV TSDNKVQYDV VFHGVLTDNA DISNNKAPIA KVTGPSTGAV
GRNIEFSGKD SKDEDGKIVS YDWDFGDGAT SRGKNSVHAY KKTGTYNVTL
KVTDDKGATA TESFTIEIKN EDTTTPITKE MEPNDDIKEA NGPIVEGVTV
KGDLNGSDDA DTFYFDVKED GDVTIELPYS GSSNFTWLVY KEGDDQNHIA
SGIDKNNSKV GTFKATKGRH YVFIYKHDSA SNISYSLNIK GLGNEKLKEK
ENNDSSDKAT VIPNFNTTMQ GSLLGDDSRD YYSFEVKEEG EVNIELDKKD
EFGVTWTLHP ESNINDRITY GQVDGNKVSN KVKLRPGKYY LLVYKYSGSG
NYELRVNK

Molecular formula:

$C_{5101}H_{7775}N_{1329}O_{1610}S_{14}$: 113,926.16

Amino acid sequence

Class II collagenase

AVDKNNATAA VQNESKRYTV SYLKTLNYYD LVDLLVKTEI ENLPDLFQYS
SDAKEFYGNK TRMSFIMDEI GRRAPQYTEI DHKGIPTLVE VVRAGFYLGF
HNKELNEINK RSFKERVIPS ILAIQKNPNF KLGTEVQDKI VSATGLLAGN
ETAPPEVVNN FTPIIQDCIK NMDRYALDDL KSKALFNVLA APTYDITEYL
RATKEKPENT PWYGKIDGFI NELKKLALYG KINDNNSWII DNGIYHIAPL
GKLHSNNKIG IETLTEVMKI YPYLSMQHLQ SADQIERHYD SKDAEGNKIP
LDKFKKEGKE KYCPKTYTFD DGKVIIKAGA RVEEEKVKRL YWASKEVNSQ
FFRVYGIDKP LEEGNPDDIL TMVIYNSPEE YKLNSVLYGY DTNNGGMYIE
PDGTFFTYER KAEESTYTLE ELFRHEYTHY LQGRYAVPGQ WGRTKLYDND
RLTWYEEGGA ELFAGSTRTS GILPRKSIVS NIHNTTRNNR YKLSDTVHSK
YGASFEFYNY ACMFMDYMYN KDMGILNKLN DLAKNNDVDG YDNYIRDLSS
NHALNDKYQD HMQERIDNYE NLTVPFVADD YLVRHAYKNP NEIYSEISEV
AKLKDAKSEV KKSQYFSTFT LRGSYTGGAS KGKLEDQKAM NKFIDDSLKK
LDTYSWSGYK TLTAYFTNYK VDSSNRVTYD VVFHGYLPNE GDSKNSLPYG
KINGTYKGTE KEKIKFSSEG SFDPDGKIVS YEWDFGDGNK SNEENPEHSY
DKVGTYTVKL KVTDDKGESS VSTTTAEIKD LSENKLPVIY MHVPKSGALN
QKVVFYGKGT YDPDGSIAGY QWDFGDGSDF SSEQNPSHVY TKKGEYTVTL
RVMDSSGQMS EKTMKIKITD PVYPIGTEKE PNNSKETASG PIVPGIPVSG
TIENTSDQDY FYFDVITPGE VKIDINKLGY GGATWVVYDE NNNAVSYATD
DGQNLSGKFK ADKPGRYYIH LYMFNGSYMP YRINIEGSVG R

Molecular formula:

$C_{5063}H_{7726}N_{1314}O_{1579}S_{22}$: 112,970.80

Review Results

May 18, 2015

[Brand name]	Xiaflex Inj.
[Non-proprietary name]	Collagenase (Clostridium Histolyticum)
[Applicant]	Asahi Kasei Pharma Corporation
[Date of application]	July 31, 2014

[Results of review]

Based on the submitted data, the Pharmaceuticals and Medical Devices Agency (PMDA) has concluded that the efficacy of the product in the treatment of Dupuytren's contracture has been demonstrated and its safety is acceptable in view of its observed benefits.

As a result of its regulatory review, PMDA has concluded that the product may be approved for the indication and dosage and administration as shown below, with the following conditions.

[Indication] Dupuytren's contracture

[Dosage and administration] The usual adult dosage is 0.58 mg of Collagenase (Clostridium Histolyticum) given as an injection into a cord with contracture of a metacarpophalangeal joint or proximal interphalangeal joint. If a satisfactory response has not been achieved, injections may be repeated up to 3 times per cord at 1-month intervals.

[Conditions for approval] The applicant is required to:

- Develop and appropriately implement a risk management plan; and
- Take appropriate measures to ensure that the product is used only by physicians trained on the product and fully versed in its safety and efficacy, and therapeutic procedures.

Review Report (1)

April 3, 2015

I. Product Submitted for Registration

[Brand name]	Xiaflex Inj.
[Non-proprietary name]	Collagenase (Clostridium Histolyticum)
[Applicant]	Asahi Kasei Pharma Corporation
[Date of application]	July 31, 2014
[Dosage form/Strength]	Lyophilized powder for solution for injection: One vial contains 0.9 mg of Collagenase (Clostridium Histolyticum)[1] to be reconstituted prior to administration.
[Proposed indication]	Dupuytren's contracture with a palpable cord
[Proposed dosage and administration]	The usual adult dosage is 0.58 mg of collagenase clostridium histolyticum given as an injection into a cord with contracture of a metacarpophalangeal joint or proximal interphalangeal joint. If a satisfactory response has not been achieved, injections may be repeated into the cord with contracture located on the same joint up to 3 times at approximately 4-week intervals.

II. Summary of the Submitted Data and Outline of the Review by Pharmaceuticals and Medical Devices Agency

The submitted data and the review thereof by the Pharmaceuticals and Medical Devices Agency (PMDA) are as summarized below.

1. Origin or history of discovery, use in foreign countries, and other information

Dupuytren's contracture is a progressive fibroproliferative disorder affecting the palmar fascia. Abnormal deposition of collagen produced by myofibroblasts and other cells causes the formation of nodules and contracture cords in the palmar fascia. As the disease progresses, resultant flexion contracture causes difficulty in extending the finger, which prevents patients with Dupuytren's contracture from performing everyday tasks.[2] Flexion contracture often involves joints of several adjacent fingers, and may occur in any of the metacarpophalangeal (MP), proximal interphalangeal (PIP), and distal interphalangeal (DIP) joints. In Japan, Dupuytren's contracture is mainly treated by surgery. Surgical procedures, which involve skin incisions of the palm and fingers to cut the tissue, or excise the affected tissue, are highly invasive and carry the risk of surgical complications such as neural and arterial injury.[3]

Xiaflex for Injection is an injectable preparation developed by Auxilium Pharmaceuticals, Inc. in the United States, containing Collagenase (Clostridium Histolyticum) (CCH), a collagenolytic enzyme

[1] The vial, provided with diluent, is overfilled to ensure that a volume of the reconstituted solution containing the labeled dose (0.58 mg) can be withdrawn from it.
[2] *Dupuytren's Disease,* p.59-116, Martin Dunitz Ltd;2000, and the like
[3] *J Bone Joint Surg Am.* 2007;89:189-198, and the like

derived from a bacterium of the genus Clostridium, *Clostridium histolyticum,* as the active ingredient. Collagenase (Clostridium Histolyticum) contains 2 types of collagenases: class I and class II collagenases. These two types of collagenases have different substrate specificities in a way that their actions on collagen are complementary, which is considered to contribute to efficient collagen hydrolysis. When injected into a cord of Dupuytren's contracture, CCH is expected to disrupt the predominantly collagenous cord, thus achieving a therapeutic effect. This idea prompted the development of the product.

CCH was first approved in the United States in February 2010, and has been approved for the treatment of Dupuytren's contracture in 35 countries including the United Kingdom and Canada as of January 2015.

The Japanese name of Dupuytren's contracture used by the Japanese Society for Surgery of the Hand and other organizations in Japan is employed in the Japanese original text of this review report.

2. Data relating to quality

2.A Summary of the submitted data

2.A.(1) Drug substance

2.A.(1).1) Preparation and control of cell substrates

The drug substance, Collagenase (Clostridium Histolyticum), is manufactured through fermentation of *Clostridium histolyticum* (*C. histolyticum*) under anaerobic conditions. [redacted] The purity study (phage assay) of the master cell bank (MCB), the working cell bank (WCB), and culture fluid after fermentation showed that they were free of adventitious microbial agents.

While there is no plan to generate a new MCB, the WCB may be newly prepared on an as-needed basis.

2.A.(1).2) Manufacturing process

The manufacturing process of the drug substance consists of the following steps: seed culture, fermentation, harvesting, hydrophobic interaction chromatography, ultrafiltration [1], ion-exchange chromatography, ultrafiltration [2 and 3], and mixing. [redacted]

The manufacturing process of the drug substance was subject to process validation in the scale of commercial production, and all steps of the process were found to be appropriately controlled.

2.A.(1).3) Adventitious agent safety evaluation

[redacted]

The purity of the MCB, WCB, and culture fluid after fermentation was studied, and the results showed that they were free of adventitious microbial agents. [redacted]

C. histolyticum is a toxin-producing strain secreting toxins α, β, γ, δ, and ε, and among them, α-toxin and ε-toxin exhibit hemolytic activity. Collagenase (Clostridium Histolyticum) is β-toxin. [redacted][4] α- and ε-toxins production can be controlled by ensuring that no hemolytic activity is detected in the process control. Production of γ-toxins can be controlled by the manufacturing conditions.

2.A.(1).4) Manufacturing process development (comparability)

Major changes in the manufacturing process during the development of the drug substance are shown below (different versions of manufacturing processes are referred to as Initial manufacturing process, Process I, and Process III [the proposed manufacturing process]).

- Changes from the Initial manufacturing process to Process I: [redacted]
- Changes from Process I to Process III: [redacted]

Following these manufacturing process changes, comparability studies on quality attributes were conducted, and it was verified that the quality attributes of the pre- and post-change drug substance are comparable.

[4] Because the amino acid sequences of α-, δ-, and ε-toxins produced by *C.histolyticum* have not been identified, proteins that exhibit similar effects (i.e., α-toxin from *C.septicum* for α-toxin; thermolysin, a protease produced by *Bacillus thermoproteolyticus*, for δ-toxin; and perfringolysin, produced by *C.perfringens*, for ε-toxins) were used for comparison.

2.A.(1).5) Characterization

(a) Structure and composition

Primary structure of the drug substance was evaluated by amino acid composition analysis, and N-terminal amino acid sequencing and peptide mapping by Edman degradation. The results showed that the amino acid sequences of AUX-I and AUX-II matched their respective theoretical sequences.

[redacted]

[redacted]

Near ultraviolet circular dichroism spectra showed that AUX-I and AUX-II have different signals, indicating that their tertiary structures are different.

Differential scanning calorimetry showed that thermal denaturation of AUX-I and AUX-II occurred at [redacted]°C and [redacted]°C, respectively. Ultracentrifugal analysis showed that AUX-I and AUX-II have different sedimentation rates.

The amount of zinc bound to AUX-I, AUX-II, and Collagenase (Clostridium Histolyticum) (a mixture of AUX-I and AUX-II, with a mixing ratio of about 1:1) were measured by inductively coupled plasma mass spectrometry (ICP-MS). The molar ratio of zinc to protein was [redacted] for AUX-I, [redacted] for AUX-II, and [redacted] for Collagenase (Clostridium Histolyticum), each showing approximately 1:1 ratio, the theoretical molar ratio of zinc to protein.

(b) Physicochemical properties

Liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF/MS) gave a molecular weight of 113,940 for AUX-I and 112,980 for AUX-II, both of which were consistent with the theoretical molecular weights.

Isoelectric points of AUX-I and AUX-II were measured by capillary isoelectric focusing (cIEF), which were [redacted] and [redacted], respectively. Collagenase (Clostridium Histolyticum) had two main peaks that corresponded to the isoelectric points of AUX-I and AUX-II. [redacted]

[redacted]

[redacted] The position of the main band of Collagenase (Clostridium Histolyticum) was between those of AUX-I and AUX-II.
[redacted]

[redacted]

AUX-I and AUX-II eluted from reverse-phase high-performance liquid chromatography (RP-HPLC) as a single major peak at different retention times. Collagenase (Clostridium Histolyticum) showed 2 major peaks corresponding to AUX-I and AUX-II, and the observed mixing ratio was consistent with the target mixing ratio of approximately 1:1. [redacted]

On size-exclusion high-performance liquid chromatography (SEC-HPLC), AUX-I and AUX-II eluted as a single major peak at different retention times. Collagenase (Clostridium Histolyticum) showed 2 major peaks corresponding to AUX-I and AUX-II, and the observed mixing ratio was consistent with the target mixing ratio of approximately 1:1. It was also shown that an aggregate eluted before the major peaks of AUX-I and AUX-II.

(c) Biological properties

The biological activity of AUX-I is expressed as the relative potency of a test sample against the drug substance reference material. Class I soluble rat collagen (SRC) substrate was degraded and the N-terminal of the degradation product was labelled with fluorescamine. The relative potency of Collagenase (Clostridium Histolyticum) was [redacted].

The biological activity of AUX-II is expressed as the relative potency of a test sample against the drug substance reference material. A peptide substrate (glycyl-prolyl-alanine, GPA) was degraded and the N-terminal of the degradation product was labelled with fluorescamine. The relative potency of Collagenase (Clostridium Histolyticum) was [redacted].

(d) Product-related substances

Acidic molecular species are identified as product-related substances, which are controlled by cIEF in the specifications of the drug substance.

(e) Impurities

i) Process-related impurities

[redacted]

[redacted]

ii) Product-related impurities

[redacted]

2.A.(1).6) Control of drug substance

The proposed specifications of the drug substance include content (protein content [ultraviolet-visible spectrophotometry, UV-Vis]), description, identification (western blot and RP-HPLC), pH, purity (sodium dodecyl sulfate polyacrylamide gel electrophoresis [SDS-PAGE] and RP-HPLC, aggregates [SEC-HPLC], acidic molecular species [cIEF], host cell protein [enzyme-linked immunosorbent assay, ELISA], and DNA [threshold method]), bacterial endotoxins (colorimetric assay), microbial limit (total viable count, membrane filter method), potency (SRC assay and GPA assay), and assay (protein content [UV-Vis] and the content of AUX-I and AUX-II [RP-HPLC]). [redacted]

2.A.(1).7) Stability of drug substance

Table 1 outlines the main stability studies of the drug substance.

Table 1. Outline of the stability studies on the drug substance

Study	Number of batches [a)]	Storage conditions	Storage form	Storage period
Long-term	**6**	**-70 ± 10°C**	[redacted]	**3 batches: 36 months 3 batches: 12 months [b)]**
Stress	**3**	**-20 ± 5°C**		**36 months**
	1	**5 ± 3°C**		**3 months**
	1	**25 ± 2°C/60 ± 5%RH**		**3 months**
Photostability	**1**	**5 ± 3°C, overall illumination of 1.2 million lux·h and an integrated near ultraviolet energy of 200 W·h/m^2**		**—**

a) manufactured with Process III
b) ongoing (up to 36 months)

No significant change in quality was observed during the long-term study. [redacted]

Photostability was also evaluated, and the results showed that the drug substance was photostable.

[redacted]

2.A.(2) Drug product

2.A.(2).1) Description and composition of the drug product, and formulation development

The drug product is a lyophilized powder for solution for injection containing 0.9 mg of the drug substance in 1 vial. The drug product contains sucrose, trometamol, and hydrochloric acid as excipients. Prior to the lyophilization process, vials were overfilled to ensure sufficient quantity of injectable formulation (0.25 or 0.20 mL) containing 0.58 mg of the drug substance when reconstituted with a specified volume of supplied diluent depending on the injection site.

A diluent (3 mL) consisting of 0.9% sodium chloride and 0.03% calcium chloride hydrate will be supplied in borosilicate glass vials for reconstruction. Cartons are used for secondary packaging.

2.A.(2).2) Manufacturing process

The manufacturing process of the drug product comprises drug solution preparation, sterile filtration, filling, lyophilization, sealing, inspection, and packaging. Drug solution preparation, sterile filtration, and filling have been defined as critical process steps. The manufacturing process of the drug product was subject to process validation in the scale of commercial production, and all steps of the process were found to be adequately controlled.

The manufacturing process of the diluent to be provided with the drug product comprises drug solution preparation, sterile filtration, filling, sterilization, inspection, and packaging. Sterile filtration, filling, and sterilization have been defined as critical process steps. The manufacturing process of the diluent was subject to process validation in the scale of commercial production, and all steps of the process were found to be adequately controlled.

2.A.(2).3) Manufacturing process development

In the course of development of the drug product, formulation and lyophilization process of the drug product were improved, and the manufacturing site and manufacturing scale for commercial production have been changed. The quality attribute studies showed that the pre- and post-change drug products are comparable.

2.A.(2).4) Control of drug product

The proposed specifications of the drug product include content, description (appearance, color, clarity and opalescence), identification (western blot and RP-HPLC), pH, purity (SDS-PAGE, RP-HPLC, and aggregates [SEC-HPLC]), water content, bacterial endotoxins (colorimetric assay), uniformity of dosage unit (mass variation), foreign insoluble matter, insoluble particulate matter, sterility, potency (SRC assay

and GPA assay), and assay (protein content). Reconstitution time, osmotic pressure, and assay (content of AUX-I and AUX-II [RP-HPLC]) were specified additionally during the review process.

The proposed specifications of the diluent include content (sodium chloride and calcium chloride hydrate), description, bacterial endotoxins (colorimetric assay), foreign insoluble matter, insoluble particulate matter, sterility, and assay (end-point detection of titration).

2.A.(2).5) Stability of drug product

Table 2 outlines the main stability studies for the drug product.

Table 2. Outline of the stability studies on the drug product

Study	Manufacturing process	Number of batches	Storage condition	Storage form	Storage period
Long-term	Process III	3	5 ± 3°C	Rubber stopper and glass vial	36 months
Accelerated	Process III	3	25 ± 2°C/60 ± 5%RH		36 months
Stress	Process III	3	30 ± 2°C/65 ± 5%RH		24 months
Photostability	Process III	1	5 ± 3°C, overall illumination of 1.2 million lux·h and an integrated near ultraviolet energy of 200 W·h/m^2	Rubber stopper and glass vial or rubber stopper and glass vial in cartons	—

No significant change in quality was observed during the long-term testing period.

[redacted]

In the photostability study, the drug product in primary packaging showed no appreciable change in any items tested after light exposure, indicating that the drug product is photostable.

The reconstitution stability study, performed after the drug product was reconstituted with the supplied diluent, showed that the drug product is stable for 72 hours when the reconstituted solution is left to stand in glass vials at room temperature for 30 minutes and then stored at 2°C to 8°C.

The following stability studies of the diluent to be provided with the drug product were performed: long-term studies for 48 months at 5°C, and 36 months at 25°C/60%RH; mid-term study for 36 months at 30°C/65%RH; and accelerated study for 6 months at 40°C/75%RH. The results demonstrated that the diluent is stable at room temperature up to 36 months.

Based on the above, a shelf-life of 36 months has been proposed for the drug product when stored in glass vials at 2°C to 8°C.

2.A.(3) Reference materials

The drug substance/drug product reference materials are selected from the drug substance batches, and the AUX-I and AUX-II reference materials are AUX-I and AUX-II obtained as intermediates in the manufacturing process of the drug substance batches selected as drug substance/drug product reference materials, and stored at -60°C or below. The proposed specifications for the drug substance/drug product reference materials include assay (specific activity SRC assay and specific activity GPA assay) in addition to the specifications for the drug substance. The proposed specifications for AUX-I and AUX-II reference materials include content (protein content), description, identification (peptide mapping and RP-HPLC), pH, purity (aggregation [SEC-HPLC] and RP-HPLC), and assay (specific activity SRC assay [for AUX-I reference materials only], specific activity GPA assay [for AUX-II reference materials only], and protein content). When a new reference material is established, the material is tested to ensure that it conforms to the specifications; the following characterization are also analyzed: amino acid composition, N-terminal and C-terminal amino acid sequencing, molecular weight, far-ultraviolet circular dichroism spectra, infrared absorption spectra, differential scanning calorimetry, dynamic light scattering, and cIEF for AUX-I/AUX-II reference materials; and dynamic light scattering and cIEF for the drug substance/drug product reference materials.

2.B Outline of the review by PMDA

Based on the submitted data and the following discussion, PMDA concluded that the quality of the drug substance and the drug product is adequately controlled.

2.B.(1) Control of MCB and WCB

The applicant's explanation:

In order to ensure appropriate manufacturing and quality control, information only on fermentation and subsequent processes was included in the application since the drug substance would be manufactured through the process that would use microorganisms existing in nature or modified by conventional methods (classical fermentation).

PMDA's view:

PMDA asked the applicant to include the information on the establishment of MCB and the manufacturing covering the maintenance of WCB and subsequent processes in the application and to assure effective production and quality control, according to the classification of APIs produced by biotechnology processes for the following reasons:

1. Collagenase (Clostridium Histolyticum) is a high molecular weight protein and not a low molecular weight substance, which is mentioned as an example of a product of classical fermentation defined in "Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients" (PMSB/ELD Notification

No. 1200 issued by the Director-General of the Pharmaceutical and Medical Safety Bureau, Ministry of Health, Labour and Welfare; dated November 2, 2001); and
2. Changes in manufacturing processes, including cell clones used, improved the purity of the product, meaning that changes in cell clones may affect the quality data of Collagenase (Clostridium Histolyticum) obtained during the development of manufacturing process.

The applicant's response:
The information on the establishment of MCB and the maintenance of WCB would be included in the application to assure appropriate production and quality control.

PMDA accepted the applicant's response.

3. Non-clinical data

3.(i) Summary of pharmacology studies

3.(i).A Summary of the submitted data

The applicant submitted the following data for primary pharmacodynamic studies: a study on collagen degradation characteristics of Class I Collagenase (Clostridium Histolyticum) (AUX-I) and Class II collagenase (Clostridium Histolyticum) (AUX-II); and a published *in vitro* study which assessed the effects of clostridial collagenase on human Dupuytren's cords obtained from patients.

No safety pharmacology studies have been performed for the following reasons: in clinical studies, no systemic exposure to AUX-I or AUX-II was observed following injection of Collagenase (Clostridium Histolyticum) into Dupuytren's cords [see "4.(ii).A.(2) Japanese phase III study" and "4.(ii).A.(3) Foreign phase I study"]; in repeated-dose toxicity studies in rats and dogs, injecting Collagenase (Clostridium Histolyticum) subcutaneously into areas near corresponding clinical injection sites, the levels of systemic exposure to AUX-I and AUX-II were low, and no findings suggestive of systemic toxicity were observed [see "3.(iii).A Summary of the submitted data"].

3.(i).A.(1) Primary pharmacodynamics

3.(i).A.(1).1) Characteristics of collagen degradation in AUX-I and AUX-II (4.2.1.1-1, Study H-ADTR-2008-01)

AUX-I 0.13, 0.23, and 0.33 μg/mL, AUX-II 0.13, 0.23, and 0.33 μg/mL, equal mixtures of AUX-I and AUX-II 0.26, 0.46, and 0.66 μg/mL were added to rat tail-derived collagen type I, and the mixtures were incubated at 25°C for 150 minutes. The mixtures were analyzed by SDS-PAGE.[5] Rat tail-derived collagen type I was degraded by AUX-I and AUX-II into fragments with different molecular weights. The amount of degraded collagen, and the amount of collagen fragments generated increased with increasing concentrations of AUX-I and AUX-II. The first collagen fragment was detected 30 minutes after adding AUX-I, and 10 minutes after adding AUX-II, regardless of the concentrations of AUX-I

[5] The drug substance manufactured with Process III was used.

and AUX-II. In the equal mixtures of AUX-I and AUX-II, some degraded fragments detected were different from those observed in AUX-I or AUX-II alone.

AUX-I, AUX-II, or equal mixture of AUX-I and AUX-II was added to rat tail-derived collagen type I or to recombinant human collagen type I, and after incubation at 25°C, the mixtures were analyzed by SDS-PAGE. The applicant stated that the SDS-PAGE showed that the Collagenase (Clostridium Histolyticum)'s collagen degradation characteristics of rat tail-derived collagen type I and recombinant human collagen type I were similar.

3.(i).A.(1).2) Action of Collagenase (Clostridium Histolyticum) **on human Dupuytren's cords obtained from patients (4.2.1.1-3, *The Journal of Hand Surgery.* 1996;21A:490-495, reference data)**
Collagenase (Clostridium Histolyticum)[6] or vehicle[7] 0.5 mL (containing 3600 units [U] [weight unknown because the information on the batch used is unavailable]) was injected into Dupuytren's cords obtained from patients during fasciectomy, and after incubation at 37°C for 24 hours, loads were applied at both ends of each cord to achieve an elongation of 10%, 15%, 20%, or 25% in a tensile testing machine. In Collagenase (Clostridium Histolyticum)-injected cords, the mean tensile modulus of elasticity[8] decreased by 93% compared to that of vehicle-injected cords, and rupture occurred in 3 of the 10 Collagenase (Clostridium Histolyticum)-injected cords but not in the 10 vehicle-injected cords.

Collagenase (Clostridium Histolyticum), at a dose of 150, 300, or 600 U, or vehicle[7] was injected in a volume of 0.2 mL into Dupuytren's cords obtained from patients during fasciectomy, and after incubation at 37°C for 24 hours, loads were applied at both ends until rupture occurs at a constant displacement rate of 0.9 mm/s in a tensile testing machine. The mean tensile strength[9] decreased with increasing dose of Collagenase (Clostridium Histolyticum), and the tensile strength in the contracture cords, to which Collagenase (Clostridium Histolyticum) was injected at doses of 300 U and 600 U, was lower than the estimated lower limit of normal human finger extensor forces.[10] Collagen degradation was observed at the injection site of all Collagenase (Clostridium Histolyticum)-injected cords, with levels increasing in a dose-dependent manner.

