Assessment report

Xiapex

Common name: Collagenase *clostridium histolyticum*

Procedure No. EMEA/H/C/2048

Assessment Report as adopted by the CHMP with
all information of a commercially confidential nature deleted.

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Table of contents

List of abbreviations

Abbreviations and Terms	Definition
AA4500	Collagenase clostridium histolyticum for injection
ADAs	Anti-drug antibodies
BMI	Body Mass Index
BTC	Biospecifics Technologies Corporation
Clinical improvement	A ≥50% reduction from baseline in degree of contracture (fixed-flexion deformity) after an injection.
Clinical success	A reduction in contracture (flexion deformity) to ≤5° of normal as measured by finger goniometry after an injection.
CMH	Cochran-Mantel-Haenszel test
Contracture	Shortening, thickening, and fibrosis of the palmar fascia, producing a fixed-flexion deformity of a finger ending in reduced extension of the joint.
CRPS	Complex Regional Pain Syndrome
CSR	Clinical study report
DASH	Disabilities of the Arm, Shoulder and Hand questionnaire
DIP	Distal intraphalangeal
EU	European Union
Fixed-flexion deformity/ contracture (degree of flexion)	The angle of the joint when the finger is passively extended (ie, straightened) as far as possible toward the neutral position of zero degrees (ie, full extension or normal extension).
Full extension angle	The angle of a joint when the finger is straightened (extended) as far as possible toward the neutral position of zero degrees (expressed in degrees)
Full flexion angle	The maximum angle of a joint when the finger is bent (flexed) as close to the palm as possible (expressed in degrees)
GCP	Good Clinical Practices
HED	Human equivalence dose
ICH	International Conference on Harmonization
ITT	Intent-to-treat
mg	Milligram
MP	Metacarpophalangeal
NA	Not applicable
NC	Not computable
Passive movement	The examiner's movement of the joint through an arc of motion.
PIP	Proximal interphalangeal
PNF	Percutaneous needle fasciotomy
PK	Pharmacokinetic
PT	Preferred term
Recurrence of contracture	Recurrence was evaluated for joints that achieved a reduction in contracture to 5° or less as measured by finger goniometry after an injection during the 12-month study period across the double-blind and open-label phases of AUX-CC-857 and AUX-CC-859. The investigator determined there was recurrence when the joint contracture increased to at least 20° and had a palpable cord. The recurrence was recorded as an AE.
ROM	Range of motion: Difference between full flexion angle and full extension angle (in degrees).
SD	Standard deviation

1. Background information on the procedure

1.1 Submission of the dossier

The applicant Pfizer Limited submitted on 16 December 2009 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Xiapex, through the centralised procedure under Article 3 (2) (a) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 29 September 2009.

The applicant applied for the following indication: treatment of Dupuytren's contracture in adult patients with a palpable cord.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC. The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies.

1.2 Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision EMEA/415970/2009 P/139/2009 for the following conditions:

- Dupuytren's Contracture,
- Peyronie's Disease

on the granting of a product-specific waiver.

Information relating to Orphan Market Exclusivity

Similarity

Not applicable.

Market Exclusivity

Not applicable.

Scientific Advice

The applicant did not seek scientific advice at the CHMP.

Licensing status

Collagenase clostridium histolyticum filed under the tradename Xiaflex has been given a Marketing Authorisation in the United States of America on 02 February 2010 for the treatment of Dupuytren's contracture in adults.

The product was not licensed in any country at the time of submission of the application.

1.3 Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Dr. Martina Weise (DE)	Co-Rapporteur: Dr. Pierre Demolis (FR)

- The application was received by the EMA on 16 December 2009.
- The procedure started on 21 January 2010.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 09 April 2010. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 09 April 2010.
- During the meeting in May 2010, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 20 May 2010.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 20 August 2010.
- A GMP inspection was carried out at the following sites: Auxilium Pharmaceuticals, Inc. at 102 Witmer Road, Horsham, PA 19044, USA, and KBI BioPharma, Inc., 1101 Hamlin Road, Durham, NC 27704, USA, between 25-29 October 2010 by the competent authorities of Spain and Germany, and were found to operate in compliance with EU GMP.
- The summary report of the GCP inspection carried out at the following sites: Dr. Stephan Wilbrand (Department of Hand Surgery, Uppsala University Hospital - Sweden), Dr. Jeff Karrasch (Peninsula Specialist Centre, Kippa Ring - Australia) and Dr. Anthony Houston (Caboolture Clinical Research Centre,Caboolture - Australia) respectively between 31 May-2 June 2010, 8-10 June 2010 and 11, 15 &16 June 2010 was issued on 10 August 2010.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 01 October 2010.
- During the CHMP meeting in October 2010, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP consolidated List of Outstanding Issues on 15 November 2010.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of outstanding issues to all CHMP members on 26 November 2010.
- During the meeting in December 2010, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Xiapex on 16 December 2010. The applicant provided the letter of undertaking on the follow-up measures to be fulfilled post-authorisation on 16 December 2010.

2. Scientific discussion

2.1 Introduction

Problem statement

Dupuytren's disease is a fibroproliferative disorder affecting the palmar fascia. It is thought to be a genetic disease of autosomal dominant transmission with variable penetrance and with an origin in the Celtic races of northern Europe. Migration is believed to have disseminated the disease throughout the world and while the disease is most common in Scandinavia and the British Isles, it is also present in Australia and North America. Dupuytren's disease is estimated to affect approximately 15 to 30 million people in the EU with prevalence in the general population estimated at 3 to 6 percent. Men are more frequently affected than women with an incidence that is 7 to 10 times higher, however this difference disappears with increasing age. It is a condition which has been linked to many risk factors including a history of smoking, alcohol consumption, epilepsy, diabetes mellitus, carpal tunnel syndrome, history of manual labour, and hand injury. However, the data supporting the association with these risk factors is inconsistent and remains controversial. Dupuytren's contractures often span several adjacent joints. Four stages of contracture have been described. Stage I is characterized by contracture of the metacarpophalangeal (MP) joint of the ring finger. Stage II is composed of contractures of the MP and proximal interphalangeal (PIP) joints of the ring finger and the MP of the little finger. Contractures of the MP and PIP of the ring finger and MP and PIP of the little finger and MP of the long finger characterize stage III. Hyperextension of the distal interphalangeal (DIP) joints of the little and ring finger are seen in Stage IV.

As the disease progresses beyond an early proliferative stage, it results in joint contracture due to the formation of a fibrotic cord comprised of collagen. The resultant flexion contracture results in significant functional disability of the hand which prevents Dupuytren's patients from performing everyday tasks (e.g. face washing, shaking hands, driving, playing sports, playing musical instruments) and hence causes a burden on quality of life.
Currently, no pharmacological therapy exists in the treatment of this affection. Surgery (mainly open fasciectomy or fasciotomy) or percutaneous needle fasciotomy (PNF) are the only available options to treat the disease.
Standard of care varies across European countries; some of them use only fasciectomy procedures while others use both fasciectomy and PNF. Overall, the most common surgical procedure in Europe is fasciectomie whereas PNF represents between 4 to 12% of treated patient, depending on the country.

The goal of surgery is to remove and/or release the fibrotic cord and correct the contracture allowing extension of the affected finger(s). However, surgical procedures are not curative in that remaining non-affected fascia may still develop Dupuytren´s disease later on. In addition, surgery can be complex, and may result in significant perioperative and/or postoperative complications which can delay full recovery. In a recent study of limited fasciectomy in 261 cases, Coert et al. reported an overall rate of complications of 26% (Coert JH et al, 2006). Surgical complications occur intraoperatively, as well as during the early and late postoperative periods and include inflammation, haematoma, ischaemic skin necrosis, wound infection, granuloma formation, neuropraxia, neurovascular injury, flexor tendon/ligament injury, scar contracture, persistent PIP flexion contracture, DIP hyperextension deformity, joint stiffness, poor flexion and grip strength, pain, circulatory disturbance and complex regional pain syndrome (CRPS). Higher incidences of surgical complications have been reported in diabetic patients including the typical diabetic stiff hand. Complications are even more complex in repeat surgery in the case of recurrence. In addition, surgery is not always the best option for individual patients because of the existence of comorbidities,

operative risks, prolonged recovery period and requirement for extensive hand therapy; or simply because the disease is not sufficiently advanced to warrant surgery.

PNF is a medical technique that shares many similarities with collagenase injections. In PNF, patients are treated in an outpatient setting under local anaesthesia using 1 ml or less of lidocaine 1% and epinephrine 1: 100,000 per treatment site. After disinfection and draping, the cord responsible for the flexion contracture of the ray is sectioned at as many levels as possible in the palm and fingers, depending on the location and extent of the disease, using a 25 gauge needle mounted on a 10 ml syringe. After division of the cord, the affected finger is passively extended to pull the ends of the sectioned cord apart and to obtain maximal release of the contracture. Patients are encouraged to start flexing and extending their fingers immediately after treatment and to start using their hands normally after 24h. No splint is needed or physiotherapy given.
PNF is a less invasive treatment which provides similar efficacy than surgery but it seems that recurrences occur more frequently and at relatively early stage than surgery. Consequently, this technique could be used in less advanced disease, in elderly patients, in patients with co-morbidities, or in postponing selective fasciectomy.

About the product

Xiapex (also referred to as AA4500) contains a fixed mixture of two purified collagenases produced by *Clostridium histolyticum*. It is a novel pharmacological treatment targeting Dupuytren's contractures through injection into the fibrotic cords. The collagenase AUX-I (Clostridial type I collagenase, formerly known as ABC-I) and collagenase AUX-II (Clostridial type II collagenase, formerly known as ABC-II) act in a complimentary manner to digest the collagen subtypes that predominate in the diseased Dupuytren's cord (mostly type I and III collagens). The drug product is provided as a lyophilized powder for reconstitution in a 3 mL Type I borosilicate glass vial. Each single-use vial of drug product is filled at a target of 0.9 mg protein per vial. The drug product is reconstituted using sterile diluent.
Xiapex must be administered by a physician appropriately trained in the correct administration of the product and experienced in the diagnosis and management of Dupuytren's disease. The injection of the collagenase into the Dupuytren's cord followed by a finger extension procedure 24 hours after the injection where needed, allows for the local disruption of the cord.

Proposed indication

Dupuytren's contracture: Xiapex is indicated for the treatment of Dupuytren's contracture in adult patients with a palpable cord.

Proposed dosing regimen

The recommended dose of Xiapex is 0.58 mg per injection into a palpable Dupuytren's cord. The volume of reconstituted Xiapex to be administered into the Dupuytren's cord differs depending on the type of joint being treated.

2.2 Quality aspects

Introduction

The active substance is composed of mixture of two purified collagenases (AUX – I and AUX – II) produced by *Clostridium histolyticum in* a fixed ratio obtained as secreted enzymes in the fermentation of a non-recombinant strain of *Clostridium histolyticum*. The individual enzymes are purified as individual intermediates prior the generation of a drug substance bulk.

The finished medicinal product is provided as a lyophilized powder for reconstitution in a 3 mL Type I borosilicate glass vial. Each vial contains 0.9 mg of clostridial collagenase.
For reconstitution, a Sterile Diluent in a single-use glass vial will be presented.

The recommended dose is 0.58 mg per injection. For cords affecting metacarpophalangeal joints, each dose is reconstituted with 0.39 ml diluent and administered in an injection volume of 0.25 ml. For cords affecting proximal interphalangeal joints, each dose is reconstituted with 0.31 ml diluent and administered in an injection volume of 0.20 ml.

The proposed indication for Xiapex (also referred to as AA4500) is: "Treatment of Dupuytren's Contracure in adult patients with palpable cord" (a collagen disorder resulting in formation of collagen nodules and cords which cause fixed-flexion contracture of one or more digits of the hand).
Xiapex is a novel pharmacological treatment targeting Dupuytren's contractures through the injection into the fibrotic cords.

Active Substance

The active substance is a mix of two collagenases (Collagenase I (AUX-I) and collagenase II (AUX-II)) in a fixed ratio. Five toxins are generally secreted by *Clostridium histolyticum*: toxins α, β, γ, δ and ε. AUX-I and AUX-II belong to the β -toxins which comprise several subtypes; the production strain was specifically selected to avoid the expression of the other toxins.

Manufacture

The active substance is manufactured, tested and released by Auxilium Pharmaceuticals, Inc. (Horsham, USA). The history of the production strain going back to a strain generated in the 1950ties and deposited at ATCC is sufficiently transparent and traceable.

Drug substance manufacture employs a two-tiered system comprising a MCB and a WCB. No mutagenic agents have been employed in the establishment of the cell banks. Information on establishment and control of the final cell bank system is found acceptable. Stability of the cell substrate during commercial process fermentation has not yet been completely evaluated. Genome sequencing data of the LIVCA cells are required to evaluate cell bank stability for the current manufacturing process.

Clostridium histolyticum is known to synthesize toxins. Evaluation of the potential presence of clostridial exosubstances in the active substance has been performed. Establishment and control of the final cell bank system and the used raw materials has been presented in a satisfactory manner.

The manufacturing process consists of fermentation of the non-recombinant bacterium *Clostridium histolyticum* under anaerobic conditions followed by several purification steps.

Satisfactory information on the manufacture of the active substance and the used raw materials has been presented. The active substance is stored at ≤-60°C.

Control of materials

The control of the manufacturing process is sufficiently transparent and in-process controls are adequately included.

An acceptable definition for Critical Performance Parameters and Key Performance Indicators as well as a rationale for the classification of the IPCs in critical and non-critical process controls has been provided. The basis of the risk assessment performed to identify the critical in-process controls has been explained.

The applicant has confirmed that the validated operating ranges and parameters will be used as acceptance limits for the fermenter and manufacturing process.

RP-HPLC, SDS-PAGE, CGE, SE-HPLC, AUC, and imaged cIEF have been used for purity analysis.

Product-related impurities have been investigated for product fragments or truncations and aggregates.

In general, the characterisation programme of the applicant is considered adequate.

Process development and validation

The process validation/evaluation was generally sufficiently documented and included analysis of consecutive full-scale GMP batches. The process was found capable of consistent operation within predetermined ranges producing product meeting the acceptance criteria.

Information on process-related impurities is overall acceptable.

The development of the active substance manufacture process has evolved via some distinct but related processes. The information provided is found satisfactory.

Comparability studies of the materials manufactured are appropriate and analytical methods have been adequately described and validated.

Specification

The characterisation of AUX-I, AUX-II and active substance included confirmation of the primary structure, higher order structure, purity, impurities, post-translational modifications and biological activity. To evaluate the primary structure, mass spectrometry, N-terminal sequencing and amino acid analysis have been used. Peptide mapping has been performed.

The specification as proposed by the applicant is acceptable.
The specification includes tests and acceptance criteria for: inter alia pH, endotoxins and bioburden, Identity by SDS-PAGE (Coomassie stain) and RP-HPLC, Concentration by A 280, Purity by SEC-HPLC, RP-HPLC and SDS-PAGE (Coomassie stain), Potency assays for AUX-I and AUX-II, residual DNA and by Threshold, HCPs by SDS-PAGE (Silver stain).

Stability

All currently available results met the proposed specification limits with the exception of results for one parameter at several time points. The decreasing trend in this parameter over time is under investigation. The applicant has committed to report additional results from on-going root cause analyses.

An interim acceptance criterion for this parameter has been implemented in the meantime

Based on the stability data provided, shelf-life of 9 months with storage at ≤ -60°C will be set. Both the interim criterion and active substance shelf-life will remain in place until the root cause analyses are closed and permanent limits and shelf-life are fixed.

In accordance with EU GMP guidelines[1], any confirmed out of specification result, or significant negative trend, should be reported to the Rapporteur and the EMA.

Comparability Exercise for Active Substance

N/A

[1] 6.32 of Vol. 4 Part I of the Rules Governing Medicinal Products in the European Union

Finished Medicinal Product

The finished medicinal product (XIAPEX) is a lyophilisate in vial containing 0.9 mg of clostridial collagenase to be reconstituted with a solvent ("Sterile Diluent") containing calcium chloride dihydrate and sodium chloride in water for injections. The powder may be reconstituted with 0.39mL or 0.31mL of solvent depending on the indication.

Concerning appropriate dose to be administered to the patient the data provided is acceptable.

Pharmaceutical Development

Regarding pharmaceutical development comprehensive investigations were performed to justify the choice of the components of the formulation mixture and their quantities. The results presented support final composition to be adequate for suitable drug product quality and stability.

Substantial studies were carried out to optimise the lyophilisation process with regard to the critical quality attributes. The conclusions drawn by the applicant are supported.

The stability studies have demonstrated that the container closures system is compatible with the lyophilised finished medicinal product with respect to strength, potency and purity. Therefore, the container closure system is regarded suitable for the drug product.

A risk analysis was performed on the analytes identified from an extraction study on all components in direct contact with the finished medicinal product with respect to possible impact on product and patient. Leachables were tested with no critical findings.

Manufacture of the product

The finished medicinal product is manufactured by Hollister-Stier Laboratories LLC (Spokane, USA). The finished medicinal product release testing is performed by Pfizer Manufacturing Belgium (Puurs, Belgium).

The manufacturing process including in-process controls is described in sufficient detail in the application file and the current IPCs and their limits are regarded sufficient to control the manufacture of drug product.

The batch sizes of drug substance used for process validation cover sufficiently the commercial batch size range. All in-process and final product testing results were within specifications. Validation data supports that the production process consistently delivers product of the intended quality.

Product Specification

The specification as proposed by the applicant is considered acceptable. Most of the analytical methods are identical to those used for the active substance, and the reference standards for finished medicinal product and active substance are the same.

The specification limits for pH and reconstitution time has been tightened as required and a quantitative method has been included in the specification for the determination of product-related impurities.

Stability of the product

Stability studies with finished medicinal product have been conducted per ICH Q1A (R2) and ICH Q5C guidelines.

Based on the stability data provided, the drug product shelf life of 24 months when stored at 2-8 °C is considered acceptable.

Based on data obtained from the reconstitution stability studies, and upon considerations regarding microbiological quality, proposed product labelling will indicate that reconstituted drug product can be kept at room temperature for up to one hour, or refrigerated at 2ºC to 8°C for up to 4 hours prior to administration.
In accordance with EU GMP guidelines[2], any confirmed out of specification result, or significant negative trend, should be reported to the Rapporteur and the EMA.

Adventitious agents

The *Clostridium histolyticum* clone used as the culture source for production of MCB was initially propagated and preserved in a meat-based medium then further propagated using a non-animal derived medium peptone (plant-based).

The MCB and WCB were generated in animal component-free medium. Only one animal-derived, but non-ruminant-derived raw material, peptone, is used during fermentation, culture medium is sterilized by moist heat (>121°C exposure for not less than 20 minutes) prior to use.

Sterile diluent

Sterile Diluent contains 0.03% (2 mM) of calcium chloride dihydrate and 0.9% (154 mM) of sodium chloride in water for injections.

The manufacturing process, including in-process controls, is described in sufficient detail in the application file. The parameters and limits laid down in the finished medicinal product specifications are acceptable.

The applicant's conclusion of a shelf life of 30 months over the temperature range of 2-30°C for Sterile Diluent is acceptable. However, considering that the approved shelf-life for the powder is 24 months, the shelf-life of the finished medicinal product (powder and diluent) is 24 months at 2-8C.

GMP Status: The sites Auxilium Pharmaceuticals, Inc. at 102 Witmer Road, Horsham, PA 19044, USA, and KBI BioPharma, Inc., 1101 Hamlin Road, Durham, NC 27704, USA, Auxilium Pharmaceuticals, Inc., 40 Valley Stream Parkway, Malvern, PA 19355, USA and at 420 Babylon Road, Horsham, PA 19044, USA have been inspected and accepted.

Comparability Exercise for Finished Medicinal Drug Product

N/A

GMO

N/A

Discussion on chemical, pharmaceutical and biological aspects

During the procedure of Xiapex, three Major Objections related to Quality were initially identified. These Major Objections concern to the following:

1) Control of process related impurities by the intended commercial process was not addressed.

[2] 6.32 of Vol. 4 Part I of the Rules Governing Medicinal Products in the European Union

2) Validation data were insufficient to assess the suitability of the analytical methods (DS/DP).

3) Significant trend in one stability indicating parameter after active substance storage at – 70º C was observed which resulted in a consequent proposal to widen the specification.

The applicant, during the review process was able to provide in a satisfactory manner the majority of information requested.

Characterisation of the production strain with regard to exotoxins has been adequately addressed. Whole genome sequencing has been performed and additional tests to confirm the absence of toxins has been added. However, strategies to assess and control the potential presence of product related impurities had to be amended. Regarding validation issues, analytical method validation summaries have been provided and all identified. Outstanding Issues have been resolved. With regard to active substance stability testing, root cause analysis for one parameter is ongoing. Shelf-life limits for this parameter have been deleted from the active substance specifications.

Thus, at the time of the responses to the List of Outstanding issues, the applicant was able to satisfactorily address the quality questions. The applicant has committed to provide further information through Post Authorisation Commitments.

Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues which were not considered to impact on the Risk-benefit balance of the product. The applicant committed to resolve these as Post Authorisation Commitments after the opinion, within an agreed timeframe to the issues related to generating further data and experience in defining the stability profile, analysing impurities and setting of specifications. In this regard, the applicant will take into account scientific and technical progress.

2.3 Non-clinical aspects

Introduction

AA4500 is a mixture of two purified collagenolytic enzymes (AUX-I (Clostridial type I collagenase, *colG*) and AUX-II (Clostridial type II collagenase, *colH*)) in a fixed mass ratio), isolated from the culture medium of *Clostridium histolyticum*. These enzymes are generated by the homologous expression of two separate chromosomal genes, *colG* and *colH*, which produce the class I and class II collagenases, respectively. Both enzyme classes are metalloproteinases, requiring the metal cofactors Zn and Ca for full activity, but they differ from each other in terms of domain structure, substrate affinity, catalytic efficiency and preferred cleavage site on the collagen molecule.

The use of class I and class II collagenase *Clostridium histolyticum* for the treatment of Dupuytren's contracture was originally developed by Biospecifics Technologies Corporation (BTC). Following licensing by Auxilium Pharmaceuticals, Inc, the manufacturing process was optimized (Process 3) and the product was then referred to as AA4500. To distinguish between the different materials used in the development of AA4500, material developed by BTC is designated either "*early BTC process*" or "*BTC Process 1*" (a second process iteration) and the drug product produced by Auxilium is designated "*AA4500 – Process 3*".

All pivotal toxicity studies utilized *AA4500 – Process 3* material and were conducted in accordance with GLP.

Pharmacology

Primary pharmacodynamic studies

AA4500 is a mixture of two purified collagenolytic enzymes in a fix ratio (AUX-I and AUX-II) isolated from the culture medium of *Clostridium histolyticum*. Both classes of collagenases readily hydrolyze gelatin (denatured collagen) and small collagen peptides, but whereas class II has higher affinity for small collagen fragments, class I cleaves insoluble triple helical collagen with higher affinity than class II collagenases. These properties account for the ability of the two classes of enzymes to digest collagen (particularly types I and III), the most relevant target in Dupuytren's contracture.
The collagenolytic activity of AUX-I and AUX-II has been shown to be comparable to purified commercial collagenase in terms of the collagen digestion patterns and their combined complimentary effect on collagen degradation.
Application of purified commercial collagenase or early BTC process to normal or diseased tissues results in histologically detectable alterations in collagenous structures. At the doses evaluated, collagen lysis was focused to the site of application independently of the dose used, indicating limited diffusion of the enzymes to the surrounding fibrous tissues. Preservation of elastic fibers and superficial neurovascular structures was observed even in areas where collagen had been completely dissolved. Despite the fact that clostridium collagenase has been shown to have activity against soluble type IV collagen *in vitro* and some types of basement membrane in *ex vivo* preparations, significant lysis of basement membranes has not been reported in the tissue explant studies (with the exception of those in the small venules) or following local administration in animal studies. With administration into looser tissues, collagen lysis may be more widespread but is still well contained. Overall, this suggests that there is only a low potential for direct damage to normal tissue structures present either within or adjacent to injected tissue following treatment of Dupuytren's contracture with AA4500.