3.(i).B Outline of the review by PMDA

3.(i).B.(1) Pharmacological action of Collagenase (Clostridium Histolyticum)

The applicant's explanation on the pharmacological action of Collagenase (Clostridium Histolyticum):
Dupuytren's cords are composed primarily of collagen types I and III.[11] The results of the study (4.2.1.1-1) of collagen degradation of AUX-I and AUX-II showed the collagenolytic effect of Collagenase

[6] The drug substance manufactured with the Initial manufacturing process was used.
[7] A 0.9% sodium chloride solution containing 0.2 mmol/L calcium chloride
[8] The observed load (stress) and elongation (strain) were plotted, and the slope of the regression line was defined as the mean tensile modulus.
[9] The mean tensile strength was defined as the load at the time of contracture cord rupture.
[10] Estimated normal human finger extensor force is 4.1, 3.06, 3.0, 2.7×10^6 MPa for index finger, middle finger, ring finger and little finger, respectively (*J Hand Surg.* 1978;3:571-578).
[11] *Tohoku Exp Med*. 1984;142:437-443

(Clostridium Histolyticum) on collagen type I. Based on a report[12] that the same level of enzyme activity was observed in rat tail collagen type I and human placental collagen type III when they were hydrolyzed by clostridial collagenase, Collagenase (Clostridium Histolyticum) is considered to degrade collagen type III in a similar way it does collagen type I. Based also on the published literature (4.2.1.1-3) which evaluated Collagenase (Clostridium Histolyticum)'s effects on Dupuytren's cords obtained from patients during fasciectomy, efficacy of Collagenase (Clostridium Histolyticum) in the treatment of Dupuytren's contracture can be expected.

The results of the study (4.2.1.1-1) of collagen degradation characteristics of AUX-I and AUX-II showed that some fragments detected in the equal mixture of AUX-I and AUX-II were not detected in AUX-I or AUX-II alone. There is a report[13] that an equal mixture of AUX-I and AUX-II has a higher degradation activity on Achilles-tendon collagen compared to AUX-I or AUX-II alone. Therefore, Collagenase (Clostridium Histolyticum), which consists of AUX-I and AUX-II as active ingredients, can degrade collagen more efficiently than AUX-I or AUX-II alone.

Collagen was reported to have subtypes of at least I to XXVIII[14]; therefore, the action of clostridial collagenase on subtypes of collagen other than types I and III were evaluated based on the published literature. Collagen type IV is one of the major collagens consisting basement membrane of blood vessels and nerves, and a study reported[15] that human placental soluble collagen type IV and bovine lens basement membrane collagen type IV were degraded. On the other hand, a study reported[16] that clostridial collagenase did not act on collagen type IV in the basement membrane synthesized by Engelbreth-Holm-Swarm tumors in mice. Another study reported[17] that clostridial collagenase did not have an effect on neurovascular tissue in the rat femur, and elastic tissue, blood vessels, or nerve fiber in the human corpus cavernosum. Some studies reported[18] that collagen types II, V, XII, XIV, and XVII were degraded by clostridial collagenases, but no reports were found on the collagenase activities of other subtypes.

According to the results of clinical studies and post-marketing safety information in foreign countries, injection site swelling, pain, and injection site hemorrhage were observed following administration of Collagenase (Clostridium Histolyticum), and it is considered that the following factors may be involved in these adverse events: physical load caused by injection puncture or finger extension procedure; inflammatory response caused by tissue injury and collagen degradation fragments resulting from Collagenase (Clostridium Histolyticum)'s degradation of collagen contained in Dupuytren's cords and

[12] *J Protein Chem.* 1992;11: 99-107
[13] Biochemistry. 1964;3:1737-1741
[14] *J Cell Sci.* 2007;120: 1955-1958, *Pathol Biol (Paris).* 2005;53:430-442
[15] *Biochem Int.* 1991;24:397-404, *J Cell Biochem.* 1987;35:31-49
[16] *Infect Immun.* 1998;66:4851-4855
[17] *Urological Research.* 1982;10:135-140
[18] *J Protein Chem* 1992;11:99-107, *Int Wound J.* 2010;7:87-95, *Eur J Biochem.* 1992;207:847-856, *J Biol Chem.* 1992;267:15759-15764, *J Clin Invest.* 1991;87:734-738

tissue around the injection site[19]; Collagenase (Clostridium Histolyticum)'s effect on promoting vascular permeability.[20]

PMDA's view:
Based on the submitted data on primary pharmacodynamic studies and the applicant's responses, Collagenase (Clostridium Histolyticum) is expected to be effective in the treatment of Dupuytren's contracture.

However, swelling, pain, and hemorrhage at the injection sites have been reported in clinical studies, although causal relationship between the reported events and the collagenolytic effect of Collagenase (Clostridium Histolyticum) has not been supported. Therefore, healthcare professionals should be advised to closely observe the injection sites after administration and take appropriate actions whenever necessary [see "4.(iii).B.(3) Safety"].

3.(ii) Summary of pharmacokinetic studies

3.(ii).A Summary of the submitted data

The pharmacokinetics of CCH following intravenous or subcutaneous administration to rats and dogs was studied based on the toxicokinetics in the toxicology studies. AUX-I and AUX-II in plasma were measured by ELISA. The lower limit of quantification (LLOQ) of AUX-I and AUX-II were both 5.00 ng/mL in rat plasma, and 12.5 ng/mL and 37.5 ng/mL in dog plasma, respectively. ELISA was used to measure anti-AUX-I and anti-AUX-II antibodies in rat serum, and electro-chemiluminescence immunoassay for the same measurement in dog plasma. The LLOQ of anti-AUX-I and anti-AUX-II antibodies were 166 ng/mL and 1000 ng/mL, respectively, in rat serum, and were both 50 ng/mL in dog plasma. Tissue distribution, metabolism, or excretion were not studied for the following reasons: clinical studies showed that CCH was not detected in plasma following its local administration; and following administration, CCH, being a protein preparation, is assumed to be degraded into peptides and amino acids without being metabolized by cytochrome P450 or other drug-metabolizing enzymes. Unless otherwise specified, male and female animals were used and CCH used in the studies was made by manufacturing Process III. Note that 10,000 U of CCH is equivalent to 0.58 mg of CCH.

3.(ii).A.(1) Absorption

3.(ii).A.(1).1 Intravenous administration (4.2.3.2-2, Study [redacted] 1007-1671)

CCH 0, 500, 2240, or 5000 U/dose were administered by intravenous injection to rats on alternate days for 16 days, and the toxicokinetics following the first injection, the fourth injection (Day 7), and the eighth injection (Day 15) was evaluated. Table 3 shows the pharmacokinetic parameters of AUX-II in plasma following the first and fourth injections. Area under the plasma activity/concentration-time curve to infinity (AUC_{inf}) and elimination half-life ($t_{1/2}$) for AUX-I following the first and fourth injections,

[19] *J Burn Care Rehabil.* 1999;20:282-291, *J Exp Med.* 1976;143:1299-1307, *J Leukoc Biol.* 1986;39:255-266, *Mediators Inflamm.* 2005;2005:31-38
[20] *Spine (Phila Pa 1976).* 1985;10:562-566

and those for AUX-I and AUX-II following the eighth injection were not calculated because much of the data were below the LLOQ. Anti-AUX-I and anti-AUX-II antibodies were observed in nearly all animals in the ≥500 U/dose treatment groups.

Table 3. Pharmacokinetic parameters of AUX-II in rat plasma following first and fourth intravenous injection[a)]

Administration group (U/dose)	n	Following the first injection		Following the fourth injection	
		AUC_{inf} (ng·h/mL)	$t_{1/2}$ (h)	AUC_{inf} (ng·h/mL)	$t_{1/2}$ (h)
0	6	NC	NC	NC	NC
500	24	1.72×10^2	0.176	1.55×10^2	0.175
2240	24	1.24×10^3	0.485	1.05×10^3	1.02
5000	24	4.18×10^3	0.226	2.29×10^3	0.344

a) Standard deviation was not calculated because it was based on the mean of plasma concentration data obtained from multiple animals per blood sampling time point.
NC, not calculable

3.(ii).A.(1).2) Subcutaneous administration (4.2.3.2-3 and 4.2.3.2-7; Studies [redacted]-696003, [redacted]-696006)

CCH 0, 259, 517, or 776 U/dose were administered by subcutaneous injection to rats every 14 days for 12 weeks, and toxicokinetics following the first and fourth injections (Day 42) was evaluated. Table 4 shows the pharmacokinetic parameters of AUX-II in plasma following the first injection. Pharmacokinetic parameters were not calculated because plasma concentrations of AUX-I following the first injection, and plasma concentrations of AUX-I and AUX-II following the fourth injection were below the LLOQ in many specimens. Anti-AUX-I and anti-AUX-II antibodies were observed in all animals in the ≥259 U/dose treatment groups.

Table 4. Pharmacokinetic parameters of AUX-II in rat plasma following the first subcutaneous injection[a)]

Administration group (U/dose)	n	Male		Female	
		C_{max} (ng/mL)	AUC_{last} (ng·h /mL)	C_{max} (ng/mL)	AUC_{last} (ng·h/mL)
0	3	BLQ	NC	BLQ	NC
259	9	NC	NC	NC	NC
517	9	14.8	7.71	18.2	16.9
776	9	26.4	14.9	17.0	9.23

a) Standard deviation was not calculated because it was based on the mean of plasma concentration data obtained from multiple animals per blood sampling time point.
BLQ, below the lower limit of quantification; NC, not calculable

CCH 0, 2586, 3879, or 6466 U/dose were administered by subcutaneous injection to dogs (n = 5/group) every 4 weeks for 12 weeks, and toxicokinetics was evaluated. Pharmacokinetic parameters were not calculated because plasma concentrations of AUX-I and AUX-II following the first and fourth injection were below the LLOQ in many specimens. Anti-AUX-I and anti-AUX-II antibodies were observed in all animals in the ≥2586 U/dose treatment groups.

3.(ii).B Outline of the review by PMDA

PMDA's view:

There is no particular concern regarding the non-clinical pharmacokinetics of CCH given that CCH is a drug that acts locally in the area of Dupuytren's cords, and CCH was not detected in plasma following

administration in patients with Dupuytren's contracture in a foreign phase I study and a Japanese phase III study [see "4.(ii).A.(2) Japanese phase III study" and "4.(ii).A.(3) Foreign phase I study"].

3.(iii) Summary of toxicology studies

3.(iii).A Summary of the submitted data

The following studies were conducted as toxicology studies of Collagenase (Clostridium Histolyticum): single-dose toxicity studies, repeated dose toxicity studies, genotoxicity studies, reproductive and developmental toxicity studies, local tolerance studies, and other toxicity studies (antigenicity studies). Data of toxicology studies that did not comply with GLP, and of toxicology studies in which intrapenile administration was employed in the course of developing the drug for Peyronie's disease were also submitted as reference. Note that 10,000 U of Collagenase (Clostridium Histolyticum) is equivalent to 0.58 mg of Collagenase (Clostridium Histolyticum).

3.(iii).A.(1) Single-dose toxicity

3.(iii).A.(1).1) Single-dose toxicity studies in mice (4.2.3.1-1, 4.2.3.1-2, and 4.2.3.1-3; Studies [redacted]-001, [redacted]-002, [redacted]-003 [reference data])

A single intramuscular injection of 80, 160, 320, 640, or 1280 U/dose,[21] or a single intraperitoneal injection of 20, 40, 80, 160, or 320 U/dose[22] of Collagenase (Clostridium Histolyticum)[6] was administered into male Swiss mice. In animals that received Collagenase (Clostridium Histolyticum) intramuscularly, local reactions at the injection site (e.g., skin ulcer, hemorrhage, and skeletal muscle necrosis) were observed in the ≥160 U/dose groups, and deaths occurred in the ≥640 U/dose groups. In animals that received Collagenase (Clostridium Histolyticum) intraperitoneally, deaths occurred in the ≥40 U/dose groups. Necropsy revealed intrapleural and intraperitoneal hemorrhage. The approximate lethal dose was thus determined to be 640 U/dose for intramuscular administration, and 40 U/dose for intraperitoneal administration.

A single-dose of Collagenase (Clostridium Histolyticum)[6] was administered by intraperitoneal injection to male Swiss mice in another 2 studies and the approximate lethal dose was determined to be 80 U/dose in both of them.

3.(iii).A.(1).2) Single-dose toxicity studies in rats (4.2.3.1-4 and 4.2.3.1-5; Studies [redacted]00014 and [redacted]00018 [reference data])

Although no single-dose toxicity studies have been conducted using rats, the acute toxicity of Collagenase (Clostridium Histolyticum) was evaluated in the following toxicology studies.

Collagenase (Clostridium Histolyticum)[5] 5000, 10,000, or 20,000 U/dose[23] was injected into the caudal vein of female Sprague-Dawley (SD) rats once daily for 3 days. Some animals were found dead or

[21] Equivalent to 4000, 8000, 16,000, 32,000, and 64,000 U/kg
[22] Equivalent to 1000, 2000, 4000, 8000, and 16,000 U/kg
[23] Equivalent to 20,000, 40,000, and 80,000 U/kg

sacrificed moribund due to deterioration in general condition in the ≥10,000 U/dose groups. Pathological findings of these animals included intrapleural and intraperitoneal hemorrhage, subcapsular hemorrhage and subcapsular necrosis of the liver, pulmonary hemorrhage, and alveolar edema/emphysema. The approximate lethal dose was thus determined to be 10,000 U/dose.

Collagenase (Clostridium Histolyticum)[5] 50, 150, 500, 1500, or 2240 U/dose[24] was injected into the caudal vein of female SD rats once daily for 3 days. No noteworthy findings were observed in the study.

3.(iii).A.(1).3) Single-dose toxicity studies in dogs

While no single-dose toxicity studies have been conducted using dogs, a single intravenous injection of 4000 U/dose, or a single intraperitoneal injection ranging from 1575 to 3950 U/dose of CCH[6] was administered into dogs, and no changes related to drug toxicity were reported.[25]

3.(iii).A.(2) Repeat-dose toxicity

3.(iii).A.(2).1) Sixteen-day repeated intravenous administration toxicity study in rats (4.2.3.2-1, Study [redacted]00006)

Collagenase (Clostridium Histolyticum)[5] 0 (placebo[26]), 50, 150, or 500 U/dose[27] was injected into the caudal vein of male and female SD rats on alternate days for 16 days (a total of 8 doses on Day 1, 3, 5, 7, 9, 11, 13, and 15). Discoloration of the tail was observed in the ≥150 U/dose groups; perivascular hemorrhage and chronic perivascular inflammation of the injection site were observed in the ≥500 U/dose groups. Based on the above findings, the no observed adverse effect level (NOAEL) for systemic toxicity was determined to be 500 U/dose, and the NOAEL for the injection site to be 150 U/dose.

3.(iii).A.(2).2) Sixteen-day repeated intravenous administration toxicity study in rats (4.2.3.2-2, Study [redacted]1007-1671)

Collagenase (Clostridium Histolyticum)[5] 0 (vehicle[28]), 500, 2240, or 5000 U/dose[29] was injected into the caudal vein of male and female SD rats on alternate days for 16 days (a total of 8 doses on Day 1, 3, 5, 7, 9, 11, 13, and 15). All dose groups included subgroups of animals for the recovery study, except for the Collagenase (Clostridium Histolyticum) 2240 U/dose group. The animals in the subgroups had a 14-day washout period following 16 days of treatment, and the reversibility was evaluated. Deaths occurred in 2 of 27 female animals in the 5000 U/dose group. One of 27 males and 1 of 27 females in the Collagenase (Clostridium Histolyticum) 5000 U/dose group were sacrificed moribund due to deterioration of condition at the injection site. In the Collagenase (Clostridium Histolyticum) treatment groups, perivascular edema, hemorrhage, and inflammation were observed at the injection site. Discoloration of the tail, increased alanine aminotransferase, increased aspartate aminotransferase,

[24] Equivalent to 200, 600, 2000, 6000, and 8960 U/kg
[25] *Spine*. 1980;5:126-32
[26] 10 mM Tris, 60 mM Sucrose, pH 8.0
[27] Equivalent to 200, 600, and 2000 U/kg
[28] Normal saline containing 0.03% calcium chloride
[29] Equivalent to 2000, 8960, and 20,000 U/kg

discoloration of the injection site, chronic inflammation of the liver, biliary hyperplasia, and injection site ulcer were observed in the ≥2240 U/dose groups; mild anemia, increased reticulocyte count, increased liver weight, masses, elevated lesions, dark color lesions, etc., in the liver, hemorrhage of the liver, hematoma, hepatocellular necrosis and fibrosis, extramedullary hemopoiesis in the spleen, perivascular necrosis at the injection site, and increased spleen weight were observed in the Collagenase (Clostridium Histolyticum) 5000 U/dose group. At the end of the washout period, signs of recovery from the above conditions were observed. Based on the above findings, the NOAELs for both systemic toxicity and injection site were determined to be 500 U/dose.

3.(iii).A.(2).3) Twelve-week repeated subcutaneous administration toxicity study in rats (4.2.3.2-3, Study [redacted]-696003)

Collagenase (Clostridium Histolyticum)[5] 0 (placebo[26]), 259, 517, or 776 U/dose[30] was administered by subcutaneous injection into the metatarsophalangeal area of the right hindlimb of male and female SD rats every 14 days for a total of 7 injections (on Day 0, 14, 28, 42, 56, 70, and 84).[31] All dose groups included subgroups of animals for the recovery study. The animals in the subgroups had a 4-week washout period following 12 weeks of treatment, and the reversibility was evaluated. In the Collagenase (Clostridium Histolyticum) treatment groups, swelling and discoloration (red and purple) of the right hindlimb, suppression of body weight gain, dark-red discoloration or cyst formation in the right popliteal lymph node, injection site hemorrhage, inflammation, edema, collagen degradation (interstitial tissue and tendon), fibrosis, neovascularization, skeletal muscle necrosis and vascular fibrinoid necrosis at the injection site, lymphatic sinus expansion in the right popliteal lymph node, red blood cells in the lymphatic sinus and pigmented macrophage were observed. Elevated globulin levels, and enlargement of the right popliteal lymph node were observed in the groups that received 517 U/dose; and low albumin to globulin ratio, and hemorrhage in the arterial wall of the injection site were observed in the 776 U/dose group. At the end of the washout period, interstitial fibrosis at the injection site, pigmented macrophages in the right popliteal lymph node, and lymphatic sinus expansion were observed in the Collagenase (Clostridium Histolyticum) treatment groups. The finding of fibrosis suggests healing was ongoing at the injection site, and findings observed at the lymph node were considered to be a secondary change associated with hemorrhage and inflammation at the injection site. Based on the above findings, the NOAEL for the systemic toxicity was determined to be 776 U/dose; however, the NOAEL for the injection site was not determined.

3.(iii).A.(2).4) Repeated subcutaneous administration toxicity study in dogs (4.2.3.2-7, Study [redacted]-696006)

Collagenase (Clostridium Histolyticum)[5] 0 (placebo[26]), 2586, 3879, or 6466 U/dose [32] was subcutaneously injected into the metacarpophalangeal area of the right forelimb of male and female beagle dogs once every 4 weeks for a total of 4 doses (on Day 0, 28, 56, and 84).[31] All dose groups

[30] Equivalent to 1036, 2068, and 3104 U/kg
[31] The day of the first injection was defined as "Day 0" for this study only.
[32] Equivalent to 323, 485, and 808 U/kg

included subgroups of animals for the recovery study. The animals in the subgroups had a 4-week washout period following the fourth injection. The reversibility of toxicity in the animals was evaluated. In the Collagenase (Clostridium Histolyticum) treatment groups, swelling of the right forelimb, increased circumference of the right forelimb, discoloration (red and purple) of the right forelimb, hardening of the injection site, injection site hemorrhage, inflammation, edema, fibrosis, neovascularization, collagen degradation of fibrosis and interstitial tissue, infiltration of red blood cells, granulocytes, and macrophages in the lymphatic sinus of the right popliteal lymph node, and pigmented macrophages were observed. Angionecrosis and thrombus at the injection site were observed in the ≥3879 U/dose groups. At the end of the washout period, signs of recovery from the above conditions were observed. Based on the above findings, the NOAEL for systemic toxicity was determined to be 6466 U/dose; however, the NOAEL for the injection site was not determined.

3.(iii).A.(3) Genotoxicity (4.2.3.3.1-1, 4.2.3.3.1-2, and 4.2.3.3.2-1; Studies 200003-[redacted]-00591, 200001-[redacted]-00391, and 200002-[redacted]-00491 [reference data])

A reverse mutation assay with bacteria, a chromosomal aberration assay using human lymphocytes, and a mouse bone marrow micronucleus study were conducted, and the results suggested that Collagenase (Clostridium Histolyticum) is not genotoxic.

3.(iii).A.(4) Carcinogenicity

Based on its biological activity and the fact that Collagenase (Clostridium Histolyticum) is a protein, Collagenase (Clostridium Histolyticum) is not considered carcinogenic.

3.(iii).A.(5) Reproductive and developmental toxicity

Neither embryo-fetal development studies using non-rodent species nor studies to determine the effects on pre- and postnatal development, including maternal function were conducted because systemic exposure to Collagenase (Clostridium Histolyticum) has not been observed in the clinical setting.

3.(iii).A.(5).1) Rat study of fertility and early embryonic development to implantation (4.2.3.5.1-1, Study [redacted]00012)

Collagenase (Clostridium Histolyticum)[5] 0 (placebo[26]), 250, 750, or 2240 U/dose[33] was injected into the caudal vein of male and female SD rats in the following manner: in males, once on alternate days from 28 days prior to mating, through the mating period, until several days before the day of necropsy; in females, once on alternate days from 15 days prior to mating until the end of mating period, and once a day on gestation days 0, 3, 5, and 7 during pregnancy. Male and female rats in the same dose group were mated. Swelling and discoloration of the tail were observed in the ≥750 U/dose group; and reduced body weight gain, decreased food consumption were observed in the 2240 U/dose group. No effects of Collagenase (Clostridium Histolyticum) administration on reproductive functions and early embryogenesis were observed. Based on the above findings, the NOAEL was determined to be 750

[33] Equivalent to 1000, 3000, and 8960 U /kg

U/dose for general toxicity, 2240 U/dose for reproductive functions, and 250 U/dose for the injection site.

3.(iii).A.(5).2) Rat embryo-fetal development study (4.2.3.5.2-1, Study [redacted]00009)

Collagenase (Clostridium Histolyticum)[5] 0 (placebo[26]), 250, 750, or 2240 U/dose[33] was injected into the caudal vein of pregnant SD rats once daily from gestation days 7 to 17, and cesarean section was performed on gestation day 21. Discoloration, swelling, and skin excoriation of the tail, abnormal phonation, and hyper-reactivity to handling were observed in 1 of 25 animals in the 2240 U/dose group, and this animal was euthanized on gestation day 8. Effects of Collagenase (Clostridium Histolyticum) on maternal animals were discoloration and swelling of the tail (the injection site), discoloration of the base of the tail and hindlimb, and swelling of the base of the tail were observed in the ≥750 U/dose groups; in contrast, no effects of Collagenase (Clostridium Histolyticum) were observed on the number of corpora lutea, number of implantation sites, number of early embryo resorptions, rate of early embryo resorption, number of viable fetuses, fetal body weight, fetal sex ratio, and external surface of viable fetuses, internal organs, and skeletal structure of fetuses. Based on the above findings, the NOAEL was determined to be 2240 U/dose for maternal general toxicity and embryo fetal development, 250 U/dose for the injection site.

3.(iii).A.(6) Local tolerance

3.(iii).A.(6).1) Single subcutaneous (intra-fat pad) administration local tolerance study in rats (4.2.3.6-1, Study [redacted]95-2384 [reference data])

A single subcutaneous dose of 1 mL of Collagenase (Clostridium Histolyticum)[6] 0 (vehicle[28]), 500, or 1000 U/mL was injected into 2 sites of each female Zucker rat (5 injections each [0.2 mL/injection] at the right and left inguinal fat pads). The animals were necropsied 48 hours after Collagenase (Clostridium Histolyticum) administration. Dose-dependent, gross disruption of the fat pads, hemorrhage, and inflammation in the Collagenase (Clostridium Histolyticum) treatment groups, and fat cell disruption in the 1000 U/mL group were observed, thus NOAEL for the injection site was not determined.

3.(iii).A.(6).2) Single subcutaneous administration local tolerance study in rats (4.2.3.6-2, Study [redacted]-696001)

A single subcutaneous dose of 5, 10, 20, or 50 μL/dose[34] of Collagenase (Clostridium Histolyticum) 3 mg/mL,[5] or 10 μL/dose[35] of Collagenase (Clostridium Histolyticum) 1.5 mg/mL[5] was injected into the metatarsophalangeal area of the right hindlimb to male and female SD rats, and necropsies were performed 3 days after administration. In the control group, placebo[26] and vehicle[28] were injected into the right hindlimb and left hindlimb, respectively (50 μL/dose each). All dose groups included subgroups of animals for the recovery study. The animals in the subgroups had 28-day washout period after the final injection, and the reversibility was evaluated. Two of 5 males, and 1 of 5 females in the 2586

[34] Equivalent to 259, 517, 1034, and 2586 U/dose
[35] Equivalent to 259 U/dose

U/dose (50 μL/dose) group were sacrificed moribund on the day of administration or the next day due to skin laceration with exposed tendon in the hindlimb. In the Collagenase (Clostridium Histolyticum) treatment groups, swelling and discoloration (purple) of the right hindlimb, injection site hemorrhage, inflammation, edema, collagen degradation (interstitial tissue or tendon), enlargement of the right popliteal lymph node, dark-red discoloration, red blood cells in the lymphatic sinus were observed. In the ≥517 U/dose (10 μL /dose) groups, right popliteal lymph node hemorrhage was observed. In the ≥1034 U/dose (20 μL /dose) groups, red exudate, decreased mobility, and swelling or flexion of toes were observed. In the 2586 U/dose (50 μL /dose) group, decreases in body weight and food consumption, skin laceration, injection site ulcer, acute inflammation in the right popliteal lymph node were observed. There was no significant difference in the toxicity findings between the 2 different concentrations (3 and 1.5 mg/mL). At the end of the washout period, interstitial fibrosis at the injection site was observed in the Collagenase (Clostridium Histolyticum) group, which was considered to suggest an ongoing healing process at the injection site. Based on the above findings, the maximum tolerated dose (MTD) was determined to be 1034 U/dose, and the NOAEL for the injection site was not determined.

3.(iii).A.(6).3) Single intradermal administration local tolerance study in guinea pigs (4.2.3.6-3, Study [redacted]-004, reference data)

A single intradermal dose ranging from 0.18 to 0.21 mL (300 U/kg) of Collagenase (Clostridium Histolyticum) 590 U/mL[6] was injected into the left side of male and female guinea pigs, and an equivalent dose of vehicle[28] was injected into the right side of the same animals as the control. Erythema was observed in 2 of 3 animals/sex within 0.5 hours of administration, but recovered within 24 hours except in 1 male in which moderate erythema persisted for 4 days following administration. Based on the above findings, Collagenase (Clostridium Histolyticum) was considered to cause mild local irritation.

3.(iii).A.(6).4) Single subcutaneous and intratendon administration local tolerance study in dogs (4.2.3.6-4, Study [redacted]-696005)

A single subcutaneous injection of 2586, 7759, or 12,931 U/dose of Collagenase (Clostridium Histolyticum)[5] was administered into the metacarpal area of the right forelimb to male and female beagle dogs. Also, a single injection of 1293, 2586, or 5172 U/dose of Collagenase (Clostridium Histolyticum) was administered by intratendon injection into the superficial digital flexor tendon of the right forelimb to male and female beagle dogs. In the control group, placebo[26] was administered similarly by intratendon injection into the left forelimb or subcutaneous injection into the right forelimb. Three days after administration, necropsies were performed in 3 animals/sex/group in the control groups and Collagenase (Clostridium Histolyticum) intratendon-injection groups, and in 2 animals/sex/group in the Collagenase (Clostridium Histolyticum) subcutaneous-injection groups; the remaining animals (n = 2/sex/group) were necropsied after the 8-week recovery period. Swelling and discoloration (red or purple) in the right forelimb, and decreased mobility were observed in the intratendon and subcutaneous injection groups; and skin laceration was observed in the subcutaneous injection groups. Other findings included injection site hemorrhage, inflammation, and collagen degradation in interstitial tissue and tendon in the intratendon and subcutaneous injection groups; and epidermal ulceration in the right front

paw, and arterial wall hemorrhage at the injection site in the subcutaneous-injection groups. At the end of the recovery period, fibrosis of the injection site was observed in the Collagenase (Clostridium Histolyticum) treatment groups, which was interpreted as a sign of ongoing healing process at the injection site.

3.(iii).A.(6).5) Subcutaneous administration local tolerance study in mini-pigs (4.2.3.6-5, Study [redacted]-696007 [reference data])

Varying concentrations of Collagenase (Clostridium Histolyticum),[5] ranging from 26 to 2586 U/mL (0.0015 to 0.15 mg/mL), were administered subcutaneously at a dose of 0.05, 0.1, or 0.2 mL to male and female Gottingen mini-pigs at 12 injection sites/animal. Necropsies were performed on 2 animals/sex/group and 1 animal/sex/group approximately 24 hours and 48 hours after administration, respectively. Dark red discoloration of subcutaneous tissue at the injection sites, collagen degradation in dermis, hemorrhage, and acute inflammation were observed at all concentrations. Other findings included muscle fiber necrosis of the dermo-muscular layer at concentrations of ≥52 U/mL; perivascular edema, vessel wall edema, neovascularization, fibrosis, angionecrosis, and thrombus at concentrations of ≥155 U/mL; injection site swelling at concentrations of ≥259 U/mL; and artery wall hemorrhage at concentrations of ≥517 U/mL.

3.(iii).A.(7) Other toxicity

3.(iii).A.(7).1) Antigenicity study in guinea pigs (4.2.3.7.1-1 and 4.2.3.7.1-2; Studies [redacted]-005 and [redacted]-006 [reference data])

Collagenase (Clostridium Histolyticum)[6] 120 U/dose was administered intraperitoneally to male and female guinea pigs for a total of 4 injections, on Day 1, 3, 5, and 8 (sensitization). Then, on Day 15, 120 U/dose of Collagenase (Clostridium Histolyticum) was administered intracardially (induction) to evaluate systemic anaphylaxis. To animals of the control group, vehicle[28] was administered intraperitoneally for a total of 4 injections, on Day 1, 3, 5, and 8, and then on Day 15, 120 U/dose of Collagenase (Clostridium Histolyticum) was administered intracardially. Redness of the auricle (for 5 to 7 minutes after injection), hyperpnea, and hyperkinesia (for 2 minutes after injection) were observed in the Collagenase (Clostridium Histolyticum) treatment group. However, these findings were considered to be attributed to acute effects due to intracardiac administration rather than being caused by antigenic factors because redness of the auricle (0.5 to 1 minute after injection), hyperpnea, and hyperkinesia were observed in the control group. Collagenase (Clostridium Histolyticum)[6] 120 U/dose was administered intraperitoneally to male and female guinea pigs for a total of 4 injections, on Day 1, 3, 5, and 8 (sensitization). Then, on Day 15, 120 U/dose of Collagenase (Clostridium Histolyticum)[6] was administered intraperitoneally (induction) to evaluate systemic anaphylaxis. To animals of the control group, vehicle[28] was administered intraperitoneally for a total of 4 injections, on Day 1, 3, 5, and 8, and then on Day 15, 120 U/dose of Collagenase (Clostridium Histolyticum) was administered intraperitoneally. No findings suggestive of Collagenase (Clostridium Histolyticum) antigenicity were observed by induction through intraperitoneal administration. The above findings suggested that Collagenase (Clostridium Histolyticum) do not induce systemic anaphylaxis.

3.(iii).A.(7).2) Repeated dose intrapenile toxicity study in dogs (4.2.3.2-6, Study [redacted]520 [reference data])

Repeated intrapenile injections of Collagenase (Clostridium Histolyticum)[5] 0 (vehicle[28]), 110, 345, or 1145 U/dose[36] in 3 cycles,[37] for a total of 9 injections (on Day 1, 3, 5, 29, 31, 33, 57, 59, and 61) were administered into the tunica albuginea of male beagle dogs. All dose groups included subgroups of animals for the recovery study. The animals in the subgroups had a 4-week washout period following 3 cycles of treatment, and the reversibility was evaluated.

Serious local reactions (e.g., penile swelling and open abscess), as well as decreases in locomotor activity and body weight, which are attributable to the above local reactions, were observed at the injection sites in 1 of 6 animals in the 345 U/dose group, and this animal was sacrificed moribund on Day 8. Further, 1145 U/dose was replaced by lower dose of 841 U/dose[38] for the second and third cycles due to excessive local reactions observed in the first cycle (hereinafter the dose is referred to as 1145/841 U/dose).

Discoloration of the prepuce, penile swelling, skin discoloration of the inguinal area (red lesion and red pigmentation), enlargement of inguinal lymph node, tunica albuginea hemorrhage, neovascularization, and inflammation, and subcutaneous tissue hemorrhage and inflammation were observed in the Collagenase (Clostridium Histolyticum) treatment groups. Other findings included contusion in the ≥345 U/dose groups; red penile node; masses on the penis, prepuce, or glans penis; scab and red spots on the penis; discoloration of the inguinal lymph node (red pigmentation); red blood cells in the lymphatic sinus and lymphoid hyperplasia of the inguinal lymph node in the 1145/841 U/dose group. At the end of the washout period, signs of recovery from the above conditions were observed. Based on the above findings, the NOAEL for systemic toxicity was determined to be 1145/841 U/dose, and the NOAEL for the injection site was determined to be 110 U/dose given that no serious local reactions were observed.

3.(iii).A.(7).3) Single intrapenile dose local tolerance study in dogs (4.2.3.2-6, Study [redacted]520 [reference data])

A single dose of Collagenase (Clostridium Histolyticum)[5] 0 (vehicle[28]), 1145, or 2055 U/dose was administered into the tunica albuginea, corpus cavernosum, vein-artery-nerve complex, or urethra of male beagle dogs. Three days after administration, 2 of 3 animals in the control group, and 3 of 5 animals in each Collagenase (Clostridium Histolyticum) group were necropsied; and after the 4-week recovery period, the remaining dogs in the control group, 1 of 3 animals, and 2 of 5 animals in each Collagenase (Clostridium Histolyticum) group were necropsied.

[36] Equivalent to 14, 43, and 143 U/kg
[37] A cycle consists of 3 injections on alternate days in the first week, and the following 3-week washout period.
[38] Equivalent to 105 U/kg

Discoloration of the prepuce, and penile swelling were observed in the Collagenase (Clostridium Histolyticum) groups. Other histopathological findings included hemorrhage, inflammation, and necrosis in the corpus cavernosum, tunica albuginea, or subcutaneous tissue; collagen degradation in the tunica albuginea, and neovascularization. At the end of the washout period, signs of recovery from the above conditions were observed.

3.(iii).B Outline of the review by PMDA

PMDA's view:

Toxicity studies of Collagenase (Clostridium Histolyticum) showed local reactions at injection sites, primarily inflammatory changes; however, these are attributable to the pharmacological actions of Collagenase (Clostridium Histolyticum), and therefore, there is no concern from toxicological viewpoint based on the submitted data.

4. Clinical data

4.(i) Summary of biopharmaceutic studies and associated analytical methods

4.(i).A Summary of the submitted data

Table 5 shows the formulations used in the clinical studies submitted as evaluation data for the application. The comparability of the primary structure, molecular weight, potency, etc., between the formulations was demonstrated [see "2.A.(1).4) Manufacturing process development (comparability)"]. The proposed formulation is manufactured with Process III, which was used in studies including a Japanese phase III study.

Table 5. Formulations used in the clinical studies (evaluation data)

Manufacturing process of drug substance	Clinical study
Initial process	Foreign phase II study: DUPY-202
Process I	Foreign phase III study: DUPY-303 and DUPY-404
Process III	Foreign phase I study: AUX-CC-855 Japanese phase III study: AK160-III-1 Foreign phase III study: AUX-CC-851/852, AUX-CC-853, AUX-CC-854, AUX-CC-856, AUX-CC-857, AUX-CC-858, and AUX-CC-859

ELISA was used to measure the plasma concentrations of Class I collagenase (Clostridium Histolyticum) (AUX-I) and Class II collagenase (Clostridium Histolyticum) (AUX-II), anti-AUX-I antibody, anti-AUX-II antibody, and cross-reactivity with human matrix metalloproteinase (MMP). The LLOQ for the plasma concentrations of AUX-I and AUX-II were 5.0 ng/mL and 25.0 ng/mL, respectively. The collagenolytic activities of AUX-I and AUX-II in the neutralizing antibody were evaluated by fluorescence intensity measurement with fluorescein-labeled bovine collagen substrate.

4.(ii) Summary of clinical pharmacology studies

4.(ii).A Summary of the submitted data

4.(ii).A.(1) Studies using human biomaterials (5.3.1.4-36 and 5.3.1.4-37, Studies MBR10-112 and MBR10-113 [reference data])

The effects of human α2-macroglobulin and human plasma on the activity of Collagenase (Clostridium Histolyticum) were investigated, because Collagenase (Clostridium Histolyticum) has been reported[39] to be inactivated by serum α2-macroglobulin in many animal species including humans.

AUX-I or AUX-II was mixed with human α2-macroglobulin at a concentration equivalent to its human serum concentration, and 24 hours later, the enzyme activity decreased by approximately 90% in both AUX-I and AUX-II. AUX-II was inactivated more rapidly than AUX-I. Further, as a result of mixing with human plasma, the enzyme activity of AUX-I and AUX-II immediately after mixing decreased by 32.7% and 43.5%, respectively, and the inhibition increased over time.

4.(ii).A.(2) Japanese phase III study (5.3.5.2-1, Study AK160-III-1 [May 2012 to January 2014])

See "4.(iii).A.(1) Japanese phase III study" for the outline of the study.

The plasma concentrations of AUX-I and AUX-II following a single injection of 0.58 mg of CCH into the patient's Dupuytren's cord affecting the metacarpophalangeal (MP) joint or the proximal interphalangeal (PIP) joint were measured at 10 and 30 minutes, and 1 and 2 hours post-dose, and approximately 24 hours post-dose (after finger extension procedure). The plasma concentrations were less than the LLOQ for all subjects at all time points.

The production of anti-AUX-I and anti-AUX-II antibodies in the serum were studied at 30 days after each of 5 injections (from the first to fifth), and at 90, 180, 270, and 360 days after the first injection. The results showed that anti-AUX-I and anti-AUX-II antibodies were positive in 89.1% (90 of 101 subjects), and 80.2% (81 of 101 subjects), respectively, at 30 days after the first dose injection of CCH. From the third injection onward, all subjects were positive for both anti-AUX-I and anti-AUX-II antibodies.

If anti-AUX-I antibody or anti-AUX-II antibody was positive, neutralizing antibody was measured at 90 or 360 days after the first injection [see "4.(iii).B.(2).6) Effects of antibodies"].

If anti-AUX-I antibody or anti-AUX-II antibody was positive, cross-reactivity was measured at 180 or 360 days after the first injection, and the results showed that there was no cross-reactivity against MMP-1, MMP-2, MMP-3, MMP-8, or MMP-13.

4.(ii).A.(3) Foreign phase I study (5.3.3.2-1, Study AUX-CC-855 [[redacted] 20[redacted] to [redacted] 20[redacted]])

[39] *J Bacteriol.* 1968;96:1969-1976, *Biochem J.* 1974;139: 359-368, *J Biol Chem.* 1989;264:393-401

An open label study was conducted in patients aged ≥18 years with Dupuytren's contracture (Table 6) at 1 foreign institution to investigate the pharmacokinetics and safety following single dose injection of CCH (target sample size, 12 to 16; ≥4 subjects each for the MP and PIP joints).

Table 6. Main inclusion and exclusion criteria

Main inclusion criteria • **Patients with a diagnosis of Dupuytren's contracture, with a flexion contracture of at least one finger, other than the thumb (a contracture of ≥20° and ≤100° for the MP joint, and ≥20° and ≤80° for the PIP joint), caused by a palpable cord.** • **Patients who are positive for the "table top test," meaning that a person cannot simultaneously place the affected finger(s) and palm flat against a table top.** **Main exclusion criteria** • **Patients who received one or more of the following treatments for Dupuytren's contracture within 90 days prior to the initial injection of the study drug into the selected joint: fasciectomy or surgical fasciotomy, needle aponeurotomy/fasciectomy, or injection of verapamil and/or interferon.** • **Patients who received an anti-coagulant within 7 days prior to study drug administration, or have been on treatment with an anti-coagulant (except for aspirin ≤150 mg/day).**

A single dose of 0.58 mg of CCH, reconstituted with diluent[40] to form a liquid formulation of 0.25 mL for MP joints or 0.20 mL for PIP joints, was injected into the Dupuytren's cord. Approximately 24 hours after study drug injection, a finger extension procedure to facilitate cord disruption was performed by the investigator. The observation period was set to 30 days after study drug administration.

All 16 subjects who received injection (MP joint 9 and PIP joint 7 subjects) were included in the pharmacokinetic and safety analysis set.

The pharmacokinetic results showed that the plasma concentrations of AUX-I and AUX-II were below the LLOQ in all subjects at all time points as follows: 5, 10, 20, and 30 minutes; 1, 2, 4, 8, and 12 hours; approximately 24 hours (after the finger extension procedure); and 7 and 30 days after administration.

Adverse events and adverse events for which a causal relationship to the study drug could not be ruled out (adverse reactions) both occurred in 100.0% (16 of 16 subjects). The adverse events that occurred in ≥2 subjects were injection site haemorrhage (93.8% [15 of 16 subjects]), injection site swelling (87.5% [14 of 16 subjects]), injection site pain (81.3% [13 of 16 subjects]), paraesthesia and hypertension (12.5% [2 of 16 subjects] each). The adverse reactions that occurred in ≥2 subjects were injection site haemorrhage (93.8% [15 of 16 subjects]), injection site swelling (87.5% [14 of 16 subjects]), injection site pain (81.3% [13 of 16 subjects]), and paraesthesia (12.5% [2 of 16 subjects]). There were no adverse events that resulted in death or led to discontinuation of administration. A serious adverse event, rupture of the flexor digitorum profundus muscle in the right little finger, occurred in 6.3% (1 of 16 subjects), and its causal relationship to the study drug could not be ruled out.[41]

4.(ii).A.(4) Other foreign clinical studies

[40] The diluent was 0.9% sodium chloride solution containing 2-mM calcium chloride. Similar diluents were used in other studies.
[41] The outcome was "improved."

See the sections in "4.(iii).A. Summary of the submitted data" for the outlines of the following studies.

In foreign clinical studies (Studies AUX-CC-854, AUX-CC-856, AUX-CC-857, AUX-CC-858, and AUX-CC-859), production of anti-AUX-I antibody and anti-AUX-II antibody in the serum was investigated. They were measured at 30 days after each injection (from the first up to the eighth injection at maximum), and 180, 270, and 360 days after the first injection.

Thirty days after the first injection, 91.6% of subjects (802 of 876 subjects) were positive for anti-AUX-I antibodies, and 85.7% of subjects (751 of 876 subjects) were positive for anti-AUX-II antibodies. All subjects were positive for both anti-AUX-I and anti-AUX-II antibodies from the fourth injection onward.[42]

Neutralizing antibodies were studied in Study AUX-CC-857 [see "4.(iii).B.(2).6) Effects of antibodies"].

The cross-reactivity of anti-AUX-I antibodies and anti-AUX-II antibodies against MMP-1, MMP-2, MMP-3, MMP-8, and MMP-13 were measured in the following serum specimens: 71 serum specimens tested positive for anti-AUX-I antibodies or anti-AUX-II antibodies in Study AUX-CC-860 (a follow-up study for Studies AUX-CC-854, AUX-CC-856, AUX-CC-857, AUX-CC-858, and AUX-CC-859); and 299 serum specimens tested positive for anti-AUX-I antibodies or anti-AUX-II antibodies after retreatment with Collagenase (Clostridium Histolyticum) in Study AUX-CC-862. No cross-reactivity was observed against MMP-1, MMP-2, MMP-3, MMP-8, or MMP-13 in any of the specimens.

4.(ii).B Outline of the review by PMDA

PMDA's view:

No clinically significant differences exist between Japanese and foreign study data that may raise any particular concerns in the pharmacokinetics of AUX-I and AUX-II in plasma, or in emergence of anti-AUX-I antibodies or anti-AUX-II antibodies after CCH administration. Effects of anti-AUX-I antibodies, anti-AUX-II antibodies, and neutralizing antibodies on the efficacy and safety will be discussed in the next sections [see "4.(iii).B.(2).6) Effects of antibodies" and "4.(iii).B.(3).3) Anti-drug antibodies and anaphylaxis"].

4.(iii) Summary of clinical efficacy and safety

4.(iii).A Summary of the submitted data

The applicant submitted efficacy and safety evaluation data including the results of 12 Japanese and foreign clinical studies. Among these, the main Japanese and foreign clinical studies are listed in Table 7. Foreign clinical studies that are not listed in Table 7 were used for the safety evaluation. Two foreign

[42] From the second injection onward, the following numbers of subjects were analyzed: 498 subjects (second), 338 subjects (third), 197 subjects (fourth), 127 subjects (fifth), 33 subjects (sixth), 21 subjects (seventh), and 9 subjects (eighth).

phase III studies (Studies AUX-CC-860[43] and AUX-CC-867[44]), and a foreign phase IV study (Study AUX-CC-862[45]) were submitted as reference data.

Table 7. Outlines of submitted major evaluation data

Study	Study Identifier	Location	Study design	Dose [a], number of subjects	Number of injectable doses/patient /study period	Dose, Clinical success rate [b]
PII	DUPY-202	US	Randomized, double-blind, placebo-controlled	Placebo, 17 subjects 2500 U, 18 subjects 5000 U, 22 subjects 10,000 U, 23 subjects	1 dose/month	Placebo, 0% 2500 U, 50.0% 5000 U, 45.5% 10,000 U, 78.3%
			Open-label, uncontrolled	10,000 U, 51 subjects	5 doses/ up to 12 months after the final injection	10,000 U, 62.2% (23/37 subjects)
			Sub-study open-label, uncontrolled	10,000 U, 10 subjects	5 doses/ up to 12 months after the final injection	—
PIII	AK160-III-1	Japan	Open-label, uncontrolled	0.58 mg, 102 subjects	5 doses/360 days	0.58 mg, 85.7% (66/77 subjects)
	AUX-CC-854	Europe	Open-label, uncontrolled	0.58 mg, 386 subjects	5 doses/9 months	0.58 mg, 58.4% (343/587 joints)
	AUX-CC-856	US	Open-label, uncontrolled	0.58 mg, 201 subjects	5 doses/9 months	0.58 mg, 52.7% (154/292 joints)
	AUX-CC-857	US	Randomized, double-blind, placebo-controlled	Placebo, 104 subjects 0.58 mg, 204 subjects	3 doses/90 days	Placebo, 6.8% (7/103 subjects) 0.58 mg, 64.0% (130/203 subjects)
	AUX-CC-858	US	Open-label, uncontrolled; extension study of AUX-CC-857	0.58 mg, 160 subjects	5 doses/9 months	0.58 mg, 50.5% (264/523 joints)
	AUX-CC-859	Australia	Randomized, double-blind, placebo-controlled	Placebo, 21 subjects 0.58 mg, 45 subjects	3 doses/90 days	Placebo, 4.8% (1/21 subjects) 0.58 mg, 44.4% (20/45 subjects)
		Australia	Open-label, uncontrolled	0.58 mg, 42 subjects	5 doses/9 months	0.58 mg, 50.7% (68/134 subjects)

a) 10,000 U is equivalent to 0.58 mg.
b) Extension deficit of ≤5°

4.(iii).A.(1) Japanese phase III study (5.3.5.2-1, Study AK160-III-1 [May 2012 to January 2014], hereinafter referred to as the Japanese study)

An open-label, uncontrolled, multicenter study was conducted in patients aged ≥20 years with Dupuytren's contracture (Table 8) at 30 institutions in Japan to evaluate the efficacy and safety of CCH (target sample size, 69 subjects; 46 subjects with the MP joint and 23 subjects with the PIP joint at the primary joint). Prior to the start of the study, instruction manuals and DVDs containing information on the injection procedure of CCH were provided to, and a briefing session with medical experts was held for investigators.

[43] A study following up subjects who received CCH in Studies AUX-CC-854, AUX-CC-856, AUX-CC-857/858 (Study AUX-CC-858 is the extension study of Study AUX-CC-857), and AUX-CC-859 up to 2 to 5 years after the first injection.
[44] A global phase III study conducted in the US, EU, and other countries after the market launch. The dosage and administration (0.58 mg of CCH was injected into contracture cords simultaneously at 2 injection sites of the same hand) are different from the original proposed dosage and administration.
[45] An open-label, non-randomized, uncontrolled, long-term follow-up study (started after the post-4 year-evaluation of Study AUX-CC-860), in which CCH 0.58 mg was administered into recurrent Dupuytren's cord affecting joints up to 3 times at approximately 28-day intervals.

Table 8. Main inclusion and exclusion criteria

Main inclusion criteria • **Patients with a diagnosis of Dupuytren's contracture, with a flexion contracture of at least one finger, other than the thumb (a contracture of 20° to 100° for the MP joint, and 20° to 80° for the PIP joint), caused by a palpable cord.** • **Patients who are positive for the "table top test," meaning that a person cannot simultaneously place the affected finger(s) and palm flat against a table top.** **Main exclusion criteria** • **Patients who received one or more of the following treatments for Dupuytren's contracture within 90 days prior to the initial injection of the study drug into the primary joints: fasciectomy or surgical fasciectomy, needle aponeurotomy/fasciotomy, or injection of verapamil and/or interferon.** • **Patients who received an anti-coagulant within 7 days prior to study drug administration, or have been on treatment with an anti-coagulant (except for aspirin ≤150 mg/day).**

A single dose of 0.58 mg of CCH, reconstituted with diluent to form a liquid formulation of 0.25 mL for the MP joint or 0.20 mL for the PIP joint, was administered into the Dupuytren's cord affecting primary joints.[46] If clinical success (extension deficit[47] of ≤5°) was not achieved, CCH could be injected up to 3 times per joint at 30-day intervals, and up to 5 times in total per subject. The follow-up period was set as up to 360 days after the first injection.[48] A finger extension procedure to facilitate cord disruption was performed as needed by the study investigator on the day following injection.

All 102 treated subjects were included in the safety analysis set and full analysis set (FAS), and the remaining subjects (n = 77) after excluding those who were enrolled in Step 3[49] (n = 25) were included in the primary analysis set for efficacy (FAS Step 1-2). Treatment was discontinued in 3 subjects.[50]

Efficacy of CCH as shown by the percentage of subjects who achieved clinical success in their primary joint 30 days after the final injection was 85.7% (66 of 77 subjects). The lower limit of the confidence interval was higher than 30%, the target set in advance (Table 9).

Table 9. Clinical success for the primary joints 30 days after the final injection (FAS Step 1-2)

	All joints	MP joint	PIP joint
Percentage of subjects who achieved clinical success [95% CI]	**85.7% (66/77 subjects) [75.9, 92.6]**	**93.6% (44/47 subjects) [82.5, 98.7]**	**73.3% (22/30 subjects) [54.1, 87.7]**
Number of injections (mean ± SD)	**1.2 ± 0.48 times**	**1.2 ± 0.46 times**	**1.3 ± 0.52 times**

Safety results show that the incidence of adverse events and the incidence of adverse reactions were both 98.0% (100 of 102 subjects). Table 10 shows adverse events and adverse reactions that occurred with an incidence of ≥2%. Two events resulted in death (gastric cancer and lung neoplasm malignant, 1

[46] A joint into which the study drug is injected first. In studies except Study DUPY-202, if there were contracture cords in the MP and PIP joints, and both met the inclusion criteria, the cord in the MP joint was selected.

[47] The angle of the joint when the finger is straightened as far as possible was measured by finger goniometry. Similar methods were used in other studies.

[48] All subjects were required to have study visits on 90, 180, 270, and 360 days after the first injection, or at the time of withdrawal.

[49] Step 1: At a limited number of medical institutions, physicians who were trained for CCH treatment in the US administered the study drug to 6 subjects.
Step 2: CCH was administered to patients from other medical institutions than Step 1, and to other patients in the same medical institutions in Step 1, and data for a total of 69 subjects (including data of Step 1) were compiled.
Step 3: If the target number of joints (the sum of primary joints and non-primary joints: ≥50 for each of MP joints and PIP joints) may not be achieved, patients with Dupuytren's cords in the joints targeted would be enrolled to supply the deficient number of joints.

[50] The reasons for treatment discontinuations included 2 adverse events (gastric cancer and lung neoplasm malignant, both resulted in death), and 1 request from the subject.

subject each), for which a causal relationship to the study drug was ruled out. Serious adverse events occurred in 13.7% (14 of 102 subjects), and included cataract (3 subjects); gastric cancer,[51] duodenal ulcer perforation, intestinal obstruction, colon cancer, pneumothorax/infectious pleural effusion, bile duct stone, spondylolysis, lung neoplasm malignant,[51] idiopathic thrombocytopenic purpura, myocardial infarction, and subarachnoid haemorrhage (1 subject each). A causal relationship to the drug substance was ruled out for all of the above events.