Secondary pharmacodynamic studies

Because AA4500 is not intended for systemic use and systemic exposure was either not quantifiable or limited at the first few hours after the initial dose following clinically relevant routes of administration, no systemic secondary pharmacodynamic effects have been evaluated or noted in clinical or animal studies. However, local secondary pharmacodynamic effects following administration of AA4500, including increased vascular permeability, inflammatory responses and regenerative changes, reflecting enhanced wound healing, may result from the release of small, pharmacologically active peptide fragments from collagen.
Degradation of a number of extracellular matrix components, including collagen, has been shown to expose biologically active sites that are not normally exposed in the mature secreted matrix protein ("matricryptic sites") which initiate a number of physiologic responses that are important in regeneration and repair processes.
The mentioned effects are thus expected and may be considered local indirect responses to the primary pharmacologic activity of AA4500.

Safety pharmacology programme

The absence of systemic levels of AA4500 following local injection by clinically relevant routes in either human subjects or in animal toxicology studies precluded the need for safety pharmacology evaluation. Additionally, no evidence of effects indicative of potential safety pharmacology concerns

(e.g. effects on the cardiovascular or central nervous systems) was apparent following administration of single or repeated IV doses of AA4500 in rats.

Pharmacodynamic drug interactions

In vitro inactivation of clostridial collagenase by some antibiotics has been described in the literature. A potential interaction with tetracycline derivatives may result from chelation of metal cofactors (Ca and Zn) essential to the activity of AA4500, although direct inhibition of purified clostridial collagenase by tetracycline derivatives at pharmacologically relevant concentrations has not been demonstrated. The only documented drug interaction is inactivation of clostridial collagenase by anthracycline and anthroquinolone antibiotics (e.g. adriamycin, daunarubricin, and related compounds).

Pharmacokinetics

Methods

AA4500 contains a fixed ratio mixture of two collagenases, AUX-I and AUX-II (for material produced by Process 3 at either the Cobra or Horsham manufacturing facilities (with corresponding components referred to as ABC-I and ABC-II for BTC Process 1 material originating from BTC)) with no relevant antigenic cross-reactivity. For this reason, antibody-based assays (double antibody sandwich ELISA/competitive RIA) were developed to detect each collagenase separately. Analytical methods for the purpose of quantification of AA4500 have been validated for rat serum, rat plasma, and dog plasma. Similar methods were developed and/or validated to quantify AUX-I and AUX-II in human plasma for clinical pharmacokinetic evaluations. To evaluate the incidence and magnitude of anti-AA4500 antibody formation, additional antibody based methods were developed to specifically detect anti-AUX-I and anti-AUX-II antibodies in rat serum and dog plasma.

Absorption

Overall, the results of the pharmacokinetic portions of the toxicology studies indicated the following:

- concerning IV administration in rats:
  - Both AUX-I and AUX-II are short-lived in systemic circulation at dose levels ranging between 50 and 5000 U/dose. In general, AUX-I levels fell below the limit of quantification at ≤30 minutes postdose, while AUX-II levels fell below the limit of quantification at ≤2 hours post dose. For both components, calculated half-life values were short, generally ranging from approximately 6 to 30 minutes.
  - In general, exposure to both AUX-I and AUX-II increased in proportion to dose or slightly greater than in proportion to dose and no significant gender-related differences in exposure were observed.
  - Exposure to both AUX-I and AUX-II (as assessed by changes in Cmax, AUC, and/or number of samples with quantifiable analyte levels) decreases after repeated doses, most likely due to the appearance of anti-drug antibodies.
  - Exposure to AUX-I/ABC-I and AUX-II/ABC-II was variable within each study and across studies. For example, following IV application to rats, AA4500-Process 3 Cobra material resulted in approximately 3 to 5-fold greater systemic exposure of AUX-I/ABC-I and AUX-II/ABC-II compared to the BTC Process 1 material (Study DLB00006) and AA4500-Process 3 Cobra material and AA4500-Process 3 Horsham resulted in more than 2-fold greater Cmax-values for AUX-II/ABC-II than the BTC Process 1 material (Study 1007-1671). However, due to the short half-life, limited quantifiable data and the large degree of variability, definite conclusions regarding comparability of different drug manufacturing lots could not be made on basis of these data. However, additional non-clinical data are not considered necessary.

- concerning local administration of AA4500 into the rat or dog paw:
    - Minimal to no quantifiable systemic exposure was detected following the injection into rat or dog paws.
- concerning local administration of AA4500 into the dog penis:
    - Minimal to no quantifiable systemic exposure was detected following injection into a dense fibrous connective tissue structure, the tunica albuginea of the penis.
    - Injection into highly vascular structures (those which allow direct access to the systemic circulation such as corpus cavernosum) resulted on some occasions in quantifiable levels of AA4500 components in the systemic circulation, but only at very low plasma concentrations that disappear rapidly (usually quantifiable only at 5 minutes postdose).

Distribution

No tissue distribution studies have been performed with AA4500. No significant systemic exposure has been observed, either in animal studies or human subjects (AUX-CC-855), following local administration of AA4500 suggesting very little if any tissue distribution away from the site of injection. After IV administration, volume of distribution estimates were low which is consistent with the nature and size of AUX-I and AUX-II (proteins of approximately 110 kD).

Metabolism

No metabolism studies have been performed with AA4500 in any species. As a protein, AA4500 (AUX-I, AUX-II) is not a substrate for cytochromes P450 or other drug metabolizing enzyme pathways, and thus no active metabolites or species differences in metabolites are expected.
The AA4500 clearance mechanism has not been directly assessed but it is proposed to involve an interaction with α-2-macroglobulin, a serum protein which acts as a substrate/inhibitor for various proteases including endogenous collagenolytic MMPs.

Excretion

No studies on the excretion of AA4500 have been performed. Significant systemic exposure does not occur either in animal studies or human subjects (AUX-CC-855) following local administration of AA4500 and thus excretion into either the urine or feces following transport from the injection site is not likely. Furthermore, both AUX-I and AUX-II are too large (~ 110 kd) to pass through the glomerular filtration barrier intact and be excreted in the urine.

Pharmacokinetic drug interactions

No pharmacokinetic drug interaction studies have been performed with AA4500. AA4500 is not a substrate for cytochromes P450 or other drug metabolizing enzyme pathways, and therefore interaction with other drugs by competition for metabolism or induction or inhibition of cytochromes P450-mediated metabolism should not occur. Furthermore, since no relevant systemic exposure results from local administration, there should be no competition for protein binding sites and/or clearance of other protein therapeutics by receptor-mediated endocytosis.

Toxicology

Design of toxicological testing program

Toxicological studies have been performed with AA4500 (*Clostridium histolyticum* collagenase) produced by BTC and by Auxilium Pharmaceuticals Inc during the course of development. They include single and repeat-dose studies by a clinically relevant route (SC injection into the paw) in rats and dogs and repeated dose IV administration (general toxicity, reproductive and developmental toxicity

studies) in rats. The rat and dog are considered appropriate non-clinical species for addressing the safety of AA4500 given that: (1) AA4500 is a combination of two types of bacterial collagenases which are equally pharmacologically active as well as immunogenic in humans, rats, and dogs; (2) The rat and dog are conventional rodent and non-rodent toxicity species; and (3) the anatomic structures of rat and dog paw are analogous to those of concern for inadvertent administration in the human hand. In addition, a range of nonpivotal studies are also summarized in support of this application, along with relevant peer-reviewed literature. All pivotal toxicology studies were conducted to GLPs.

The following summarises the studies conducted by the Applicant. For the results see section the discussion section 3.3.6:

Single dose toxicity

Studies SS-001, SS-002, SS-003. Single-dose non-GLP toxicity studies have been conducted in mice and 3-day toxicity studies in rats. In both species, acute death was associated with evidence of hemorrhage into body cavities (pleural and/or peritoneal spaces) and occurred at lower relative doses in mice (≥80 U/animal, equivalent to ≥4000 U/kg or ≥12000 U/m^2) than in rats (≥5000 U/animal, equivalent to ≥20000 U/kg or ≥140000 U/m^2).

Single-Dose Subcutaneous Toxicity Study with a 28-Day Recovery in Rats

Study WIL-696001: AA4500 – Process 3 Horsham was administered once by s.c. injection in the metatarsal-phalangeal area of the right hind paw to rats. Dose levels were 0.015, 0.03, 0.06, or 0.15 mg/animal (7280, 14560, 28840, or 72520 U/m^2). On day 1 and day 28 rats were euthanized following a nondosing (recovery) period.

Single-Dose Intratendon and Deep Subcutaneous Toxicity Study with an 8-Week Recovery in Dogs

Study WIL-696005: AA4500 – Process 3 Horsham was administered once by intratendon injection in the superficial digital flexor of the right forelimb and by deep subcutaneous injection in the metacarpal area of the right forelimb. Dose levels of intratendon injection were 0.075, 0.15, or 0.3 mg/animal (corresponding to doses of 1290, 2590, or 5170 U/animal), respectively, and by deep subcutaneous injection were 0.15, 0.45, or 0.75 mg/animal (corresponding to doses of 2590, 7760, or 12930 U/animal) respectively. Recovery was assessed in a group on animals after 8 weeks.

Intrapenile Toxicity/Local Tolerance Study in Dogs (Non-pivotal) – Single dose phase

Study 520: Dogs received AA4500 by single intrapenile injection into the tunica albuginea, corpus cavernosum, urethra, or VAN complex and were necropsied at either 48 hours or 28 days following injection. Toxicokinetics were evaluated following the first dose on Day 1.

Repeat dose toxicity

A. LOCAL APPLICATION

3-Month Subcutaneous Rat With a 28-Day Recovery

Study WIL-696003: AA4500 was administered once every other week for 3 months (7 total doses) by s.c injection into the metatarsal-phalangeal area of the hindlimb. The dose levels used were 0.015, 0.03, or 0.045 mg/animal (7280, 14560, or 21728 U/m^2). At each dose level, a group of animals was randomly selected for an additional 28-days of nondosing recovery period after the final

administration. Toxicokinetic analyses were also performed. Blood samples were collected from all animals for the investigation of anti-AUX-I and anti-AUX-II antibody titers at predose, at the primary sacrifice and at the end of the recovery period.

Intrapenile Toxicity/Local Tolerance Study in Dogs (Non-pivotal) – Repeat dose phase

Study 520: In the repeat-dose phase of this GLP-study, dogs received injections of 0, 140, 430, or 1430 U/animal (0, 336, 1032, or 3432 U/m^2) into the tunica albuginea 3x weekly every four weeks for a total of three cycles (nine doses). The high dose was lowered to approximately 1050 U/animal (2520 U/m^2) for the second and third cycles due to excessive local reactions. Necropsy was performed at either 24 hours or 28 days following their last injection for histologic evaluation of tissue damages. Systemic exposures were evaluated during the study.

A 3-Month (Once Monthly) Deep Subcutaneous Toxicity Study With a 28-Day Recovery Period in Beagle Dogs

Study WIL-696006: AA4500 was administered once every month for 3 months (4 total doses) by deep s.c. injection into the peri-digital flexor tendon fascia/connective tissue area of the right forelimb. The doses employed were 0, 0.15, 0.225, and 0.375 mg/animal (0, 259, 388, or 647 U/kg; and 6216, 9312, or 15528 U/m^2). At each dose level, a group of animals was randomly selected for an additional 28 days of nondosing recovery period after the final administration. Toxicokinetic analyses were also performed. Blood samples were collected from all animals for the investigation of anti-AUX-I and anti-AUX-II antibody titers at predose, at the primary sacrifice and at the end of the recovery period.

B. INTRAVENOUS APPLICATION

3-Day IV Toxicity Studies in Rats (Non-pivotal)

Studies DLB00014 and DLB00018: Two repeat dose range-finding studies were conducted with AA4500 – Process 3 Cobra by the IV route (non-GLP).

In Study DLB00014, female rats were administered AA4500 daily by IV infusions (slow bolus) once daily for 3 days at dose levels of 5000, 10000, or 20000 U/animal (corresponding to doses of 140000, 280000, or 560000 U/m^2). Animals were observed further for two days after the cessation of treatment.

Study DLB00006: GLP study to evaluate the potential toxicity of AA4500 when administered by IV bolus injection every other day during a 16-day period. In addition, the study aimed to demonstrate the comparability of AA4500– Process 3 Cobra and the BTC Process 1 material.

16-Day IV Toxicity Study Followed by 14-Day Recovery in Rats

Study 1007-1671: Rats were administered an IV bolus dose of AA4500 Process 3-Horsham every 48 hours for 16 days at 0, 0.029, 0.13, or 0.29 mg protein/animal (0, 14000, 62720, or 140000 U/m^2). Recovery and toxicokinetics were investigated in additional groups of animals.

Genotoxicity

The applicant reported the results of studies conducted with the BTC collagenase.

Type of test/study ID/GLP	Test article	Test system	Concentrations/Dose Metabolising system	Result
Gene mutations in bacteria 200003-M-00591 GLP: yes	BTC collagenase	Salmonella strains TA1535, TA1537, TA98, TA100	213 to 3400 U/plate +/- S9	Negative
Chromosomal aberrations in mammalian cells 200001-M-00391 GLP: yes	BTC collagenase	Human peripheral blood lymphocytes	7.9 to 1700 U/mL +/- S9	Negative
Chromosomal aberrations *in vivo* 200002-M-00491 GLP: yes	BTC collagenase	Mouse, micronuclei in bone marrow (5/sex/group)	21.4 and 42.8 U/animal	Negative

AA4500 (early BTC process) was not mutagenic in a *Salmonella typhimurium* Reverse Mutation Assay and was not clastogenic either in a human lymphocyte chromosomal aberrations assay (or in vivo in a mouse micronucleus study.

Carcinogenicity

In accordance with ICH guidance (ICH, 1995 and ICH, 1997) carcinogenicity studies with AA4500 were not conducted. ICH (1997) indicates that standard carcinogenicity bioassays are generally not required for biotechnology derived pharmaceuticals, particularly proteins with no known growth factor activity.

Reproduction Toxicity

Two studies on fertility and early embryonic development as well as an embryofetal development study have been submitted. Both studies were conducted in rats (Crl:CD(SD) and in compliance with GLP. The intravenous route of administration was selected to maximize systemic exposure and to better define systemic and reproductive toxicity and immunogenicity.

Fertility and early embryonic development

In Study DLB00012, rats were administered 0, 0.0145, 0.0435, 0.13 mg/animal (0, 250, 750, or 2240 U/animal; 0, 1000, 3000, or 8960 U/kg; and 0, 7000, 21000, or 62720 U/m2) as an IV bolus every other day.

Embryo-fœtal development

In Study DLB00009, female rats were given 0, 0.0145, 0.0435, or 0.13 mg/animal (0, 250, 750, or 2240 U/animal; 0, 1000, 3000, or 8960 U/kg; and 0, 7000, 21000, or 62720 U/m2) by IV bolus administration, once daily from Day 7 through 17 of gestation.

Prenatal and postnatal development, including maternal function

A pre- and postnatal study was not conducted given that systemic exposure was very limited or not quantifiable and lack of systemic toxicities following administration of AA4500 by a clinically relevant route (rat and dog paw studies). In addition, AA4500 had no adverse effects on reproduction and early embryonic development; processes considered sensitive to MMP inhibition (a likely target of AA4500 antibodies should cross reactivity to endogenous MMPs occur).

Studies in which the offspring (juvenile animals) are dosed and/or further evaluated

As Dupuytren's contracture is extremely rare in patients less than 18 years of age, use of AA4500 in neonates, infants, and children is extremely unlikely. Therefore, studies in juvenile animals are of no clinical relevance and hence have not been conducted with AA4500.

Local Tolerance

A range of additional studies/publications are available on the early BTC material that provides information in support of the local findings with the commercial drug product. Generally, injection into a variety of sites and locations resulted in qualitatively similar findings to those seen in the pivotal studies. These include a GLP local tolerance study in Zucker rats (Study 95-2384; Tabulated Summary 2.6.7.16) and a non-GLP local tolerance study in guinea pigs (Study SS-004; Tabulated Summary 2.6.7.16), studies with the early material BTC following injection into the tail tendon of rats (& Hurst, 1996), perineural injection in rats (et al, 1992) and rabbits (et al, 1985) and following dermal administration to pigs (et al, 1986).

Other toxicity studies

Antigenicity

As a foreign bacterial protein, AA4500 is antigenic following administration in both animals and humans. Antibody titers to the components of AA4500 have been consistently reported in the clinical and nonclinical program.

Dependence

Given the lack of systemic exposure, the large molecular weight (limiting access to the CNS and brain) and the intermittent exposure of the proposed dosing regime, dependence studies with AA4500 have not been conducted.

Metabolites

No metabolism studies have been performed with AA4500 in any species. AA4500 is not a substrate for cytochromes P450 or other drug metabolizing enzyme pathways, and thus no active metabolites or species differences in metabolites are expected.

Studies on impurities

Impurities in the drug product have been qualified through the nonclinical and clinical testing program for AA4500, as a result no additional toxicity studies have been conducted on impurities within AA4500 drug product.

Ecotoxicity/environmental risk assessment

In accordance with the CHMP guidance EMEA/CHMP/SWP/4447/00, proteins are exempted because they are unlikely to result in a significant risk to the environment.
AA4500 is comprised of two proteins, the enzyme collagenases AUX-I and AUX-II, which are unlikely to result in a significant risk to the environment. Therefore, an ERA is not provided in this MAA.

Discussion on non-clinical aspects

A potential interaction with tetracycline derivatives may result from chelation of metal cofactors (Ca and Zn) essential to the activity of AA4500, although direct inhibition of purified clostridial collagenase by tetracycline derivatives at pharmacologically relevant concentrations has not been demonstrated. The only documented drug interaction is inactivation of clostridial collagenase by anthracycline and anthroquinolone antibiotics (e.g. adriamycin, daunarubricin, and related compounds). Given the therapeutic use of these antibiotic agents, concomitant treatment with AA4500 is unlikely to occur. The SmPC adequately reflects that use of Xiapex in patients who have received tetracycline antibiotics (e.g. doxycycline) within 14 days prior to receiving an injection of Xiapex is not recommended.

Following either IV or local repeat-dose administration of AA4500, anti-AUX-I and anti-AUX-II antibodies were detected in nearly 100% of the animals. Increased antibody titers were observed as early as 7 days following the first dose of AA4500. Neutralizing capacity and potential interactions of these ADAs with endogenous MMPS were not further characterized on the non-clinical level. Since the collagenolytic activity of AA4500 appeared to be maintained in non-clinical studies even in the presence of large ADA titers and since systemic exposure of AA4500 was low or non-quantifiable following single or repeated administration by a clinically relevant route, a relevant impact of these ADAs on pharmacodynamic activity and systemic disposition of AA4500 is considered unlikely. However, since results of animal studies on ADA induction may not be predictive for the clinical situation, a detailed characterization of AA4500-ADAs in patient samples available from the clinical studies is considered important (see clinical section).

Single-dose local toxicity studies by SC injection into the hindlimb of rats or SC and intratendon injection into the forelimb of dogs demonstrated that AA4500-induced adverse effects are localized to the site of injection and the draining lymph node and are qualitatively similar across both species and sex.
Swelling and discoloration of the limb (injection site and the draining lymph node) were consistently reported, with hemorrhage, edema, inflammation, collagen lysis and fibroplasia/neovascularization, arterial intramural hemorrhage, and sinus erythrocytosis in lymph nodes noted on histologic evaluation. The lymph node findings were considered secondary to the AA4500-induced injection site changes. No relevant effects on adjacent nerve bundles were observed in these studies.
After injection of higher doses of AA4500 SC into the rat or dog paws, some of the clinical findings extended beyond the initial site of application. In the single dose rat study (at ≥29 and ≥5x the HED on U/kg and U/m^2 basis respectively), inflammation was observed extending to the periosteum of the metatarsal bones. However, this finding was not observed in the repeat dose study in rats or the single and repeat dose studies in dogs.

Examination of the recovery phase animals indicated essentially complete recovery of the gross findings with ongoing healing processes of all AA4500-related tissue changes.
At dose levels well in excess of the clinical dose (73x the HED on a body weight basis; 13x on U/m^2 basis) skin lacerations (with exposed tendons) resulted in the premature euthanasia of several rats.

The findings in the repeat-dose studies in rats and dogs were consistent with those reported in the single dose studies. No systemic toxicity and limited to no quantifiable systemic exposure was observed following repeated local administration of AA4500 by clinically relevant routes of exposure into rats and dogs. Further support for the lack of systemic toxicity and limited systemic exposure was provided by the results of the repeat-dose intra-penile toxicity study in dogs.
In general, the clinical observations were less severe and resolved more rapidly following repeated administration in dogs. Ongoing healing processes were reported at the end of the 28-day recovery period in both species. Similar findings were reported following intrapenile injection into dogs.
Treatment related findings following direct injection into superficial digital flexor tendon of the paw or other dense collagenous structures (tunica albuginea) resulted in less severe/extensive findings and more complete recovery than SC injection into the rat and dog paw or application into the looser fibrous connective tissue structures (corpus cavernosum, urethra, and VAN complex) of the dog penis.
Thus, adverse effects observed after single and repeated administration by the relevant route of injection into the paw of dogs and rats in non-clinical studies were local in nature, and were reversible or showed evidence of ongoing resolution and healing. This is consistent with the spectrum of adverse effects observed in clinical studies, and supports the characterization in the SmPC that the non-clinical data reveal no special hazard for humans not addressed by the available clinical data.

Clinically, the worst scenario following misapplication of AA4500 into the local vasculature would be injection of the full clinical dose of AA4500 into the local vein and/or artery surrounding the Dupuytren's cord. The misapplication would be expected to trigger similar systemic effects as induced by an IV injection.
In repeat-dose IV studies in rats (Studies DLB00006 and 1007-1671), AA4500 did not induce systemic toxicity at ≤500 U/animal (2.5x HED based on U/m^2). Locally, at 500 U/animal, the only notable clinical sign was blue, red, and/or dark discoloration and/or wound at the injection site. However, repeat IV administration at ≥2240 U/animal (11x HED, based on U/m^2) resulted in:
- dose-dependent liver findings (Study 1007-1671). The hepatic lesions were reversed partially with evidence of ongoing healing process by the end of the recovery period. Histologic findings are consistent with minimal to mild chronic inflammation and perivascular hemorrhage/edema.
- a dose-related exacerbation of perivascular hemorrhage/edema and inflammation with complete reversal of the perivascular hemorrhage/edema and partial reversal of the inflammation by the end of the recovery period.
- Administration of even higher doses (5000 U/animal, 25x HED, based on U/m^2), resulted in mortality, probably related to adverse effects on the liver.
In conclusion, according to the rat IV studies, following misapplication of a partial or full clinical dose into the local vasculature, no systemic toxicity is to be expected. At ≥11x HED, the systemic toxicity would likely be related to hepatic findings. However, commonly observed injection site reactions, such as perivascular oedema/hemorrhages and inflammation are expected locally at all doses right after injections. Based on the pharmacology of AA4500, the walls of the small-size and mid-size veins, which are relatively relatively rich in collagen, are expected to be directly affected by AA4500. In contrast, mid-size or large arteries, as well as nerve fibers are unlikely direct targets of AA4500 given that they are relatively poor in collagen content.