Table 10. Adverse events and adverse reactions that occurred with an incidence of ≥2%

	Adverse event (102 subjects)		Adverse reaction (102 subjects)			Adverse event (102 subjects)		Adverse reaction (102 subjects)	
	Incidence	Number of subjects	Incidence	Number of subjects		Incidence	Number of subjects	Incidence	Number of subjects
All events	98.0%	100	98.0%	100	Subcutaneous haematoma	2.9%	3	2.0%	2
Injection site pain	76.5%	78	76.5%	78	Erythema	2.9%	3	1.0%	1
Injection site bruising	45.1%	46	45.1%	46	Tenosynovitis	2.9%	3	0.0%	0
Injection site swelling	34.3%	35	34.3%	35	Constipation	2.9%	3	0.0%	0
Contusion	29.4%	30	29.4%	30	Cataract	2.9%	3	0.0%	0
Injection site laceration	15.7%	16	15.7%	16	Pain in extremity	2.0%	2	2.0%	2
Nasopharyngitis	15.7%	16	0.0%	0	Blood pressure decreased	2.0%	2	2.0%	2
Injection site oedema	11.8%	12	11.8%	12	Lymph node pain	2.0%	2	2.0%	2
Injection site haematoma	10.8%	11	10.8%	11	Alanine aminotransferase increased	2.0%	2	1.0%	1
Local swelling	8.8%	9	8.8%	9	Blood alkaline phosphatase increased	2.0%	2	1.0%	1
Laceration	8.8%	9	7.8%	8	Blood glucose increased	2.0%	2	1.0%	1
Injection site pruritus	5.9%	6	5.9%	6	Epicondylitis	2.0%	2	0.0%	0
Lymphadenitis	5.9%	6	5.9%	6	Acute sinusitis	2.0%	2	0.0%	0
Haemorrhage subcutaneous	5.9%	6	4.9%	5	Periodontitis	2.0%	2	0.0%	0
Glucose urine present	5.9%	6	1.0%	1	Periarthritis	2.0%	2	0.0%	0
Injection site vesicles	4.9%	5	4.9%	5	Back pain	2.0%	2	0.0%	0
Oedema peripheral	4.9%	5	4.9%	5	Pigmentation disorder	2.0%	2	0.0%	0
Hypertension	4.9%	5	1.0%	1	Large intestine polyp	2.0%	2	0.0%	0
Injection site haemorrhage	3.9%	4	3.9%	4	Enterocolitis	2.0%	2	0.0%	0
Pruritus	3.9%	4	2.9%	3	Epistaxis	2.0%	2	0.0%	0

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4.(iii).A.(2) Foreign phase II study (5.3.5.1-1, Study DUPY-202 [19 to 20])

A randomized, double-blind, placebo-controlled, dose-response study[52] was conducted in patients aged ≥18 years with Dupuytren's contracture (Table 11) at 2 foreign institutions (target sample size, 72) to evaluate efficacy and safety of Collagenase (Clostridium Histolyticum).

[51] The same event that resulted in death

[52] The double-blind phase was followed by the open-label phase. In addition, 2 sub-studies (open-label) were conducted separately.

Table 11. Main inclusion and exclusion criteria

Main inclusion criteria • **Patients with a diagnosis of Dupuytren's contracture, with a flexion contracture of at least one finger (a contracture of ≥20°), caused by a palpable cord.** • **Patients who are positive for the "table top test." This means that a person cannot simultaneously place the affected finger(s) and palm flat against a table top.** **Main exclusion criteria** • **Patients who underwent a surgical procedure for Dupuytren's contracture within 30 days prior to enrolment in the clinical study.** • **Patients who have IgE antibody of >15 ng/mL against collagenase**

A single dose of placebo or 2500 U, 5000 U, or 10,000 U[53] of Collagenase (Clostridium Histolyticum), reconstituted with diluent to form a liquid formulation of 0.25 mL for the MP joint or 0.20 mL for the PIP joint, was injected into the Dupuytren's contracture cord in the primary joint[46] (double-blind phase). After the evaluation of all subjects were completed 30 days after injection, a maximum of 5 additional Collagenase (Clostridium Histolyticum) 10,000 U injections were allowed to be administered, into a contracture cord of the primary joint if clinical success (extension deficit of ≤5°) had not been achieved, or into a contracture cord of another joint if clinical success had been achieved (open-label phase)[54] at intervals of 4 to 6 weeks in an open-label manner in the same procedure as in the double-blind phase. A finger extension procedure to facilitate cord disruption was performed by the study investigator as needed on the day after administration. The observation period was set to 12 months after administration. All 80 subjects who received the injection (17 subjects in the placebo group; 18, 22, and 23 subjects in the 2500 U, 5000 U, and 10,000 U groups, respectively) were included in the intent-to-treat (ITT) population in the double-blind phase, and were included in the efficacy and safety analysis sets. Ten of the 37 subjects who achieved clinical success, and 41 of the 43 subjects who did not achieve clinical success in the double-blind phase entered the open-label phase.

Table 12 shows the primary endpoint, the percentage of subjects whose cords in the primary joint achieved clinical success 30 days after administration (double-blind phase).

Table 12. Clinical success rate 30 days after injection into the primary joints (double-blind phase, ITT, LOCF)

	Placebo group (17 subjects)	2500 U group (18 subjects)	5000 U group (22 subjects)	10,000 U group (23 subjects)
All joints	**0% (0/17 subjects)**	**50.0% (9/18 subjects)**	**45.5% (10/22 subjects)**	**78.3% (18/23 subjects)**
MP joint	**0% (0/10 subjects)**	**50.0% (7/14 subjects)**	**40.0% (6/15 subjects)**	**81.3% (13/16 subjects)**
PIP joint	**0% (0/7 subjects)**	**50.0% (2/4 subjects)**	**57.1% (4/7 subjects)**	**71.4% (5/7 subjects)**

In the open-label phase, Collagenase (Clostridium Histolyticum) 10,000 U was injected (up to 3 times) into the primary joints that did not achieve clinical success during the double-blind phase (37 of 43 subjects); of these, clinical success was achieved in 62.2% (23 of 37subjects). Further, 10,000 U of Collagenase (Clostridium Histolyticum) was injected (up to 3 times into non-primary joints) into 52

[53] 10,000 U is equivalent to 0.58 mg.
[54] If collagenase-specific IgE was >15 ng/mL, or 0 to 15 ng/mL, scratch test on the skin was performed, and if positive, CCH was not permitted to be administered, and the subject was excluded from the study.

non-primary joints of 34 subjects[55] (1 joint in 20 subjects, 2 joints in 10 subjects, and 3 joints in 4 subjects). After the final injection, 55.8% (29 of 52 joints) achieved clinical success.

In the double-blind phase,[56] the incidence of adverse events were 64.7% (11 of 17 subjects) in the placebo group, 77.8% (14 of 18 subjects) in the 2500 U group, 81.8% (18 of 22 subjects) in the 5000 U group, and 91.3% (21 of 23 subjects) in the 10,000 U group; and the incidence of adverse reactions were 41.2% (7 of 17 subjects) in the placebo group, 77.8% (14 of 18 subjects) in the 2500 U group, 81.8% (18 of 22 subjects) in the 5000 U group, 87.0% (20 of 23 subjects) in the 10,000 U group. Adverse events and adverse reactions that occurred in ≥2 subjects of at least 1 group are shown in Tables 13 and 14, respectively. No deaths occurred. Serious adverse events occurred in 1 subject in the 2500 U group (pneumothorax), and 1 subject in the 10,000 U group (lung adenocarcinoma), and a causal relationship of these events to the study drug was unknown.[57] No adverse events led to discontinuation of Collagenase (Clostridium Histolyticum) treatment.

Table 13. Adverse events that occurred in ≥2 subjects of at least 1 group (double-blind phase)

	Placebo group (17 subjects)		2500 U group (18 subjects)		5000 U group (22 subjects)		10,000 U group (23 subjects)	
	Incidence	Number of subjects	Incidence	Number of subjects	Incidence	Number of subjects	Incidence	Number of subjects
All adverse events	64.7%	11	77.8%	14	81.8%	18	91.3%	21
Oedema peripheral	5.9%	1	55.6%	10	50.0%	11	65.2%	15
Tenderness	11.8%	2	55.6%	10	45.5%	10	65.2%	15
Ecchymosis	0.0%	0	55.6%	10	40.9%	9	39.1%	9
Skin laceration	0.0%	0	0.0%	0	4.5%	1	17.4%	4
Upper respiratory tract infection	0.0%	0	0.0%	0	9.1%	2	8.7%	2
Contusion	0.0%	0	5.6%	1	4.5%	1	8.7%	2
Lymphadenopathy	0.0%	0	0.0%	0	0.0%	0	8.7%	2
Pain in extremity	0.0%	0	5.6%	1	9.1%	2	0.0%	0
Swelling	0.0%	0	11.1%	2	4.5%	1	0.0%	0
Hypertension	11.8%	2	0.0%	0	4.5%	1	0.0%	0

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[55] Ten subjects achieved clinical success in the double-blind phase, and 24 subjects did not achieve clinical success in the double-blind phase (20 of them also received injections into the primary joints).

[56] From the day of the first injection in the double-blind phase to the day before the first injection in the open-label phase.

[57] The subject here was aged 83 years, and 10,000 U of Collagenase (Clostridium Histolyticum) was administered at 6-week intervals. Missing information included date of onset, date resolved, and the severity of lung adenocarcinoma, and investigator's description on the causal relationship with the study drug.

Table 14. Adverse reactions that occurred in ≥2 subjects of at least 1 group (double-blind phase)

	Placebo group (17 subjects)		2500 U group (18 subjects)		5000 U group (22 subjects)		10,000 U group (23 subjects)	
	Incidence	Number of subjects	Incidence	Number of subjects	Incidence	Number of subjects	Incidence	Number of subjects
All adverse reactions	41.2%	7	77.8%	14	81.8%	18	87.0%	20
Tenderness	11.8%	2	55.6%	10	45.5%	10	65.2%	15
Oedema peripheral	5.9%	1	50.0%	9	50.0%	11	60.9%	14
Ecchymosis	0.0%	0	50.0%	9	40.9%	9	39.1%	9
Skin laceration	0.0%	0	0.0%	0	4.5%	1	13.0%	3
Contusion	0.0%	0	5.6%	1	4.5%	1	8.7%	2
Lymphadenopathy	0.0%	0	0.0%	0	0.0%	0	8.7%	2
Pain in extremity	0.0%	0	5.6%	1	9.1%	2	0.0%	0
Swelling	0.0%	0	11.1%	2	4.5%	1	0.0%	0

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Both adverse events and adverse reactions occurred in 96.1% (49 of 51 subjects) in the open-label phase. Table 15 shows the adverse events and adverse reactions that occurred in ≥2 subjects. There was one death (chronic obstructive pulmonary disease), for which a causal relationship to the study drug was ruled out. Serious adverse events other than death occurred in 15.7% (8 of 51 subjects) and included myocardial infarction (2 subjects); benign prostatic hyperplasia, blood pressure increased, coronary artery occlusion, iliac artery stenosis,[58] malignant melanoma, and prostate cancer (1 subject each). For all the serious adverse events and adverse reactions, a causal relationship to the study drug was ruled out. Iliac artery stenosis[58] led to discontinuation of treatment in 1 subject.

Table 15. Adverse events and adverse reactions that occurred in ≥2 subjects (open-label phase)

	Adverse event (51 subjects)		Adverse reaction (51 subjects)			Adverse event (51 subjects)		Adverse reaction (51 subjects)	
	Incidence	Number of subjects	Incidence	Number of subjects		Incidence	Number of subjects	Incidence	Number of subjects
All events	96.1%	49	96.1%	49	Pruritus	5.9%	3	5.9%	3
Oedema peripheral	82.4%	42	82.4%	42	Pain in extremity	5.9%	3	3.9%	2
Tenderness	74.5%	38	74.5%	38	Limb injury	5.9%	3	0.0%	0
Ecchymosis	62.7%	32	62.7%	32	Injection site oedema	3.9%	2	3.9%	2
Lymphadenopathy	31.4%	16	31.4%	16	Injection site haemorrhage	3.9%	2	3.9%	2
Oedema	23.5%	12	23.5%	12	Lymphangitis	3.9%	2	3.9%	2
Skin laceration	19.6%	10	17.6%	9	Skin haemorrhage	3.9%	2	3.9%	2
Hypertension	13.7%	7	5.9%	3	Blood pressure increased	3.9%	2	2.0%	1
Contusion	11.8%	6	11.8%	6	Myocardial infarction	3.9%	2	0.0%	0
Blood blister	7.8%	4	7.8%	4	Urinary tract infection	3.9%	2	0.0%	0
Haematoma	7.8%	4	7.8%	4	Benign prostatic hyperplasia	3.9%	2	0.0%	0
Injection site pain	5.9%	3	5.9%	3	Hypothyroidism	3.9%	2	0.0%	0
Pain	5.9%	3	5.9%	3					

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[58]The same event that resulted in death.

A sub-study and lidocaine sub-study[59] were conducted in patients aged ≥18 years with Dupuytren's contracture (Table 11) to determine pharmacokinetics, and Collagenase (Clostridium Histolyticum) 10,000 U was injected up to 4 times in the same manner as in the double-blind and open-label phases. Five subjects in each group (10 subjects in total) received Collagenase (Clostridium Histolyticum), and were included in the safety analysis set. Both adverse events and adverse reactions occurred in 100% (10 of 10 subjects). The adverse events that occurred in ≥2 subjects were as follows: oedema peripheral (10 subjects); tenderness (9 subjects); ecchymosis (8 subjects); oedema (4 subjects); limb injury (3 subjects); injection site pain, skin laceration, and hypothyroidism (2 subjects each). The adverse reactions that occurred in ≥2 subjects were as follows: oedema peripheral (10 subjects); tenderness, ecchymosis (8 subjects each); oedema (4 subjects); injection site pain, and limb injury (2 subjects each). There were no deaths, and no adverse events that led to discontinuation of treatment. A serious adverse event occurred in 1 subject (lobar pneumonia), but a causal relationship to the study drug was ruled out.

4.(iii).A.(3) Foreign phase III studies

4.(iii).A.(3).1) Foreign phase III confirmatory study (5.3.5.1-2, Study AUX-CC-857 [August 2007 to April 2008])

A randomized, double-blind, placebo-controlled, parallel-group study was conducted in patients aged ≥18 years with Dupuytren's contracture (in the same way as the Japanese study, as shown in Table 8) at 16 institutions outside Japan to evaluate the efficacy and safety of CCH (target sample size, 216 subjects; 144 subjects with the MP joint and 72 subjects with the PIP joint for the primary joint).

Placebo or CCH 0.58 mg, reconstituted with diluent to form a liquid formulation of 0.25 mL for the MP joint or 0.20 mL for the PIP joint, was injected into Dupuytren's cord of the primary joint.[46] The second dose was injected into the contracture cord in the primary joint if clinical success (extension deficit of ≤5°) had not been achieved after 30 days from the first injection, or into the cord of the secondary joint if clinical success had been achieved. The third dose was injected into the cord of the same joint as the second injection if clinical success still had not been achieved after 30 days from the second injection (60 days after the first injection), or into the cord of the tertiary joint if clinical success had been achieved (no more than 3 injections in total). The observation period was set to 90 days after the first injection.[60] A finger extension procedure to facilitate cord disruption was performed as needed by the study investigator on the day after injection.

All 308 subjects (104 subjects in the placebo group, and 204 subjects in the CCH group) who received injections were included in the intent-to-treat (ITT) population, and also in the safety analysis set. Two of the ITT subjects[61] were excluded, and the remaining 306 subjects (103 subjects in the placebo group,

[59] An exploratory study was conducted to investigate whether lidocaine injection alleviates pain from contracture cord disruption on the next day of Collagenase (Clostridium Histolyticum) administration.

[60] Subjects who completed evaluation during the 90-day observation period from the first injection were eligible to be enrolled in Study AUX-CC-858 (9-month open-label, extension study, up to 5 injections).

[61] "The extension deficit angle after the first injection was not measured" in 1 subject (placebo group), and "at the screening and on the day of first injection, the extension deficit was <5°" in 1 subject (CCH group).

and 203 subjects in the CCH group) were included in the modified-intent-to-treat (MITT) population, and efficacy analysis were performed using this population (primary efficacy analysis set). Treatment was discontinued in 17 subjects.[62]

The primary endpoint was the percentage of subjects who achieved clinical success in their primary joint 30 days after the final injection, and the results were 6.8% (7 of 103 subjects) in the placebo group, and 64.0% (130 of 203 subjects) in the CCH group, indicating that the clinical success rate is significantly higher in the CCH group than in the placebo group ($P < 0.001$, Cochran-Mantel-Haenszel test) (Table 16).

Table 16. Clinical success in the primary joint 30 days after the final injection (MITT)

	Placebo group	CCH group	*P*-value
All joints			
Percentage of subjects who achieved clinical success	**6.8% (7/103 subjects)**	**64.0% (130/203 subjects)**	**<0.001[a)]**
Number of injections (mean ± SD)	**2.9 ± 0.4**	**1.7 ± 0.8**	
MP joint			
Percentage of subjects who achieved clinical success	**7.2% (5/69 subjects)**	**76.7% (102/133 subjects)**	**<0.001[b)]**
Number of injections (mean ± SD)	**2.9 ± 0.4**	**1.7 ± 0.8**	
PIP joint			
Percentage of subjects who achieved clinical success	**5.9% (2/34 subjects)**	**40.0% (28/70 subjects)**	**<0.001[b)]**
Number of injections (mean ± SD)	**2.8 ± 0.6**	**1.8 ± 0.8**	

a) Cochran-Mantel-Haenszel test stratified by baseline severity and joint type (two-sided 5% significance level)
b) Cochran-Mantel-Haenszel test stratified by baseline severity (two-sided 5% significance level)
Tests were performed for all joints data. Only if the hypothesis was rejected, the closed testing procedure was used for the MP joint test first, and then for the PIP joint test.

Based on the safety results, the incidence of adverse events was 47.1% (49 of 104 subjects) in the placebo group, and 97.1% (198 of 204 subjects) in the CCH group; the incidence of adverse reactions was 21.2% (22 of 104 subjects) in the placebo group, and 96.6% (197 of 204 subjects) in the CCH group. The adverse events and adverse reactions that occurred with an incidence of ≥2% in at least 1 group are shown in Tables 17 and 18, respectively. No deaths occurred. The incidence of serious adverse events was 1.0% (1 of 104 subjects; cholecystitis acute) in the placebo group, and 3.4% (7 of 204 subjects: tendon rupture [2 subjects]; complex regional pain syndrome, spinal fusion surgery, myocardial infarction, panic attack, and ligament disorder [1 subject each]). A causal relationship to the study drug could not be ruled out for tendon rupture (2 subjects) and complex regional pain syndrome (1 subject) in the CCH group.[63] Adverse events led to discontinuation of treatment in 3 subjects (injection site pain, dizziness, and complex regional pain syndrome; 1 subject each), and a causal relationship to the study drug could not be ruled out for any of the events.

[62] Discontinuation occurred in 4 subjects in the placebo group ("withdrawal of consent" in 3 subjects, and "no visit to the institution" in 1 subject), and in 13 subjects in the CCH group ("withdrawal of consent" and "no visit to the institution" in 4 subjects each, "adverse events" in 3 subjects, and "personal reasons" in 2 subjects).

[63] The outcomes were reported as "resolved" for all events.

Table 17. Adverse events that occurred with an incidence of ≥2% in at least 1 group

	Placebo group (104 subjects)		CCH group (204 subjects)			Placebo group (104 subjects)		CCH group (204 subjects)	
	Incidence	Number of subjects	Incidence	Number of subjects		Incidence	Number of subjects	Incidence	Number of subjects
All adverse events	47.1%	49	97.1%	198	Erythema	0.0%	0	6.4%	13
Oedema peripheral	3.8%	4	72.5%	148	Injection site pruritus	0.0%	0	5.4%	11
Contusion	1.9%	2	51.5%	105	Blister	0.0%	0	5.4%	11
Injection site haemorrhage	3.8%	4	37.3%	76	Axillary pain	0.0%	0	4.9%	10
Injection site pain	4.8%	5	32.4%	66	Inflammation	0.0%	0	3.9%	8
Pain in extremity	2.9%	3	31.9%	65	Arthralgia	0.0%	0	3.9%	8
Tenderness	0.0%	0	26.5%	54	Blood blister	0.0%	0	3.4%	7
Ecchymosis	1.0%	1	25.0%	51	Nasopharyngitis	8.7%	9	2.9%	6
Injection site swelling	3.8%	4	21.1%	43	Joint swelling	0.0%	0	2.9%	6
Pruritus	1.0%	1	10.8%	22	Headache	2.9%	3	2.5%	5
Skin laceration	0.0%	0	10.8%	22	Swelling	0.0%	0	2.5%	5
Lymph node pain	0.0%	0	10.3%	21	Injection site vesicles	1.0%	1	2.0%	4
Lymphadenopathy	0.0%	0	9.8%	20	Sinusitis	2.9%	3	1.0%	2

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Table 18. Adverse reactions that occurred with an incidence of ≥2% in at least 1 group

	Placebo group (104 subjects)		CCH group (204 subjects)			Placebo group (104 subjects)		CCH group (204 subjects)	
	Incidence	Number of subjects	Incidence	Number of subjects		Incidence	Number of subjects	Incidence	Number of subjects
All adverse reactions	21.2%	22	96.6%	197	Lymphadenopathy	0.0%	0	9.8%	20
Oedema peripheral	3.8%	4	72.5%	148	Erythema	0.0%	0	6.4%	13
Contusion	1.9%	2	51.0%	104	Injection site pruritus	0.0%	0	5.4%	11
Injection site haemorrhage	3.8%	4	37.3%	76	Blister	0.0%	0	5.4%	11
Injection site pain	4.8%	5	32.4%	66	Axillary pain	0.0%	0	4.9%	10
Pain in extremity	2.9%	3	30.9%	63	Inflammation	0.0%	0	3.9%	8
Tenderness	0.0%	0	26.5%	54	Arthralgia	0.0%	0	3.4%	7
Ecchymosis	1.0%	1	25.0%	51	Blood blister	0.0%	0	3.4%	7
Injection site swelling	3.8%	4	21.1%	43	Joint swelling	0.0%	0	2.9%	6
Pruritus	1.0%	1	10.8%	22	Headache	1.0%	1	2.5%	5
Skin laceration	0.0%	0	10.8%	22	Swelling	0.0%	0	2.5%	5
Lymph node pain	0.0%	0	10.3%	21	Injection site vesicles	1.0%	1	2.0%	4

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4.(iii).A.(3).2) Foreign phase III extension study (5.3.5.2-4, Study AUX-CC-858 [20 to 20])

An open-label, extension study (target sample size, 216) to investigate the safety and efficacy of CCH was conducted at 16 institutions outside Japan in patients who completed the visit 90 days after the first injection in the Study AUX-CC-857.

In Study AUX-CC-858, CCH was administered to those who had received <3 injections of CCH into Dupuytren's cords among subjects who did not achieve an extension deficit of ≤5°in Study AUX-CC-857, or those who had CCH-untreated Dupuytren's cords in Study AUX-CC-857.

A single dose of 0.58 mg of CCH, reconstituted with diluent to form a liquid formulation of 0.25 mL for the MP joint or 0.20 mL for the PIP joint, was injected into Dupuytren's cords. If clinical success (extension deficit of ≤5°) was not achieved, CCH injection was allowed up to 3 times per joint at 30-day intervals, and up to 5 times in total per subject. The follow-up period was set to 9 months for all subjects regardless of CCH administration.[64] A finger extension procedure to facilitate cord disruption was performed as needed by the study investigator on the day following injection.

The ITT population was defined as all subjects originally enrolled in Study AUX-CC-857 and then participated in Study AUX-CC-858 (286 subjects; 100 subjects in the placebo group, and 186 subjects in the CCH group in Study AUX-CC-857). In Study AUX-CC-858, 160 subjects received 425 CCH injections (total of 283 joints), whereas 126 subjects did not receive CCH (5 subjects from the placebo group, and 121 subjects from the CCH group in Study AUX-CC-857). Further, 299 subjects, who received at least 1 dose of CCH in Study AUX-CC-857 or Study AUX-CC-858 (542 joints in total; 314 MP joints, and 228 PIP joints) were included in the CCH treated population. Treatment was discontinued in 29 subjects.[65]

The incidences of adverse events and adverse reactions were both 97.7% (292 of 299 subjects) in the CCH treated population.[66] Table 19 shows the adverse events and adverse reactions that occurred with an incidence of ≥2%. There were no adverse events that led to death or discontinuation of treatment in Study AUX-CC-858. Serious adverse events occurred in 5.0% (15 of 299 subjects) in the pooled data of those 2 studies, and 9 subjects experienced them in Study AUX-CC-858 (1 subject each for acute myocardial infarction, abdominal pain/nausea, nephrolithiasis, chronic myeloid leukaemia, clostridial infection, pneumonitis, meningioma, asthenia/syncope, and finger deformity). A causal relationship to the study drug could not be ruled out for finger deformity.[67]

[64] All subjects were required to visit the institutions at 90 days, 6 months, and 9 months after study treatment was initiated.
[65] The reasons for discontinuation were as follows: "no visit to the institution" (13 subjects), "personal reasons" (12 subjects), "withdrawal of consent" (2 subjects), and "management reasons" (2 subjects).
[66] The data for Studies AUX-CC-857 and AUX-CC-858 were pooled for analyses.
[67] The outcome is "unknown" (the event was still ongoing as of 2009 after the completion of the study).

Table 19. Adverse events and adverse reactions that occurred with an incidence of ≥2% (CCH treated population, data were consolidated with those for Study AUX-CC-857)

	Adverse event (299 subjects)		Adverse reaction (299 subjects)			Adverse event (299 subjects)		Adverse reaction (299 subjects)	
	Incidence	Number of subjects	Incidence	Number of subjects		Incidence	Number of subjects	Incidence	Number of subjects
All events	97.7%	292	97.7%	292	Axillary pain	5.7%	17	5.7%	17
Oedema peripheral	80.3%	240	79.9%	239	Pain	5.7%	17	5.4%	16
Contusion	59.5%	178	59.2%	177	Dupuytren's contracture	5.7%	17	2.0%	6
Pain in extremity	40.5%	121	39.1%	117	Blister	5.4%	16	5.4%	16
Injection site haemorrhage	36.5%	109	36.5%	109	Joint swelling	4.7%	14	4.7%	14
Injection site pain	34.1%	102	34.1%	102	Arthralgia	4.7%	14	3.7%	11
Tenderness	28.8%	86	28.4%	85	Swelling	4.3%	13	4.3%	13
Ecchymosis	27.8%	83	27.8%	83	Nasopharyngitis	4.3%	13	0.3%	1
Injection site swelling	23.4%	70	23.4%	70	Injection site erythema	3.7%	11	3.7%	11
Pruritus	17.1%	51	16.7%	50	Inflammation	2.7%	8	2.7%	8
Skin laceration	14.0%	42	13.0%	39	Injection site vesicles	2.7%	8	2.7%	8
Lymphadenopathy	10.0%	30	10.0%	30	Hypoaesthesia	2.7%	8	1.7%	5
Lymph node pain	10.0%	30	10.0%	30	Injection site warmth	2.3%	7	2.3%	7
Blood blister	9.4%	28	9.4%	28	Headache	2.3%	7	2.3%	7
Injection site pruritus	8.4%	25	8.4%	25	Rash	2.0%	6	2.0%	6
Erythema	8.4%	25	8.0%	24	Influenza	2.0%	6	0.0%	0

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The percentage of joints that achieved clinical success within 30 days after the final CCH injection was 50.5% (264 of 523 joints in total; 66.6% for the MP joints [199 of 299 joints], and 29.0% for the PIP joints [65 of 224 joints]).

4.(iii).A.(3).3) Foreign phase III confirmatory study (5.3.5.1-3, Study AUX-CC-859 [August 2007 to September 2008])

A study consisting of a randomized, double-blind, placebo-controlled, parallel-group phase (double-blind phase) followed by an open-label extension phase (open-label phase) was conducted in patients aged ≥18 years with Dupuytren's contracture (in the same way as in the Japanese study, as shown in Table 8) at 5 institutions outside Japan (target sample size, 60; 20 subjects in the placebo group, and 40 subjects in the CCH group) to investigate the efficacy and safety of CCH.

In the double-blind phase, placebo or CCH 0.58 mg, reconstituted with diluent to form a liquid formulation of 0.25 mL for the MP joint or 0.20 mL for the PIP joint, was injected into Dupuytren's cord of the primary joint.[46] The second dose was injected into the contracture cord in the primary joint if clinical success (extension deficit of ≤5°) had not been achieved, or into the cord of the secondary joint if clinical success had been achieved after 30 days from the injection. The third dose was injected into the cord of the same joint as the second dose was if clinical success still had not been achieved after 30 days from the second dose (60 days from the first dose), or into the cord of the tertiary joint if clinical success had been achieved (no more than 3 doses in total). The observation period for the double-blind phase was 90 days after the first dose. Subjects who completed the 90-day evaluation following the first injection (30 days after the third injection) were eligible to be enrolled in the 9-month open-label phase

of the study (12 months in total including the double-blind phase). In the open-label phase, if joints to be treated had not achieved clinical success, the subjects were allowed to receive up to 3 injections of 0.58 mg of CCH per joint at 30-day intervals up to 5 injections in total (up to 8 injections in total including those in the double-blind phase), in the same manner as in the double-blind phase. The follow-up period was set to 12 months after the first injection.[68] A finger extension procedure to facilitate cord disruption was performed as needed by the study investigator on the day following injection.

The ITT population for the double-blind phase was all 66 treated subjects (21 and 45 subjects in the placebo and CCH groups, respectively), and efficacy and safety analyses were performed using this population. In the double-blind phase, 2 subjects in the placebo group discontinued the treatment.[69] The ITT population for the open-label phase was the 64 subjects (19 and 45 subjects in the placebo and CCH groups, respectively) who completed the double-blind phase, and enrolled in the open-label phase. Among the ITT subjects, 63 subjects, who received at least 1 CCH injection in the double-blind or open-label phase, were included in the CCH treated population,[70] and this population was used for the safety analyses set of both phases.

The primary efficacy endpoint was the percentage of subjects who achieved clinical success in their primary joint 30 days after the final injection in the double-blind phase, and the results were 4.8% (1 of 21 subjects) in the placebo group, and 44.4% (20 of 45 subjects) in the CCH group, indicating that the clinical success rate is significantly higher in the CCH group than in the placebo group (P <0.001, Cochran-Mantel-Haenszel test) (Table 20).

Table 20. Clinical success for the primary joint 30 days after the final injection (double-blind phase, ITT)

	Placebo group	CCH group	*P*-value
All joints			
Percentage of subjects who achieved clinical success	4.8% (1/21 subjects)	44.4% (20/45 subjects)	<0.001[a)]
Number of injections (mean ± SD)	2.8 ± 0.6	1.7 ± 0.8	
MP joints			
Percentage of subjects who achieved clinical success	9.1% (1/11 subjects)	65.0% (13/20 subjects)	0.003[b)]
Number of injections (mean ± SD)	2.7 ± 0.7	1.5 ± 0.7	
PIP joints			
Percentage of subjects who achieved clinical success	0% (0/10 subjects)	28.0% (7/25 subjects)	0.069[b)]
Number of injections (mean ± SD)	2.8 ± 0.6	1.8 ± 0.8	

a) Cochran-Mantel-Haenszel test stratified by baseline severity and joint type (two-sided 5% significance level)
b) Cochran-Mantel-Haenszel test stratified by baseline severity (two-sided 5% significance level)
Tests were performed for all joints data. Only when the hypothesis was rejected, the closed testing procedure will be used, first for the MP joint, and then for the PIP joint.

The incidence of adverse events for the double-blind phase was 57.1% (12 of 21 subjects) in the placebo group, and 100.0% (45 of 45 subjects) in the CCH group. The incidence of adverse reactions was 38.1% (8 of 21 subjects) in the placebo group, and 100.0% (45 of 45 subjects) in the CCH group. The adverse events and adverse reactions that occurred in ≥2 subjects in at least 1 group are shown in Tables 21 and

[68] Study visits were required 1, 7, and 30 days after each injection, and 6, 9, and 12 months after the first injection in the double-blind phase.
[69] Discontinuation was due to "withdrawal of consent" by the subject in both cases.
[70] Of the 64 subjects who completed the double-blind phase and entered the open-label phase, 1 subject in the placebo group did not receive CCH.

22, respectively. No adverse events resulted in death, or led to treatment discontinuation. During the double-blind phase, a serious adverse event (ligament injury) occurred in 1 of 45 subjects (2.2%) in the CCH group, and its causal relationship to the study drug could not be ruled out, however, the outcome was reported as "resolved."

Table 21. Adverse events that occurred in ≥2 subjects in at least 1 group (double-blind phase)

	Placebo group (21 subjects)		CCH group (45 subjects)			Placebo group (21 subjects)		CCH group (45 subjects)	
	Incidence	Number of subjects	Incidence	Number of subjects		Incidence	Number of subjects	Incidence	Number of subjects
All adverse events	57.1%	12	100.0%	45	Dizziness	0.0%	0	6.7%	3
Oedema peripheral	9.5%	2	77.8%	35	Blood blister	0.0%	0	6.7%	3
Contusion	9.5%	2	73.3%	33	Arthralgia	4.8%	1	4.4%	2
Pain in extremity	14.3%	3	48.9%	22	Neck pain	4.8%	1	4.4%	2
Injection site haemorrhage	0.0%	0	42.2%	19	Injection site pruritus	0.0%	0	4.4%	2
Injection site pain	9.5%	2	37.8%	17	Injection site vesicles	0.0%	0	4.4%	2
Injection site swelling	14.3%	3	35.6%	16	Groin pain	0.0%	0	4.4%	2
Lymphadenopathy	0.0%	0	24.4%	11	Hypoaesthesia	0.0%	0	4.4%	2
Tenderness	0.0%	0	13.3%	6	Headache	9.5%	2	2.2%	1
Axillary pain	0.0%	0	11.1%	5	Upper respiratory tract infection	9.5%	2	2.2%	1
Pruritus	0.0%	0	11.1%	5	Haematuria	9.5%	2	2.2%	1
Paraesthesia	4.8%	1	8.9%	4					

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Table 22. Adverse reactions that occurred in ≥2 subjects in at least 1 group (double-blind phase)

	Placebo group (21 subjects)		CCH group (45 subjects)			Placebo group (21 subjects)		CCH group (45 subjects)	
	Incidence	Number of subjects	Incidence	Number of subjects		Incidence	Number of subjects	Incidence	Number of subjects
All adverse reactions	38.1%	8	100.0%	45	Pruritus	0.0%	0	11.1%	5
Oedema peripheral	9.5%	2	77.8%	35	Paraesthesia	4.8%	1	6.7%	3
Contusion	9.5%	2	73.3%	33	Blood blister	0.0%	0	6.7%	3
Pain in extremity	9.5%	2	48.9%	22	Arthralgia	4.8%	1	4.4%	2
Injection site haemorrhage	0.0%	0	42.2%	19	Dizziness	0.0%	0	4.4%	2
Injection site pain	9.5%	2	37.8%	17	Injection site pruritus	0.0%	0	4.4%	2
Injection site swelling	14.3%	3	35.6%	16	Injection site vesicles	0.0%	0	4.4%	2
Lymphadenopathy	0.0%	0	24.4%	11	Groin pain	0.0%	0	4.4%	2
Tenderness	0.0%	0	13.3%	6	Hypoaesthesia	0.0%	0	4.4%	2
Axillary pain	0.0%	0	11.1%	5					

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The incidence of adverse events and the incidence of adverse reactions throughout the 2 phases were both 100.0% (63 of 63 subjects). Table 23 shows the adverse events and adverse reactions that occurred in ≥2 subjects in at least 1 group. There were no adverse events that resulted in death or led to discontinuation of treatment.

Serious adverse events occurred in 11.1% (7 of 63 subjects) (Dupuytren's contracture/sensory disturbance, Dupuytren's contracture operation, orbital cyst, cholecystitis/abdominal hernia

repair/umbilical hernia repair, atrial fibrillation, pneumonia, and inguinal hernia repair [1 subject each]) in the open-label phase, and a causal relationship to the study drug could not be ruled out for Dupuytren's contracture/sensory disturbance, however, their outcomes were reported as "resolved."

Table 23. Adverse events and adverse reactions that occurred in ≥2 subjects (through the 2 phases, in the CCH treated population)

	Adverse event (63 subjects)		Adverse reaction (63 subjects)			Adverse event (63 subjects)		Adverse reaction (63 subjects)	
	Incidence	Number of subjects	Incidence	Number of subjects		Incidence	Number of subjects	Incidence	Number of subjects
All events	100.0%	63	100.0%	63	Blood blister	4.8%	3	4.8%	3
Oedema peripheral	85.7%	54	85.7%	54	Neck pain	4.8%	3	1.6%	1
Contusion	73.0%	46	73.0%	46	Feeling hot	3.2%	2	3.2%	2
Pain in extremity	57.1%	36	54.0%	34	Dupuytren's contracture	3.2%	2	3.2%	2
Injection site pain	44.4%	28	44.4%	28	Joint swelling	3.2%	2	3.2%	2
Injection site haemorrhage	42.9%	27	42.9%	27	Groin pain	3.2%	2	3.2%	2
Injection site swelling	36.5%	23	36.5%	23	Hypoaesthesia	3.2%	2	3.2%	2
Tenderness	31.7%	20	31.7%	20	Nausea	3.2%	2	3.2%	2
Pruritus	20.6%	13	20.6%	13	Raynaud's phenomenon	3.2%	2	3.2%	2
Lymphadenopathy	20.6%	13	20.6%	13	Skin laceration	3.2%	2	1.6%	1
Axillary pain	12.7%	8	11.1%	7	Axillary mass	3.2%	2	1.6%	1
Injection site vesicles	7.9%	5	7.9%	5	Headache	3.2%	2	1.6%	1
Paraesthesia	7.9%	5	4.8%	3	Haematuria	3.2%	2	1.6%	1
Dizziness	7.9%	5	3.2%	2	Hypersensitivity	3.2%	2	1.6%	1
Upper respiratory tract infection	6.3%	4	0.0%	0	Pneumonia	3.2%	2	0.0%	0
Lower respiratory tract infection	6.3%	4	0.0%	0	Pharyngolaryngeal pain	3.2%	2	0.0%	0
Injection site pruritus	4.8%	3	4.8%	3	Blood pressure increased	3.2%	2	0.0%	0
Arthralgia	4.8%	3	4.8%	3	Conjunctivitis	3.2%	2	0.0%	0

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4.(iii).A.(3).4) Foreign phase III study (5.3.5.2-2, Study AUX-CC-854 [September 2007 to December 2008])

A multi-center, uncontrolled, open-label study was conducted in patients aged ≥18 years with Dupuytren's contracture[71] (in the same way as in the Japanese study, as shown in Table 8) at 20 institutions outside Japan (target sample size, 240) to investigate the safety and efficacy of CCH.

CCH 0.58 mg, reconstituted with diluent to form a liquid formulation of 0.25 mL for the MP joint or 0.20 mL for the PIP joint, was injected into Dupuytren's cords. If clinical success (extension deficit of ≤5°) was not achieved, CCH could be injected up to 3 times per joint at 30-day intervals, and up to 5 times in total per subject. The follow-up period was set to 9 months after the first injection.[72] A finger extension procedure was performed as needed by the study investigator to facilitate cord disruption on the day after injection.

[71] In addition, patients who had received 1 or 2 injections of CCH in Study AUX-CC-851 or Study AUX-CC-853, randomized, placebo-controlled, double-blind studies in non-Japanese patients, or in Study AUX-CC-855, were also eligible to be enrolled in the study.
[72] All subjects were required to visit study institutions 90 days, 6 months, and 9 months after the first injection.

All 386 treated subjects were included in the ITT population, and this population was used for the safety analyses. Two ITT subjects who had a baseline extension deficit of ≤5° were excluded, and the data of the remaining 384 subjects were used for efficacy analysis. Treatment was discontinued in 28 subjects.[73]

The incidence of adverse events was 99.0% (382 of 386 subjects), and incidence of adverse reactions was 98.2% (379 of 386 subjects). Table 24 shows the adverse events and adverse reactions that occurred with an incidence of ≥2%. One death occurred (acute myocardial infarction), but a causal relationship to the study drug was ruled out. Serious adverse events occurred in 10.1% (39 of 386 subjects) (Table 25), and a causal relationship to the study drug could not be ruled out for deep vein thrombosis and tendonitis (1 subject each).[74] Pancreatic carcinoma and gastrointestinal carcinoma (1 subject each) led to discontinuation of treatment, and a causal relationship to the study drug was ruled out for both events.

Table 24. Adverse events and adverse reactions that occurred with an incidence of ≥2%

	Adverse event (386 subjects)		Adverse reaction (386 subjects)			Adverse event (386 subjects)		Adverse reaction (386 subjects)	
	Incidence	Number of subjects	Incidence	Number of subjects		Incidence	Number of subjects	Incidence	Number of subjects
All events	99.0%	382	98.2%	379	Dizziness	3.9%	15	1.8%	7
Oedema peripheral	75.6%	292	75.6%	292	Nasopharyngitis	3.6%	14	0.5%	2
Contusion	65.5%	253	65.0%	251	Paraesthesia	3.4%	13	2.6%	10
Pain in extremity	49.0%	189	46.6%	180	Erythema	3.1%	12	2.3%	9
Injection site pain	35.0%	135	35.0%	135	Pain	2.8%	11	2.6%	10
Injection site haemorrhage	29.0%	112	29.0%	112	Local swelling	2.6%	10	2.6%	10
Tenderness	26.7%	103	25.9%	100	Joint swelling	2.6%	10	2.3%	9
Injection site swelling	26.2%	101	26.2%	101	Lymph node pain	2.6%	10	2.3%	9
Blood blister	9.8%	38	9.8%	38	Joint stiffness	2.6%	10	2.1%	8
Lymphadenopathy	9.8%	38	9.8%	38	Shoulder pain	2.6%	10	0.8%	3
Skin laceration	9.8%	38	8.3%	32	Dupuytren's contracture	2.6%	10	0.5%	2
Axillary pain	9.6%	37	9.6%	37	Lower respiratory tract infection	2.6%	10	0.0%	0
Haematoma	9.6%	37	9.1%	35	Swelling	2.3%	9	2.3%	9
Pruritus	9.3%	36	9.3%	36	Back pain	2.3%	9	0.0%	0
Arthralgia	6.7%	26	4.7%	18	Sinusitis	2.3%	9	0.0%	0
Injection site vesicles	6.5%	25	6.5%	25	Oedema	2.1%	8	2.1%	8
Upper respiratory tract infection	4.1%	16	0.5%	2	Hypoaesthesia	2.1%	8	2.1%	8
Headache	3.9%	15	2.6%	10					

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[73] The reasons for discontinuation were as follows: "no visit to the institution" (13 subjects), "withdrawal of consent" (7 subjects), "adverse events" (2 subjects), "personal reasons" (2 subjects), "deviation from the protocol" (2 subjects), "management reasons" (1 subject), and "death" (1 subject).

[74] The outcome for deep vein thrombosis was reported as "improved," while that for tendonitis is unknown (because the subject declined to participate in the follow-up survey).

Table 25. Serious adverse events

Pneumonia, inguinal hernia, prostate cancer, and Dupuytren's contracture operation (2 subjects each); lower respiratory tract infection, pleural effusion, urinary incontinence, colostomy closure/large intestine anastomosis, convulsion, atrial fibrillation, skin cancer, Dupuytren's contracture operation/tendon sheath incision, pancreatic carcinoma, renal abscess, carpal tunnel decompression, coronary arterial stent insertion, deep vein thrombosis, muscle injury, gastrointestinal carcinoma, atrial fibrillation/atrial flutter/chest pain, sick sinus syndrome, atrial fibrillation/pleural effusion/pneumonia/renal failure acute, hand fracture/ligament injury, pancreatitis acute, cholelithiasis/sinusitis, cervical vertebral fracture, deep vein thrombosis/cellulitis, syncope, benign prostatic hyperplasia, acute myocardial infarction, tendonitis, joint arthroplasty, cerebrovascular disorder, peripheral occlusive disease/thrombosis, and herpes zoster/pneumonia (1 subject each).

The percentage of joints which achieved clinical success 30 days after the final injection was 58.4% (343 of 587 joints) (Table 26).

Table 26. Clinical success 30 days after the final injection

	All joints	MP joint	PIP joint
Percentage of joints which achieved clinical success	58.4% (154/292 joints)	70.8% (243/343 joints)	41.0% (100/244 joints)
Number of injections (mean ± SD)	1.4 ± 0.69	1.3 ± 0.67	1.5 ± 0.72

4.(iii).A.(3).5) Foreign phase III study (5.3.5.2-3, Study AUX-CC-856 [October 2007 to October 2008])

A multi-center, open-label, uncontrolled study was conducted in patients aged ≥18 years with Dupuytren's contracture[75] (in the same way as in the Japanese study, as shown in Table 8) at 14 institutions outside Japan (target sample size, ≥100) to investigate the safety and efficacy of CCH.

CCH 0.58 mg, reconstituted with diluent to form a liquid formulation of 0.25 mL for the MP joint or 0.20 mL for the PIP joint, was injected into Dupuytren's cords. If clinical success (extension deficit of ≤5°) was not achieved, CCH could be injected up to 3 times per joint at 30-day intervals, and up to 5 times in total per subject. The follow-up period was set to 9 months after the first injection.[76] A finger extension procedure to facilitate cord disruption was performed as needed by the study investigator on the day following injection.

All 201 treated subjects were included in the ITT population, and this population was used for the safety analysis. One ITT subject who had baseline extension deficit of ≤5° was excluded, and the data of the remaining 200 subjects were used for efficacy analysis. Treatment was discontinued in 33 subjects.[77]

The incidence of adverse events was 98.0% (197 of 201 subjects), and the incidence of adverse reactions was 93.5% (188 of 201 subjects). Table 27 shows the adverse events and adverse reactions that occurred with an incidence of ≥2%. Two deaths occurred (myocardial infarction, acute myocardial infarction [1 subject each]), but a causal relationship to the study drug was ruled out for both events. The following

[75] In addition, patients who had received 1 or 2 injections of CCH in Study AUX-CC-851 or Study AUX-CC-853, randomized, double-blind, placebo-controlled studies in non-Japanese patients, or in Study AUX-CC-855, were also eligible to be enrolled in the study.
[76] All subjects were required to visit the study institution 90 days, 6 months, and 9 months after the first injection.
[77] The reasons for discontinuation: "withdrawal of consent" (17 subjects), "no visit to the institution" (10 subjects), "death" (2 subjects), "personal reasons" (2 subjects), "adverse events" (1 subject) and "management reasons" (1 subject).

serious adverse events occurred in 4.5% (9 of 201 subjects): finger amputation/peritonitis/small intestinal perforation/enterococcal sepsis/renal failure/myocardial infarction/respiratory failure/intervertebral discitis/osteomyelitis, small intestinal obstruction/compression fracture/fall/atrial fibrillation, deep vein thrombosis/embolism, clavicle fracture, lung neoplasm malignant, thyroid cancer, Rocky mountain spotted fever, renal cell cancer, and chest pain (1 subject each). A causal relationship to the study drug was ruled out for all serious adverse events. One adverse event led to discontinuation of treatment (lung neoplasm malignant), but a causal relationship to the study drug was ruled out.

Table 27. Adverse events and adverse reactions that occurred with an incidence of ≥2%

	Adverse event (201 subjects)		Adverse reaction (201 subjects)			Adverse event (201 subjects)		Adverse reaction (201 subjects)	
	Incidence	Number of subjects	Incidence	Number of subjects		Incidence	Number of subjects	Incidence	Number of subjects
All events	98.0%	197	93.5%	188	Swelling	5.0%	10	4.0%	8
Oedema peripheral	75.1%	151	73.1%	147	Arthralgia	5.0%	10	2.5%	5
Injection site pain	57.7%	116	56.2%	113	Injection site vesicles	4.5%	9	4.5%	9
Injection site haemorrhage	54.2%	109	51.7%	104	Dupuytren's contracture	4.5%	9	1.5%	3
Contusion	49.8%	100	49.3%	99	Nasopharyngitis	4.5%	9	0.0%	0
Injection site swelling	28.4%	57	27.4%	55	Axillary pain	4.0%	8	4.0%	8
Tenderness	23.9%	48	22.9%	46	Joint swelling	3.0%	6	2.0%	4
Pain in extremity	23.9%	48	21.9%	44	Musculoskeletal stiffness	2.5%	5	2.5%	5
Ecchymosis	21.4%	43	20.4%	41	Lymphadenopathy	2.5%	5	2.0%	4
Skin laceration	15.9%	32	11.4%	23	Pain	2.5%	5	1.5%	3
Pruritus	11.9%	24	11.4%	23	Back pain	2.5%	5	0.0%	0
Injection site pruritus	9.5%	19	9.5%	19	Sinusitis	2.5%	5	0.0%	0
Haematoma	6.0%	12	5.0%	10	Blister	2.0%	4	2.0%	4
Erythema	6.0%	12	3.5%	7	Headache	2.0%	4	0.5%	1
Blood blister	5.0%	10	5.0%	10	Rash	2.0%	4	0.0%	0

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The percentage of joints which achieved clinical success 30 days after the final injection was 52.7% (154 of 292 joints) (Table 28).

Table 28. Clinical success 30 days after the final injection

	All joints	MP joint	PIP joint
Percentage of joints which achieved clinical success	52.7% (154/292 joints)	67.0% (126/188 joints)	26.9% (28/104 joints)
Number of injections (mean ± SD)	1.4 ± 0.70	1.3 ± 0.62	1.6 ± 0.80

4.(iii).B Outline of the review by PMDA

4.(iii).B.(1) Clinical data package

The present application includes clinical studies described in "4.(iii).A Summary of the submitted data." The applicant justified the use of foreign study data in this application as follows.

The use of foreign study data can be justified in terms of intrinsic and extrinsic ethnic factors in the treatment of Dupuytren's contracture as follows:

(a) Intrinsic ethnic factors

- There are no ethnic differences in the composition of collagen fibers constituting Dupuytren's cords.[78] Since CCH exert its effects through enzymatic reactions, it cannot be influenced by ethnic differences from the viewpoint of the mechanism of action.
- After being injected into a contracted cord, CCH is considered to act directly on the affected area at injection site without being absorbed into the systemic circulation, or being influenced by metabolizing enzyme, etc. Therefore, ethnic differences in pharmacokinetics are not expected to cause major problems [see "4.(ii).A.(2). Japanese phase III study" and "4.(ii).A.(3) Foreign phase I study"].
- Foreign clinical studies showed that the efficacy and safety of CCH were not influenced by patient characteristics such as sex or physical constitution.

(b) Extrinsic ethnic factors

- Although there were no clear diagnostic guidelines in or out of Japan at the time when clinical studies were conducted,[79] any hand surgeon, regardless of nationality, could diagnose Dupuytren's contracture from its typical sign of palpable cords in the palm.
- There are no major differences in surgical indications and outcomes in and out of Japan.
- Therapeutic procedures could be standardized among clinical studies conducted in and out of Japan.

From the above discussions, neither intrinsic nor extrinsic ethnic factors are considered likely to affect the use of CCH.

An open-label, uncontrolled design was used in the Japanese clinical study for the following reasons.

In Japan, the number of patients with Dupuytren's contracture is uncertain because epidemiologic studies on this disease have rarely been conducted. However, given that Dupuytren's contracture is mainly treated by surgery in Japan, the number of patients was estimated from the annual number of operations. The obtained number of patients was 1800 according to the Survey of Medical Care Activities in Public Health Insurance conducted by the Ministry of Health, Labour and Welfare and 2800 according to the data in the Diagnosis Procedure Combination (DPC) system (both for 2012), which suggested that the number of patients with Dupuytren's contracture, for whom CCH is indicated, is fairly limited in Japan. Even if the Japanese study was open-labeled and uncontrolled, a certain reliability in terms of efficacy evaluation can be obtained for the following reasons: advanced Dupuytren's contracture is unlikely to subside spontaneously; the results of foreign phase III studies indicated that placebo effects were small and fairly constant among clinical studies; and the efficacy of CCH can be evaluated objectively by contracture based on finger goniometry.

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[78] *J Exp Med.* 198;142:437-443; *Eur J Clin Invest.* 1980;10:9-16; and *J Bone Joint Surg Am.* 1981;63:787-797

[79] In Europe, a diagnosis/treatment guideline was published in 2013 (*Plast Reconstr Surg.* 2013;132:964e-976e)

From the above discussions, the Japanese study was designed on an open-label, uncontrolled basis from the viewpoint of feasibility, but otherwise by emulating the designs used in the main foreign clinical studies, and the results of foreign studies were included in the clinical data package.