After single dose IP administration to mice, deaths occurred already at an AA4500 dose of 6000 U/m^2, corresponding to a HED of about 1 (Non-GLP Study SS-001). Severe hemorrhage in the peritoneal cavity was a consistent observation, probably based on direct local irritation of exposed visceral organs. Therefore, data generated by the IP route are not considered relevant for the estimation of potential risks associated with the appropriate clinical use or inadvertent misapplication of AA4500 in humans.

Studies on the genotoxic potential with material from an early development batch of Collagenase *Clostridium histolyticum* (early BTC process) were negative. Studies on the genotoxic potential of Collagenase *Clostridium histolyticum* produced with the final manufacturing process (AA4500 process 3) have not been performed. This however, is consistent with ICH S6.

Consistent with ICH S6, conventional studies on the carcinogenic potential are not considered appropriate. As AA4500 also does not target any critical receptor types or specific immunological mechanisms, further investigations are not considered necessary.

There is no explicit regulatory guidance for reproductive toxicity studies of products such as AA4500 that exhibit limited or nonquantifiable systemic exposure and no systemic toxic effects. Therefore a typical ICH-compliant reproductive toxicity program was not conducted with AA4500.
In rats a fertility and early embryonic development study as well as an embryofetal development study has been performed. A prenatal and postnatal study was not deemed necessary by the applicant due to the limited or not quantifiable systemic exposure after local application of AA4500 into the paws of rats and dogs. No second species was used for the study on embryofetal development.
In the rat studies males and females of the parent generation showed clinical signs at the injection site in the mid and high dose group. In male rats soft and/or liquid feces were observed in the mid and high dose group additionally. Toxicokinetic investigations showed that Cmax of AUX-I and AUX-II were lower in female rats compared to male rats. Mating and fertility were not influenced by treatment and no effects on sperm parameters, estrus cycle and uterine parameters were detected in these studies. The prenatal development of the F1-generation was not affected by treatment.

Since high anti-AUX-II titers were noted in a few control males in the fertility and early embryonic development study, misdosing of these control animals cannot be ruled out. However, after removal of the control animals in question, the data still support the original interpretation. Therefore, the observed presence of positive ADA titers in some control and pretreatment samples is not expected to have a relevant impact on study interpretation.

The applicant does not provide an environmental risk assessment. As the active ingredient collagenase clostridium histolyticum is composed of two proteins an environmental risk assessment is not required.

Conclusion on the non-clinical aspects

Overall the non-clinical program conducted by the Applicant meets the general requirements and the data are acceptable. Furthermore the SmPC adequately reflects the non-clinical findings.

2.4 Clinical aspects

Introduction

A total of 13 clinical studies were conducted with AA4500 (one Phase 1, three Phase 2, and nine Phase 3) in subjects with Dupuytren's contracture and have been completed. These include one Phase 1 and seven Phase 3 studies conducted by Auxilium Pharmaceuticals, Inc. The studies in the clinical

development programme were designed to evaluate pharmacokinetics, establish an efficacy dose response, and an adequate efficacy and safety profile in subjects with Dupuytren's contracture. Process optimization was undertaken in order to improve the quality (impurity profile) and scalability of the resultant drug substance and drug product for future commercial purposes.
Primary support for efficacy and safety of AA4500 in subjects with Dupuytren's contracture comes from study AUX-CC-857 (US study) and study AUX-CC-859 (Australian study), which are considered as pivotal.
Study DUPY-303 provides supportive efficacy data but cannot be considered as pivotal, contrary to the orginal claim of the Applicant, since it is a monocentric study performed in only 35 patients. This was accepted by the Applicant.
Study AUX-CC-854 was the only study in which European subjects were enrolled. Efficacy results are presented for the subset of European subjects who were enrolled in the open-label study (n=137) to further contextualize the results from the double-blind placebo-controlled study population.

One long term extension trial (AUX-CC-860: "Long-term follow-up of subjects treated with AA4500 in studies AUX-CC-854, AUX-CC-856, AUX-CC-857/AUX-CC-858, and AUX-CC-859") is ongoing. This study follows subjects who received AA4500 in the Phase 3 double blind and open label studies for 2 to 5-years and will evaluate durability of response in joints with measurable improvement, progression of disease in joints that were not treated with AA4500 or did not have measurable improvement, and assess the long-term safety in patients previously treated with AA4500. A detailed clinical study protocol for study AUX-CC-860 (Amendment 5) was submitted by the Applicant.

The comparability of the materials manufactured by different processes has been assessed adequately. The Applicant also confirmed that Phase 3 study AUX-CC-855 was conducted using the final commercial formulation prepared according to Process 3.

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.
The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Study	Design	# Centers/ Location(s)	Study Drug and Dose	Patients Entered/Completed
		MAIN STUDIES		
AUX-CC-857	Phase 3, multi-center, double-blind, placebo controlled study (subjects could receive up to 3 injections of AA4500)	16/USA	**AA4500:** 0.58 mg **Placebo**	**AA4500:** 204/191 **Placebo**: 104/100
AUX-CC-859	Phase 3, multi-center, double-blind, placebo-controlled followed by open-label extension (subjects could receive up to 3 injections of AA4500 in DB phase and up to 5 injections OL phase; max of 3 per joint)	5/Australia	**AA4500:** 0.58 mg **Placebo**	**Double-blind:** **AA4500:** 45/45 **Placebo:** 21/19 **Open Label:** **AA4500:** 64/58
		SUPPORTIVE STUDIES		
DUPY-303	Phase 3, single-center, double-blind, placebo controlled study (subjects could receive up to 3 injections of AA4500)	1/USA	**AA4500:** 10,000 U2 **Placebo**	**AA4500 10,000 U:** 23/21 **Placebo:** 12/12
AUX-CC-854	Phase 3, multi-center, open-label study (subjects could receive up to 5 injections of AA4500; max of 3 per joint)	20/ Australia, United Kingdom, Switzerland, Sweden, Denmark, and Finland	**AA4500:** 0.58 mg	386/358

Pharmacokinetics

Pharmacokinetic studies have only been conducted in subjects with Dupuytren's contracture. Additionally, because of no quantifiable systemic exposure following a single injection of AA4500 into Dupuytren's cords intrinsic factor, extrinsic factor, and population pharmacokinetic studies were not considered necessary and/or possible.

Absorption

Study AUX-CC-855 was a Phase 1, open-label pharmacokinetic study in subjects with Dupuytren's contracture. The objectives of this study were to determine if there was systemic exposure following a single injection of AA4500 0.58 mg directly into the Dupuytren's cord affecting either the metacarpophalangeal (MP) joint or proximal interphalangeal (PIP) joint and to determine the safety of AA4500.

Sixteen subjects were enrolled and treated with one injection of AA4500 0.58 mg. Fifteen (93.8%) of the 16 subjects completed the study. One subject was lost-to-follow up after being in the study for eight days. This subject had a reduction in contracture from 60 degrees at baseline to 10 degrees on Day 7.

AUX-I and AUX-II levels were below quantifiable limits in every subject at every time point through the first 24 hours, on Day 7, or on Day 30 following administration of a single 10,000U (equivalent to 0.58 mg) injection of AA4500 into a Dupuytren's cord. All samples were below the lower limit of quantification (i.e., ≤ 5 ng/mL for AUX-I and ≤ 25 ng/mL for AUX-II).

In conclusion, there was no quantifiable systemic exposure following a single injection of 10,000U (equivalent to 0.58 mg) of AA4500 into the cord of the affected finger in subjects with Dupuytren's contracture.

Distribution

No tissue distribution studies have been performed with AA4500, as the absence of significant systemic exposure either in animal studies or human subjects following local administration of AA4500 indicates that AA4500 primarily remains confined to the tissues near the injection site and/or is rapidly inactivated either before or upon reaching the systemic circulation.

Elimination

Excretion

Excretion was not formally examined after treatment with AA4500 because there is no quantifiable systemic exposure following a single injection of AA4500 in humans. However, literature data on the elimination of the collagenolytic matrix metalloproteinases (MMPs), the endogenous mammalian functional analogs of AA4500, and commercial collagenase clostridium histolyticum suggest the elimination of AA4500 in human as follows: 1) Systemically, AA4500 is inactivated by endogenous serum components, i.e., complex formation with α-2-macroglobulin (α2M), a serum protein that serves as a substrate/inhibitor for proteases of a variety of types, resulting in steric inhibition by preventing access of macromolecular substrates to the active site of the enzyme, followed by removal of circulating α2M-protease complexes primarily in the liver both by hepatocytes and Kupffer cells) 2) Locally, inactivation and uptake of AA4500 occur due to the local release of α2M by fibroblasts and tissue macrophages, followed by endocytosis and lysosomal proteolysis of the resulting complexes by fibroblasts, tissue macrophages and other in flammatory cells.

Metabolism

AA4500 is not a substrate for cytochrome P450 or other drug metabolizing enzyme pathways, and thus no active metabolites are expected. Therefore, no metabolism studies have been performed with AA4500.

Dose proportionality and time dependencies

Dose and Time Dependencies were evaluated in *in vitro* studies. A definitive dose response was established, and a dose of at least 3600 units (equivalent to 0.21 mg) was needed to disrupt Dupuytren's cords in vitro (Starkweather et al 1996).

Special populations

The Phase 3 clinical studies evaluated the safety and efficacy of AA4500 in a subject population that is representative (e.g., in age, gender, and race) of the intended target population. As systemic exposure to AA4500 after intralesional injection into Dupuytren's cords is below quantifiable level, no pharmacokinetic studies are deemed necessary to evaluate the effects of age, gender and race on AA4500 or in subjects with impaired hepatic or renal function.

Pharmacokinetic interaction studies

Pharmacokinetic drug interactions have not been evaluated. AA4500 is not a substrate for cytochrome P450 or other drug metabolizing enzyme pathways; therefore P450 related metabolic drug interaction is not expected. The potential competition for protein binding sites and/or clearance of other protein

therapeutics by receptor-mediated endocytotic pathways at the systemic level is unlikely, as systemic exposure to AA4500 after intralesional injection into Dupuytren's cords is below quantifiable level.

Immunogenicity

The majority of AA4500 treated subjects developed anti-AUX-I and anti-AUX-II antibodies by injection 3 and all developed antibodies by injection 4. Of note, 11% and 22% of the samples in study AUX-CC-857 were positive for neutralizing antibodies for AUX-I and AUX-II, respectively. There is a theoretical possibility of cross-reactivity of anti-product antibodies (anti-AUX-I and anti-AUX-II) with endogenous human matrix metalloproteinases (MMPS) with similar homology. An MMP inhibitor-associated musculoskeletal syndrome (MSS) has been described in literature after oral treatment with matrix metalloproteinase inhibitors in knee osteoarthritis which includes shoulder arthralgia, myalgia, and stiffness, as well as hand oedema, palmar fibrosis, tendons/thickening nodules (reminiscent of the early development of Dupuytren's contracture) (Krzeski et al., 2007).

In the feasibility study, an in vitro study conducted in 5 patients, there was no clear direct (experiments with human serum from 5 patients) or indirect evidence to indicate that the anti-AUX-I or anti-AUX-II antibodies cross-react with or neutralize endogenous mammalian MMPs. However, one patient with anti-AUX-II antibodies had a positive inhibition results versus 5 MMP indicative of a possible cross-reaction. Therefore, on the basis of these data the potential for cross-reactivity of anti-product antibodies with these endogenous human MMPs cannot be really excluded (see also safety section). In addition, there was indirect evidence in some of the nonclinical repeat dose studies that there may be the potential for ADAs to neutralize AA4500, although the neutralizing potential was without a significant effect on the pharmacologic activity of AA4500 in animals; correspondingly, a clinical benefit from treatment is observed in patients even after multiple injections, however efficacy results appear most favourable for the first joint treated. The Applicant has provided a number of analyses that indicate a persistence of efficacy even in the presence of high and increasing anti-drug antibody titres which supports the lack of a neutralising antibody effect. There is no correlation between efficacy outcomes and antibody titres.

There was no evidence of AA4500-related severe systemic hypersensitivity or anaphylaxis in either the nonclinical or clinical setting and there was no correlation with the incidence, severity and duration of adverse events with antibody titre.

Pharmacodynamics

Mechanism of action

Collagenases are proteinases that hydrolyze native collagen under physiological conditions. Injection of Xiapex into a Dupuytren's cord, which is comprised mostly of interstitial collagen types I and III, results in enzymatic disruption of the cord.

Primary and Secondary pharmacology

Primary pharmacology

The pharmacologic activity of AA4500 involves selective lysis of collagen at the site of injection (i.e., the Dupuytren's cord). Because its therapeutic activity is localized and does not require or result in quantifiable systemic exposure, the primary pharmacodynamic activity of AA4500 cannot be evaluated in subjects and, therefore, such studies have not been undertaken. Instead, evidence for primary pharmacodynamic activity of clinical relevance has been obtained from in vitro studies using excised Dupuytren's cords.

Secondary pharmacology

No systemic secondary pharmacodynamic effects have been evaluated or noted in clinical or animal studies.

Discussion on clinical pharmacology

Because AA4500 is not intended for systemic use and systemic exposure was either non-quantifiable or limited only at the first few hours following the initial dose via clinically relevant routes of administration, there are no systemic primary or secondary pharmacodynamic actions of relevance and no safety pharmacology concerns. Application of purified commercial collagenase or early BTC process to collagenous tissues in explant culture results in a dose and time dependent lysis of collagen fibers, with smaller fibers being more susceptible to lysis. The collagen lysis was focused to the site of application independently of dose used. Elastic fibers and superficial neurovascular structures are well preserved and no damage to tissue adjacent to collagenase injection was observed. However, clostridium collagenase has been shown to have activity against soluble type IV collagen in vitro and some types of basement membranes in ex vivo preparations. Significant lysis of basement membranes has not been reported in the tissue explant studies or following local administration in animal studies with a dose of ≥ 10,000 units which is reassuring but explant studies were not performed with the commercial drug product.
Due to the therapeutic use and non-quantifiable systemic bioavailability significant interactions are unlikely. However, use of doxycycline or other tetracycline derivatives during the 14 days before the first dose of study drug was an exclusion criterion which is adequately reflected in the SmPC.

No systemic secondary pharmacodynamic effects have been evaluated or noted in clinical or animal studies. However, local secondary pharmacodynamic effects resulting from administration of AA4500 may result from the release of small, pharmacologically active peptide fragments from collagen. These secondary pharmacodynamic effects include vascular permeability, inflammatory responses and regenerative changes reflecting enhanced wound healing. These effects are expected and may be considered indirect responses to the primary pharmacologic activity of AA4500. Since collagen fragments generated from purified type I collagen by clostridium collagenase have been shown to have potent bradykinin-like effects on skin capillaries (increased permeability) adjacent structures to the injection site are likely to be affected by the secondary pharmcodynamic properties of AA4500. From animal studies there is no morphological evidence of primary and secondary pharmacodynamics effects on tissues other than those adjacent to the site of injection. These findings are consistent with observations in clinical studies, ie, no evidence of systemic bradykinin-mediated AEs. Therefore, the risk of systemic bradykinin-like effects can be considered minimal.

Pharmacokinetic studies have been conducted only in the target population. This is acceptable since data from study AUX-CC-855 indicate that there is no quantifiable systemic exposure following a single injection of AA4500 0.58 mg into the cord of the treated finger in subjects with Dupuytren's contracture or following the subsequent procedure to disrupt the cord. Consequently no tissue distribution and metabolism studies have been performed. However, there is a theoretical possibility that AA4500 might diffuse into adjacent tissues, especially after cord disrupture. This might be important for antibody development and immunologic reactions in adjacent tissues. Furthermore, adjacent structures to the injection site are likely to be affected by the secondary pharmcodynamic properties of AA4500.
Literature data support elimination of α2M-proteases complexes from the circulation in the liver and/or spleen and locally by fibroblasts, tissues macrophages and other inflammatory cells and in the absence

of systemic plasma levels renal clearance is very unlikely. Based on these considerations, a suitable urine assay was not developed nor validated and no urine samples were collected which is acceptable.

The majority of AA4500 treated subjects developed anti-AUX-I and anti-AUX-II antibodies by injection 3 and all developed antibodies by injection 4. The Applicant's strategy to investigate anti-drug antibodies is not convincing.
Screening, confirmatory and titer determination assay have been developed based on the identical ELISA technique. The established assay format however is not optimised in terms of sensitivity. Nevertheless, as a strong immune response against the bacterial-derived collagenases AUX-I and AUX-II is expected, the assay sensitivity is considered sufficient for confirmation of anti-drug antibodies (ADAs). This is confirmed by the clinical results indicating ~85 % ADA incidence in patients after first injection and 100% after third or fourth injection.

Since the protein components in Xiapex (AUX-I and AUX-II) have some sequence homology with human matrix metalloproteinases (MMPs), Anti-product antibodies to the protein components of Xiapex may have the potential to interfere with these endogenous human proteins. Such cross-reactivity was assessed via limited in vitro data in a feasibility study from only 5 patients and additional cross-reactivity experiments using a validated assay were provided. However, assay results are not considered to be reliable. On the basis of these data cross-reactivity cannot be really excluded. Whereas formation of ADAs is expected due to the fact that AUX-I and AUX-II are of bacterial origin, potential cross-reactivity of these antibodies represents a serious risk for adverse effects, e.g. development of musculoskeletal syndrome that should be monitored routinely by meaningful assays. Currently, the detection of cross reactivity of anti-AUX-I or anti-AUX-II antibodies versus the selected MMPs is restricted to binding assays. This is not considered sufficient. For the time being this is addressed in the educational program, the labelling and the RMP.
The potential for cross-reactivity should be additionally investigated in terms of inactivation of endogenous MMPs by neutralising ADAs. A respective assay that is based on enzymatic activity of the MMPs should be developed and validated. The Applicant agreed to develop respective assays as post-approval commitment.
As the full immune response in some patients (~15%) does not occur until the third or fourth injection, cross-reactivity should be also investigated at this stage of treatment. The Applicant provided a respective commitment.

11% and 22% of the samples in study AUX-CC-857 were positive for neutralizing antibodies for AUX-I and AUX-II, respectively. The Applicant has provided a number of analyses that indicate a persistence of efficacy even in the presence of high and increasing anti-drug antibody titres which supports the lack of a neutralising antibody effect. There is no correlation between efficacy outcomes and antibody titres. Extrinsic factors such as the type of joint treated may explain the difference in response between the first joint treated and the subsequent ones.
Regarding the neutralising potential of ADAs versus AUX-I and AUX-II, data on the determination of the optimised concentrations of enzymes and substrate were not provided. It is acknowledged that varying amounts of alpha-2-macroglobulin in the sera to be tested may disturb the assay. The Applicant should however develop a suitable assay to assess potential neutralising activity of NAbs not only in terms of binding but also in terms of enzymatic and thus pharmacodynamic activity of the drug substance. The assay setup should not only be based on literature but on experimental data. The applicant committed to initiate feasibility studies to evaluate the potential impact of Nabs and /or anti-AUX-I or –II on enzymatic activity of endogenous MMPs.

Conclusions on clinical pharmacology

Data from study AUX-CC-855 indicate that there is no quantifiable systemic exposure following a single injection of AA4500 0.58 mg into the cord of the treated finger in subjects with Dupuytren's contracture or following the subsequent procedure to disrupt the cord.
Adjacent structures to the injection site are likely to be affected by the primary and secondary pharmacodynamic properties of AA4500. However, the risk of systemic bradykinin-like effects can be considered minimal.
Investigation of immunogenicity particularly with respect to the neutralising potential of ADAs versus the drug substance and cross-reactivity to endogenous MMPs needs to be further investigated. For the time being this is addressed in the educational program, the labelling and the RMP. Respective commitments to develop meaningful assays were provided by the Applicant.

2.5 Clinical efficacy

Dose response studies

Dose-response studies

An early open-label study (Badalamente and Hurst, 2000) and study DUPY-101 were available as publications only. A clinical effect was observed at a dose level of 10,000 U (equivalent to 0.58 mg) (Badalemente al 2002).

Study DUPY-202
In a Phase 2, double-blind, randomized, placebo-controlled, dose response study (DUPY-202) 80 subjects with Dupuytren's contracture and fixed-flexion deformity of at least 20° to 30° in a single finger were randomized to receive a single injection of placebo or one of three dose levels of AA4500 (ie, 2500 U, 5000 U, or 10,000 U [equivalent to 0.58 mg]), followed approximately 24 hours later with passive extension of the treated finger. Study drug was injected into the cord affecting the MP joint (N=55; injection volume=0.25 mL) or the PIP joint (N=25; injection volume=0.20 mL). Single injections of AA4500 2500 U, 5000 U, and 10,000 U (equivalent to 0.58 mg), followed after approximately 24 hours by a finger extension procedure to facilitate cord disruption, were all statistically superior ($p \leq 0.002$) to placebo in reducing baseline contracture to 5° or less in the primary joint (2500 U, 50.0%; 5000 U, 45.5%, 10,000 U [equivalent to 0.58 mg], 78.3%; placebo, 0%). Similar percent responses were observed when the primary joint was analyzed separately by joint type (MP or PIP). However the 10,000 U (0.58 mg) dose was the only dose in which the difference from placebo was statistically significant for both MP (81.3%) and PIP (71.4%) joints.
Based on the efficacy and safety results from these studies, the AA4500 dose of 0.58 mg per injection into the Dupuytren's cord was selected for Phase 3.

Main studies

The efficacy of Xiapex 0.58 mg was mainly assessed in two randomized, double-blind, placebo-controlled studies in adult patients with Dupuytren's contracture of the MP and/or PIP joints: studies AUX-CC-857 and AUX-CC-859

Study AUX-CC-857: A Phase 3, double-blind, randomized, placebo-controlled study of the safety and efficacy of AA4500 in the treatment of subjects with Dupuytren's contracture.

Methods

Study AUX-CC-857 was a 90-day double-blind, randomized, placebo-controlled study. Before double-blind treatment, eligible subjects were stratified by:

- primary joint type (144 MP joints and 72 PIP joints) and
- severity of the primary joint contracture (i.e., ≤ 50° or > 50° for MP joint and ≤ 40° or > 40° for PIP joint)

Then, patients were randomized in a 2:1 ratio to either AA4500 0.58 mg or placebo.

Upon completion of the double-blind phase (i.e., 90-day evaluation after the first injection), all subjects entered an open-label extension study where they were followed for an additional 9 months (Study AUX-CC-858).

Subjects who required further treatment because they either did not achieve clinical success (the cord affecting the primary joint received placebo or another cord received < 3 injections of AA4500) during the double-blind phase or they had untreated cords that were affecting other joints had the option to receive up to 5 additional injections of AA4500 in the open label extension study.

Study Participants

Study centres: 16 centers in the United States

Criteria for inclusion: Men and women of at least 18 years of age with a diagnosis of Dupuytren's contracture with a fixed-flexion (i.e., ≥ 20º but ≤ 80º for PIP joint; ≥ 20º but ≤ 100º for MP joint) deformity of at least one finger, other than the thumb, that was caused by a palpable cord and a positive "table top test" defined as the inability to simultaneously place the affected finger(s) and palm flat against a table top.

Treatments

A treatment cycle comprised injection, manipulation and 30-days of follow up.
AA4500 0.58 mg was administered in a volume of 0.25 mL for cords affecting MP joints and 0.20 mL for cords affecting PIP joints. Study drug was injected directly into the cord affecting the joint using a hubless syringe with an attached 27 gauge ½ inch needle using a standardized technique. No dose adjustments or modifications were permitted under the protocol. If needed, the day after injection, the joints were manipulated to rupture the cords. Patients were given a splint to wear nightly for up to 4 months. They were no physical therapy.
If the primary joint met the primary endpoint with one or two injections, a secondary joint could be treated, if primary and secondary joints met the primary endpoint with one injection each, a tertiary joint could be treated.
Patients could receive a maximum of 3 injections of study drug during the double-blind phase and up to 5 additional injections during the open-label phase of the study.
Each injection was separated by approximately 30 days. A cord affecting a joint could receive up to a maximum of three injections of AA4500.

Objectives

The primary objective was to evaluate the efficacy and safety of up to three injections of AA4500 as compared to placebo in reducing the degree of contracture (fixed-flexion deformity) in the primary joint of subjects with advanced Dupuytren's disease. The primary joint was either metacarpophalangeal (MP) or proximal interphalangeal (PIP).

The secondary objective was to evaluate the efficacy and safety of up to three injections of AA4500 as compared to placebo in reducing the degree of contracture (flexion deformity) in multiple joints (MP and PIP) of subjects with Dupuytren's disease.

Outcomes/endpoints

The primary endpoint was the proportion of subjects who achieved a reduction in contracture of their primary joint to 5° or less at the Day 30 evaluation after the last injection of study drug.

Secondary endpoints:
- Clinical improvement: defined as a ≥50% reduction from baseline in degree of contracture after an injection.
- Mean percent change in degree of contracture
- Time to clinical success
- Change in range of motion
- Subject global assessment of treatment satisfaction
- Physician global assessment

Sample size

The sample size calculation for each joint type (MP and PIP) with the estimated response rates above, significance level of 0.05 and power of 80% is presented as follows:

Joint	Response rate Active vs Placebo	Sample Size Active/Placebo
MP	80% vs 10%	14/7
PIP	70% vs 10%	18/9

Randomisation

Primary joints were randomized in a 2:1 ratio to receive either AA4500 0.58 mg (equivalent to 10,000 U) or placebo. The randomization was stratified by joint type and also by the severity of the primary joint contracture (i.e., ≤ 50° or > 50° for an MP joint and ≤ 40° or > 40° for a PIP joint). This study design ensured that the efficacy and safety of AA4500 would be evaluated in all degrees of joint contracture.

Blinding (masking)

This study was double-blind whereby the investigator, study subject, and other study personnel were blinded to the study medication. Reconstitution of the appropriate study drug vial was performed by a designated reconstitution person. All precautions were taken to ensure that the blinding was maintained throughout the double-blind study period.

Statistical methods

- **Statistical methods**

The intent-to-treat (ITT) population was defined as all randomized subjects who received at least one injection. All safety analyses were based on the ITT population. Modified intent-to-treat: ITT subjects were excluded from this population if they did not have fixed flexion measurements after the first

injection or had both screening and Treatment 1, Day 0 fixed-flexion measurements between 0 and 5 degrees. Efficacy analyses were performed using this population.
Per Protocol: Modified ITT subjects were excluded from this population if their primary joint:
1) had a baseline contracture less than 20° or greater than 100° for MP (80° for PIP); 2) received incorrect study medication; 3) received reduced number < 3 injections and did not reach clinical success but still had a palpable cord, and did not stop treatment due to an adverse event; 4) received more than 3 doses of study medication into the primary joint in the double-blind phase; and/or 5) did not receive the Day 30 evaluation after the last injection. Efficacy analyses were performed using this population.
The primary efficacy variable and other variables expressing proportions were analyzed by a Cochran-Mantel-Haenszel (CMH) test, which was stratified by baseline severity and joint type for all primary joints.

Study AUX-CC-859: A Phase 3, Double-Blind, Randomized, Placebo-Controlled Study of the Safety and Efficacy of AA4500 in the Treatment of Subjects With Dupuytren's Contracture Followed by an Open-Label Extension Phase.

Methods

This 12-month study had two phases; a 90-day double-blind, randomized, placebo-controlled phase, and a nine-month open-label extension phase.
Before treatment, eligible subjects were stratified by:

- the primary joint type (30 MP joints and 30 PIP joints)
- and severity of the primary joint contracture (i.e., ≤ 50° or > 50° for MP joint and ≤ 40° or > 40° for PIP joint) and then randomized in a 2:1 ratio to either AA4500 0.58 mg or placebo.

Upon completion of the double-blind phase (i.e., 90-day evaluation after the first injection), all subjects were eligible to enter the open-label extension phase of the study in which they were followed for an additional nine months.

Subjects who required further treatment because they either did not achieve reduction in contracture to 5° or less (the cord affecting the primary joint received placebo or another cord received < three injections of AA4500 0.58 mg) during the double-blind phase or they had untreated cords that were affecting other joints had the option to receive up to five additional injections of AA4500 0.58 mg in the open-label extension phase, with individual joints receiving a maximum of three injections.

Study Participants

Study centers: 5 sites in Australia enrolled subjects in the study.
Criteria for inclusion: Healthy male or female subjects ≥ 18 years of age with a diagnosis of Dupuytren's contracture with a fixed-flexion (i.e., ≥ 20º but ≤ 80º for PIP joint; ≥ 20º but ≤ 100º for MP joint) deformity of at least one finger, other than the thumb, which was caused by a palpable cord.

Treatments

A treatment cycle comprised injection, manipulation and 30-days of follow up.

AA4500 0.58 mg was administered in a volume of 0.25 mL for cords affecting MP joints and 0.20 mL for cords affecting PIP joints. Study drug was injected directly into the cord affecting the joint using a

hubless syringe with an attached 27 gauge ½ inch needle using a standardized technique. No dose adjustments or modifications were permitted under the protocol.
If needed, the day after injection, the joints were manipulated to rupture the cords.
Patients were given a splint to wear nightly for up to 4 months. There was no physical therapy.

If the primary joint met the primary endpoint with one or two injections, a secondary joint could be treated, if primary and secondary joints met the primary endpoint with one injection each, a tertiary joint could be treated.

Duration of treatment:
Patients could receive a maximum of 3 injections of study drug during the double-blind phase and up to 5 additional injections during the open-label phase of the study.
Each injection was separated by approximately 30 days. A cord affecting a joint could receive up to a maximum of three injections of AA4500.

Objectives

The primary objective was to evaluate the efficacy and safety of up to three injections of AA4500 as compared to placebo in reducing the degree of contracture (fixed-flexion deformity) in the primary joint of subjects with advanced Dupuytren's disease. The primary joint was either metacarpophalangeal (MP) or proximal interphalangeal (PIP).

The secondary objective was to evaluate the efficacy and safety of up to three injections of AA4500 as compared to placebo in reducing the degree of contracture (flexion deformity) in multiple joints (MP and PIP) of subjects with Dupuytren's disease.

The tertiary objective was to evaluate the recurrence rate in joints that were successfully treated during the 12-month study period.

Outcomes/endpoints

The primary endpoint was the proportion of subjects who achieved a reduction in contracture of their primary joint to 5° or less at the Day 30 evaluation after the last injection of study drug.

Secondary endpoints:
- Clinical improvement: defined as a ≥50% reduction from baseline in degree of contracture after an injection.
- Mean percent change in degree of contracture
- Time to clinical success
- Change in range of motion
- Subject global assessment of treatment satisfaction
- Physician global assessment

Sample size

A total of 54 subjects was sufficient to ensure 80% power at a 2-sided significance level of 0.05 to detect the response rates shown in the following table.

Joint	Response rate Active vs Placebo	Sample Size Active/Placebo
MP	80% vs 10%	16/8
PIP	70% vs 10%	20/10

Randomisation

Primary joints were randomized in a 2:1 ratio to receive either AA4500 0.58 mg (equivalent to 10,000 U) or placebo. The randomization was stratified by joint type and also by the severity of the primary joint contracture (i.e., ≤ 50° or > 50° for an MP joint and ≤ 40° or > 40° for a PIP joint). This study design ensured that the efficacy and safety of AA4500 would be evaluated in all degrees of joint contracture.

Blinding (masking)

The first phase of this study was double-blind whereby the investigator, study subject, and other study personnel were blinded to the study drug. Reconstitution of the appropriate study drug vial was performed by a designated reconstitution person. All precautions were taken to ensure that the blinding was maintained throughout the double-blind study period. The second phase of the study was open-label.

Statistical methods

The intent-to-treat (ITT) population was defined as all randomized subjects who received at least one injection. All safety analyses were based on the ITT population. Modified intent-to-treat: ITT subjects were excluded from this population if they did not have fixed flexion measurements after the first injection or had both screening and Treatment 1, Day 0 fixed-flexion measurements between 0 and 5 degrees. Efficacy analyses were performed using this population.

Per Protocol: Modified ITT subjects were excluded from this population if their primary joint:

1) had a baseline contracture less than 20° or greater than 100° for MP (80° for PIP); 2) received incorrect study medication; 3) received reduced number < 3 injections and did not reach clinical success but still had a palpable cord, and did not stop treatment due to an adverse event; 4) received more than 3 doses of study medication into the primary joint in the double-blind phase; and/or 5) did not receive the Day 30 evaluation after the last injection. Efficacy analyses were performed using this population.

The primary efficacy variable and other variables expressing proportions were analyzed by a Cochran-Mantel-Haenszel (CMH) test, which was stratified by baseline severity and joint type for all primary joints.

Results

Study AUX-CC-857

Participant flow

Subject Disposition, Double-Blind Phase - Intent-to-Treat Population			
	AA4500 N=204	**Placebo N=104**	**Total N=308**
Intent-to-treat (ITT)	204 (100.0)	104 (100.0)	308 (100.0)
Modified intent-to-treat[a]	203 (99.5)	103 (99.0)	306 (99.4)
Per protocol[b]	182 (89.2)	91 (87.5)	273 (88.6)
Completed double-blind phase, N (%)	191 (93.6)	100 (96.2)	291 (94.5)
Discontinued double-blind phase, N (%):	13 (6.4)	4 (3.8)	17 (5.5)
Withdrew consent	4 (2.0)	3 (2.9)	7 (2.3)
Lost to follow-up	4 (2.0)	1 (1.0)	5 (1.6)
Adverse events	3 (1.5)	0	3 (1.0)
Other	2 (1.0)	0	2 (0.6)

Number of injections during double-blind, N (%):			
1	61 (29.9)	4 (3.8)	65 (21.1)
2	46 (22.5)	7 (6.7)	53 (17.2)
3	97 (47.5)	93 (89.4)	190 (61.7)
Number of joints treated during double-blind, N (%):			
Primary	138 (67.6)	102 (98.1)	240 (77.9)
Primary and secondary	49 (24.0)	2 (1.9)	51 (16.6)
Primary, secondary, and tertiary	17 (8.3)	0	17 (5.5)
Total number of joints treated	287	106	393
Days in Study			
N	204	104	308
Mean (SD)	92.2 (18.0)	92.0 (17.9)	92.2 (18.0)
Median	92.0	92.0	92.0
Min, Max	2, 161	2, 149	2, 161

[a] Intent-to-treat subjects were excluded from this population if they did not have fixed-flexion measurements after the first injection or had both screening and Treatment 1, Day 0 fixed-flexion measurements between 0 and 5 degrees.
[b] Modified intent-to-treat subjects were excluded from this population if their primary joint: 1) had a baseline contracture less than 20° or greater than 100° for MP (80° for PIP); 2) received incorrect study medication; 3) received reduced number < 3 injections and did not reach clinical success but still had a palpable cord, and did not stop treatment due to an adverse event; and/or 4) did not receive the Day 30 evaluation after the last injection.

Recruitment

Date first patient enrolled: 28 August 2007
Date last patient completed: 14 April 2008

Conduct of the study

The protocol was amended twice, however the changes were minor.

Baseline data

Demographics and Baseline Disease Severity – Intent-to-Treat Population

	AA4500 N=204	**Placebo N=104**	**Total N=308**
Age			
N	204	104	308
Mean (SD)	62.3 (9.7)	63.3 (9.1)	62.7 (9.5)
Median	63.0	64.0	63.0
Min, Max	33, 89	42, 83	33, 89
Gender, N (%)			
Male	171 (83.8)	74 (71.2)	245 (79.5)
Female	33 (16.2)	30 (28.8)	63 (20.5)
Race, N (%)			
White	203 (99.5)	104 (100.0)	307 (99.7)
Hispanic	1 (0.5)	0	1 (0.3)
Total contracture Indexa			
N	204	104	308
Mean (SD)	149.1 (127.6)	149.3 (111.4)	149.1 (122.2)
Median	105.0	119.0	115.0
Min, Max	20, 860	20, 489	20, 860
Hand with ≥ 1 contracture[b], N (%)			
Left	54 (26.5)	28 (26.9)	82 (26.6)
Right	69 (33.8)	40 (38.5)	109 (35.4)
Both	81 (39.7)	36 (34.6)	117 (38.0)
Mean number of affected joints[b] per subject			
Mean (SD)	3.0 (2.2)	3.0 (2.1)	3.0 (2.2)

Min, Max	1, 13	1, 10	1, 13
Mean number of affected MP joints[b] per subject			
Mean (SD)	1.6 (1.5)	1.7 (1.4)	1.7 (1.5)
Min, Max	1, 6	1, 6	1, 6
Mean number of affected PIP joints[b] per subject			
Mean (SD)	1.4 (1.3)	1.3 (1.3)	1.3 (1.3)
Min, Max	1, 7	1, 6	1, 7

a Sum of fixed-flexion contractures (ie, ≥ 20 ° caused by a Dupuytren's cord) for all 16 joints measured at screening.
b Number of joints at screening with fixed-flexion contractures ≥ 20° caused by a Dupuytren's cord.

Numbers analysed

216 subjects were planned and 308 were enrolled and treated (AA4500 204; placebo 104).

Study AUX-CC-859

Participant flow

Subject Disposition, Double-Blind Phase - Intent-to-Treat Population

	AA4500 0.58 mg (N=45)	Placebo (N=21)	Total (N=66)
Intent-to-treat	45 (100.0)	21 (100.0)	66 (100.0)
Per protocol[a]	43 (95.6)	21 (100.0)	64 (97.0)
Completed double-blind phase, N (%)	45 (100.0)	19 (90.5)	64 (97.0)
Discontinued double-blind phase, N (%):	0	2 (9.5)	2 (3.0)
Withdrew consent	0	2 (9.5)	2 (3.0)
Number of injections during double-blind, n (%):			
1	11 (24.4)	1 (4.8)	12 (18.2)
2	7 (15.6)	1 (4.8)	8 (12.1)
3	27 (60.0)	19 (90.5)	46 (69.7)
Number of joints treated during double-blind, n (%):			
Primary	23 (51.1)	20 (95.2)	43 (65.2)
Primary and secondary	17 (37.8)	1 (4.8)	18 (27.3)
Primary, secondary, and tertiary	5 (11.1)	0	5 (7.6)
Total number of joints treated	72	22	94
Days in study			
N	45	21	66
Mean (SD)	90.6 (8.6)	80.1 (17.6)	87.3 (13.0)
Median	86.0	85.0	85.0
Min, Max	81, 121	28, 94	28, 121

[a] Intent-to-treat subjects were excluded from this population if their primary joint: 1) had a baseline contracture less than 20° or greater than 100° for MP (80° for PIP); 2) was mistreated due to incorrect randomization;3) received too much or too little study drug; and/or 4) did not receive the Day 30 evaluation

Recruitment

Date first subject enrolled: 24 August 2007

Data last subject completed: 29 September 2008

Conduct of the study

This protocol was amended three times, however the changes were minor.

Baseline data

Table 11: Demographics and Baseline Disease Severity – Intent-to-Treat Population

	AA4500 0.58 mg (N=45)	Placebo (N=21)	Total (N=66)
Age			
N	45	21	66
Mean (SD)	63.0 (7.8)	65.5 (11.1)	63.8 (9.0)
Median	63.0	67.0	64.0
Min, Max	45, 88	41, 86	41, 88
Gender, N (%)			
Male	39 (86.7)	17 (81.0)	56 (84.8)
Female	6 (13.3)	4 (19.0)	10 (15.2)
Race, N (%)			
White	45 (100.0)	21 (100.0)	66 (100.0)
Total contracture Index[a]			
N	45	21	66
Mean (SD)	174.7 (107.2)	150.1 (84.0)	166.9 (100.4)
Median	145.0	140.0	142.5
Min, Max	25, 525	50, 335	25, 525
Hand with ≥ 1 contracture[b], N (%)			
Left	12 (26.7)	5 (23.8)	17 (25.8)
Right	11 (24.4)	4 (19.0)	15 (22.7)
Both	22 (48.9)	12 (57.1)	34 (51.5)
Mean number of affected joints[b] **per subject**			
Mean (SD)	3.4 (2.3)	3.0 (1.5)	3.3 (2.1)
Min, Max	1, 11	1, 5	1, 11
Mean number of affected MP joints[b] **per subject**			
Mean (SD)	1.5 (1.6)	1.5 (1.5)	1.5 (1.5)
Min, Max	0, 7	0, 5	0, 7
Mean number of affected PIP joints[b] **per subject**			
Mean (SD)	2.0 (1.6)	1.4 (1.2)	1.8 (1.5)
Min, Max	0, 7	0, 4	0, 7

[a] Sum of fixed-flexion contractures (ie, ≥ 20 ° caused by a Dupuytren's cord) for all 16 joints measured at screening.
[b] Number of joints at screening with fixed-flexion contractures ≥ 20° caused by a Dupuytren's cord.

Numbers analysed

60 subjects were planned; 66 (45 AA4500 0.58 mg and 21 placebo) subjects were analyzed in the double-blind phase; 64 subjects were analyzed in the open-label phase (42 subjects received open-label AA4500 0.58 mg); 63 subjects received at least one injection of AA4500 0.58 mg during either the double-blind or open-label phase.

Study DUPY-303

Study DUPY-303 was a Phase 3, double-blind, randomized, placebo-controlled study.

Primary joints were randomized to receive 0.58 mg (10,000 units) of AA4500 or placebo in a ratio of 2:1 in favor of AA4500.

Study medication was injected into the cord affecting the MP joint or PIP joint. If the first injection was a clinical success, subjects could receive up to 2 additional injections for treatment of a secondary and tertiary joint, if indicated.

Study treatment consisted of a single treatment visit (to a maximum of 3 treatments) and follow-up visits at 1 day, 1 week, 2 weeks, and 1 month after each injection. Additional follow-up visits were 60

days and 90 days after the first injection. At each follow-up visit, subjects were evaluated for degree of contracture (flexion deformity).

Upon completing the Day 90 follow-up, subjects who desired further treatment for unsuccessfully treated joints or previously untreated joints were permitted to enroll in the open-label extension study, DUPY-404. Subjects who responded to treatment under DUPY-303 and who did not participate in DUPY-404 were evaluated at 6, 9, and 12 months after their last injection for durability of response. These subjects were also evaluated annually from 2 to 5 years after treatment to assess contracture recurrence and/or need for surgery.

DUPY-303 was conducted at a single center in the US with only 35 patients (planned enrollment included 116 subjects) compared to study AUX-CC-857 which enrolled 308 patients at 16 centers in the US and study AUX-CC-857 which enrolled 66 patients at 5 centers in Australia. Apart from that, study DUPY-303 was performed with a study drug that was produced by an earlier manufacturing process whereas studies AUX-CC-857 and AUX-CC-859 were performed with AA4500 produced by Auxilium's optimized manufacturing process. In addition in study DUPY-303 only 1 investigator performed all the injections compared to up to 7 to 33 investigators (principal investigators and sub-investigators) in the other studies. The efficacy results of trials with fewer centers, patients and investigators may not be as generalisable as efficacy results of trials with more centers, patients and investigators.Therefore, data from study DUPY-303 are considered only as supportive. This was accepted by the Applicant and data are not included in the SmPC.

Study AUX-CC-854

Study AUX-CC-854 was the only study in which European subjects were enrolled. Efficacy results are presented for the subset of European subjects who were enrolled in the open-label study (n=137) to further contextualize the results from the double-blind placebo-controlled study population.

This was a 9-month, open-label study that investigated subjects who had Dupuytren's contracture in an MP or PIP joint that resulted in a fixed flexion deformity of at least 1 finger, other than the thumb, that was ≥ 20° as measured by finger goniometry and was suitable for injection and evaluation. The study was conducted at 20 sites in Australia, Sweden, Denmark, Finland, Switzerland, and the United Kingdom. Subjects were either naïve to AA4500 treatment or had received 1 or 2 injections of AA4500 for the treatment of Dupuytren's contracture in a previous study (AUX-CC-853). Subjects were followed for safety on Days 1, 7, and 30 after each injection, with injections separated by 30 days. All subjects had follow-up visits for safety evaluations on Day 90, Month 6, and Month 9.

Subjects were eligible to receive a maximum of 5 injections of AA4500 0.58 mg, with individual joints receiving a maximum of 3 injections. Approximately 24 hours after each injection of study drug, a finger extension procedure to facilitate cord disruption was conducted, if needed.

This open label study is considered relevant because the pivotal double-blind studies did not enrol patients from European countries. In AUX-CC-854 patients had similar baseline characteristics and comparbale severity of the disease to those in pivotal studies AUX-CC-857 and AUX-CC-859. In addtion, similar variables to assess the efficacy of the treatment with AA4500 were included in the study design. This allows comparisons of the exploratory results for treatment effects between populations. No formal sample size estimation was performed for AUX-CC-854. It should be noted that a primary joint was not designated in study AUX-CC-854. In this study, analyses assessed responses per numbers of joints rather than numbers of subjects, i.e. the results from all treated joints in study AUX-CC-854 are summarized rather than all treated subjects, since there could be more than one joint analyzed per subject. Comparisons of secondary endpoints between the Phase 3, double-blind, placebo-controlled studies and to European subjects in Study AUX-CC-854 are made where possible throughout.

Outcomes and estimation

Primary endpoint: Reduction of Contracture to 5° or less in the Primary Joint

The primary efficacy endpoint of the reduction in contracture to 5° or less in the primary joint (i.e., clinical success) was assessed by goniometry. The primary efficacy endpoint was achieved in both pivotal trials (AUX-CC-857, and AUX-CC-859); that is, AA4500 was statistically superior to placebo with respect to the percentage of subjects who achieved a reduction in contracture of their primary joint to 5° or less after the last injection in each study ($p < 0.001$). This pertains also to the supportive study (DUPY-303).