PMDA considers as follows:
The applicant's explanations are understandable, and the establishment of a clinical data package including the results of both the Japanese open-label, uncontrolled study and foreign studies to evaluate the efficacy and safety of CCH is acceptable, given that the number of patients with Dupuytren's contracture, for whom CCH is indicated, is limited in Japan.

4.(iii).B.(2) Efficacy
PMDA evaluated the primary efficacy of CCH focusing mainly on the results of the Japanese study, and the foreign studies, Studies AUX-CC-857 and AUX-CC-859. Based on the following discussions 1) to 6), PMDA considers that the efficacy of CCH has been demonstrated; however, the issue will be finalized taking account of the comments from the Expert Discussion. Recurrence of contracture after CCH treatment, and retreatment with CCH after recurrence were evaluated using the results of foreign open-label studies, Studies AUX-CC-854 and AUX-CC-856 [see "4.(iii).B.(4) Recurrence after treatment with CCH and re-treatment"].

4.(iii).B.(2).1) Primary endpoint
In the Japanese study and Studies AUX-CC-857 and AUX-CC-859 (double-blind phase), the primary endpoint was defined as "the percentage of subjects who achieved clinical success (extension deficit ≤5°) for their primary joint 30 days after the final injection." The applicant explained the rationale for selecting above-mentioned the primary endpoint as follows:

Dupuytren's contracture is a disorder caused by abnormal deposition of collagen in the palmar fascia, and resultant formation of a cord that causes flexion contracture in the finger, causing difficulty in extending the finger and leading to a deterioration in hand function. The objective of treatment for Dupuytren's contracture is to improve finger extension, and reduce difficulty in performing everyday tasks. The extension deficit is an objective indicator that is determined based on the angle measured by a finger goniometer, and clinical success is defined as a condition in which limitation in finger extension is almost eliminated. "Clinical success" is considered to be a valid indicator because of its clinical relevance, its objectivity, and the fact that a similar indicator has been used for the evaluation of surgical outcomes. Thirty days after injection was selected for the point of evaluation because in Study DUPY-202, the percentage of subjects who had achieved clinical success continued to increase over time up until 30 days after injection.

PMDA considers as follows:

There is no particular problem with the rationales for selecting the primary endpoint and the point of evaluation in the Japanese study and Studies AUX-CC-857 and AUX-CC-859. Table 29 shows the results of the Japanese study and Studies AUX-CC-857 and AUX-CC-859 (double-blind phase), and "the percentage of subjects who achieved clinical success in their primary joint 30 days after the final injection" was significantly higher in the CCH group than in the placebo group. In the Japanese study, "the percentage of subjects who achieved clinical success in their primary joint 30 days after the final injection" was 85.7% (66 of 77 subjects) (95% confidence interval [CI], 75.9%-92.6%). The lower limit of the 95% CI is higher than the prescribed limit of 30%, which suggests that the results of the Japanese study were not inferior to those for Studies AUX-CC-857 and AUX-CC-859. Based on the above, the efficacy of CCH for the treatment of Dupuytren's contracture has been demonstrated.

Table 29. Clinical success in the primary joint 30 days after the final injection (primary efficacy analysis sets for each study)

	The Japanese study	AUX-CC-857		AUX-CC-859	
	CCH group (77 subjects)	Placebo group (103 subjects)	CCH group (203 subjects)	Placebo group (21 subjects)	CCH group (45 subjects)
Percentage of subjects who achieved clinical success	85.7% (66 subjects) [75.9%, 92.6%][b)]	6.8% (7 subjects)	64.0% (130 subjects)	4.8% (1 subjects)	44.4% (20 subjects)
P-value		<0.001[a)]		<0.001[a)]	
Number of injections (mean ± SD)	1.2 ± 0.48	2.9 ± 0.4	1.7 ± 0.8	2.8 ± 0.6	1.7 ± 0.8

a) Cochran-Mantel-Haenszel test stratified by baseline severity and joint type (two-sided 5% significance level).
b) [95% confidence interval]

The applicant explained the trend that the clinical success rate was relatively higher in the Japanese study than in Studies AUX-CC-857 and AUX-CC-859 as follows:

The use of local anesthesia during a finger extension procedure was not recommended in Studies AUX-CC-857 and AUX-CC-859, while it was allowed if necessary in the Japanese study. As a result, the percentage of use of local anesthesia differed greatly: 3.4% (7 of 203 subjects), 0% (0 of 45 subjects), and 90.9% (70 of 77 subjects) in Study AUX-CC-857, Study AUX-CC-859, and the Japanese study, respectively. It is considered that study investigators were able to exert more force during the finger extension procedures with the use of local anesthesia, resulting in a higher clinical success rate.

PMDA considers that the applicant's explanation is understandable.

4.(iii).B.(2).2) Response by affected joint

(a) MP and PIP joints

Table 30 shows the results of the Japanese study, and Studies AUX-CC-857 and AUX-CC-859 (double-blind phase) classified by joint type. Although the results for the PIP joint in Study AUX-CC-859 failed to show statistical significance, the percentage of subjects who achieved clinical success in their primary joint 30 days after the final injection was higher in the CCH group than in the placebo group for the both

joint types in Studies AUX-CC-857 and AUX-CC-859. The results of the Japanese study indicated that the percentage of subjects who achieved clinical success in their primary joint 30 days after the final injection was no lower than that in Studies AUX-CC-857 or AUX-CC-859 for either joint type.

Table 30. Clinical success in primary joint 30 days after the final injection by joint type (primary efficacy analysis set for each study)

MP joints					
	The Japanese study	AUX-CC-8597		AUX-CC-859	
	CCH group (47 subjects)	Placebo group (69 subjects)	CCH group (133 subjects)	Placebo group (11 subjects)	CCH group (20 subjects)
Percentage of subjects who achieved clinical success	93.6% (44 subjects) [82.5%, 98.7%][b)]	7.2% (5 subjects)	76.7% (102 subjects)	9.1% (1 subjects)	65.0% (13 subjects)
P-value[a)]		<0.001		<0.001	
Number of injections (mean ± SD)	1.2 ± 0.46	2.9 ± 0.4	1.7 ± 0.8	2.7 ± 0.7	1.5 ± 0.7
PIP joints					
	The Japanese study	AUX-CC-857		AUX-CC-859	
	CCH group (30 subjects)	Placebo group (34 subjects)	CCH group (70 subjects)	Placebo group (10 subjects)	CCH group (25 subjects)
Percentage of subjects who achieved clinical success	73.3% (22 subjects) [54.1%, 87.7%][b)]	5.9% (2 subjects)	40.0% (28 subjects)	0% (0 subjects)	28.0% (7 subjects)
P-value[a)]		<0.001		0.069	
Number of injections (mean ± SD)	1.3 ± 0.52	2.8 ± 0.6	1.8 ± 0.8	2.8 ± 0.6	1.8 ± 0.8

a) Cochran-Mantel-Haenszel test stratified by baseline severity (two-sided 5% significance level).
b) [95% confidence interval]

The applicant's explanation:

The percentage of subjects who achieved clinical success in their primary joint 30 days after the final injection was higher in MP joints than in PIP joints in all studies for the following reasons:

According to an epidemiologic survey and other reports in and out of Japan, surgical procedures also provide better results in MP joints than in PIP joints.[80] The inferior surgical outcomes in PIP joints are attributable to the following factors: the PIP joint has an anatomically complex structure, preventing perfect removal of a contracted cord; if prolonged, contracture in the PIP joint would not be improved only by cord removal; and the PIP joint is more prone to develop other types of secondary joint contracture than Dupuytren's contracture. Furthermore, among subjects whose joint became impalpable after 1 or 2 injections in the Japanese study, Study AUX-CC-857, or Study AUX-CC-859 (double-blind phase), the percentage of subjects who did not achieve clinical success was 23.3% (7 of 30 subjects), 15.7% (11 of 70 subjects), and 28.0% (7 of 25 subjects), respectively where the PIP joint was the primary joint; and 4.3% (2 of 47 subjects), 10.5% (14 of 133 subjects), and 15.0% (3 of 20 subjects), respectively, where the MP joint was the primary joint. Based on the above results, even if Dupuytren's cord in the PIP joint is disrupted to the extent it becomes impalpable following injection of CCH, in many cases, contracture does not improve to the normal range (extension deficit of ≤5°) due to the effect of secondary joint contracture.

[80] *Dupuytren's Disease Biology and Treatment.* Churchill Livingstone, 1990;387-412; *The Journal of Japanese Society for Surgery of the Hand.* 1991;8:769-773.

PMDA considers the applicant's explanation is understandable.

(b) Efficacy based on each finger

Table 31 shows the results of the Japanese study, Study AUX-CC-857, and Study AUX-CC-859 (double-blind phase) classified by finger of MP joints and PIP joints. In the Studies AUX-CC-857 and AUX-CC-859, the percentage of subjects who achieved clinical success in their primary joint 30 days after the final injection was higher in the CCH group than in the placebo group where the primary joint was in the little finger or ring finger. The percentage of subjects who achieved clinical success in their primary joint 30 days after the final injection into the little finger and ring finger in the Japanese study was not lower than that in Studies AUX-CC-857 and AUX-CC-859. The results for the index finger and middle finger were difficult to evaluate because of the paucity of data.

PMDA's view:

Paucity of data in some fingers should be taken into consideration; however, the percentage of subjects who achieved clinical success was higher in the CCH group than in the placebo group for all fingers; therefore, there is no need to limit the finger into which CCH is injected. Yet, data obtained from the studies for the middle finger and index finger were limited; therefore, data should be continuously collected via post-marketing survey, or from other sources to study efficacy for each finger.

Table 31. Percentage of subjects who achieved clinical success in their primary joint 30 days after the final injection by finger (primary efficacy analysis set for each study)

MP joint					
Finger	The Japanese study	AUX-CC-857		AUX-CC-859	
	CCH group (47 subjects)	Placebo group (69 subjects)	CCH group (133 subjects)	Placebo group (11 subjects)	CCH group (20 subjects)
Little finger	96.3% (26/27 subjects)	3.1% (1/32 subjects)	68.1% (47/69 subjects)	16.7% (1/6 subjects)	75.0% (9/12 subjects)
Ring finger	94.4% (17/18 subjects)	9.7% (3/31 subjects)	86.0% (49/57 subjects)	0.0% (0/4 subjects)	57.1% (4/7 subjects)
Middle finger	50.0% (1/2 subjects)	16.7% (1/6 subjects)	83.3% (5/6 subjects)	0.0% (0/1 subjects)	0.0% (0/1 subjects)
Index finger	–	–	100.0% (1/1 subjects)	–	–
PIP joint					
Finger	The Japanese study	AUX-CC-857		AUX-CC-859	
	CCH group (30 subjects)	Placebo group (34 subjects)	CCH group (70 subjects)	Placebo group (10 subjects)	CCH group (25 subjects)
Little finger	66.7% (12/18 subjects)	8.0% (2/25 subjects)	38.9% (21/54 subjects)	0.0% (0/8 subjects)	26.3% (5/19 subjects)
Ring finger	80.0% (8/10 subjects)	0.0% (0/4 subjects)	40.0% (4/10 subjects)	0.0% (0/1 subjects)	33.3% (1/3 subjects)
Middle finger	100.0% (1/1 subjects)	0.0% (0/1 subjects)	25.0% (1/4 subjects)	0.0% (0/1 subjects)	0.0% (0/1 subjects)
Index finger	100.0% (1/1 subjects)	0.0% (0/4 subjects)	100.0% (2/2 subjects)	—	50.0% (1/2 subjects)

—: no data on treated subjects

4.(iii).B.(2).3) Severity

Table 32 shows the results of the MP and PIP joints in the Japanese study, Study AUX-CC-857, and Study AUX-CC-859 (double-blind phase) by severity (baseline extension deficit). The percentage of

subjects who achieved clinical success in their primary joint 30 days after the final injection in Studies AUX-CC-857 and AUX-CC-859 was higher in subjects who had a smaller baseline extension deficit and was higher in the CCH group than in the placebo group regardless of severity. The percentage of subjects who achieved clinical success in their primary joint 30 days after the final injection classified by severity in joints in the Japanese study was not markedly lower than that of Studies AUX-CC-857 and AUX-CC-859.

Table 32. Percentage of subjects who achieved clinical success in their primary joint 30 days after the final injection by the level of extension deficit (primary efficacy analysis set for each study)

MP joint					
Extension deficit	The Japanese study	AUX-CC-857		AUX-CC-859	
	CCH group (47 subjects)	Placebo group (69 subjects)	CCH group (133 subjects)	Placebo group (11 subjects)	CCH group (20 subjects)
≤50°	94.3% (33/35 subjects)	11.6% (5/43 subjects)	88.9% (72/81 subjects)	14.3% (1/7 subjects)	70.0% (7/10 subjects)
>50°	91.7% (11/12 subjects)	0.0% (0/26 subjects)	57.7% (30/52 subjects)	0.0% (0/4 subjects)	60.0% (6/10 subjects)
PIP joint					
Extension deficit	The Japanese study	AUX-CC-857		AUX-CC-859	
	CCH group (30 subjects)	Placebo group (34 subjects)	CCH group (70 subjects)	Placebo group (10 subjects)	CCH group (25 subjects)
≤40°	71.4% (10/14 subjects)	11.1% (1/9 subjects)	81.0% (17/21 subjects)	0.0% (0/2 subjects)	40.0% (2/5 subjects)
>40°	75.0% (12/16 subjects)	4.0% (1/25 subjects)	22.4% (11/49 subjects)	0.0% (0/8 subjects)	25.0% (5/20 subjects)

4.(iii).B.(2).4) Surgical history

Table 33 shows the results for the Japanese study, Study AUX-CC-857, and Study AUX-CC-859 (double-blind phase) by the presence or absence of previous surgery in the primary joint. Although the number of subjects who had undergone previous surgery was small, the percentage of subjects who achieved clinical success in their primary joint 30 days after the final injection was approximately the same in Study AUX-CC-857, and slightly lower in subjects who had undergone surgery in the Japanese study and Study AUX-CC-859.

PMDA's view: Given that a certain percentage of subjects who had undergone surgery achieved clinical success, there is no need to limit subjects to whom CCH is administered. Data should be continuously collected via post-marketing survey etc., to evaluate the efficacy of CCH in patients who have undergone surgery previously.

Table 33. Percentage of subjects who achieved clinical success 30 days after the final injection by the presence or absence of previous surgery in the primary joint (primary efficacy analysis set for each study)

The Japanese study		AUX-CC-857 (CCH group)			AUX-CC-859 (CCH group)		
With surgery	Without surgery	With surgery	Without surgery	Unknown	With surgery	Without surgery	Unknown
62.5% (5/8 subjects)	88.4% (61/69 subjects)	62.5% (10/16 subjects)	65.7% (109/166 subjects)	52.4% (11/21 subjects)	33.3% (3/9 subjects)	45.5% (15/33 subjects)	66.7% (2/3 subjects)

4.(iii).B.(2).5) Number of injections into the same joint

Injections into the primary joint were allowed up to 3 times in the Japanese study, Study AUX-CC-857, and Study AUX-CC-859 (double-blind phase). The percentage of subjects in the CCH group who achieved clinical success 30 days after the final injection by number of injections they received (1, 2, or 3 injections) is shown in Table 34.

PMDA's view:

Up to 3 injections per joint are acceptable because while more than half of the subjects achieved clinical success after the first injection in all 3 studies, some subjects achieved clinical success after the third injection, [see "4.(iii).B.(8) Dosage and administration"].

Table 34. Percentage of subjects in the CCH group who achieved clinical success 30 days after the final injection by number of injections

Number of injections	The Japanese study (66 subjects)	AUX-CC-857 (130 subjects)	AUX-CC-859 (20 subjects)
1	83.3% (55 subjects)	60.8% (79 subjects)	60.0% (12 subjects)
2	13.6% (9 subjects)	26.9% (35 subjects)	30.0% (6 subjects)
3	3.0% (2 subjects)	12.3% (16 subjects)	10.0% (2 subjects)

4.(iii).B.(2).6) Effects of antibodies

In Japanese and foreign clinical studies, the effects of anti-AUX-I and anti-AUX-II antibodies were evaluated. Figures 1 and 2 show the percentage of subjects who were tested positive for these antibodies and their antibody titers in the Japanese study, demonstrating that both increased with the increasing number of injections. This trend is consistent with those seen in foreign studies (AUX-CC-854, AUX-CC-856, AUX-CC-857, AUX-CC-858, and AUX-CC-859).

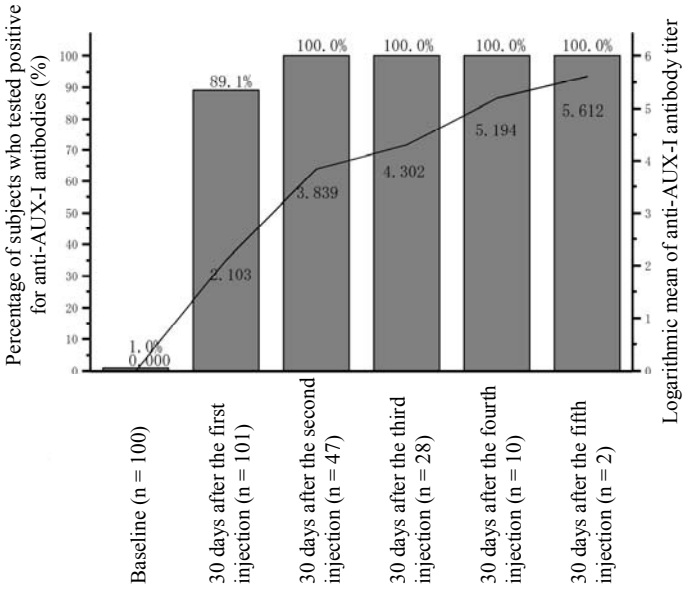

Figure 1. Percentage of subjects who tested positive for anti-AUX-I antibodies and anti-AUX-I antibody titers

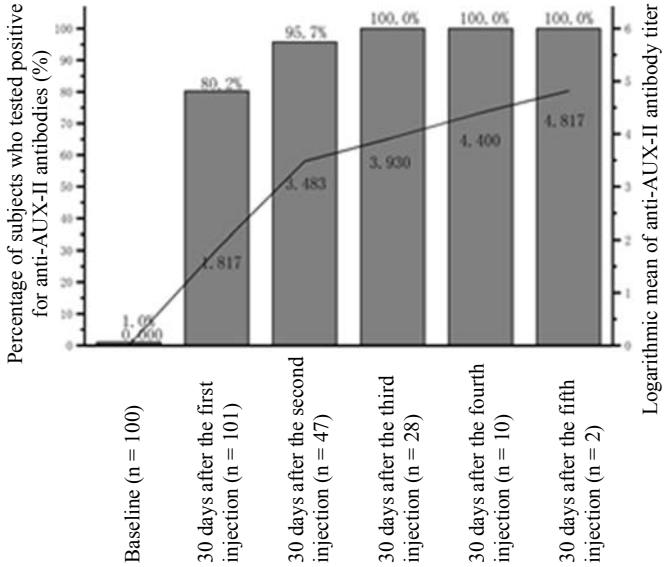

Figure 2. Percentage of subjects who tested positive for anti-AUX-II antibodies and anti-AUX-II antibody titers

In Study AUX-CC-857 and the Japanese study, production of neutralizing antibodies for AUX-I and AUX-II was studied. In Study AUX-CC-857, specimens collected before the first injection and 90 days after the first injection were measured. Out of all the specimens positive for anti-AUX-I antibodies or anti-AUX-II antibodies, 11.0% (22 of 200 specimens) and 21.6% (44 of 204 specimens) were positive for neutralizing antibodies, respectively. In the Japanese study, the measured specimens were those that tested positive for anti-AUX-I antibodies or anti-AUX-II antibodies 90 days or 360 days after the first injection. Out of all the specimens positive for anti-AUX-I antibodies or anti-AUX-II antibodies, 19.8% (20 of 101 specimens) and 3.0% (3 of 99 specimens) measured 90 days after the first injection, and 24.7% (24 of 97 specimens) and 17.6% (16 of 91 specimens) measured 360 days after the first injection, were positive for neutralizing antibodies for AUX-I and AUX-II, respectively.

Table 35 shows the percentage of subjects who achieved clinical success in their primary joint 90 days after the first injection by the presence or absence of neutralizing antibodies (by number of injections) in AUX-CC-857 and the Japanese study. Although further analysis is difficult to perform due to the small number of subjects positive for neutralizing antibodies, the results did not indicate any obvious relationship between the results of neutralizing antibodies and clinical success.

Table 35. Percentage of subjects who achieved clinical success in their primary joint 90 days after the first injection by the presence or absence of neutralizing antibodies (30 days after each injection)

Number of injections	The Japanese study[a)]				AUX-CC-857 (MITT) (CCH group)			
	Anti-AUX-I neutralizing antibody		Anti-AUX-II neutralizing antibody		Anti-AUX-I neutralizing antibody		Anti-AUX-II neutralizing antibody	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
1	65.0% (13/20 subjects)	72.8% (59/81 subjects)	66.7% (2/3 subjects)	70.8% (68/96 subjects)	30.0% (6/20 subjects)	38.1% (64/168 subjects)	23.8% (10/42 subjects)	41.1% (60/146 subjects)
2	71.4% (5/7 subjects)	41.7% (5/12 subjects)	0.0% (0/0 subjects)	52.6% (10/19 subjects)	33.3% (4/12 subjects)	34.9% (29/83 subjects)	26.9% (7/26 subjects)	37.7% (26/69 subjects)
3	0.0% (0/0 subjects)	66.7% (2/3 subjects)	0.0% (0/0 subjects)	66.7% (2/3 subjects)	25.0% (2/8 subjects)	36.1% (13/36 subjects)	23.5% (4/17 subjects)	40.7% (11/27 subjects)
Total	90.0% (18/20 subjects)	81.5% (66/81 subjects)	66.7% (2/3 subjects)	83.3% (80/96 subjects)	60.0% (12/20 subjects)	63.1% (106/168 subjects)	50.0% (21/42 subjects)	66.4% (97/146 subjects)

a) Subjects who were tested for neutralizing antibody.

Table 36 shows the percentage of subjects who achieved clinical success in their primary joint in Study AUX-CC-857, Study AUX-CC-859, and the Japanese study by number of injections. The results did not suggest any decreasing trend in the clinical success rate with increasing number of injections.

Table 36. Percentage of subjects who achieved clinical success in their primary joint 30 days after the injection by number of injections (primary efficacy analysis set for each study)

Number of injections	AUX-CC-857		AUX-CC-859		The Japanese study
	Placebo group	CCH group	Placebo group	CCH group	CCH group
1	1.0% (1/103 subjects)	38.9% (79/203 subjects)	4.8% (1/21 subjects)	26.7% (12/45 subjects)	71.4% (55/77 subjects)
2	1.0% (1/100 subjects)	35.4% (35/99 subjects)	0.0% (0/19 subjects)	27.3% (6/22 subjects)	56.3% (9/16 subjects)
3	5.5% (5/91 subjects)	35.6% (16/45 subjects)	0.0% (0/18 subjects)	25.0% (2/8 subjects)	100.0% (2/2 subjects)
Total	6.8% (7/103 subjects)	64.0% (130/203 subjects)	4.8% (1/21 subjects)	44.4% (20/45 subjects)	85.7% (66/77 subjects)

4.(iii).B.(3) Safety

Based on the following discussions 1) to 3), PMDA considers that the safety of CCH is acceptable; however, a final decision will be made by taking into account the comments made in the Expert Discussion.

(iii).B.(3).1) Summary of adverse events reported in clinical studies

Table 37 shows the incidence of adverse events and other events in the Japanese study, Study AUX-CC-857, and Study AUX-CC-859. The incidence of adverse events and adverse reactions were approximately the same in the Japanese study and in the foreign studies. Deaths occurred in the Japanese study, and serious adverse events occurred at a higher rate in the Japanese study than in the foreign studies; however, a causal relationship to CCH was ruled out for all these events.

Table 37. Incidence of adverse events and other events in the Japanese study and the foreign studies

	The Japanese study (Overall period)	AUX-CC-857 (double-blind phase)		AUX-CC-859 (double-blind phase)	
	CCH group (102 subjects)	Placebo group (104 subjects)	CCH group (204 subjects)	Placebo group (21 subjects)	CCH group (45 subjects)
Adverse event	98.0%	47.1%	97.1%	57.1%	100.0%
Adverse reaction	98.0%	21.2%	96.6%	38.1%	100.0%
Death	2.0% [a)]	0.0%	0.0%	0.0%	0.0%
Serious adverse event	13.7%	1.0%	3.4%	0.0%	2.2%
Serious adverse reactions	0.0%	0.0%	1.5%	0.0%	2.2%

a) Gastric cancer and lung neoplasm malignant (1 subject each); a causal relationship to CCH was ruled out for these events.

4.(iii).B.(3).2) Common adverse events

Table 38 shows the adverse events that occurred with an incidence of ≥10% in at least one of the following study categories: the Japanese study, foreign study total, and foreign double-blind study total.

The applicant's explanation on the common adverse events observed in the Japanese and foreign studies: Almost all adverse events were injection site adverse events. The majority of these events occurred within 30 days after the final injection, and was classified as adverse reactions that were mild or moderate in severity.

Table 38. Adverse events that occurred with an incidence of ≥10% in at least 1 group

		The Japanese study		Foreign study total[a)]		Foreign double-blind study total[b)]			
		CCH group (102 subjects)		CCH group (1082 subjects)		Placebo group (137 subjects)		CCH group (272 subjects)	
		Incidence	Number of subjects	Incidence	Number of subjects	Incidence	Number of subjects	Incidence	Number of subjects
Injection site adverse events	Injection site pain	76.5%	78	40.9%	442	9.5%	13	39.3%	107
	Injection site bruising	45.1%	46	28.0%	303	2.2%	3	25.7%	70
	Injection site swelling	34.3%	35	24.3%	263	5.1%	7	21.7%	59
	Injection site laceration	15.7%	16	13.1%	142 [c)]	0.0%	0	9.2%	25 [c)]
	Injection site oedema	11.8%	12	0.8%	9	0.7%	1	1.1%	3
	Injection site haematoma	10.8%	11	3.5%	38	0.0%	0	3.3%	9
	Local swelling	8.8%	9	64.2%	695	4.4%	6	56.6%	154
	Oedema peripheral	4.9%	5	17.5%	189	0.7%	1	21.7%	59
	Injection site haemorrhage	3.9%	4	6.0%	65	0.7%	1	10.7%	29
Other adverse events	Contusion[f)]	29.4%	30	54.9%	594	2.9%	4	50.4%	137
	Nasopharyngitis	15.7%	16	4.1%	44	7.3%	10	3.3%	9
	Lymphadenopathy	5.9%	6 [d)]	11.2%	121	0.0%	0	15.1%	41
	Pruritus	3.9%	4 [e)]	12.8%	138	0.7%	1	12.1%	33
	Pain in extremity[f)]	2.0%	2	38.4%	415	3.6%	5	33.1%	90
	Tenderness[f)]	1.0%	1	29.5%	319	0.0%	0	23.2%	63
	Ecchymosis[f)]	0.0%	0	18.1%	196	0.0%	0	23.2%	63

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a) Of the 12 submitted studies, 11 studies were included (the Japanese study was excluded).

b) Studies DUPY-303 (randomized, double-blind, placebo-controlled study in non-Japanese patients), AUX-CC-857, and AUX-CC-859 (double-blind phase).

c) Incidence of adverse events that are termed "laceration."

d) Incidence of adverse events that are termed "lymphadenitis."

e) In the Japanese study, injection site pruritus occurred in 5.9% (6 of 102 subjects).

f) Although most of them were suspected to be injection site adverse events, it was difficult to determine so based only on the reported terms of the events; therefore they were included in the "other adverse events" category.

Among injection site adverse events, 15 serious adverse events occurred in 13 of 1082 subjects in the foreign study total: tendon rupture (3 subjects), Dupuytren's contracture operation (2 subjects), ligament rupture, ligament rupture/hand fracture, finger amputation, Dupuytren's contracture/sensory disturbance, complex regional pain syndrome, finger deformity, tendonitis, and ligament disorder (1 subject each). Of the above adverse events, tendon rupture (3 subjects), ligament rupture, Dupuytren's contracture/sensory disturbance, complex regional pain syndrome, finger deformity, and tendonitis (1 subject each) were determined to be adverse reactions. Of the above adverse reactions, tendon rupture (3 subjects) in Studies AUX-CC-855 and AUX-CC-857/AUX-CC-858, and ligament rupture (1 subject) in Study AUX-CC-859 were presumably caused by exposure to CCH, and these events required surgical interventions. These adverse reactions occurred in the little finger PIP joints; therefore, the injection procedure for the little finger PIP joints was revised in clinical studies conducted after Studies AUX-CC-855 and AUX-CC-857/AUX-CC-858. Subsequent to the introduction of the revised injection procedure, no serious adverse events in the little finger PIP joints have been reported. [see "4.(iii).B.(8) Dosage and administration"].

Common non-injection site adverse events included lymphadenopathy and lymphadenitis, which were likely to be caused by secondary inflammation of lymph nodes as a result of an inflammatory reaction occurring at the local injection site due to the pharmacological action of CCH. Lymphadenopathy and lymphadenitis reported in clinical studies in and out of Japan are typically mild in severity, and recovered without intervention.

PMDA's view:
Tendon rupture and ligament rupture need special attention as adverse reactions of CCH. Therefore, it is important that physicians be fully familiar and compliant with the injection procedure of CCH, and that a scheme be established to ensure proper use of CCH, including implementation of a training program for physicians [see "4.(iii).B.(9) Measures to ensure proper use"].

4.(iii).B.(3).3) Anti-drug antibodies and anaphylaxis
The applicant's explanation on the effects of the antibodies of CCH on safety, and adverse events including anaphylaxis:
In the Japanese study, following the first injection of CCH, anti-AUX-I and anti-AUX-II antibodies were positive in 89.1% and 80.2% of the subjects, respectively [see "4.(iii).B.(2).6) Effects of antibodies"]. In foreign studies (AUX-CC-854, AUX-CC-856, AUX-CC-857, AUX-CC-858, and AUX-CC-859), following the first CCH injection, anti-AUX-I and anti-AUX-II antibodies were positive in 91.6% and 85.7% of the subjects, respectively. While the antibody titers increased with the increasing number of injections, the occurrence of adverse events (incidence, duration, and severity) did not change with the number of CCH injections.

In the Japanese study, anaphylaxis or other events classified as severe systemic hypersensitivity did not occur. Injection site pruritus (5.9% [6 of 102 subjects]), pruritus (3.9% [4 of 102 subjects]), and urticaria (1.0% [1 of 102 subjects]) were observed, and all of these were mild in severity. In the foreign studies (foreign study total), anaphylaxis or other severe systemic hypersensitivity did not occur. Adverse events that occurred in ≥1% of the subjects in the CCH group were as follows: pruritus (12.8% [138 of 1082 subjects]), and injection site pruritus (5.3% [57 of 1082 subjects]), and the majority of these events were mild to moderate in severity. Pruritus (4 subjects) and injection site pruritus (1 subject) were severe but localized. In Study AUX-CC-867, a foreign study,[44] anaphylactic reaction occurred in 1 subject after injection of CCH. It occurred approximately 15 minutes after CCH was injected into a cord at 2 sites in the same hand, and recovered approximately 4 hours later with supportive care. This subject had received 5 injections of CCH in Study AUX-CC-856, which was approximately 5 years before the anaphylactic reaction occurred.

PMDA's view:
While many subjects tested positive for the antibodies after receiving CCH, no significant relationship has been identified between the antibodies and adverse events including those related to allergic reaction. However, given that 1 case of anaphylactic reaction occurred in Study AUX-CC-867, it is necessary to

observe patients closely after injection, and take appropriate steps if abnormalities are observed. Information on the occurrence of adverse events including anaphylaxis should be collected continuously through post-marketing survey with careful attention.

4.(iii).B.(4) Recurrence of Dupuytren's contracture after treatment with CCH and retreatment

4.(iii).B.(4).1) Recurrence of Dupuytren's contracture after treatment with CCH

In clinical studies, recurrences in successfully treated joints among those treated with CCH were evaluated according to the method shown in Table 39.

Table 39. Definitions of and methods used to evaluate recurrence

	The Japanese study	AUX-CC-854, AUX-CC-856, AUX-CC-857/858, AUX-CC-859	AUX-CC-860[a)]
Definition of recurrence	**A joint evaluated by the study investigator as having at least one of the following conditions:** **• an increase in contracture (extension deficit) to ≥20° from that measured 30 days after the final injection, and a palpable cord.** **• surgical intervention was performed for a newly developed, or exacerbated Dupuytren's cord.**	**A joint evaluated by the study investigator as having the following condition:** **• an increase in contracture (extension deficit) to ≥20° from that measured 30 days after the final injection, and presence of a palpable cord.**	**A joint evaluated by the study investigator as having at least one of the following conditions:** **• an increase in contracture (extension deficit) to ≥20°from that measured 30 days after the final injection by ≥20°, and a palpable cord.** **• a surgical intervention or a treatment intervention with CCH in the market was performed for a newly developed, or exacerbated Dupuytren's cord.**
Data collection scheme	**As part of the efficacy survey**	**As an adverse event**	**As part of the efficacy survey**
Evaluation timing	**Scheduled study visit after clinical success has achieved**	**Scheduled study visit after clinical success has achieved**	**Once a year (evaluations ≥6 months apart)**
Evaluation period	**From clinical success up to 12 months after the first injection of the study drug**	**From clinical success up to 9 months or 12 months after the first injection of the study drug[b)]**	**Up to 2-5 years after the first injection of the previous studies analyzed**

a) Recurrence was evaluated for joints that achieved clinical success after injection of CCH in Studies AUX-CC-854, AUX-CC-856, AUX-CC-857/858, and AUX-CC-859.
Subjects who were enrolled in Study AUX-CC-862 during the evaluation period of Study AUX-CC-860 were discontinued from Study AUX-CC-860 at that point.

b) Studies AUX-CC-854 and AUX-CC-856 were evaluated up to 9 months; and Studies AUX-CC-857/858 and AUX-CC-859 were evaluated up to 12 months.

Study AUX-CC-860, conducted outside Japan, analyzed recurrence rates in previous foreign studies (Studies AUX-CC-854, AUX-CC-856, AUX-CC-857/858, and AUX-CC-859). Table 40 shows recurrence rates up to 9 or 12 months after the first injection in the previous foreign studies and the Japanese study. Study AUX-CC-860 followed the subjects of each previous foreign study for 5 years after injection, and the recurrence rate in joints that had achieved clinical success in these studies was 3.0% (19 of 623 joints) at 1 year after clinical success, 19.6% (122 of 623 joints) at 2 years, 35.2% (219 of 623 joints) at 3 years, 42.4% (264 of 623 joints) at 4 years, and 46.7% (291 of 623 joints) at 5 years. Five-year recurrence rate was 39.5% (178 of 451 joints) for the MP joint and 65.7% (113 of 172 joints) for the PIP joint. In the Japanese study, subjects that achieved clinical success were followed up to 1

year after the first injection, and the recurrence rate was 3.8% (5 of 131 joints) at 6 months following clinical success and 13.7% (18 of 131 joints) at 12 months following clinical success.

Table 40. The recurrence rate for joints that had achieved clinical success (up to 9 or 12 months after the first injection)

Study	The Japanese study	AUX-CC-854	AUX-CC-856	AUX-CC-857/858	AUX-CC-859
MP joints	4.1% (3/74 joints)	1.2% (3/243 joints)	2.4% (3/126 joints)	1.5% (3/199 joints)	0.0% (0/42 joints)
PIP joints	26.3% (15/57 joints)	5.0% (5/100 joints)	27.6% (8/29 joints)	12.3% (8/65 joints)	0.0% (0/26 joints)

The applicant's explanation of the reasons that the recurrence rate observed in the Japanese study tended to be higher than those in foreign studies, and that the rate in Study AUX-CC-856 tended to be higher than those in other foreign studies:

Comparison of patient characteristics between the Japanese study and foreign studies in terms of distribution of reported risk factors for recurrence (sex, complications, early onset, positive family history, and bilateral involvement of hands)[81] revealed differences in the proportions of subjects with early onset (<50 years of age at diagnosis) and those with a positive family history. Patients with early onset or a positive family history are generally considered to have a higher risk of recurrence; however, the percentage of subjects with early onset (<50 years of age at diagnosis) or a positive family history was lower in the Japanese study than in foreign studies. The distribution of risk factors was similar between Study AUX-CC-856 and other foreign studies. Therefore, the above risk factors for recurrence were considered to be unrelated to the differences in the recurrence rate between the Japanese study and foreign studies. However, as recurrence data for Japanese patients are limited, a more in-depth discussion of this issue was difficult.

The applicant's explanation of the reasons that the recurrence rate tended to be higher in the PIP joints than in the MP joints in Japanese and foreign studies:

In the PIP joint, the development of Dupuytren's contracture involves several cords (central cord, spiral cord, and lateral cord), which are entangled with nerves and blood vessels, forming a complex anatomical structure.[82] In the MP joint, the development of contracture mainly involves the pretendinous cord, forming a simpler anatomical structure than that in the PIP joint. Because of this structural difference, surgical cord removal tends to be incomplete in the PIP joint, and as a result, recurrences are more likely to occur in the PIP joint than in the MP joint. In the treatments with CCH, cord rupture also tends to be incomplete in the PIP joint, and even after achieving clinical success, residual cords can cause recurrence, which accounts for the higher frequency of recurrences in the PIP joint than in the MP joint. Furthermore, in cases of severe long-term contracture in the PIP joints, the

[81] *J Hand Surg Am.* 2006;31:1626-1634

[82] *The Journal of Japanese Society for Surgery of the Hand.* 2003;20:687-689

extensor mechanism (structural mechanism for finger extension) has deteriorated and the affected fingers are likely to remain curled even after therapeutic correction of contracture, which is considered to explain frequent recurrences.[83] The above reasons explain why recurrences after treatment with CCH are more likely to occur in the PIP joint than in the MP joint. In conclusion, the higher recurrence rate for the PIP joint than for the MP joint in the Japanese and foreign studies was considered attributable to the anatomical differences between the two joints.

PMDA's view:
The information on recurrence rates should be provided using relevant materials, and given that the recurrence data for Japanese patients are limited, it is necessary to collect data through post-marketing survey and from other sources.

4. (iii).B.(4).2) Treatment after recurrence

The applicant's explanation on the treatment after recurrence:
Foreign treatment data for recurrence after CCH injection are available in Study AUX-CC-860, in which subjects were followed for up to 5 years after CCH injection. In Study AUX-CC-860, additional treatment was performed in 34.5% (105 of 304 joints) in which recurrence was observed after clinical success was achieved after CCH injection. Treatment methods included fasciectomy, dermo-fasciectomy, percutaneous needle fasciotomy (PNF), and CCH injection. Among these treatment methods, fasciectomy and re-injection of CCH were used relatively often, with the rates being 46.7% (49 of 105 joints) and 30.5% (32 of 105 joints), respectively. The efficacy and safety of the treatment with CCH for episodes of recurrence were studied in Study AUX-CC-862. The rate of clinical success 30 days after the final injection was 64.5% (20 of 31 joints) for the MP joint, and 45.0% (9 of 20 joints) for the PIP joint. According to a study conducted outside Japan to investigate the difficulty of surgical fasciectomy after previous treatment with CCH,[84] of 9 physicians who reported on 15 subjects, 1 physician (4 subjects) reported that surgery on the recurrent cord after previous treatment with CCH injection was more difficult than surgery on the recurrent cord after previous surgery.

The applicant's explanation on the safety of CCH in retreatment as follows:
Study AUX-CC-862 was conducted in subjects enrolled in Study AUX-CC-860,[85] a follow-up study of previous studies, to investigate the safety and efficacy of CCH in the retreatment of subjects who experienced recurrence in successfully treated joints (reduction in extension deficit to ≤5°) in the previous studies. In Study AUX-CC-862, a total of 72 injections of CCH was administered into Dupuytren's cords in 52 joints (52 subjects). Adverse events occurred in 88.5% (46of 52 subjects). Adverse events that occurred with an incidence of ≥10% were as follows: oedema peripheral (61.5% [32 of 52 subjects]), contusion (44.2% [23 of 52 subjects]), pain in extremity (30.8% [16 of 52 subjects]), injection site pain and pruritus (both 19.2% [10 of 52 subjects]), injection site haematoma and

[83] *PEPARS*. 2012;61-66
[84] *J Hand Surg Eur*. 2013;39:463-465
[85] A follow-up study in subjects who received CCH in Study AUX-CC-854, AUX-CC-856, AUX-CC-857/858, or AUX-CC-859 and subjects were followed up to 2 to 5 years after the first injection of CCH.

lymphadenopathy (both 15.4% [8 of 52 subjects]), and skin laceration (13.5% [7 of 52 subjects]). All of them were known events that had occurred during the treatment period in the previous studies. One death (cerebellar infarction) occurred. Serious adverse events occurred in 3 subjects (acute coronary syndrome/acute myocardial infarction/chest pain, Dupuytren's contracture, and urosepsis; 1 subject each). These events occurred several months after the final CCH injection, and a causal relationship to CCH was ruled out for the events. Therefore, the safety of CCH in retreatment is the same as that in the treatment with CCH for the first time. Accordingly, no particular safety problems associated with retreatment with CCH have been identified.

The above information suggests that many options, including no treatment, retreatment with CCH, and various surgical procedures, have been selected for patients who were originally treated with CCH and have experienced recurrence. Therefore, at this moment, the methods for retreatment should not be restricted, but rather be determined on a case-by-case basis through consultation/discussion between physician and patient.

PMDA's view:
The results of Study AUX-CC-862 revealed that there were no specific problems associated with the occurrence of adverse events at the time of retreatment with CCH compared to initial treatment with CCH (Table 38). Based on the information provided by foreign clinical studies, restriction of retreatment with CCH is not necessary. Given that the retreatment data for Japanese patients are limited, data including re-treatment procedures for recurrence and the efficacy and safety of CCH in retreatment should be collected through post-marketing survey and from other sources.

4.(iii).B.(5) Post-marketing information from foreign countries
The applicant's explanation on the post-marketing information collected in foreign countries:
CCH was first approved in the United States for the treatment of Dupuytren's contracture in adult patients with a palpable cord. From the market launch in March 2010 to February 27, 2014, the number of patients who received CCH injections is estimated to be 59,945 based on the number of vials shipped and on the assumption of average use of 1.1 injections per joint in 1.5 joints per patient.

From the approval in the US up to February 27, 2014, a total of 2333 adverse reactions (149 serious and 2184 non-serious) have been reported. Common serious adverse reactions were tendon rupture (45 episodes) and laceration (15 episodes), while the numbers of other serious adverse reactions were all ≤3. During the survey period for the sixth Periodic Safety Update Report,[86] 228 adverse reactions (11 serious and 217 non-serious) were reported, and of the serious adverse reactions, tendon rupture was most common (5 episodes). Tendon rupture and laceration were already observed in the clinical studies, and no new safety risks were identified.

[86] The survey period was from August 28, 2013 to February 27, 2014. The number of patients who received CCH during this period is estimated to be 13,117.

PMDA's view:

Serious tendon rupture and laceration, although uncommon, have been reported outside Japan through post-marketing survey. Therefore, in addition to establishing a scheme to ensure proper use of CCH including training programs for injection, information on serious adverse events such as tendon rupture should be collected through post-marketing survey, and relevant information should be provided to healthcare professionals in clinical settings as needed [see "4.(iii).B.(9) Measures to ensure proper use"].

4.(iii).B.(6) Treatment options for Dupuytren's contracture and clinical positioning of CCH in Japan

The applicant's explanation on the therapeutic role of CCH in the treatment of Dupuytren's contracture in Japan:

In Japan, no drug has been approved for the indication of Dupuytren's contracture, and surgery is the only treatment option. Outside Japan, surgery is recommended when patients are positive for the table-top test,[87] or have a flexion contracture of > 30° in the MP joint or of > 15° in the PIP joint.[88] Likewise in Japan, surgery is considered for patients with a positive table-top test[89] or with a flexion contracture of ≥ 30° and with difficulty in performing activities of daily living,[90] or patients in the advanced stage[91] according to Rayan's classification.[92] The type of surgery is chosen according to each patient's condition. An international collaborative epidemiologic survey conducted outside Japan showed the type of surgery did not affect outcomes significantly, and the percentage of joints achieving full extension (0° contracture) was greater in the MP joint (70% to 89%) than in the PIP joint (13% to 29%). The drawbacks of surgery include the following: it is highly invasive; it requires advanced surgical skills, postoperative immobilization, rehabilitation training, and a long time to achieve functional recovery; and it carries the risk of surgical complications such as neural and arterial injury, and the second surgery in case of recurrence. The efficacy of CCH (Table 9) is comparable to that for surgery reported in the literature; in addition, CCH has been demonstrated to be effective for a wide range of patients regardless of the type of affected joints or the severity of contracture. The indications and outcomes of surgery are unlikely to differ significantly between in and out of Japan. Treatment with CCH, which consists only of its injection into the cord and the finger extension procedure, is less invasive than surgery, and has a lower risk of complications affecting hand function. Therefore, CCH is considered to be a safe treatment option.

Given that Dupuytren's contracture is diagnosed in the same way by any hand surgeon in and out of Japan from its typical sign, i.e., palpable cords in the palm, and that therapeutic procedures were

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87 *Hand Surgery*. 3rd ed. Baltimore:Williams & Wilkins, 1982;797-823, 1982

88 *J Bone Joint Surg Am.* 2007;89:189-198

89 *The Journal of Japanese Society for Surgery of the Hand.* 2010;26:516-519

90 *J Plast Surg Hand Surg.* 2010;44:306-310; *The Journal of Japanese Society for Surgery of the Hand.* 2010;26:507-509; *The Journal of Japanese Society for Surgery of the Hand.* 2011;27:665-669

91 *J Bone Joint Surg Am.* 2007;89:189-198
Early stage, changes in normal skin structure, depression of the skin; intermediate stage, formation of nodules and regression, formation of a contracture cord, appearance of flexion contracture; advanced stage, progression of flexion contracture

92 *Orthopaedic Surgery and Traumatology.* 2010;53:253-259

standardized among the clinical studies conducted in and out of Japan, CCH is expected to be used in Japan in the same manner as it is overseas where it has been marketed.

Relatively less invasive surgical procedures introduced in recent years, including arthroscopic palmar fasciotomy and percutaneous needle fasciotomy (PNF),[93] do not appear to be used extensively in Japan at present because reports on therapeutic outcomes of such procedures were not identified in Japanese literature. However, a comparison based on the foreign literature[94] indicated no major differences between CCH and PNF in terms of efficacy, safety, and the incidence of recurrence.

From the above discussions, it was concluded that CCH is a minimally invasive treatment option that would first be made widely available in Japan for patients with Dupuytren's contracture.

Having reviewed the applicant's explanations, PMDA considers that CCH offers a new treatment option for patients with Dupuytren's contracture.

4.(iii).B.(7) Indications

PMDA considers that the efficacy of CCH has been demonstrated in patients with Dupuytren's contracture based on the studies the Japanese study, Study AUX-CC-857, and Study AUX-CC-859; and the safety of CCH is acceptable provided measures to ensure proper use of CCH are thoroughly implemented. Therefore, it is appropriate to select, as the indication for CCH, "Dupuytren's contracture," the name of the disease used by the Japanese Society for Surgery of the Hand and other organizations.

Dupuytren's contracture in the thumb was excluded from the treatment target of the clinical studies to evaluate efficacy. The applicant explained the appropriateness of including Dupuytren's contracture of the thumb in the target digits to be treated as follows:

Dupuytren's contracture of the thumb was excluded from major clinical studies because the incidence of Dupuytren's contracture of the thumb is lower than that of the other digits, and evaluation of efficacy in the thumb is more difficult than that in the other fingers. Accordingly, there were only for 3 subjects (3 joints) in whom clinical outcomes in the thumb were available in all clinical studies.[95] Of these, 1 joint achieved clinical success, while the other 2 joints improved extension deficit. Severe injection site pain and pain in extremity were observed in 1 subject, and recovered within 15 days of onset. According to other reports on injection of CCH in the thumb, CCH was injected into 5 joints of 4 subjects in clinical researches,[96] in which disruption of cords was successful in all joints, and there was an improvement in

[93] In Europe, while it is not as widespread as fasciectomy, PNF is listed as one of the procedures in the diagnosis/treatment guideline for Dupuytren's contracture (*Plast Reconstr Surg*. 2013;132:964e-976e).

[94] *J Hand Surg Br.* 2003;28:427-431, 2003; *J Hand Surg Am*. 2006;31:717-725; *J Hand Surg Br.* 2006;31:498-501; *Joint Bone Spine.* 2011;78: 625-628; *J Hand Surg Eur.* 2011;36:548-552; *Plast Reconstr Surg.* 2012;129:469-477; *J Hand Surg Am.* 2012;37:651-656; *J Hand Surg Am.* 2013;38:2377-2380

[95] Study AUX-CC-853 and two phase II studies published in *J Hand Surg Am*. 2000;25:629-636 and *J Hand Surg Am* 2002;27:788-798 (1 subject each).

[96] J Bone Joint Surg Br 94: 1390-1392, 2012

the angle between the thumb and index finger. Adverse reactions were local reaction and lymphadenopathy, which recovered within 2 weeks of onset. Further, there is a report that the thickness of the cord in the thumb is approximately the same as cords in the other fingers.[97] Therefore, it is considered that CCH is effective for thumb as well as for the other fingers. Furthermore, Dupuytren's contracture of the thumb is anatomically characterized by a cord that is formed apart from the flexor tendon of the thumb, as a result, the risk of thumb tendon rupture is expected to be no higher than that of the other fingers'.

From the above discussions, while it is necessary to note that the available clinical data are limited, the applicant considered it acceptable to include Dupuytren's contracture of the thumb in the indication of CCH based on the observation that no particular problems have been found in the efficacy and safety results of CCH injection into the thumb. According to the Package Insert used in other countries, there are no restrictions placed on the CCH injection into cords in the thumb and there have been no specific reports in the post-marketing survey on serious adverse events in relation to CCH injection into the thumb.

PMDA considers as follows:
In the thumb, digital nerves are located in the midline anterior to the flexor tendon; therefore, the anatomical characteristics of the thumb is not similar to the other fingers. However, based on the applicant's explanation, there is no need to prohibit the use of CCH for a Dupuytren's cord in the thumb. Due to the fact that in Japan there is no use experience of CCH for cord treatment in the thumb, and experience is also fairly limited in other countries, it is necessary to collect data to evaluate safety and efficacy following CCH injection into a Dupuytren's cord in the thumb through post-marketing survey.

A final decision on the indication of CCH will be made taking into account the comments made in the Expert Discussion.

4.(iii).B.(8) Dosage and administration

Based on the following discussions 1) to 5), PMDA considers that a final decision on the dosage and administration will be made taking into account the comments made in the Expert Discussion. It is also important that physicians be fully understand the safety and efficacy of CCH, and its injection procedure through training programs before CCH injection into patients; therefore, it is necessary to establish a scheme to ensure such measures [see "4.(iii).B.(9) Measures to ensure proper use"].

4.(iii).B.(8).1) Dosage and administration for one Dupuytren's cord

The applicant explained the rationale for selecting the dosage and administration in the Japanese study as follows:

[97] *The Journal of Japanese Society for Surgery of the Hand*. 27: 665-669, 2011

When a single dose of placebo, 2500 U, 5000 U, or 10,000 U[98] of Collagenase (Clostridium Histolyticum) was injected into the MP or PIP joint in Study DUPY-202, a dose selection study, the results for the efficacy (Table 12) showed that the highest clinical success was demonstrated in the 10,000 U group compared to the placebo group in MP or PIP joint or all joints. The safety profiles did not differ significantly between the 3 dose levels. Based on the results, 10,000 U was selected as the dose level for the subsequent phase III studies.

Prior to phase III studies, additional injections into contracture in the same joint were tried if the first injection did not achieve clinical success. As a result, up to 3 injections per cord in the same joint were confirmed to be sufficient to achieve satisfactory efficacy with CCH. Thus in phase III studies, the maximum number of injections was set to 3. Although ≥4 injections into a cord affecting the same joint was not studied in any of the clinical studies conducted in and out of Japan, considering the results that there were relatively few patients who required 3 injections to achieve efficacy (Table 34), the maximim number of 3 injections into a cord affecting the same joint as in the approved dosage and administration outside Japan was considered appropriate.

In all clinical studies, additional injections of CCH were given at an interval of 30 days if the first injection was not enough. These studies showed that the necessary time to achieve clinical success after injection varied; some subjects achieved clinical success on the day following injection, while some subjects required 30 days. The majority of adverse events following treatment with CCH recovered at approximately 1 month after injection. Based on the above, if necessary, additional injections were given, at 1-month interval.