Reduction of Contracture to 5° or Less in the Primary Joint After the Last Injection – Studies DUPY-303, AUX-CC-857, and AUX-CC-859

	DUPY-303		AUX-CC-857		AUX-CC-859	
	AA4500 N=23	**Placebo N=12**	**AA4500 N=203**	**Placebo N=103**	**AA4500 N=45**	**Placebo N=21**
Number (%) of subjects with a reduction in contracture to ≤5°	21 (91.3)	0	130 (64.0)	7 (6.8)	20 (44.4)	1 (4.8)
p-value[a]	<0.001	-	<0.001	-	<0.001	-
Mean (SD) number of injections to achieve reduction in contracture to ≤5°	1.4 (0.7)	NC	1.5 (0.7)	2.6 (0.8)	1.5 (0.7)	1.0

NC=not computable.
Last injection=final injection of AA4500 into the cord. Individual cords could receive up to 3 injections of AA4500.
a p-value based on CMH test comparing treatment group, stratified by baseline severity group and joint type.

Reduction of contracture to 5° or Less in the Primary Joint After the Last Injection Subset by Joint Type – Studies DUPY-303, AUX-CC-857, and AUX-CC-859

	DUPY-303		AUX-CC-857		AUX-CC-859	
	AA4500	**Placebo**	**AA4500**	**Placebo**	**AA4500**	**Placebo**
Primary MP	**N=14**	**N=7**	**N=133**	**N=69**	**N=20**	**N=11**
Number (%) of subjects who achieved reduction in contracture to ≤5°	12 (85.7%)	0	102 (76.7%)	5 (7.2%)[b]	13 (65.0%)	1 (9.1%)
p-valuea	**<0.001**	-	**<0.001**	-	**0.003**	-
Mean (SD) number of injections to achieve reduction in contracture to ≤ 5°	1.3 (0.6)	NC	1.6 (0.8)	2.8 (0.5)	1.4 (0.7)	1.0
Primary PIP	**N=9**	**N=5**	**N=70**	**N=34**	**N=25**	**N=10**
Number (%) of subjects who achieved reduction in contracture to ≤5°	9 (100.0%)	0	28 (40.0%)	2 (5.9%) [b]	7 (28.0%)	0
p-value[a]	**<0.001**	-	**<0.001**	-	**0.069**	-
Mean (SD) number of injections to achieve reduction in contracture to ≤ 5°	1.6 (0.9)	NC	1.3 (0.5)	2.0 (1.4)	1.7 (0.8)	NC

NC=not computable. Last injection=final injection of AA4500 into the cord. Individual cords could receive up to 3 injections of AA4500.
a p-value based on Cochran-Mantel-Haenszel test comparing treatment group, stratified by baseline severity group and joint type.
b Subjects 1154-2715 and 1182-4309 were inadvertently administered AA4500 at the 2nd and 3rd injection, respectively.

Overall 64,0% of all AA4500 treated primary joints achieved a reduction of contracture to 5° or less in Study AUX-CC-857 (76,7% for MP joints and 40% for PIP joints), and 44,4% in Study AUX-CC-859 (65% for MP joints and 28% for PIP joints). The effect of AA4500 at PIP joints is less clearly demonstrated and did not reach statistical significance in Study AUA-CC-859. Similarly in the European subjects from study AUX-CC-854, 50.5% of all treated joints (MP+PIP) achieved a reduction in contracture to 5° or less after the last injection; 60.5% of treated MP joints and 37.9% of treated PIP joints achieved clinical success after last injection.

Reduction of Contracture to 5° or Less After the Last Injection by Joint Type - Studies DUPY 303, AUX-CC-857, AUX CC-859 and European Subjects from AUX-CC-854

	AA4500				Placebo		
Parameter	**DUPY-303 (N=23)**	**AUX-CC-857 (N=203)**	**AUX-CC-859 (N=45)**	**AUX-CC-854 EUR (N=137)**	**DUPY-303 (N=12)**	**AUX-CC-857 (N=103)**	**AUX-CC-859 (N=21)**
MP+PIP	91.3% (21/23)	64.0% (130/203)	44.4% (20/45)	50.5% (108/214)	0 (0/12)	6.8% (7/103)	4.8% 1/21)
MP	85.7% (12/14)	76.7% (102/133)	65.0% (13/20)	60.5% (72/119)	0 (0/7)	7.2% (6/59)	9.1% (1/11)
PIP	100% (9/9)	40.0% (28/70)	28.0% (7/25)	37.9% (36/95)	0 (0/5)	5.9% (2/34)	0 (0/10)

In both the Phase 3 double-blind, placebo-controlled studies and the European subjects from study AUX-CC-854, most subjects required one or two injections of AA4500 for a reduction in contracture of the primary joint to 5° or less. In study AUX CC 859, more primary PIP joints treated with up to three injections had a reduction in contracture to 5° or less compared with placebo (7 joints [28.0%] vs. 0 joints); however, the difference between the groups was not statistically significant (p=0.069). Compared to US subjects from study AUX-CC-857, Australian subjects from study AUX-CC-859 may had more severe disease at baseline as indicated by a greater proportion of affected PIP joints (55.6% versus 34.3%), greater proportion of affected PIP joints in the little finger (42.2% versus 26.5%); more bilateral disease (51% versus 38%) and more medical history of prior surgery for Dupuytren's disease (53% versus 38%). Apart from that there was a higher proportion of individuals older than 65 years or 75 years in the placebo group in study AUC-CC-859.
In addition, 72.2% (13/18) of PIP joints did not receive the full AA4500 treatment regimen, most commonly because there was "no palpable cord to inject" after only one or two injections of AA4500 0.58 mg.

Secondary Efficacy endpoints

Results of the secondary efficacy endpoints in studies 857 and 859 were consistent with the results of the primary efficacy endpoints in demonstrating treatment benefit with AA4500. This pertains also to the European patient population.

Clinical Improvement

Clinical improvement was defined as a ≥50% reduction from baseline in degree of contracture after an injection.
A significantly higher proportion of subjects who were treated with AA4500 in Studies AUX-CC- 857 and AUX-CC-859 had a 50% or greater improvement in the baseline contracture of their primary joint

compared with subjects treated with placebo (p<0.001). Clinical improvement was 84.7% versus 11.7% (p<0.001) in Study AUX-CC- 857 and 77.8% versus 14.3% (p<0.001) in Study AUX-CC-859 for AA4500 and placebo, respectively. Similar results were observed for primary MP joints treated with AA4500 in both studies and for primary PIP joints in AUX-CC-857.

In the pooled data across the three double-blind, placebo-controlled studies 84.5% of all AA4500 treated primary joints (MP+PIP) showed clinical improvement; 94.0% for primary MP joints and 69.2% for PIP joints. Clinical improvement was not a pre-specified secondary endpoint in supportive Study DUPY-303 but the fixed flexion contractures (FFC) measures were collected allowing one to analyze this endpoint in DUPY-303, and to include DUPY-303 in the pooled analysis of this endpoint. However, since it was agreed that study DUPY-303 should not be considered as pivotal data can only be considered supportive.

For subjects enrolled in the AUX-CC-854 study similar results were observed, with clinical improvement in 80.8% of all joints (MP + PIP) after the last injection. By joint type, clinical improvement was observed in 91.6% of MP joints and 67.4% of PIP joints after the last injection of AA4500.

Mean percent change in degree of contracture

In all three Phase 3 double-blind, placebo-controlled studies, subjects who received AA4500 had a significant mean percent reduction from baseline in the degree of contracture of their primary joint (>70%) compared with subjects who received placebo (p<0.001) at Day 30 following the last injection. Similar results were observed for primary MP joints (>84%) treated with AA4500 in the three studies and for primary PIP joints (>64%) in Studies AUX-CC-857 and DUPY-303. In the pooled data set, subjects treated with AA4500 showed a 79.2 percent reduction in baseline contracture of their primary joint compared to an 8.6% reduction in baseline contracture subjects who were treated with placebo in the Phase 3 double-blind, placebo-controlled studies, and 74.2% of all joints (MP + PIP) for European subjects enrolled in the AUX-CC-854 study.

By joint type, percent reduction in baseline contracture was 87.3% for MP joints and 66.2% for PIP joints in all three Phase 3 double-blind, placebo-controlled studies; and 84.1% for MP joints and 61.8% for PIP joints in the European subjects enrolled in the AUX-CC-854 study.

Time to clinical success

In all three Phase 3 studies, time to achieve clinical success (a reduction in contracture of the primary joint to 5° or less), was significantly (p<0.001) shorter in the AA4500 group compared with the placebo group. The estimated median time to clinical success in AA4500 treated patients was 8, 56 and 57 days in DUPY-303, AUX-CC-857 and AUX-CC-859 respectively.

The median time to achieve clinical success was not determined for European subjects from the AUX-CC-854 study. However, 60.5% of AA4500 treated MP joints and 37.9% of AA4500 treated PIP joints achieved clinical success by Day 30 following the last injection.

Change in Range of Motion

Normalisation of ROM is considered as surrogate for functional outcome of surgical procedures. Normal ROM varies with body habitus, age and genetic background, however ROMs of 90 degrees for finger MP joints, and 100 degrees for finger PIP joints are considered normal. In all three Phase 3 double-blind, placebo-controlled studies, subjects treated with AA4500 showed a significantly greater increase in ROM compared with subjects who were treated with placebo (p<0.001) restoring near normal ROM. Mean ROM after the last injection improved to 87.9°, 83.7°, and 79.5° in MP joints and to 101.1°, 74.9°, and 72.8° in PIP joints in Studies DUPY-303, AUX-CC-857 and AUX-CC-859, respectively. Similarly in the European subjects from the AUX-CC-854 study, ROM after the last injection improved to 82.4° for MP joints, 80.9° for PIP joints, and 81.7° for MP+PIP joints.

Normalisation was not reached in the pivotal trials but results for this endpoint were consistent in showing a treatment benefit in favour of AA4500.

Subject Global Assessment of Self Satisfaction

Subjects receiving AA4500 reported a greater level of treatment satisfaction. This pertains also to the European population. Subject global assessment of self-satisfaction was not measured for Study DUPY-303.

Physician Global Assessment

Patient subjective rating is endorsed by observer results. Physicians reported that the majority of subjects treated with AA4500 were either very much improved or much improved as compared to placebo (86% vs. 3% and 80% vs. 5%, respectively; $p<0.001$). Similarly, in the European subjects enrolled in the AUX-CC-854 study, investigators assessed 84.9% of subjects as very much improved or much improved.

Except for the latter secondary efficacy endpoint, all efficacy endpoints are structural criteria; none of them measures the functional hand status whereas the aim of treatment in DD is not only to disrupt the contracted cords but to recover hand function and activities of daily living which are generally altered by the contractures.

Ancillary analyses

Persistence of efficacy

Across studies AUX-CC-854, AUX-CC-856, AUX-CC-857, AUX-CC-858 and AUX-CC-859 recurrence of contracture was evaluated in primary joints that achieved the primary endpoint (i.e., a reduction in contracture to 5° or less). In these studies, recurrence was defined as an increase in joint contracture to at least 20° in the presence of a palpable cord, as determined by the investigator, at any time during the double-blind phase or open-label extension phase, and in the open-label studies.

In the pooled controlled and uncontrolled portions of these studies, of the 830 AP4500- treated cords that achieved clinical success, twenty-nine subjects (30 joints) experienced a recurrence after treatment with AA4500. Of the 30 joints, 21 were PIP joints and 9 were MP joints and of the 21 PIP joints that had a recurrent contracture 15 were in the little finger. Twelve subjects had a prior history of surgery for Dupuytren's disease; 8 surgeries were PIP joints and 4 were MP joints. In only one of the 12 subjects was the prior surgery conducted on the same joint as was treated with AA4500.

Overall, a low recurrence rate of 3.7% was observed by approximately 9 to 12 months after subjects received the first dose of AA4500. The average time of follow-up after success was 220.7 days in the original application. Meanwhile 2 year data (730 days) from study AUX-CC-860 are available and as expected the recurrence rate has increased. The nominal rate of recurrence is 15.9 % in the presence of a palpable cord and 19.9 % with or without a palpable cord. Kaplan-Meier estimates of recurrences which take into account that not all 838 joints were followed-up for one year (i.e., the 207 non-recurrent joints with less than 365 days of follow-up), were 25.2 % and 28.7 % respectively. There was a higher proportion of recurrent PIP joints than MP joints at year 1 and year 2. Because no non-surgical treatments are currently available, the incidence of contracture recurrence in the AA4500 studies was compared to the reported incidence of contracture recurrence following fasciectomy and/or PNF for Dupuytren's contracture in literature. Depending on the definition of recurrence, and the duration of follow-up, highly variable recurrence rates have been reported. The incidence was 2% (1.5 years) to 51% (3.1 years) following fasciectomy and up to 65% (2.75 years) following PNF.

In summary, the recurrence rate has increased following 2 years of follow up compared to 1 year, but overall the recurrence rates with or without a palpable cord in A4500 treated joints continue to be comparable to the rates observed in the literature for surgical intervention and appear favorable compared to those reported for PNF.

Few subjects that were treated with AA4500 required surgical intervention in study AUX-CC-860.

While the recurrence profile continues to be favourable at year 2 post treatment, the observation period is still too short to conclude on the persistence of effect of Xiapex on the treated joint. Recurrence data from study AUX-CC-860 will continue to be provided annually through interim clinical study reports at Years 3, 4, and 5 post-treatment.

Summary of main studies for trial AUX-CC-857

Title: A Phase 3, double-blind, randomized, placebo-controlled study of the safety and efficacy of AA4500 in the treatment of subjects with Dupuytren's contracture

Study identifier	Protocol number: AUX-CC-857		
Design	A Phase 3, double-blind, randomized, placebo-controlled study. Population stratified by the primary joint type (144 MP joints and 72 PIP joints) and by severity of the primary joint contracture (i.e., ≤ 50 o or > 50 o for MP joint and ≤ 40 o or > 40o for PIP joint) and then randomized in a 2:1 ratio to either AA4500 0.58 mg or placebo. Upon completion of the double-blind phase (i.e., 90 day evaluation after the first injection), all subjects entered an open-label extension study where they were followed for an additional 9 months (Study AUX-CC-858). Subjects who required further treatment because they either did not achieve clinical success (the cord affecting the primary joint received placebo or another cord received < 3 injections of AA4500) during the double-blind phase or they had untreated cords that were affecting other joints had the option to receive up to 5 additional injections of AA4500 in the open label extension study. Multicenter study, 16 centers in the United States		
	Duration of main phase:		90 day
	Duration of Run-in phase:		not applicable
	Duration of Extension phase:		9 months (Study AUX-CC-858)
Hypothesis	Superiority		
Treatments groups	AA4500 0.58 mg		Treatment: Clostridial collagenase 0.58 mg Duration: Up to 3 injections Number randomized: 204
	Placebo		Treatment: Placebo Duration: Up to 3 injections Number randomized: 104
Endpoints and definitions	Primary endpoint	Reduction in contracture	Proportion of subjects who achieved a reduction in contracture of their primary joint to 5° or less at the Day 30 evaluation after the last injection of study drug.
Database lock	(28/08/2007-14/04/2008)		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Modified intent-to-treat: ITT subjects were excluded from this population if they did not have fixed flexion measurements after the first injection or had both screening and Treatment 1, Day 0 fixed-flexion measurements between 0 and 5 degrees. Efficacy analyses were performed using this population.		
Descriptive statistics and estimate variability	90 days Treatment group	AA4500 0.58 mg	Placebo
	Number of subject	203	103

	Reduction in contracture	130 (64.0%)	7 (6.8%)
	By joint type		
	Primary MP	102 (76.7%)	5 (7.2%)
	Primary PIP	28 (40.0%)	2 (5.9%)
Effect estimate per comparison	Primary endpoint: Proportion of subjects who achieve clinical success of the primary joint after the last injection.	Comparison groups	AA4500 0.58 mg versus Placebo
		P-value (Cochran-Mantel-Haenszel)	<0.001

Summary of main studies for trial AUX-CC-859

Title: A Phase 3, double-blind, randomized, placebo-controlled study of the safety and efficacy of AA4500 in the treatment of subjects with Dupuytren's contracture Followed by an Open-Label Extension Phase

Study identifier	Protocol number: AUX-CC-859		
Design	This 12-month study had two phases; a 90-day double-blind, randomized, placebo-controlled phase, and a nine-month open-label extension phase. Before treatment, eligible subjects were stratified by the primary joint type (30 MP joints and 30 PIP joints) and by severity of the primary joint contracture (i.e., ≤ 50° or > 50° for MP joint and ≤ 40° or > 40° for PIP joint) and then randomized in a 2:1 ratio to either AA4500 0.58 mg or placebo. Upon completion of the double-blind phase (i.e., 90-day evaluation after the first injection), all subjects were eligible to enter the open-label extension phase of the study in which they were followed for an additional nine months. Subjects who required further treatment because they either did not achieve reduction in contracture to 5° or less (the cord affecting the primary joint received placebo or another cord received < three injections of AA4500 0.58 mg) during the double-blind phase or they had untreated cords that were affecting other joints had the option to receive up to five additional injections of AA4500 0.58 mg in the open-label extension phase, with individual joints receiving a maximum of three injections.		
	Multicenter study, 5 centers in Australia		
	Duration of main phase:	90 day	
	Duration of Run-in phase:	not applicable	
	Duration of Extension phase:	9 months	
Hypothesis	Superiority		
Treatments groups	AA4500 0.58 mg		Treatment: Clostridial collagenase 0.58 mg Duration: Up to 3 injections Number randomized: 45
	Placebo		Treatment: Placebo Duration: Up to 3 injections Number randomized: 21
Endpoints and definitions	Primary endpoint	Reduction in contracture	Proportion of subjects who achieved a reduction in contracture of their primary joint to 5° or less at the Day 30 evaluation after the last injection of study drug.
Database lock	(24/08/2007-29/09/2007)		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Modified intent-to-treat: ITT subjects were excluded from this population if they did not have fixed flexion measurements after the first injection or had both screening and Treatment 1, Day 0 fixed-flexion measurements between 0 and 5 degrees. Efficacy analyses were performed using this population. 90 days		
Descriptive statistics and estimate variability	Treatment group	AA4500 0.58 mg	Placebo
	Number of subject	45	21
	Reduction in contracture	20 (44.4%)	1 (4.8%)
	By joint type		
	Primary MP	13 (65.0%)	1 (9.1%)
	Primary PIP	7 (28.0%)	0
Effect estimate per comparison	Primary endpoint: Proportion of subjects who achieve clinical success of the primary joint after the last injection.	Comparison groups	AA4500 0.58 mg versus Placebo
		P-value (Cochran-Mantel-Haenszel)	<0.001

Analysis performed across trials (pooled analyses and meta-analysis)

Efficacy data from the 3 Phase 3, double-blind, placebo-controlled studies (DUPY-303, AUX-CC-857, and AUX-CC-859) were combined and summarized with descriptive statistics for selected intrinsic and extrinsic factors and for the effect of joint type and baseline severity on outcome.

Efficacy by Joint Type and Baseline Disease Activity

In the combined analysis of data from all three double-blind, placebo-controlled studies, 254 MP joints (low severity [≤ 50°] N = 154 and high severity [> 50°] N=100) and 153 PIP joints (low severity [≤ 40°] N=44 and high severity [> 40°] N=109) were studied. The efficacy results by joint type and baseline severity indicate that AA4500 is efficacious in both MP and PIP joints and across a broad range of severity of joint contractures. On average, less severely contracted MP and PIP joints had a reduction in contracture to <10° after treatment with AA4500. Severely contracted MP and PIP joints responded to treatment with AA4500 to a lesser extent (to 12° for MP joints of high severity and 25.6° for PIP joints of high severity).

Efficacy on MP joints was well demonstrated whereas for PIP joints, efficacy was not so obvious. Indeed, in study 857, a statistically significant proportion of patients met clinical success for PIP joints but in study 859, the statistical difference versus placebo was not reached. Although surgical procedures provide also less good efficacy results in PIP joints than in MP joints (due to a greater difficulty to treat little joints), the efficacy of collagenase injections in PIP joints seems not so well established than for MP joints.

Since study DUPY-303 is not considered as a pivotal study, data were presented also separately for studies AUX-CC-857 and AUX-CC-859 and are consistent with the pooled analysis.

Clinical studies in special populations

No clinical studies were conducted in special populations. As systemic exposure to AA4500 after intralesional injection into Dupuytren's cords is below quantifiable level, no clinical studies are deemed necessary to evaluate the effects of age, gender and race on AA4500 or in subjects with impaired hepatic or renal function.

Subgroup analysis across placebo-controlled studies for age, weight, BMI, gender and history of diabetes was undertaken. In the combined analysis of data from all three double-blind, placebo-controlled studies, no notable differences in efficacy were observed by age category (< 45, 45-54 , 55-64, 65-74, ≥ 75 years), weight quartile (first, second, third, fourth quartile), body mass index (BMI) category (normal, overweight, obese), and history of diabetes (yes, no). The only exception was country location and gender. When all primary joints were analyzed by country location, a higher percentage of subjects treated in the United States had a reduction in contracture of the primary joint to 5° or less compared with subjects treated in Australia (66.8% versus 44.4%). While the percentage of subjects with a reduction in contracture to 5° or less was similar between the United States and Australia for primary MP joints, the success rate for primary PIP joints tended to be higher in the United States compared with Australia. Australian subjects however may have had more severe disease at baseline.

A higher percentage of females (70.7%) treated with AA4500 had a reduction in contracture of the primary joint to 5° or less compared with male (61.7%) subjects. This difference may be attributed to females having less severe disease as evidenced by fewer joints of high severity compared to males (45.5% vs. 52.7%) and lower overall baseline fixed flexion contracture in MP joints (41.4° vs 48.7°) and PIP joints (51.5° vs. 55.4°). No conclusions could be drawn about race, as only one of the 409 subjects who were treated in the three double-blind, placebo-controlled studies was non-white. The low number of non-white subjects was to be expected as Dupuytren's disease has a genetic predisposition to occur in men of Northern European ancestry. The percentage of patients with a history of diabetes who had a reduction in contracture to 5° or less was similar to that of subjects without a history (63,6% and 63,1% respectively). However, no final conclusion can be drawn since the overall number of diabetic patients was low (22 of 271).

Change in Surgical Eligibility Status

In general, the criteria established for surgical intervention of Dupuytren's disease specify fixed-flexion contractures of >30° for MP joints and >15° for PIP joints. Using these criteria in the two Phase 3, double-blind, placebo-controlled studies (AUX-CC-857 and AUX-CC- 859), 85.6% of subjects (131 of 153) with primary MP joints experienced a reduction in contracture to <30◦ with 75.2% (115 of 153) achieving clinical success (reduction to <5◦). Mean change in contracture was 41.3◦ at Day 30 following the last injection for all 153 subjects. Approximately 52.6% of subjects (50 of 95) with primary PIP joints experienced a reduction in contracture to <15◦ with 36.8% (35 of 95) achieving clinical success (reduction to <5◦). Mean change in contracture was 31.3◦ at Day 30 following the last injection for all 95 subjects. Taken together these results indicate that surgical intervention would be either no longer indicated and/or delayed in the majority of the subjects that received injections of AA4500 to treat their Dupuytren's contracture.

Supportive studies

Studies DUPY-101, DUPY 202, DUPY 404, AUX-CC-851/852, AUX-CC-853, AUX-CC-854, AUX-CC-856, AUX-CC-858 and AUX-CC-859 were considered as supportive. AUX-CC-851/852 and AUX-CC-853 were terminated early due to a manufacturing issue. Data from these latter studies were not included in the pooled analyses of efficacy but were included in the safety analyses.