Injection volume was adjusted for the MP joint to 10,000 U/0.25 mL during the early phase II study overseas based on the advice from the US Food and Drug Administration. The injection volume for the PIP joint, which is anatomically smaller in size than the MP joint, was determined to be 10,000 U/0.20 mL. Thereafter, the above injection volume, 10,000 U/0.25 mL or 10,000 U/0.20 mL, was used in all clinical studies conducted in and out of Japan, and treatment results were favorable.

The injection procedure used in clinical studies in and out of Japanese was as follows:
The needle was inserted only into the cord to make sure that normal tissues around the site would not be exposed to CCH. An injection volume of 0.25 mL for a cord affecting the MP joint, and 0.20 mL for a cord affecting the PIP joint was injected at 3 sites, 2 to 3 mm apart from one another (approximately one-third of the injection volume into each site). In Studies AUX-CC-855, AUX-CC-857/858, and AUX-CC-859, tendon rupture of the PIP joint of the little finger (3 subjects) and ligament rupture (1 subject) requiring surgical intervention occurred. Therefore, the injection procedure in the protocol was changed as follows: when injecting into the PIP joint of the little finger, do not inject into the area located ≥4 mm distal to the palmar digital crease, and the insertion depth should not exceed 3 mm. The Japanese

[98] 10,000 U is equivalent to 0.58 mg.

study also used the same injection method. It was further required that the insertion depth should not exceed 3 mm in all fingers. Following the change, no serious injection site adverse events occurred in any of the fingers. It was also determined that local anesthesia at the time of CCH injection should not be used in any studies conducted in or out of Japan in case the needle tip is inadvertently inserted into the nerve and the patient cannot detect any sensory disturbance. Moreover, a local anesthesia injection can be as painful as a CCH injection.

Based on the above discussions, the injection method to be used after the market launch in Japan was decided to be the same as that employed in the Japanese study.

4.(iii).B.(8).2) Maximum number of injections for each patient

The applicant explained the maximum number of injections per patient as follows:

In foreign clinical studies (Studies AUX-CC-857/858 and AUX-CC-859), the maximum number of injections per patient was set at 8; however, study results showed that most patients did not require so many injections. Consequently, in the Japanese study, the maximum number of injections per patient was set at 5. Table 41 shows the maximum number of injections per patient in these studies. Based on the results of the Japanese study, few patients were expected to require multiple injections after the market launch in Japan.

Table 41. Maximum number of injections per patient in the clinical studies conducted in and out of Japan

	Number of subjects	1	2	3	4	5	6	7	8
The Japanese study	102	53.9% (55 subjects)	18.6% (19 subjects)	17.6% (18 subjects)	7.8% (8 subjects)	2.0% (2 subjects)	—	—	—
AUX-CC-857/858	299	30.1% (90 subjects)	17.1% (51 subjects)	20.4% (61 subjects)	12.0% (36 subjects)	12.7% (38 subjects)	3.0% (9 subjects)	2.0% (6 subjects)	2.7% (8 subjects)
AUX-CC-859	63	25.4% (16 subjects)	17.5% (11 subjects)	14.3% (9 subjects)	7.9% (5 subjects)	14.3% (9 subjects)	6.3% (4 subjects)	7.9% (5 subjects)	6.3% (4 subjects)

—: not available

Adverse events [see "4.(iii).B.(3).2) Common adverse events"] that occurred with an incidence of ≥10% in the Japanese study by number of injections (adverse events that occurred from each injection to 30 days after injection[99]) are shown in Table 42. The incidence of adverse events did not increase with increasing number of injections. In the foreign studies, the incidence of adverse events did not show a trend to increase with the increasing number of injections either, except for pruritus and injection site pruritus.

[99] For the first injection, if adverse events occurred on the day of injection, they were included in the first injection events, regardless of the onset time being before or after the injection; from the second injection onward, if adverse events occurred on the day of injection, they were included in the previous injection if the onset time was before injection; from the day after 30 days after each previous injection to the next injection, adverse events were included in the previous injection events.

Table 42. Incidence of adverse events that was ≥10% by number of injections (Japanese study)

	First (102 subjects)		Second (47 subjects)		Third (28 subjects)		Fourth (10 subjects)		Fifth (2 subjects)	
	Incidence	Subject (n)	Incidence	Subject (n)	Incidence	Subject (n)	Incidence	Subject (n)	Incidence	Subject (n)
All adverse events	93.1%	95	93.6%	44	89.3%	25	80.0%	8	100.0%	2
Injection site pain	67.6%	69	63.8%	30	39.3%	11	50.0%	5	0.0%	0
Injection site bruising	40.2%	41	27.7%	13	21.4%	6	30.0%	3	50.0%	1
Injection site swelling	28.4%	29	29.8%	14	28.6%	8	30.0%	3	50.0%	1
Contusion	26.5%	27	25.5%	12	17.9%	5	0.0%	0	0.0%	0
Injection site laceration	13.7%	14	2.1%	1	7.1%	2	0.0%	0	0.0%	0
Nasopharyngitis	7.8%	8	4.3%	2	3.6%	1	20.0%	2	0.0%	0
Injection site oedema	10.8%	11	8.5%	4	7.1%	2	10.0%	1	0.0%	0
Injection site haematoma	8.8%	9	8.5%	4	7.1%	2	10.0%	1	0.0%	0

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No safety or efficacy issues stemming from the number of injections have been reported overseas, while there are no specifications or limitations on the maximum number of injections per patient after the market launch.

Based on the above discussions, as is the case in foreign countries, it is considered unnecessary to specify the maximum number of injections per patient in Japan.

With respect to 1) and 2), PMDA considers as follows:
The applicant's explanation on the rationale for the dosage and administration (including the maximum number of injections per cord affecting the same joint) in the Japanese study is understandable. In Studies AUX-CC-857 and AUX-CC-859, foreign phase III double-blind confirmatory studies, the efficacy and safety of CCH were demonstrated, and they also were in the Japanese study, in which similar dosage and administration were employed.

At this point, it is not necessary to limit the number of injections per patient under normal use conditions, yet data on the number of injections should be collected through post-marketing survey.

From above discussions, it is appropriate to determine the dosage and administration of CCH based on phase III clinical studies in and out of Japan as follows:
A total of 0.58 mg of CCH, reconstituted with diluent to form a liquid formulation of 0.25 mL for the MP joint or 0.20 mL for the PIP joint, is injected into a Dupuytren's cord. If a satisfactory response has not been achieved, injections may be repeated into the cord affecting the same joint at 1-month intervals, up to 3 times. Injection volume adjustment and injection procedure should also conform to those used in clinical studies in and out of Japan.

4.(iii).B.(8).3) Administration to more than one cord (cords affecting different joints of the same finger, or cords in different fingers)

The applicant explained that in the Japanese study, when subjects had more than one Dupuytren's cord (cords affecting different joints of the same finger, or cords in different fingers), one of the joints was selected for treatment with CCH. Therefore, there was no experience in treating more than one cord concurrently. For this reason, only one cord affecting one joint was to be treated at a time after the market launch.

PMDA's view:

At this point, there is no problem with the applicant's decision that CCH should be administered to one joint at a time when multiple cords are present, and should not be administered into 2 or more cords concurrently.

4.(iii).B.(8).4) Timing of finger extension procedure after injection

The applicant's explanation on the timing of the finger extension:

In clinical studies in and out of Japan, investigators were allowed to perform the finger extension procedure on the day following CCH injection if extension of the finger did not occur after injection. Around the time when marketing approvals were granted in foreign countries (February 2010 in the US, and February 2011 in Europe), the finger extension procedure in routine clinical practice was to be performed on the day following injection (approximately 24 hours later). However, after the market launch of CCH, there were occasions when the finger extension procedure was delayed in actual clinical practice, for example, when physicians could not see the patients for two consecutive days. Consequently, in Study AUX-CC-867, which was conducted recently outside Japan, finger extension procedures were also performed on other days than the day following injection to investigate the effect of timing (24, 48, and 72 hours after injection) of the finger extension procedure on the efficacy and safety of CCH. The results showed that the efficacy and safety were not influenced by the timing of the finger extension procedure in the range from 24 to 72 hours after injection.[100] Accordingly, the timing of the finger extension procedure prescribed in the US Package Insert was modified to "24 to 72 hours following an injection" (October 2014), which is currently in practice.

Based on the above, the finger extension procedure was to be performed between 24 hours to 72 hours after injection in Japan.

[100] The rate of clinical success for the finger extension procedure 24 hours after an injection was 65.5% (213 of 325 joints) for the MP joint and 29.4% (62 of 211 joints) for the PIP joint; 48 hours after an injection the rate of clinical success was 62.9% (237 of 377 joints) for the MP joint and 32.0% (70 of 219 joints) for the PIP joint; and 72 hours after an injection the rate of clinical success was 66.5% (129 of 194 joints) for the MP joint and 21.3% (26 of 122 joints) for the PIP joint.
The percentage of subjects who developed adverse reactions up to Day 61 visit after injection of CCH in subjects who underwent the finger extension procedure 24, 48, and 72 hours after an injection, was 93.7% (251 of 268 subjects), 97.7% (292 of 299 subjects), and 93.0% (147 of 158 subjects), respectively.

PMDA considers that it is desirable for the finger extension procedure to be performed on the day following injection even after the market launch in the same way as prescribed in the Japanese study; however, the issue will be finalized, taking into account the comments made in the Expert Discussion.

4.(iii).B.(8).5) Patient education

In the Japanese study, as in the foreign clinical studies, after injection, patients were to wear a splint on the treated finger at night, and to perform finger extension and flexion exercises at home under the instruction of a physician. The applicant explained this as follows:

As was done in the clinical studies conducted in and out of Japan, patients are recommended to perform finger extension and flexion exercises at home for several months after CCH injection. Patients are also recommended to wear the splint provided by the physician on the treated hand at night for several months after CCH injection. Information on finger exercises and wearing a splint at home should be included in the materials for physicians and in those for patients, and the materials should be distributed to ensure that the safety of patients who receive treatment is protected, and maximum efficacy is achieved.

PMDA considers that in order for the above follow-up procedures to be performed, it is important to make sure, through the Package Insert, that physicians fully understand the need for patient education, and, through information materials, that patients are well informed of the above procedures using information materials.

4.(iii).B.(9) Measures to ensure proper use

In the clinical studies conducted in and out of Japan, prior to the start of study, instruction manuals and DVDs containing information on the CCH injection procedure were provided, and a briefing session with medical experts was held for study investigators to ensure that CCH would be injected into a Dupuytren's cord properly. The applicant stated that CCH must be administered only by physicians with specialized knowledge and experience in the treatment with CCH, and suggested that the physicians acquire specialized knowledge and experience in the treatment of Dupuytren's contracture, and fully understand the therapeutic procedures with CCH as detailed in the following paragraphs:

CCH should only be administered by physicians who have a thorough knowledge of hand anatomy, pathology and therapeutic procedures for Dupuytren's contracture, and specialized knowledge and experience in the treatment of Dupuytren's contracture. Specific requirements for qualification will be discussed by the management organization operated jointly by the Japanese Society for Surgery of the Hand (JSSH) and the applicant. The eligibility of physicians who wish to use CCH is determined by the management organization, and if the requirements are met, the physicians are permitted to participate in the training program on therapeutic procedures. Qualified physicians are requested to take the e-learning-based training program of the therapeutic procedures and proper use of CCH. Planned training program includes preparation of CCH, injection sites, injection procedure, method used for the finger

extension procedure, and how to handle adverse reactions. A certificate will be issue to participants if they can demonstrate that they have achieved a certain level of understanding in the confirmatory test at the end of the program. The management organization will register in the database the physicians who completed the training program and the medical institutions in which they are engaged. CCH will be distributed only to the medical institutions in which physicians who have completed the program are engaged. Such measures have been implemented in the US and in Europe after the market launch to ensure proper use of CCH.

Proper use of CCH will be assured by the establishment of the above controlled distribution system in which CCH is distributed only to the medical institutions with qualified/certified physicians, ensuring that CCH will be used only by physicians with specialized knowledge and experience.

PMDA's view:
A precise surgical procedure is indispensable for CCH injection from the viewpoint of efficacy and safety. Finger extension procedures and other follow-ups after injection are also important. Given that the measures planned by the applicant are the same as those that have been implemented after the market launch in the US and Europe [see "4.(iii).B.(5) Post-marketing information from foreign countries"], and no safety problems have been reported in these countries so far, PMDA considers there is no problem with the suggested measures; however, the issue will be finalized, taking into account the comments made in the Expert Discussion.

4.(iii).B.(10) Post-marketing investigations
The applicant is planning to implement a post-marketing survey (Table 43) to collect and assess safety- and efficacy-related data under actual use conditions so that information on the proper use of CCH can be provided to healthcare professionals in clinical settings as promptly as possible.

Table 43. Outline of use-results survey (draft)

Objectives	**Assessing adverse reactions under actual use conditions; identifying factors that may have an impact on safety and efficacy under actual use conditions**
Survey method	**Central registration system**
Target patients	**Patients with Dupuytren's contracture with a palpable cord**
Implementation period	**3 years and 9 months (registration period, 2 years)**
Observation period	**From the first injection to 30 days after the final injection for each joint (up to 18 months)**
Planned number of patients	**300 patients**
Main survey items	• **Patient characteristics (age, sex, medical history, complications, medical history/treatment history/family history of Dupuytren's contracture, hand injury history, history of allergy, etc.)** • **Status of treatment with CCH (injection site, injection dates, dose)** • **Finger extension procedure (timing of procedures, use of local anesthesia)** • **Drugs used concomitantly; combined therapy (including anticoagulants, antiplatelet drugs)** • **Adverse events, laboratory test results** • **Efficacy (extension deficit angle, flexion angle, and general evaluation by physician)** • **Condition at the end of observation period**

PMDA considers that the following information should also be collected through the post-marketing survey:

- Safety and efficacy by finger
- Safety and efficacy by surgical history
- Recurrence and method of treatment after recurrence
- Safety and efficacy of retreatment with CCH after recurrence
- Occurrence of anaphylaxis, and other reactions
- Total number of injections per patient
- Whether finger extension procedures were performed, and timing of procedure performed

A final decision on post-marketing survey will be made taking into account the comments made in the Expert Discussion.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

A document-based compliance inspection and data integrity assessment was conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application. As a result, there were no particular problems. Thus, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted application documents.

2. PMDA's conclusion on the results of GCP on-site inspection

GCP on-site inspection was conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application (5.3.5.2-1). The results showed satisfactory overall GCP compliance in the conduct of clinical studies, and PMDA concluded that there should be

no problem with conducting a regulatory review based on the submitted application documents. However, the inspection revealed the following finding regarding some medical institutions, albeit with no major impact on the overall study evaluation, and the finding was notified to the directors of the institutions in question as an issue to be redressed.

[Issue to be improved]
Medical institutions

- When the investigator resigned, the director of the institution failed to take necessary procedures such as advance notification to the sponsor.

IV. Overall Evaluation

Based on the submitted data, the efficacy of CCH in patients with Dupuytren's contracture has been demonstrated and its safety is acceptable in view of its observed benefits.

PMDA considers that CCH may be approved if it is not considered to have any particular problem based on comments from the Expert Discussions.

Review Report (2)

May 18, 2015

I. Product Submitted for Registration

[Brand name]	Xiaflex Inj.
[Non-proprietary name]	Collagenase (Clostridium Histolyticum)
[Applicant]	Asahi Kasei Pharma Corporation
[Date of application]	July 31, 2014

II. Content of the Review

The comments from the Expert Discussion and the subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) are outlined in the following sections. The expert advisors for the Expert Discussion were nominated based on their declarations etc., concerning the product submitted for registration, 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) Efficacy

PMDA concluded that the efficacy of collagenase (Clostridium Histolyticum) (hereinafter referred also to as collagenase or CCH) has been demonstrated based on the results of the Japanese clinical study and the major foreign clinical studies.

However, PMDA considered that information on the efficacy of CCH by finger (especially the middle finger and index finger), and the efficacy in patients with a history of surgery should also be collected through post-marketing survey because only a limited number of subjects were included in the clinical studies.

The above conclusion by PMDA was supported by the expert advisors.

(2) Safety

The incidence of adverse events and adverse reactions was approximately the same in the Japanese clinical study and the major foreign clinical studies (Table 37). Adverse events that were commonly observed in the clinical studies in and out of Japan were injection site adverse events (Table 38), and the majority of these were mild or moderate in severity. Deaths occurred in the Japanese study, and serious adverse events occurred at a slightly higher rate in the Japanese study than in the foreign studies; however, a causal relationship to CCH was ruled out for all these events. Further, there was no marked difference in the incidence of adverse events in multiple injections or in treatment for recurrence between the clinical studies in and out of Japan.

Based on the above, PMDA concluded that the safety of CCH is acceptable.

PMDA also considered as follows:
Serious adverse reactions including tendon rupture and ligament rupture, although uncommon, have been reported in the PIP joint of the little finger in foreign clinical studies. Therefore, CCH should be administered only by physicians who fully understand the safety and efficacy of and therapeutic procedures with CCH; it is important to establish a scheme to ensure proper use of CCH including a training program for physicians [see "4.(iii).B.(9) Measures to ensure proper use" in Review Report (1), and "(6) Measures to ensure proper use" in Review Report (2)]. Furthermore, occurrence of anaphylaxis and other reactions should be collected through post-marketing survey.

The following comments were raised by the expert advisors, and the PMDA's conclusion was supported by the expert advisors.

- Following treatment with CCH, anti-drug antibodies for CCH develop at a high rate and patients tend to remain positive for the antibodies. Therefore, when injecting CCH, it is necessary to carefully check the patient's CCH administration history, and patients should be monitored closely for anaphylaxis and other reactions.
- Tendon rupture and ligament rupture have been reported in the literature published outside Japan. Blood flow disorder and cold intolerance, which may be caused by the exposure of neuro-vascular bundle area to CCH, have also been reported. Therefore, development of these adverse events should be carefully monitored in CCH treatment.

(3) Recurrence after treatment with CCH and retreatment
PMDA's view:
The information on recurrence rates after treatment with CCH should be provided using relevant materials, and given that the recurrence data on Japanese patients are limited, it is necessary to collect the data through post-marketing survey and from other sources.

From the results of Study AUX-CC-862 and other studies, which investigated the efficacy and safety of CCH when used in retreatment for recurrence, it is not necessary to limit retreatment with CCH. However, it is necessary to collect data of retreatment after recurrence, including therapeutic regimens and the efficacy and safety of CCH in retreatment, through post-marketing survey and from other sources.

The above conclusion by PMDA was supported by the expert advisors.

(4) Indications
PMDA's view:
Given that the results of clinical studies in and out of Japan demonstrated the efficacy of CCH, the safety of CCH is acceptable provided measures to ensure proper use of CCH are implemented. Therefore, it is

appropriate to select "Dupuytren's contracture," an equivalent disease name used by the Japanese Society for Surgery of the Hand and other organizations in Japan, as the indication for CCH.

Treatment with CCH requires accurate identification of the cord position to be treated, and direct injection into the cord, and serious tendon rupture and laceration, although uncommon, have been reported in foreign clinical studies. Therefore, CCH should be administered only by physicians who have a thorough knowledge of hand anatomy, and who fully understand the safety and efficacy of CCH, and injection procedure and post-injection finger extension procedures through prior training programs before administering CCH to patients. Caution statements to this effect should be included in the "Precautions for Indication" section.

Based on the above, PMDA concluded that the "Indication" section should be described as follows:

[Indication]
Dupuytren's contracture

[Precautions for Indication]
1. Collagenase (Clostridium Histolyticum) should only be administered by physicians who have a thorough knowledge and treatment experience in Dupuytren's contracture, and who have completed the training program and fully understand the safety and efficacy of and therapeutic procedures with collagenase through prior training program.
2. Collagenase should be injected into a palpable cord.

The above conclusion by PMDA was supported by the expert advisors.

(5) Dosage and administration

PMDA considered that the dosage and administration of CCH should be described as follows conforming to the phase III studies conducted in and out of Japan.

[Dosage and administration]
The usual adult dosage is 0.58 mg as collagenase (Clostridium Histolyticum) given as an injection into a cord with contracture of a metacarpophalangeal joint or proximal interphalangeal joint. If a satisfactory response has not been achieved, injections may be repeated up to 3 times per cord at 1-month intervals.

PMDA considered that the injection volume adjustment, injection procedure (puncture method), and timing of the finger extension procedure after injection of CCH should be established according to those used in the Japanese study. The maximum number of injections per patient does not have to be limited at this point, although information should be collected through post-marketing survey. CCH should also be injected into one joint at a time when multiple cords are present (cords affecting different joints of

the same finger, or cords in different fingers), and should not be injected into 2 or more cords concurrently.

After injection with CCH, patients are required to perform finger flexion and extension exercises by themselves and wear a splint. Instructions and other information on these matters should be provided in the Package Insert and other relevant materials to ensure that physicians are fully informed of these procedures. In addition, physicians need to give sufficient instructions to patients by using information materials.

The above conclusion by PMDA was supported by the expert advisors.

(6) Measures to ensure proper use

PMDA considered that CCH should only be administered by physicians who have a thorough knowledge of hand anatomy, pathology, and treatment methods for Dupuytren's contracture, and have a full understanding of injection procedure and post-injection finger extension procedures through prior training programs before administering CCH to patients. Therefore, it is crucial to establish a scheme to ensure all of the above, and this should be emphasized by including statements to this effect in the "Precautions for indication" section [see "(4) Indications"]. Conditions for approval should be established including measures needed to ensure proper use.

The following comments were raised from the expert advisors concerning the scheme to ensure proper use.

- Information on proper procedure of using CCH should be provided thoroughly in cooperation with the relevant societies and organizations.
- The treatment outcomes and prevention of serious adverse reactions including tendon rupture are greatly affected by the techniques to inject CCH; furthermore, when CCH is used, appropriate procedures in the event of tendon rupture and ligament rupture should be readily available. Therefore, physicians who administer CCH should be limited to those qualified to do so, such as hand surgeons, and those who have a thorough knowledge of hand anatomy.

Based on the above comments from the expert advisors, PMDA decided that, at this point, only hand surgeons will be permitted to use CCH, and they will be required to complete the training program on the treatment techniques before providing treatment with CCH. Information on proper use of CCH is to be disseminated through the relevant societies. The materials of the training program should be developed in close consultation with the societies and, together with registration data of physicians, need to be appropriately controlled under applicant's responsibility.

The above conclusion by PMDA was supported by the expert advisors.

(7) Risk Management Plan (draft)

PMDA considered that issues listed in "4.(iii).B.(10) Post-marketing investigations" of the Review Report (1) should also be reviewed through post-marketing survey. There is no need to contraindicate the use of CCH in the thumb at this point, which was not included in the major clinical studies; however, the statement to this effect should be included in the "Clinical studies" section of the Package Insert, and data should be collected through post-marketing survey.

Further, the following opinions were raised by the expert advisors, and the above conclusion by PMDAwas supported.

- Since the tissue of Dupuytren's cord is entangled with digital nerves, CCH should be injected carefully to prevent injury to digital nerves.
- An observation period of approximately 12 months after the final injection of CCH is necessary to collect data on the rate of recurrence in Japan.

Based on the above, PMDA asked the applicant to develop a risk management plan (draft) at this point, and the applicant submitted the risk management plan (draft) (Tables 44 and 45), and outline of a use-results survey plan (draft) (Table 46).

Table 44. Specifications on safety and efficacy in the risk management plan (draft)

Safety specification		
Important identified risks	**Important potential risks**	**Important missing information**
Tendon/ligament rupture or injury • **Local reaction** • **Anaphylaxis** • **Skin laceration**	• **Digital nerve injury** • **Cross-reaction of anti-drug antibodies** • **Injection site hemorrhage in patients with blood coagulation disorder or on anticoagulant therapy**	**Not applicable**
Efficacy specification		
Efficacy in patients with Dupuytren's contracture under actual use conditions		

Table 45. Outline of additional pharmacovigilance and risk minimization actions in the risk management plan (draft)

Additional pharmacovigilance actions	**Additional risk minimization activities**
• **Early post-marketing phase vigilance** • **Use-results survey**	• **Providing information obtained through an early post-marketing phase vigilance system** • **Ensuring that CCH is administered only by physicians who have a thorough knowledge and treatment experience in Dupuytren's contracture, and who have completed the training program and fully understand the safety and efficacy of and therapeutic procedures with CCH. (including preparation of information materials)**

Table 46. Outline of use-results survey (draft)

Objectives	**Assessing adverse reactions under actual use conditions; identifying factors that may have an impact on safety and efficacy under actual use conditions**
Survey method	**Central registration system**
Target patients	**Patients with Dupuytren's contracture**
Implementation period	**4 years (registration period, 2 years)**
Observation period	**From the first injection to 30 days after the final injection for each joint; a total of 3 follow-up surveys will be conducted once a year covering all patients registered**
Planned number of patients	**300 patients**
Main survey items	• **Patient characteristics (age, sex, medical history, complications, medical history/treatment history/family history of Dupuytren's contracture, hand injury history, history of allergy, etc.)** • **Status of treatment with CCH (injection site, injection dates, dose)** • **Finger extension procedure (timing of procedures, use of local anesthesia)** • **Drugs used concomitantly, combined therapy (including anticoagulants, antiplatelet drugs)** • **Adverse events, laboratory test results** • **Efficacy (extension deficit angle, flexion angle, and general evaluation by physician)** • **Status at recurrence and method used to treat recurrence** • **Condition at the end of observation period**

III. Overall Evaluation

As a result of the above review, PMDA has concluded that the product may be approved with the following conditions for approval, after modifying the indication and dosage and administration, as shown below. Since the drug product is a drug with a new active ingredient, the re-examination period is 8 years. The drug substance and the drug product are both classified as powerful drugs, but neither the drug substance nor the drug product is classified as a biological product or a specified biological product.

[Indication] Dupuytren's contracture

[Dosage and administration] The usual adult dosage is 0.58 mg as collagenase (Clostridium Histolyticum) given as an injection into a cord with contracture of a metacarpophalangeal joint or proximal interphalangeal joint. If a satisfactory response has not been achieved, injections may be repeated up to 3 times per cord at 1-month intervals.

[Conditions for approval] The applicant is required to:

- Develop and appropriately implement a risk management plan; and
- Take appropriate measures to ensure that the drug product is used only by physicians trained on the product and fully versed in its safety and efficacy, and therapeutic procedures.