This list should have included Study DUPY-303 for the reasons mentioned above. In these studies subjects could receive up to a total of 5 injections of AA4500 0.58 mg (10,000 U) with individual cords receiving up to three injections of AA4500. Each injection was separated by approximately 4 weeks. The primary endpoint in each study was the proportion of joints with a reduction in contracture to 5° or less after treatment.
The results for MP joints were generally comparable across all studies. The results for PIP joints were more variable than for MP joints across all studies, perhaps in part due to few number of PIP joints. Clinical Success ranges from 24.0% to 100%. Clinical improvement was more consistent with a range of 46.2% to 67.1% in studies for which that endpoint was determined. In general the results are consistent with the results of the pivotal trials.

Discussion on clinical efficacy

Design and conduct of clinical studies

Study DUPY-202 has been performed with early BTC process material which cannot be regarded representative for the commercial process manufacturing the final formulation. Therefore, the relevance of this study is put in question and data can only be considered supportive.
However, based on the overall in vitro and in vivo data the selected AA4500 dose of 0.58 mg per injection and the dosing interval seem to be supported by sufficient data. The efficacy results observed with the highest dose of 10,000 U AA4500 (equivalent to 0.58 mg) were similar to those reported for surgical intervention. Therefore, no higher dose levels were studied. This is considered acceptable.

The statistical methods for the pivotal studies AUX-CC-857, AUX-CC-859 and the supportive study DUPY-303 are appropriate. Both pivotal studies AUX-CC-857 and AUX-CC-859 had open-label extension phases to assess the effect of additional treatments for joints that did not receive 3 injections and other joints. Up to 8 injections (5 in open-label phase) were allowed. In AUX-CC-854 up to 5 injections in total were allowed.
Sample size calculations were based on 80% and 70% rates for clinical success for MP and PIP joints, respectively. Clinical success rates of 10 % for placebo injections were assumed. This is reasonable, although success rates were not achieved in the AUX-CC-859 study.
A randomization ratio of 2:1 was selected with a stratified randomization by MP/PIP joints and baseline severity of contracture. The allocation selected is reasonable as the clinical success of placebo injections was expected to be low. Furthermore, the statistical tests for primary and secondary analyses take into account the stratification used for randomisation.
Inferential statistics are based on the comparisons for primary joints. This is appropriate since the randomization is based on the first treated joint and due to the limited clinical success rate of the placebo treatment most of the patients treated with placebo were expected to be treated only on one joint.
No interim analyses were performed or specified in the protocols.
Multiple hypothesis testing for efficacy was performed with a closed testing procedure that controlled the family-wise type I error on the 5% significance level. This approach is appropriate. The First 15 hypotheses in the AUX-CC-857 and AUX-CC-859 studies are related to the last injection, the last 12 hypotheses are related to first injection. For the AUX-CC-859 the hierarchical procedure had to be stopped after the tenth level ("reduction in contracture to 5° or less" for PIP joints) and no confirmative testing for the levels higher than 10 could be performed.
The primary efficacy variable and other variables expressing proportions were analyzed by a Cochran-Mantel-Haenszel (CMH) test, which was stratified by baseline severity and joint type for all primary joints. This approach is appropriate. For the rate comparisons only the statistical results and listed

proportions in 2x2 tables are provided. No adjusted odds ratio or confidence intervals are reported. This is not optimal but acceptable, since various aspects of the data that additionally allow assessing the clinical relevance are documented by the results for other secondary variables.
A sensitivity analysis for the primary efficacy comparison with missing values rated as failures is appropriate and confirms the robustness of the results for the primary efficacy comparison.
The statistical methods for the secondary variables "change from baseline in ROM" and "Time to achieve reduction in contracture to 5° or less" are appropriate.

Efficacy data and additional analyses

A total of 407 subjects (AA4500 271 and placebo 136) were enrolled and received up to three injections of study drug into the cord affecting their primary joint in the 3 Phase 3, double-blind, placebo-controlled studies. The majority of subjects in each treatment group completed the study with few discontinuing due to adverse event (3 subjects in the AA4500 group and none in the placebo group). Baseline demographics were similar between the two treatment groups from the two double-blind, placebo-controlled pivotal studies. The subject population from these studies and the European subjects from the open-label study AUX-CC-854 were typical of subjects with Dupuytren's contracture. The majority (84.9% AA4500 0.58 mg group and 72.1% placebo group) of subjects were men, and most subjects were white (99.6% AA4500 0.58 group and 100% in both the placebo group and European subjects from AUX-CC-854). The median age of subjects overall was 63 years in the AA4500 0.58 mg treatment group, 64 years in the placebo treatment group, and 63 years in the European subjects. The subject population from the Phase 3 double-blind placebo-controlled studies and the European subjects from the open-label study AUX-CC-854 were similar with respect to years of disease (9.4 years and 8.4 years, respectively), presence of bilateral disease (38.6% and 38.7%, respectively), disease severity (modified Tubiana score of 3.1 and 2.9, respectively), fingers affected (majority of ring and little fingers), number of affected joints (73.9% and 72.9% had 1 or 2 joints affected, respectively) and number of affected fingers (89.8% and 86.2% had 1 or 2 fingers affected, respectively). 39.1 % of patients in the AA4500 0.58 mg treatment group had prior surgery for Dupuytren's disease compared to 43.3% in the placebo group and 39.4% in the European subjects.
Of note, compared to US subjects from study AUX-CC-857, Australian subjects from study AUX-CC-859 may had more severe disease at baseline as indicated by a greater proportion of affected PIP joints (55.6% versus 34.3%), greater proportion of affected PIP joints in the little finger (42.2% versus 26.5%); more bilateral disease (51% versus 38%) and more medical history of prior surgery for Dupuytren's disease (53% versus 38%). Apart from that there was a higher proportion of individuals older than 65 years or 75 years in the placebo group in study AUC-CC-859.
The severity of disease at baseline as assessed by the median total contracture index (about 50° of contracture) and the number and type of affected joints seemed to suggest that included patients have a moderate Dupuytren's disease (approximately 50% of patients had a stage II of modified Tubiana's classification). Nevertheless, other clinical features such as positive family history, recurrent disease, presence of bilateral disease, total number of digits affected, number of surgical procedures, functional disability also reflect disease severity. Of note, the standard Tubiana's classification which is defined as the sum of the FFC at baseline in all joints (MP, PIP and DIP) was not used in the pivotal studies since angles at the DIP joints were not recorded. Hence, a modified Tubiana score was used, that does not take into account contracture angles at the DIP joints. Therefore, this modified score is likely to represent an underestimate of the true disease severity.
In addition, approximately 20% of patients had a severe disease at inclusion (stages 3 and 4 of the modified Tubiana's classification). Overall, it is considered that included patients correspond to the target population.

The primary endpoint was achieved in each of the two pivotal double-blind, placebo-controlled studies, that is, AA4500 0.58 mg was statistically superior to placebo with respect to the proportion of subjects who achieved a reduction in contracture to 5° or less of their primary joint after the last injection ($p<0.001$). Overall, 64,0% of all AA4500 treated primary joints achieved a reduction in contracture of the primary joint to 5° or less in Study AUX-CC-857 (76.7% for MP joints and 40% for PIP joints), and 44,4% in Study AUX-CC-859 (65% for MP joints and 28% for PIP joints) compared to 6,8% and 4,8% respectively for placebo. In study AUX-CC 859, although more primary PIP joints treated with up to three injections had a reduction in contracture to 5° or less compared with placebo (7 joints [28.0%] versus 0 joints), the difference between the groups was not statistically significant ($p=0.069$).
In addition, 72.2% (13/18) of the subjects in the AA4500 group who did not have a reduction in contracture to 5° or less in their primary PIP joint did not receive the full treatment regimen of up to 3 injections, most commonly because there was "no palpable cord to inject" after only one or two injections of AA4500 0.58 mg. In the pooled placebo-controlled studies 271 cords affecting primary joints were treated with 455 injections.

Heterogeneity observed for the primary variable between regions and centers is of concern, as the application of AA4500 involves a complex procedure and this might influence the generalizability of the results of the studies.
The Applicant comprehensively discussed the difference in clinical success rates between regions (US and Australia) for the AUX-CC-857 and AUX-CC-859 studies and provides valid reasons for the observed differences. However, there is a considerable heterogeneity found for the clinical success rate in the AUX-CC-857 study conducted in 16 US centers. Data of tests for heterogeneity of the success rates in the different centers for the AUX-CC-857 study were provided. Due to the small sample size the power for possible tests of heterogeneity is limited. It is agreed that the results of the presented tests are not incompatible with homogeneity and that observed heterogeneity could be due to variability in a binomial endpoint given the small sample size.
For further exploration of a possible center effect and its cause without the use of statistical methods, links to baseline data for centers with the highest and lowest success rates were provided. Different baseline fixed flexion contracture may have had an influence on the success rates. It is acknowledged that the success rates in each center for the AA4500 treatment group were higher than in the placebo group.
Although no specific concerns on the heterogeneity of the results of study AUX-CC-857 remain, a carefully planned and conducted training of prescribing physicians for treatment with AA4500 seems warranted to ensure high success rates for varying baseline conditions.
Study DUPY-303 can be considered only as supportive trial since it was conducted at a single center in the US by one investigator and included only 35 patients. Generalisability of efficacy results of trials with fewer centers, patients and investigators may be questioned. Apart from that, it was performed with a study drug that was produced by an earlier manufacturing process.
The efficacy results from the double-blind, placebo-controlled Phase 3 studies were comparable to the results observed from European subjects in the AUX-CC-854 open-label study. 50.5% of all treated joints (60.5 % for MP joints and 37.9 % for PIP joints) achieved a reduction in contracture to 5° or less after the last injection. This open label study is considered relevant because the pivotal double-blind studies did not enrol patients from European countries. In AUX-CC-854 patients had similar baseline characteristics and comparable severity of the disease to those in pivotal studies AUX-CC-857 and AUX-CC-859. In addition, similar variables to assess the efficacy of the treatment with AA4500 were included in the study design. This allows comparisons of the exploratory results for treatment effects between populations. No formal sample size estimation was performed for AUX-CC-854.

It has been demonstrated that the absolute change in fixed flexion contracture was highest in subjects with severe and very severe disease whereas the primary endpoint clinical success (defined as a

reduction in contracture to 5◦ or less) was generally highest in subjects with low severity. This is also reported in literature for surgical interventions including PNF. From the data presented, it can be concluded that subjects with more severe disease also benefit from treatment with Xiapex. For PIP joints, efficacy was less clearly demonstrated than for MP joints. However, of the 95 collagenase treated PIP joints, 73 (76.8%) were in the little finger which are considered the most difficult to treat due to anatomical structure and other variables. The small sample size could also explain for a part the difference observed in efficacy. However, the Applicant presented an analysis to show that even the most severely affected PIP joints showed meaningful clinical improvement. Overall, these findings are similar to what is observed for surgical corrections or PNF outlined in published literature.
Tubiana classification is a measurement used to determine disease severity in patients with Dupuytren´s contracture and is defined as the sum of the fixed flexion contractures (FFC) at baseline in all joints (MP, PIP and DIP) which is then translated to a scale of 1 to 4 based on the calculated FFC. Since DIP joints were not recorded in the studies a modified Tubiana score was used in the analyses presented which due to the lack of the DIP joints is likely to represent an underestimate of the true disease severity.
In all studies, AA4500 met the primary endpoint demonstrating consistent efficacy in reducing contractures in both MP and PIP joints in patients with moderate (about 50% of patients had moderate disease at inclusion, i.e. a modified Tubiana score of 2) and severe disease (20% of included patients, i.e a modified Tubiana score of 3 and 4). Therefore, a wide indication to all types of severity (low and high severity) in Dupuytren's disease is considered acceptable.

The significant correction in finger deformity was associated with significant improvements in both ROM and subject global assessment of treatment satisfaction indicating clinically relevant benefit to the patients. Patient subjective ratings are endorsed by physician global assessment of improvement. However, only surrogate endpoints and indirect evidence is given to justify an improvement of clinical hand-function. The Applicant justifies the lack of functional hand assessment tools (such as DASH) which were designed and validated for conditions other than Dupuytren's contractures.
Overall, the proposed indication "treatment of Dupuytren's contracture in adult patients with a palpable cord" is supported by sufficient data.

Therefore, the responder rates of ROM, instead of mean change from baseline, might be helpful to provide more insight and are considered more relevant to assess a clinical relevance of this outcome. The efficacy profile was similar to that seen across surgical procedures, and a more thorough comparison between collagenase injections and percutaneous needle fasciotomy suggests that efficacy outcomes with AA4500 are similar to those observed following PNF with perhaps a slightly better outcome for PIP joints. No clinically meaningful differences in the efficacy of AA4500 0.58 mg were observed by age, gender, weight quartile, BMI category, or history of diabetes. However, included patient numbers for this latter subgroup was low and no final conclusion can be drawn. In addition, efficacy data from the non-primary joints evaluated in the open-label studies provide supportive evidence of the efficacy of AA4500 0.58 mg in the treatment of Dupuytren's contracture.
Because no non-surgical treatments are currently available, the incidence of contracture recurrence in the AP4500 studies was compared to the reported incidence of contracture recurrence following fasciectomy and/or PNF for Dupuytren's contracture in literature. Depending on the definition of recurrence, and the duration of follow-up, highly variable recurrence rates have been reported.
The recurrence rate of 4% is at the lower range of the reported incidence of contracture recurrence after surgery in literature (2%- 65%) and provides evidence of the durability of treatment effect within the 12-month period after the first injection of AA4500 0.58 mg. Meanwhile 2 year data (730 days) are available and as expected the recurrence rate has increased. The nominal rate of recurrence is 15.9 % in the presence of a palpable cord and 19,9 % with or without a palpable cord. In summary, the recurrence rate has increased following 2 years of follow up compared to 1 year, but overall the

recurrence rates with or without a palpable cord in A4500 treated joints continue to be comparable to the rates observed in the literature for surgical intervention and appear favorable compared to those reported for PNF.
However, more long-term follow-up data are needed and will be provided post-approval on the ongoing extension trial AUX-CC-860 through interim clinical study reports at Years 3, 4, and 5 post-treatment.

Conclusions on the clinical efficacy

In summary in both pivotal Phase 3 studies (AUX-CC-857 and AUX-CC-859), AA4500 0.58 mg was demonstrated to be effective in the treatment of Dupuytren's disease across a broad range of fixed flexion contractures (high and low severity). Therefore, a wide indication to all types of severity (low and high severity) in Dupuytren's disease is considered acceptable**.**
For PIP joints, efficacy was less clearly demonstrated than for MP joints. Severely contracted MP and PIP joints responded to treatment with AA4500 to a lesser extent**.**
The efficacy profile was comparable to the current mainstay of treatment, i.e. surgical intervention and percutaneous needle fasciotomy (PNF), a minimally invasive technique until now not so commonly used across Europe.
The recurrence rate of 4% provides evidence of the durability of treatment effect for the 9 to 12-month period after first injection. The recurrence rate has increased following 2 years of follow up (19,9%), but overall the recurrence rates with or without a palpable cord in AA4500 treated joints continue to be comparable to the rates observed in the literature for surgical intervention and appear favourable compared to those reported for PNF. However, more long-term follow-up data are needed. Recurrence data from AUX-CC-860 will continue to be provided annually through interim clinical study reports at Years 3, 4, and 5 post-treatment.
Overall, the proposed indication "treatment of Dupuytren's contracture in adult patients with a palpable cord" is supported by sufficient data.

2.6 Clinical safety

Patient exposure
Overall there were 1196 subjects who were enrolled and treated in the AA4500 clinical programme with 1082 subjects receiving at least 1 dose of AA4500 0.58 mg comprising the formal safety database. Two early development studies (DUPY-101 and Badalamente and Hurst, 2000) which collectively treated 84 subjects were excluded from the pooled analyses, as they lacked formal study databases; results from these studies are not included in the Formal Safety Database.

Seventeen (17) subjects who received less than 0.58 mg of AA4500 and participated in the dose ranging studies were not in the All Subjects With At Least 1 Dose of AA4500 0.58 mg population.

Three integrated populations were analyzed in order to characterize the safety of AA4500 0.58 mg in the treatment of Dupuytren's contracture:

- The Phase 3 Double-Blind, Placebo-Controlled population comprised 409 subjects who received at least 1 injection of double-blind study medication (137 placebo subjects, 272 AA4500 0.58 mg subjects) in Studies DUPY-303 and AUX-CC-857, and the double-blind phase of Study AUX-CC-859. Subjects in this population could have received up to 3 injections of either AA4500 0.58 mg or placebo.
- The All Subjects With At Least 1 Dose of AA4500 0.58 mg population included 1082 subjects who received at least 1 dose of AA4500 0.58 mg across 11 Phase 2 and Phase 3 studies (Studies DUPY-202, DUPY-303, DUPY-404, AUX-CC-851, AUX-CC-853, AUX-CC-854, AUX-

CC-855, AUX-CC-856, AUX-CC-857, AUX-CC-858, and AUX-CC-859; excludes DUPY-101 and Badalamente and Hurst, 2000, which lacked formal databases). Subjects in this population could have received up to 8 injections of AA4500 0.58 mg.

- The All Subjects With 12 Months Post First Dose AA4500 0.58 mg population was a subset of the All Subjects With At Least 1 Dose of AA4500 0.58 mg population and included 268 subjects whose final study visit was more than 48 weeks (336 days) after their first injection of AA4500 0.58 mg from Studies DUPY-202, DUPY-303/404, AUX-CC-85 1/852, AUX-CC-853, AUX-CC-857/858, and AUX-CC-859. This analysis set was used to evaluate the long-term safety profile of AA4500 0.58 mg.

Additionally, a safety sub-analysis was performed on those subjects from investigator sites in EU countries that participated in Study AUX-CC-854.

Adverse events

Across all populations, the majority of TEAEs and treatment-related AEs were mild or moderate in severity and occurred in the treated extremity. In the All Subjects With At Least 1 Dose of AA4500 0.58 mg population, few subjects experienced AEs during the posttreatment period (from Day 31 after last injection through the end of the study) compared to during the treatment period (32.3% vs. 97.0%). Results were similar in the other populations.

In the Phase 3 Double-Blind, Placebo-Controlled population, approximately two times as many AA4500 treated patients than placebo-treated patients had an AE (97,8% versus 54%). The majority of AA4500 associated AE were local reactions. The most frequently reported TEAEs and treatment-related AEs were oedema peripheral, contusion, injection site pain, injection site haemorrhage, and pain in extremity and were likely related to AA4500 injection. A greater proportion of AA4500 treated patients compared to the placebo group experienced pain at the injection site (39% vs. 9.5%) or pain in the extremity (33.1% vs. 3.6%).

The AE profile of AA4500 0.58 mg in the All Subjects With At Least 1 Dose of AA4500 0.58 mg population was similar to that of the AA4500 0.58 mg treatment group in the Phase 3 Double-Blind, Placebo-Controlled population (oedema peripheral, contusion, injection site pain, injection site haemorrhage, pain in extremity and tenderness). Most adverse reactions resolved within 2 weeks after injection. However, some adverse reactions such injection site vesicles and blisters had a longer median duration. Furthermore, an analysis of AEs by SOCs was consistent and revealed no new safety signals.

The adverse event profile for European subjects enrolled in Study AUX-CC-854 was similar to that for the *All Subjects With At Least 1 Dose of AA4500 0.58 mg* population.

Some exceptions to this were haematoma and injection site vesicles which occurred in a higher frequency in the European population. One explanation for differences in the incidence of specific events (eg, contusion, ecchymosis, haematoma) observed among study location may be differences in local diagnostic classification and/or choice of Preferred Term for a given AE.

Further analysis of treatment-related AEs in the All Subjects With At Least 1 Dose of AA4500 0.58 mg population demonstrated that most treatment-related AEs started on the day of injection or the day of the finger extension procedure. The overall percentage of subjects treated with AA4500 0.58 mg who reported treatment-related AEs was similar across injections 1 through 8. Moreover, with the exception of oedema peripheral and pruritus, no clinically meaningful increase or trend in duration of the most frequently reported treatment-related AEs was observed with increased number of injections of AA4500 0.58 mg. The term oedema peripheral represents the most common adverse reaction in the AA4500 clinical programme. This preferred term describes not only oedema in the treated extremity but also other oedematous states. Similarly, the PT Pruritus not only describes localized pruritus in the

treated extremity but also cases of generalized Pruritus. The Applicant provided a comprehensive table separating oedema in the treated extremity and other oedematous states and similarly localized pruritus and cases of generalized Pruritus. There was no hint for a generalised reaction.
Of subjects who had between 2 to 5 injections of AA4500 0.58 mg and who experienced oedema peripheral, the majority experienced these events at nearly every injection. For other TEAEs, the majority of subjects who received multiple injections of AA4500 0.58 mg had no consistent pattern of specific treatment-related events occurring with subsequent injections with the exception of pruritus. There were too few subjects who received between 6 and 8 injections (N=13 or 14) to draw meaningful conclusions regarding a pattern of treatment-related AE occurrence.
For the *All Subjects With At Least 1 Dose of AA4500 0.58 mg* population, TEAEs were summarized by age (< 45 years, 45-54 years, 55-64 years, 65-74 years, ≥ 75 years), gender (male, female), weight quartile (first, second, third, fourth quartile), BMI category (normal, overweight, obesity), diabetes history (history of diabetes, no history of diabetes), and study location (United States, Europe, Australia). No clinically meaningful differences were observed between or among subgroups. Due to the small sample size of non-white subjects compared to white subjects, no clinically meaningful comparisons of the incidence of TEAEs by race could be made.

Serious adverse events and deaths

Serious adverse events
A total of 94 subjects experienced at least one nonfatal SAE across all 13 studies among which were at least 88 subjects who received at least one injection of AA4500 0.58 mg. Eleven (1.0%, 11/1082) subjects who received at least 1 injection of AA4500 0.58 mg experienced a treatment-related SAE or SAE with unknown relationship to study drug.

The percentage of subjects prematurely discontinuing due to an AE was also low (0.8%, 9/1082). Three subjects had the adverse reaction of tendon rupture (3/1082) and one subject had an adverse reaction of ligament injury (flexion pulley rupture) in the little PIP joints. All of these adverse reactions occurred in little finger PIP joints and were classified as serious adverse events. The cause of the adverse reaction is thought to be the inadvertent exposure or injection of AA4500 into or around the tendon/ligament structure rather than the Dupuytren's cord. After two of these initial serious adverse reactions of tendon rupture following 734 injections had been reported, all investigators were informed of these events, the study protocols were modified and all investigators were trained with the revised injection procedure. The training introduced a refined injection technique for little finger PIP joints. Subsequent to these steps being taken, there was only one additional tendon rupture over a total of 1896 additional injections of AA4500.

There were no reports of nerve injury or division, artery damage, circulatory disturbance, wound infection, scar contracture or stiffness following treatment with AA4500. The incidence rate for neuropraxia (including paresthesia, dysesthesia) was uncommon, (less than 3%) and reversible. In addition, only one case of CRPS was reported which occurred in a female patient with a history of CRPS following surgical release of a carpal tunnel syndrome. In Study AUX-CC-856 one patient underwent amputation of the right little finger and MP joint. The patient narrative was provided and mentions injury of the right little finger which had advanced Dupuytren's disease while throwing out trash with subsequent amputation of the right little finger and MP joint. The event is not considered related to the study drug.

A literature research of surgical complications was undertaken, however a direct comparison of the incidence of SAE of injection versus surgical intervention was not feasible since literature does not specifically discuss adverse events in the context whether or not they were serious.
Overall, the numbers of serious adverse events reported as related to AA4500 from the clinical programme are low and the risks associated have been well characterised and are largely based on anticipated pharmacology of collagenase. The majority of AA4500 treatment related SAEs occurred in the treated extremity. Four of these were related to the effect of inadvertent injection or extravasation of AA4500 into or around the tendon/ligament structure rather than the Dupuytren's cord.
The number of tendon ruptures was comparable to surgery but higher than that reported for PNF. Other SAEs related to events of the hand included tendonitis, finger deformity, CRPS, sensory disturbance and Dupuytren's contracture. Events associated with fasciectomy that would be judged to be medically serious by inspection of the surgical literature also indicate surgical damage to nerves, tendons, and arteries as well as CRPS, pain, issues of wound healing, and infection which generally occurred at increased frequencies relative to treatment with AA4500.

Overall, as far as one can judge given the lack of direct comparison of the incidence of SAE and AE between collagenase injection and surgical procedures or PNF, the safety profile of collagenase seems better than surgery and generally similar to PNF.

Deaths
Seven subjects died during the course of the clinical programme. All serious adverse events leading to these deaths are not unexpected in this elderly population and none of these deaths were considered to be related to study drug. Each of the subjects was treated with AA4500 with the deaths occurring from 2 months to more than 1 year after the last dose of study drug.

Special Safety Topics

- Local Reactions

Local reactions at or around the site of injection is a nearly universal finding in subjects treated with AA4500, as captured in the PTs: Oedema peripheral, Contusion, Injection site pain, Injection site haemorrhage, Injection site swelling, Pain in extremity, Tenderness, Ecchymosis, Blood blister, Blister, Inflammation, Erythema, and Swelling. The majority of adverse reactions in subjects treated with AA4500 (0.58 mg) either began on the day of injection or on the day of the finger extension procedure (one day post-injection). Almost all treatment-related AEs resolved without intervention before the next scheduled injection of AA4500 and typically within 2 weeks.

- Skin Lesions

In some cases of Dupuytren's contracture, especially in patients with more advanced disease, the cord may become adherent to the skin overlying the cord and often becomes thin and easily damaged. Patients with Dupuytren's contractures that adhere to the skin are at higher risk for AEs of skin lesions as a result of the pharmacological effect of AA4500 and the finger extension procedure on the skin overlying the targeted cord. Event terms that describe adverse reactions linked to the breakdown of the skin overlying pathologic cords observed in clinical studies of AA4500 occurred at the following frequencies: skin laceration (120/1082; 11.1%), wound (6/1082; 0.6%), skin exfoliation (2/1082; 0.2%), skin lesion (2/1082; 0.2%), skin necrosis (1/1082; 0.1%), and injection site desquamation (3/1082; 0.3%). All but 4 events of skin laceration were mild or moderate in severity and all healed without complication.

- Injection Site Bleeding in Patients with Coagulation Disorders

In the clinical development programme for AA4500, subjects receiving anticoagulant medication (except for daily low-dose aspirin, i.e., doses up to 150 mg daily) within 7 days of enrolment were excluded. Adverse reactions involving injection site haemorrhage were frequent, occurring in 369/1082 (34.1%) of the subjects in the clinical programme, though less than 1% of these subject experienced injection site haemorrhage that was considered severe (10/1082, 0.9%). Importantly, the events with PT injection site haemorrhage are representative of ecchymosis and subcutaneous haemorrhage and do not indicate active bleeding. None of the subjects participating in the clinical programme required any invasive procedures to control bleeding.

Laboratory findings

No clinically meaningful changes were observed between baseline and final assessment for haematology, chemistry, and vital sign parameters. Mean changes in hand grip strength from baseline to the final assessment for the primary and secondary hand were not considered clinically meaningful in any analysis population.

Safety results in the *All Subjects With 12 Months Post First Dose AA4500 0.58 mg* population were similar to those in the *All Subjects With At Least 1 Dose of AA4500 0.58 mg* population with respect to AEs, clinical laboratory evaluations, vital sign results, and hand grip strength assessment.

Safety in special populations

No clinical studies were conducted in special populations. As systemic exposure to AA4500 after intralesional injection into Dupuytren's cords is below quantifiable level, no clinical studies are deemed necessary to evaluate the effects of age, gender and race on AA4500 or in subjects with impaired hepatic or renal function.

Immunological events

As expected when foreign proteins are injected into tissues and an antibody response is elicited, immune-mediated reactions have been reported with the use of AA4500. The adverse reaction profile of AA4500 includes PTs that are possibly consistent with immune-mediated reactions: Lymphadenopathy, Lymph node pain, Axillary pain, Erythema, Oedema peripheral, Pruritus, and Injection site pruritus. As noted above, the etiology of the local reactions to injection is likely partially immune-mediated, and adverse reactions coded to these PTs are possibly consistent with an immune response to the injection of AA4500. These adverse reactions were nearly always in the treated extremity, and there were no findings of diffuse adenopathy or anaphylaxis in the clinical programme that could be consistent with severe systemic hypersensitivity.
Most subjects (≥ 85.8%) had positive antibodies to AUX-I and/or AUX-II 30 days after the first injection of AA4500, with all subjects developing antibodies to both AUX-I and AUX-II by the fourth injection. Mean log titre levels were almost 2-fold higher after the second injection compared with the first. Levels continued to increase after the first three injections, appearing to plateau after the fourth injection. Among those subjects who received one injection of AA4500 0.58 mg, there was diminution of titre levels 1-year after injection. Antibody titres also decreased at 1-year after the last injection in those subjects that received more than 1 injection although the rate of decrease seemed slower than in subjects that received only 1 injection. Kinetics of titer levels of anti-AUX-I and anti-AUX-II revealed

in summary that all Injection Cohorts increase to a maximum level that depends on the number of injections received, with 4 or more injections giving approximately the same Maximum Sample mean titer levels of 5 to 5.5 (titer level of 100,000 to 300,000). Furthermore, all Injection Cohorts are showing a decrease in titer levels by the time of the Year 2 Sample but only subjects who received a total of 1 or 2 injections have started to become serum negative at Year 2.

More information on the impact of these antibodies on re-treatment after treatment pause and the impacts this might have on treatment recommendations are expected from the three Phase 3b/4 studies.

There were few immune mediated adverse events such as pruritus, lymphadenopathy, hypersensitivity and urticaria. The incidence of pruritus increased with subsequent injections but generally resolved prior to the next injection (median 4.0 days). The incidence of some of these events also seemed to increase in subjects with 90 days or more between injections. There was no evidence of severe systemic hypersensitivity or anaphylaxis in the safety population including the evaluation of patients who had experienced a drug holiday of greater than 90 or 180 days in between two doses of AA4500.

There was also no correlation between antibody titre and severity or duration of adverse events. An MMP inhibitor-associated musculoskeletal syndrome (MSS) has been described in literature after oral treatment with matrix metalloproteinase inhibitors in knee osteoarthritis which includes shoulder arthralgia, myalgia, and stiffness, as well as hand oedema, palmar fibrosis, tendons/thickening nodules (reminiscent of the early development of Dupuytren's contracture) (Krzeski et al., 2007)

In the AA4500 clinical programme, review of all treatment-emergent adverse events revealed no signals identified for a combination of AEs suggestive of the musculoskeletal syndrome. Oedema, joint stiffness and musculoskeletal stiffness were found in the treated extremity following the first dose (in the absence of anti-drug antibodies) as well as following subsequent doses. However, the patient numbers included up till now are too low to really exclude this adverse event. As discussed above in vitro data from 5 patients indicated at least a potential for the cross-reactivity of anti-product antibodies with endogenous proteins and should be further investigated to identify an unexpected serious risk of inhibiting enzymatic activity of the endogenous matrix metalloproteinases with Xiapex treatment.

In addition, exacerbation of autoimmune diseases secondary to cross-reactivity of AUX-1 and AUX-2 is a potential adverse reaction. A post-hoc analysis was performed on 33 subjects that had a pre-existing autoimmune condition (Type 1 Diabetes Mellitus (13), Rheumatoid Arthritis (7), Dry Eye (5), Hepatitis (2), scleroderma (2), Connective Tissue Disorder (2), and Inclusion Body Myositis (1)). No acute exacerbations of the underlying condition were reported except for one moderate case of exacerbation of rheumatoid arthritis in a 76 year old male which was considered not related to AA4500 by the investigator. The rate of all adverse reactions was similar in this group compared to all subjects without pre-existing autoimmune disease in the clinical programme for AA4500.

Safety related to drug-drug interactions and other interactions

Drug interaction studies have not been conducted with AA4500 0.58 mg, as human pharmacokinetic studies show that AA4500 0.58 mg is not significantly absorbed into the systemic circulation following injection into a Dupuytren's cord. Whilst there is no clinical evidence of an interaction between tetracycline and anthraquinone derivatives and Xiapex, such derivatives have been shown to inhibit matrix metalloproteinase-mediated collagen degradation at pharmacologically relevant concentrations *in vitro*. Therefore, use of Xiapex in patients who have received tetracycline antibiotics (e.g. doxycycline) within 14 days prior to receiving an injection of Xiapex is not recommended.

Discontinuation due to AES

Across the 13 studies, nine subjects had a nonfatal AE recorded as a reason for premature discontinuation (0.8%, 9/1082). Only three events (injection site pain, dizziness, complex regional pain syndrome) were considered by the investigator to be possibly related to study drug. No specific TEAEs led to premature discontinuation in more than one subject. There was no pattern in the types of AEs leading to premature discontinuation.

Post marketing experience

No post marketing data is available.

Discussion on clinical safety

Overall there were 1196 subjects who were enrolled and treated in the AA4500 clinical programme with 1082 subjects receiving at least 1 dose of AA4500 0.58 mg comprising the formal safety database. Based on the review of the nonclinical and clinical data of the AA4500 development programme, the adverse events of interest include: local reactions, potential immune-mediated events, tendon/ligament rupture or injury, skin lesions, and injection site bleeding.
Proposed activities to mitigate the risk associated with those adverse reactions are described in the Risk Management Plan.
The influence of investigator training on adverse events, especially serious adverse events like tendon ruptures is crucial for the safe application of the drug.
Investigator training in the clinical studies included intensive injection technique instructions via manuals and DVDs, workshops and investigator meetings and it has to be ensured that the training for the education of healthcare professionals in clinical practice is adequate.

The safety results studies with AA4500 0.58 mg indicate that the majority of TEAEs were non serious, mild or moderate in intensity, confined to the treated extremity, and resolved within a short period without sequelae.
In the Phase 3 Double-Blind, Placebo-Controlled population, approximately two times as many AA4500 treated patients than placebo-treated patients had an AE (97.8% versus 54%). Among subjects who received at least one dose of AA4500 0.58 mg, most patients experienced adverse reactions in the treated extremity, with the most frequently reported adverse reactions reported being: oedema peripheral, contusion, injection site pain, pain in extremity, injection site haemorrhage, and tenderness. A greater proportion of AA4500 treated patients compared to the placebo group experienced pain at the injection site (39% vs. 9.5%) or pain in the extremity (33.1% vs. 3.6%).
Eleven (1.0%, 11/1082) subjects who received at least 1 injection of AA4500 0.58 mg experienced a treatment-related SAE or SAE with unknown relationship to study drug.
The majority of treatment-related SAEs were related to events of the treated hand. Four of these were related to unintended effects of AA4500 on collagen (three tendon ruptures and one ligament injury [pulley injury]). The cause of these adverse reactions is thought to be the inadvertent exposure or injection of AA4500 into or around the tendon/ligament structure rather than into the Dupuytren's cord. A physician training plan is part of the risk minimization plan and includes tendon rupture and severe systemic hypersensitivity as specific scopes which is of utmost importance.

Due to the highly immunogenic potential of AA4500 the majority of subjects developed ADAs after the first injection and all subjects had antibodies after four or more injections. However, no adverse reactions consistent with systemic hypersensitivity or anaphylactic response were observed in the Dupuytren's disease programme. Except for pruritus and oedema peripheral, no clear increase in the number of adverse events with subsequent injections were observed. ADA titres were not predictive of the rate, severity, or duration of any of the adverse events. Potential cross-reactivity of neutralising antibodies (Nabs) against AUX-I or AUX-II to endogenous MMPs represents a serious risk for adverse effects, e.g. development of musculoskeletal syndrome which is characterized by one or more of the following signs and symptoms: arthralgia, myalgia, joint stiffness, stiffness of the shoulders, hand oedema, palmar fibrosis and thickening or nodules forming in the tendons. Adverse events did not reveal any clinical findings indicative of the musculoskeletal syndrome. This suggests that ADAs to both AUX-I and AUX-II, while present in all subjects after four or more injections, did not affect the safety profile of AA4500.
However, although in the clinical programme there was no signal identified for a combination of AE suggestive of the musculoskeletal syndrome, a potential for cross-reactivity of anti-product antibodies (anti-AUX-I and anti-AUX-II) with endogenous human matrix metalloproteinases with similar homology could not really be excluded. *In vitro* assessment of cross-reactivity has been performed in 5 patients and additional cross-reactivity experiments using a validated assay were provided. However, assay results are not considered to be reliable. Currently, the detection of cross reactivity of anti-AUX-I or anti-AUX-II antibodies versus the selected MMPs is restricted to binding assays. This is not considered sufficient. The cross-reactivity should be additionally investigated in terms of inactivation of endogenous MMPs by neutralising ADAs. The applicant committed to develop and validate a respective assay that is based on enzymatic activity of the MMPs. For the time being this is addressed in the educational program, the labelling and the RMP.

Long-term safety data after repeated administration are lacking and potential long-term effects of MMP cross-allergy need to be monitored.
Regarding the neutralising potential of ADAs versus AUX-I and AUX-II, data on the determination of the optimised concentrations of enzymes and substrate were not provided. It is acknowledged that varying amounts of alpha-2-macroglobulin in the sera to be tested may disturb the assay. This however does not release the Applicant from developing a suitable assay to assess potential neutralising activity of NAbs not only in terms of binding but also in terms of enzymatic and thus pharmacodynamic activity of the drug substance. The assay setup should not only be based on literature but on experimental data. The applicant committed to initiate feasibility studies to evaluate the potential impact of Nabs and /or anti-AUX-I or –II on enzymatic activity of endogenous MMPs.

Conclusions on the clinical safety

In conclusion, the safety results studies with AA4500 0.58 mg indicate that the majority of TEAEs were non serious, mild or moderate in intensity, confined to the treated extremity, and resolved within a short period without sequelae.

- Among subjects who received at least one dose of AA4500 0.58 mg, most patients experienced adverse reactions in the treated extremity, with the most frequently reported adverse reactions reported being: oedema peripheral, contusion, injection site pain, pain in extremity, injection site haemorrhage, and tenderness.
- The majority of treatment-related SAEs were related to events of the treated hand. Four of these were related to unintended effects of AA4500 on collagen (three tendon ruptures and one ligament injury [pulley injury]). The cause of these adverse reactions is thought to be the inadvertent exposure or injection of AA4500 into or around the tendon/ligament structure rather than into the Dupuytren's cord.

- Most subjects had positive antibodies to AUX-I and/or AUX-II by 30 days after the first injection of AA4500, with all subjects developing positive antibodies to both AUX-I and AUX-II by the fourth injection of AA4500.
- The adverse reaction profile of AA4500 includes events that are consistent with both immune-mediated reactions to collagenase clostridium histolyticum and inflammatory response to collagen breakdown peptides as a result of the collagenase activity of AA4500 on the Dupuytren's cord. These reactions were limited to the treated extremity
- There were no observed cases of severe systemic hypersensitivity or anaphylaxis in the clinical development programme for AA4500, including the evaluation of patients who had experience a drug holiday of greater than 90 or 180 days in between two doses of AA4500.
- No clinically meaningful effects on hand grip strength or laboratory or vital sign parameters were observed.
- The universal formation of anti-drug antibodies does not seem to affect the safety and efficacy profile of AA4500.
- Although in the clinical programme there was no signal identified for a combination of AE suggestive of the musculoskeletal syndrome, a potential for cross-reactivity of anti-product antibodies (anti-AUX-I and anti-AUX-II) with endogenous human matrix metalloproteinases with similar homology could not really be excluded by the existing data and should therefore be further investigated.
- More information on the development of antibody titres after treatment and the impacts this might have on re-treatment is expected from ongoing studies. The question of re-treatment using Xiapex following previous treatment will be addressed in Study AUX-CC-862-Re-treatment study that will utilize patients with recurrent joints requiring further treatment from the existing long term follow-up study AUX-CC-860.
- As far as one can judge given the lack of direct comparison of the incidence of SAE and AE between collagenase injection and surgical procedures or PNF, the safety profile of collagenase seems better than surgery and generally similar to PNF.

The SmPC adequately describes the current safety profile and the elements to mitigate the risks associated with the product.

2.7 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The MAA submitted a risk management plan, which included a risk minimisation plan.

Table Summary of the risk management plan:

Safety Concern	Pharmacovigilance	Proposed Activities Risk Minimization
Local Reactions	**Routine pharmacovigilance** **Additional pharmacovigilance activities** Risk Management Committee	**SmPC (Section 4.8):** "The most frequently reported adverse reactions during the Xiapex clinical studies were local injection site reactions such as oedema peripheral (local to the injection site), contusion (including ecchymosis), injection site haemorrhage and injection site pain. Injection site reactions were very common, occurring in the vast majority of patients, were mostly mild to moderate in severity and generally subsided within 1-2 weeks post injection. Serious adverse reactions of tendon rupture (3 cases), tendonitis (1 case), other ligament injury (1 case) and complex regional pain syndrome (1 case) related to the medicinal product were reported." **SmPC (Section 4.9):** "Administration of Xiapex at greater than recommended dosages is expected to be associated with increased local reaction at the site of injection. Routine supportive care and symptomatic treatment must be provided in the case of overdose." **PIL (Section 4):** "Like all medicines, Xiapex can cause side effects, although not everybody gets them. Most of the side effects that occurred in the clinical studies were mild or moderate in severity and were localized to the hand treated. However if any of the side effects becomes more severe or if you notice any side effects not listed in this leaflet, please tell your doctor." Physician training/educational materials will educate physicians regarding the most frequently reported local reactions and instructions will also be provided with respect to the proper documentation and reporting of adverse events occurring associated with the use of AA4500.
Tendon/Ligament Rupture or Injury	**Routine pharmacovigilance** Active surveillance: A physician safety questionnaire will be distributed and collected to determine additional information associated with post-marketing cases of tendon/ligament injury in order to enhance signal detection. **Additional pharmacovigilance activities** Risk Management Committee	**SmPC (Section 4.2):** "Xiapex must be administered by a physician appropriately trained in the correct administration of the product and experienced in the diagnosis and management of Dupuytren's disease." **SmPC (Section 4.4):** *"Tendon rupture or other serious injury to the injected extremity* Xiapex must only be injected into the Dupuytren's cord. Because Xiapex lyses collagen, care must be taken to avoid injecting into tendons, nerves, blood vessels, or other collagen-containing structures of the hand. Injection of Xiapex into collagen containing structures may result in damage to those structures, and possible permanent injury such as tendon rupture or ligament damage. When injecting a cord affecting a PIP joint of the fifth finger, the needle insertion must not be more than 2 to 3 mm in depth and not more than 4 mm distal to the palmar digital crease. Patients should be instructed to promptly contact the physician if there is trouble bending the finger after the swelling goes down (symptoms of tendon rupture).

		Patients with Dupuytren's contractures that adhere to the skin may be at higher risk of skin lesions as a result of the pharmacological effect of Xiapex and the finger extension procedure on the skin overlying the targeted cord." **SmPC (Section 4.8):** "The most frequently reported adverse reactions during the Xiapex clinical studies were local injection site reactions such as oedema peripheral (local to the injection site), contusion (including ecchymosis), injection site haemorrhage and injection site pain. Injection site reactions were very common, occurring in the vast majority of patients, were mostly mild to moderate in severity and generally subsided within 1-2 weeks post injection. Serious adverse reactions of tendon rupture (3 cases), tendonitis (1 case), other ligament injury (1 case) and complex regional pain syndrome (1 case) related to the medicinal product were reported." **PIL Section 2: Take special care with Xiapex** "This medicine must only be injected into the collagen cord in your hand by your doctor. Your doctor will take care to avoid injecting into tendons, nerves or blood vessels. Incorrect injection into tendons, nerves or blood vessels may result in bleeding or damage and possible permanent injury to these structures. If your cord to be treated is attached to the skin, you are at higher risk of the skin splitting during the finger extension procedure following the injection of Xiapex. Physician training/educational materials will be presented to physicians eligible to use AA4500. Physicians training will be available regarding the proper injection and finger extension technique, expected tissue reaction, recognition and treatment of localized reactions. Additionally, training will be provided with respect to the proper documentation and reporting of adverse events occurring associated with the use of AA4500. Fluoroquinolone antibiotics (ciprofloxacin, norfloxacin, ofloxacin, gatifloxacin, gemifloxacin, levofloxacin and moxifloxacin) have been associated with the potential risk of tendon injury, including tendonitis and tendon rupture, therefore physicians will be informed that patients receiving these medicinal products may be at greater risk from tendon related injury.
Skin Lesions	**<u>Routine pharmacovigilance</u>** Active Surveillance: A physician safety questionnaire will be distributed and collected to determine additional information associated with post-marketing cases of localized skin lesions in order to enhance signal detection.	**SmPC: Sec 4.4.** "Patients with Dupuytren's contractures that adhere to the skin may be at higher risk of skin lesions as a result of the pharmacological effect of Xiapex and the finger extension procedure on the skin overlying the targeted cord." **PIL:** Section 6.6 of the SmPC is included as a tear off page (description of the reconstitution, injection procedure, and finger extension procedures). **Physician Training/Educational Materials**: Instruction to physicians on the appropriate location for administration of AA4500 in cases where skin is adherent to the Dupuytren's cord, and to recognize that skin lesions are an expected adverse reaction in such cases. In addition, training will also be provided with respect to the proper documentation and reporting of adverse events occurring

		associated with the use of AA4500.
	Additional pharmacolovigilance activities Risk Management Committee	
Immune-Mediated Reactions	**Routine pharmacovigilance** Active surveillance: A physician safety questionnaire will be distributed and collected to determine additional information associated with post-marketing cases of immune-mediated reactions (severe systemic hypersensitivity/anaphylaxis, new onset/exacerbation of autoimmune disease(s) or musculoskeletal syndrome) in order to enhance signal detection.	**SmPC (Section 4.4): Allergic reactions** "In the double blind portion of the three phase 3 placebo-controlled clinical studies, 17 % of Xiapex-treated patients had mild allergic reactions (i.e. pruritus). Although there was no severe allergic reaction observed in the Xiapex studies (e.g. those associated with respiratory impairment, hypotension, or end-organ dysfunction) physicians must be prepared to address any severe local or systemic allergic reactions including the potential for anaphylaxis that may occur following injection Whilst there is no evidence from the clinical data of an increased risk of serious allergic reactions upon re-challenge, the potential for such reactions following repeated use cannot be excluded." **SmPC (Section 4.4): Immunogenicity** "As with any non-human protein product, patients may develop antibodies to the therapeutic protein. During clinical studies, blood samples from patients with Dupuytren's contracture were tested at multiple time points for antibodies to the protein components of the medicinal product (AUX-I and AUX-II). At 30 days post the first injection, 92 % of patients had circulating antibodies detected against AUX-I and 86 % of patients against AUX-II. After a third or fourth injection, all subjects developed positive antibodies to both AUX-I and AUX-II. No apparent correlation of antibody development to clinical response or adverse reactions was observed. Since the enzymes in Xiapex have some sequence homology with human matrix metalloproteinases (MMPs), anti-drug antibodies (ADA) could theoretically interfere with human MMPs. No safety concerns related to the inhibition of endogenous MMPs have been observed, in particular no adverse events indicating the development or exacerbation of autoimmune diseases or the development of a musculoskeletal syndrome (MSS). Whilst there is no clinical evidence from the current safety data of a musculoskeletal syndrome developing following the administration of Xiapex, the potential for it to occur cannot be excluded. If this syndrome were to develop, it would occur progressively and is characterized by one or more of the following signs and symptoms: arthralgia, myalgia, joint stiffness, stiffness of the shoulders, hand oedema, palmar fibrosis and thickening or nodules forming in the tendons." **PIL Section 2:** "Please consult a doctor immediately if you experience any signs or symptoms of a serious allergic reaction, e.g., wide spread redness or rash, swelling, tightness in the throat or difficulty breathing. You must not be given Xiapex if you know that you have had a serious allergic reaction to collagenase or any of the other ingredients."
	Additional pharmacovigilance activities Risk Management Committee Study AUX-CC-860 (Long-term follow-up of subjects treated with AA4500)	

		Physician Training/Educational Materials: Available training will educate physicians regarding the potential immune-mediated reactions and guidance on management of the condition if it occurs. In addition, training will be provided with respect to the proper documentation and reporting of adverse events occurring associated with the use of AA4500.
Severe Systemic Hypersensitivity /Anaphylaxis	**<u>Routine pharmacovigilance</u>** Active surveillance: A physician safety questionnaire will be distributed and collected to determine additional information associated with post-marketing cases of hypersensitivity/anaphylaxis in order to enhance signal detection. **<u>Additional pharmacovigilance activities</u>** Risk Management Committee	**SmPC (Section 4.4): Allergic reactions** "In the double blind portion of the three phase 3 placebo-controlled clinical studies, 17 % of Xiapex-treated patients had mild allergic reactions (i.e. pruritus). Although there was no severe allergic reaction observed in the Xiapex studies (e.g. those associated with respiratory impairment, hypotension, or end-organ dysfunction) physicians must be prepared to address any severe local or systemic allergic reactions including the potential for anaphylaxis that may occur following injection. Whilst there is no evidence from the clinical data of an increased risk of serious allergic reactions upon re-challenge, the potential for such reactions following repeated use cannot be excluded." **PIL Section 2:** "Please consult a doctor immediately if you experience any signs or symptoms of a serious allergic reaction, e.g., wide spread redness or rash, swelling, tightness in the throat or difficulty breathing. You must not be given Xiapex if you know that you have had a serious allergic reaction to collagenase or any of the other ingredients." **Physician Training/Educational Materials:** Available training will educate physicians regarding the potential risk of severe systemic hypersensitivity/anaphylaxis and guidance on management of the condition if it occurs. In addition, training will also be provided with respect to the proper documentation and reporting of adverse events occurring associated with the use of AA4500.

Injection Site Bleeding in Patients With Coagulation Disorders Including Those on Concurrent Anti-Coagulation Therapy	**<u>Routine pharmacovigilance</u>** **<u>Additional pharmacovigilance activities</u>** Risk Management Committee	**SmPC (Section 4.4):** "Xiapex must be used with caution in patients with coagulation disorders. In the three double-blind, placebo-controlled phase 3 studies, 73 % of Xiaflex treated patients reported an ecchymosis or a contusion and 38 % reported a haemorrhage at the injection site. The efficacy and safety of Xiapex in patients receiving anticoagulant medicinal products other than up to 150 mg acetylsalicylic acid per day prior to Xiapex administration is not known. Use of Xiaflex in patients who have received anticoagulants (with the exception of up to 150 mg acetylsalicylic acid daily) within 7 days prior to receiving an injection of Xiapex is not recommended." **PIL (Section 2):** **"Before you are given this medication, make sure your doctor knows:** if you have a history of problems with the normal clotting of your blood or if you are taking any medications to help control the normal clotting of your blood (known as anticoagulation medications). if you are currently taking any anticoagulation medicines, you must not receive Xiapex within 7 days of the last dose of your anticoagulation medicine. One exception is the use of up to 150 mg daily dose of acetylsalicylic acid (a substance present in many medicines used to prevent blood clotting) which can be taken." **PhysicianTraining/Educatiional Materials:** Available training will educate physicians regarding the caution to be utilized when administering AA4500 in patients with coagulation disorders. In addition, training will be provided with respect to the proper documentation and reporting of adverse events occurring associated with the use of AA4500.

Reactions related to Cross-Reactivity With Endogenous MMPs (including musculoskeletal syndrome and development/exacerbation of autoimmune disorders)	**Routine pharmacovigilance** Active Surveillance: A physician safety questionnaire will be distributed and collected to determine additional information associated with post-marketing cases of new onset/exacerbation of autoimmune disorder(s) or musculoskeletal syndrome in order to enhance signal detection. **Additional pharmacovigilance activities** Risk Management Committee Study AUX-CC-860 (Long-term follow-up of subjects treated with AA4500)	**SmPC (Section 4.4): Immunogenicity** "As with any non-human protein product, patients may develop antibodies to the therapeutic protein. During clinical studies, blood samples from patients with Dupuytren's contracture were tested at multiple time points for antibodies to the protein components of the medicinal product (AUX-I and AUX-II). At 30 days post the first injection, 92 % of patients had circulating antibodies detected against AUX-I and 86 % of patients against AUX-II. After a third or fourth injection, all subjects developed positive antibodies to both AUX-I and AUX-II. No apparent correlation of antibody development to clinical response or adverse reactions was observed. . Since the enzymes in Xiapex have some sequence homology with human matrix metalloproteinases (MMPs), anti-drug antibodies (ADA) could theoretically interfere with human MMPs. No safety concerns related to the inhibition of endogenous MMPs have been observed, in particular no adverse events indicating the development or exacerbation of autoimmune diseases or the development of a musculoskeletal syndrome (MSS). Whilst there is no clinical evidence from the current safety data of a musculoskeletal syndrome developing following the administration of Xiapex, the potential for it to occur cannot be excluded. If this syndrome were to develop, it would occur progressively and is characterized by one or more of the following signs and symptoms: arthralgia, myalgia, joint stiffness, stiffness of the shoulders, hand oedema, palmar fibrosis and thickening or nodules forming in the tendons." An in vitro study of human sera from patients with multiple Xiapex injections to assess the frequency of inhibition of human proteins by neutralizing anti-product antibodies will be provided by Q1-2011. Five assays for measuring inhibition of MMP enzyme activity (MMP-1, -2, -3, -8, and -13) have already been developed and are currently being validated. Validated assay method reports will be provided by Q2-2011. Feasibility studies which may include a sample pretreatment step to remove serum inhibitors to evaluate the potential impact of NAbs and/or anti-AUX-I or –II on enzymatic activity of endogenous MMPs will be initiated in 2011. In addition, the possibility to improve the NAb assay addressing the potential impact on enzymatic activity of the drug substance will also be assessed. A respective report will be provided by Q2-2011. **PhysicianTraining/Educational Materials**: Instruction to physicians on the potential risk of reactions related to cross-reactivity to endogenous MMPs, including musculoskeletal syndrome and the development/exacerbation of autoimmune disorders. In addition, training will also be provided with respect to the proper documentation and reporting of adverse events occurring associated with the use of AA4500.
Potential Medication Errors	**Routine Pharmacovigilance** **Additional pharmacovigilance**	**SmPC (Section 4.2):** "Xiapex must be administered by a physician appropriately trained in the correct administration of the product and experienced in the diagnosis and management of Dupuytren's disease." **PIL:** Section 6.6 of the SmPC is included as a tear off

activities: Risk Management Committee	page (description of the reconstitution, injection procedure, and finger extension procedures). <u>Physician</u> training/educational materials will be provided to physicians eligible to use AA4500. Physician training will be available regarding the proper injection and finger extension technique to minimize potential medication errors. Additionally, training will be provided with respect to the proper documentation and reporting of adverse events occurring associated with the use of AA4500.

The CHMP, having considered the data submitted in the application is of the opinion that the following risk minimisation activities are necessary for the safe and effective use of the medicinal product:

The MAH, in agreement with the competent authorities in the Member States, shall implement, prior to the launch, an educational programme for physicians aiming to ensure proper injection placement to minimize occurrence of injection-related adverse events and to inform on expected and potential risks associated with the treatment.

The physician educational programme should contain the following key elements:

- Injection technique and dosing interval.
- Proper amount of volumes for both reconstitution and injection differences in the metocarpophalangeal (MP) and proximal interphalangeal (PIP) joints.
- Recognition and treatment of severe immune-mediated reaction, including anaphylaxis.
- Information on bleeding risk in patients with coagulation disorders including those on concurrent anti-coagulation therapy.
- Information on the potential risk of matrix metalloproteinases (MMP) cross reactivity including the development of musculoskeletal syndrome and exacerbation/initiation of autoimmune disorders.
- Reminder of the need to report adverse events, including medication errors.
- The need to inform the patient about the signs and symptoms associated with the treatment and when to seek attention from the health care provider.
- The summary of product characteristics and the patient information leaflet.

User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.8 Benefit-Risk Balance

Benefits

Beneficial effects

In both pivotal Phase 3 studies (AUX-CC-857 and AUX-CC859), AA4500 met the primary endpoint demonstrating consistently efficacy in reducing contractures in both MP and PIP joints, in patients with moderate (about 50% of patients had moderate disease at inclusion, i.e. a modified Tubiana score of 2) and severe disease (20% of included patients, i.e. a modified Tubiana score of 3 and 4).

AA4500 0.58 mg was statistically superior to placebo with respect to the proportion of subjects who achieved a reduction in contracture to 5° or less of their primary joint after the last injection ($p<0.001$). Overall, 64.0% of all AA4500 treated primary joints achieved a reduction in contracture of the primary joint to 5° or less in Study AUX-CC-857 (76.7% for MP joints and 40% for PIP joints), and 44.4% in Study AUX-CC-859 (65% for MP joints and 28% for PIP joints) compared to 6.8% and 4.8% respectively for placebo. The modified Tubiana score used in the studies led likely to an underestimate of the true disease severity since DIP joints were not recorded. Therefore, a wide indication to all types of severity (low and high severity) in Dupuytren's disease is considered acceptable.
In study AUX-CC 859, although more primary PIP joints treated with up to three injections had a reduction in contracture to 5° or less compared with placebo (7 joints [28.0%] versus 0 joints), the difference between the groups was not statistically significant ($p=0.069$). For PIP joints, efficacy was less clearly demonstrated than for MP joints, probably due to the small sample size and to the fact that most PIP joints were in the little finger that is usually the most difficult to treat due to anatomical reason.
Severely contracted MP and PIP joints responded to treatment with AA4500 to a lesser extent.
However, the Applicant presented an analysis to show that even the most severely affected PIP joints showed meaningful clinical improvement that is similar to what is observed for surgical corrections outlined in published literature.
All these findings were also reported in literature for surgical interventions including PNF. Therefore, Xiapex efficacy considers similar to that of surgery and PNF

The European patient experience is limited to the 137 subjects who were enrolled in the open label study AUX-CC-854 but the patient demographics, disease characteristics and the efficacy and safety results are very similar to those from the overall population. Therefore, the data from the double-blind placebo-controlled studies carried out in the United States and Australia are fully applicable to European subjects as there are no genetic or physiological reasons why subjects in Europe would not respond in a similar manner than those in the United States and Australia.

Overall, the efficacy profile as described above was comparable to the current mainstay of treatment, i.e. surgical intervention (fasciotomy, percutaneous needle aponeurotomy (PNF)). The goal of surgery is to remove and/or release the fibrotic cord and correct the contracture allowing extension of the affected finger(s). However, surgical procedures can be complex, and may result in significant perioperative and/or postoperative complications which can delay full recovery.

The recurrence rate of around 4% provides evidence of the durability of treatment effect within the 9 to 12-month period after first injection. Meanwhile 2 year data (730 days) are available and as expected the recurrence rate has increased. The nominal rate of recurrence is 15.9 % in the presence of a palpable cord and 19.9 % with or without a palpable cord. In summary, the recurrence rate has increased following 2 years of follow up compared to 1 year, but overall the recurrence rates with or without a palpable cord in A4500 treated joints continue to be comparable to the rates observed in the literature for surgical intervention and appear favourable compared to those reported for PNF.
However, more long-term follow-up data are needed and will be provided on the ongoing extension trial AUX-CC-860 through interim clinical study reports at Years 3, 4, and 5 post-treatment.

Uncertainty in the knowledge about the beneficial effects

The Applicant comprehensively discusses the difference in clinical success rates between regions (US and Australia) for the AUX-CC-857 and AUX-CC-859 studies and provides valid reasons for the observed differences. However, there is a considerable heterogeneity found for the clinical success rate in the AUX-CC-857 study conducted in 16 US centers. Data of tests for heterogeneity of the

success rates in the different centers for the AUX-CC-857 study were provided. Due to the small sample size the power for possible tests of heterogeneity is limited. The approach to only include data of the patients receiving AA4500 is justified. It is agreed that the results of a χ^2-test for comparison of success rates are not incompatible with homogeneity and that observed heterogeneity could be due to variability in a binomial endpoint given the small sample size.
Further exploration of a possible center effect and its cause without the use of statistical methods was expected and the links to baseline data for centers with the highest and lowest success rates were provided. Different baseline fixed flexion contracture may have had an influence on the success rates.
It is acknowledged that the success rates in each center for the AA4500 treatment group were higher than in the placebo group. Although no specific concerns on the heterogeneity of the results of study AUX-CC-857 remain, a carefully planned and conducted training of prescribing physicians for treatment with AA4500 seems warranted to ensure high success rates for varying baseline conditions.
The Applicant has provided a number of analyses that indicate a persistence of efficacy even in the presence of high and increasing anti-drug antibody titres which supports the lack of a neutralising antibody effect. There is no correlation between efficacy outcomes and antibody titres. Extrinsic factors such as the type of joint treated may explain the difference in response between the first joint treated and the subsequent ones.

Long term data, particularly data on the impact of repeated treatment on the rate of recurrence, complications, and hand functionality/QoL are not available. In clinical practice, Xiapex may complement surgical treatment. Studies investigating the recurrence rate and rate of complications if Xiapex and surgical interventions are utilised sequentially, intermittently or at the same time are lacking. The Applicant commits to undertake post-marketing activities to collect and analyse such data.

Risks

Unfavourable effects

Overall there were 1196 subjects who were enrolled and treated in the AA4500 clinical programme with 1082 subjects receiving at least 1 dose of AA4500 0.58 mg comprising the formal safety database. Based on the review of the nonclinical and clinical data of the AA4500 development programme, the adverse events of interest include: local reactions, potential immune-mediated events, tendon/ligament rupture or injury, skin lesions, and injection site bleeding. In the Phase 3 Double-Blind, Placebo-Controlled population, approximately two times as many AA4500 treated patients than placebo-treated patients had an AE (97.8% versus 54%).The majority of AEs were nonserious, mild or moderate in intensity, confined to the treated extremity, and resolved within a short period without sequelae.
Among subjects who received at least one dose of AA4500 0.58 mg, most patients experienced adverse reactions in the treated extremity, with the most frequently reported adverse reactions reported being: oedema peripheral, contusion, injection site pain, pain in extremity, injection site haemorrhage, and tenderness.
Eleven (1.0%, 11/1082) subjects who received at least 1 injection of AA4500 0.58 mg experienced a treatment-related SAE or SAE with unknown relationship to study drug.
The majority of treatment-related SAEs were related to events of the treated hand. Four of these were related to unintended effects of AA4500 on collagen (three tendon ruptures and one ligament injury [pulley injury]). The cause of these adverse reactions is thought to be the inadvertent exposure or injection of AA4500 into or around the tendon/ligament structure rather than into the Dupuytren's cord.

The influence of investigator training on adverse events, especially serious adverse events like tendon ruptures is crucial for the safe application of the drug. Investigator training in the clinical studies included intensive injection technique instructions via manuals and DVDs, workshops and investigator meetings and it has to be ensured that the training for the education of healthcare professionals in clinical practice is adequate. The Applicant should maintain a list of trained, enrolled physicians and the product must only be distributed to these prescribers. These requirements as well as the minimum contents of the training program and minimum requirements for a qualified trainer should be included in Annex II B - Conditions of the Marketing Authorisation.

Due to the immunogenic potential of AA4500 the majority of subjects developed ADAs after the first injection and all subjects had antibodies after four or more injections. In study AUX-CC-857, 11% and 22% of detected antibodies had neutralising capacity against AUX-1 and AUX-II, respectively. However, no adverse reactions consistent with systemic hypersensitivity or anaphylactic response were observed in the Dupuytren's disease programme. Except for pruritus and oedema peripheral, no clear increase in the number of adverse events with subsequent injections were observed. ADA titres were not predictive of the rate, severity, or duration of any of the adverse events. Potential cross-reactivity of neutralising antibodies (Nabs) against AUX-I or AUX-II to endogenous MMPs represents a serious potential risk for adverse effects, e.g. development of musculoskeletal syndrome. An MMP inhibitor-associated musculoskeletal syndrome (MSS) has been described in literature after oral treatment with matrix metalloproteinase inhibitors in knee osteoarthritis which includes shoulder arthralgia, myalgia, and stiffness, as well as hand oedema, palmar fibrosis, tendons/thickening nodules (reminiscent of the early development of Dupuytren's contracture) (Krzeski et al., 2007)
So far, musculoskeletal syndrome has not been observed in patients treated with Xiapex. Overall, the available data suggest that ADAs to both AUX-I and AUX-II, while present in all subjects after four or more injections, did not affect the safety profile of AA4500. For the time being this is addressed in the educational program, the labelling and the RMP.

Uncertainty in the knowledge about the unfavourable effects

Although in the clinical programme there was no signal identified for a combination of AE suggestive of the musculoskeletal syndrome, a potential for cross-reactivity of anti-product antibodies (anti-AUX-I and anti-AUX-II) with endogenous human matrix metalloproteinases with similar homology cannot finally be excluded. *In vitro* assessment of cross-reactivity has been performed in 5 patients and additional cross-reactivity experiments using a validated assay were provided. However, assay results are not considered to be reliable. Currently, the detection of cross reactivity of anti-AUX-I or anti-AUX-II antibodies versus the selected MMPs is restricted to binding assays. This is not considered sufficient. The cross-reactivity should be additionally investigated in terms of inactivation of endogenous MMPs by neutralising ADAs. The applicant committed to develop and validate a respective assay that is based on enzymatic activity of the MMPs.
Long-term safety data after repeated administration are lacking and potential long-term effects of MMP cross-allergy need to be monitored.
Regarding the neutralising potential of ADAs versus AUX-I and AUX-II, data on the determination of the optimised concentrations of enzymes and substrate were not provided. It is acknowledged that varying amounts of alpha-2-macroglobulin in the sera to be tested may disturb the assay. The Applicant committed to develop a more suitable assay to assess potential neutralising activity of NAbs in terms of enzymatic and thus pharmacodynamic activity of the drug substance. For the time being this is addressed in the educational program, the labelling and the RMP.

3.9 Benefit-Risk Balance

Overall, the proposed indication "treatment of Dupuytren's contracture in adult patients with a palpable cord" is supported by sufficient efficacy data. Overall, 64.0% of all AA4500 treated primary joints achieved a reduction in contracture of the primary joint to 5° or less in Study AUX-CC-857 (76.7% for MP joints and 40% for PIP joints), and 44,4% in Study AUX-CC-859 (65% for MP joints and 28% for PIP joints) compared to 6.8% and 4.8% respectively for placebo. The success rate is similar to the current mainstay of treatment in this disease and Xiapex provides an alternative option to surgery for physicians to use in the treatment of Dupuytren's contracture. The majority of TEAEs were non serious, mild or moderate in intensity, confined to the treated extremity, and resolved within a short period without sequelae. Efficacy and safety data suggest that collagenase is intended to the same category of patients than PNF.

Importance of favourable and unfavourable effects

Overall, the proposed indication "treatment of Dupuytren's contracture in adult patients with a palpable cord" is supported by sufficient efficacy data. Overall, 64,0% of all AA4500 treated primary joints achieved a reduction in contracture of the primary joint to 5° or less in Study AUX-CC-857 (76,7% for MP joints and 40% for PIP joints), and 44,4% in Study AUX-CC-859 (65% for MP joints and 28% for PIP joints) compared to 6,8% and 4,8% respectively for placebo. The success rate is similar to the current mainstay of treatment in this disease and Xiapex provides an alternative option to surgery for physicians to use in the treatment of Dupuytren's contracture. The majority of TEAEs were non serious, mild or moderate in intensity, confined to the treated extremity, and resolved within a short period without sequelae. Efficacy and safety data suggest that collagenase is intended to the same category of patients than PNF.

Benefit-risk balance

A positive benefit risk profile has been established across a broad range of Dupuytren's contracture (MP and PIP joints of low, moderate and high severity) that supports the proposed indication of "for the treatment of patients with Dupuytren's contracture in adult patients with a palpable cord." The subjects enrolled across the clinical programme were typical to what has been described in the literature and included a wide range of contracture severity. AA4500 has been found to have an acceptable safety profile in treating Dupuytren's contractures. The majority of AEs were mild to moderate in severity, local, confined to the treated extremity, and resolved prior to the next injection, generally within 2 weeks. Due to the immunogenic potential of AA4500 the majority of subjects developed ADAs after the first injection and all subjects had antibodies after four or more injections. ADA titres were not predictive of the rate, severity, or duration of any of the adverse events and did not negatively affect efficacy.

Discussion on the benefit-risk balance

Based on the available data, efficacy and safety data show that collagenase is intended to the same category of patients than percutaneous needle aponeurotomy (PNF). The Applicant has one ongoing and three planned Phase 3b/4 clinical studies that will provide post-approval relevant data to assess long term recurrence rate and complications associated with Xiapex treatment relative to currently available treatment options, i.e. surgery and PNF. Hence, the therapeutic value of Xiapex in comparison to PNF will be better characterised.
In addition, the potential of cross-reactivity of anti-product antibodies (anti-AUX-I and anti-AUX-II) with endogenous human matrix metalloproteinases with similar homology will be further investigated.

Whereas formation of ADAs is expected due to the fact that AUX-I and AUX-II are of bacterial origin, potential cross-reactivity of these antibodies represents a serious risk for adverse effects, e.g. development of musculoskeletal syndrome that should be monitored routinely by meaningful assays. As the full immune response in some patients (~15%) does not occur until the third or fourth injection, cross-reactivity should be also investigated at this stage of treatment.
Overall, investigation of immunogenicity particularly with respect to the neutralising potential of ADAs versus the drug substance and cross-reactivity to endogenous MMPs should be further investigated.
Overall, the CHMP recommendations to investigate further post-approval the above mentioned aspects are part of the Risk management plan and are without prejudice to the CHMP conclusion on the benefit ratio of the product based on the data currently available.

2.9 Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Xiapex in the treatment of Dupuytren´s contracture in adult patients with a palpable cord was favourable and therefore recommended the granting of the marketing authorisation.