

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*  
**125338**

**PHARMACOLOGY REVIEW(S)**

Tertiary Pharmacology Review

**By:** Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology  
OND IO *Paul C. Brown 12-23-09*

**BLA:** 125338

**Submission date:**

**Drug:** Collagenase (*Clostridium histolyticum*) for injection

**Applicant:** Auxilium Pharmaceuticals Inc.

**Indication:** Patients with advanced Dupuytren's disease

**Reviewing Division:** Division of Anesthesia, Analgesia and Rheumatology Products

**Introductory Comments:** The pharm/tox reviewer and supervisor concluded that this BLA could be approved from a pharm/tox perspective. No additional nonclinical studies were recommended.

Reproductive and developmental toxicity:

A fertility and early embryonic development study and an embryofetal development study were conducted in rats by intravenous administration. There were no adverse effects noted in these studies. As noted in the supervisory pharm/tox memo, a complete battery of reproductive and developmental toxicity studies would also include an embryofetal development study in a second species and a pre/postnatal study. However, these were considered unnecessary due to the absence of adverse effects in the two rat studies and the absence of detectable systemic exposure to collagenase in the clinical setting.

The pharm/tox reviewer recommended pregnancy category C since the sponsor did not complete a pre/postnatal study so this would be an absence of animal data. The supervisor recommended category B since although the complete battery was not conducted, the complete battery was not required in this case.

Another issue that was raised is the possible interaction of antiproduct antibodies with human matrix metalloproteinases (MMP). Such an interaction could theoretically be detrimental to fetal development. Both animals and humans developed antiproduct antibodies. The presence of these antibodies appeared to have no adverse effect on animals including fetal development. Five different MMPs were tested for their ability to inhibit the binding of antibodies from sera from several patients to the two Auxilium collagenases (Aux I and Aux II). Some inhibition was noted from one patient for Aux II binding.

Carcinogenicity:

Long term carcinogenicity studies with the drug substance or product were not required since the clinical use is not considered chronic.

**Conclusions and Recommendations:**

I agree that the pregnancy labeling can be category B. While the complete battery of reproductive and developmental toxicity studies was not completed, this was acceptable

since the risk was considered low based on the lack of adverse effects and lack of systemic exposure. The potential for adverse developmental effects based on MMP inhibition is theoretical and no adverse effects were observed in animals in which antidrug antibodies were present. This issue does not appear to be sufficient to prohibit use of a pregnancy category of B.

I agree that the BLA can be approved from a pharm/tox perspective and that no additional nonclinical studies are needed at this time.



DEPARTMENT OF HEALTH AND HUMAN SERVICES  
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**SUPERVISOR'S SECONDARY REVIEW  
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION**

BLA NUMBER: 125338  
SERIAL NUMBER: 000  
DATE RECEIVED BY CENTER: 27-Feb-2009  
PRODUCT: XIAFLEX™  
(Collagenase *Clostridium histolyticum*)  
for injection

INTENDED CLINICAL POPULATION: Patients with advanced Dupuytren's  
disease

SPONSOR: Auxilium Pharmaceuticals Inc.  
REVIEW DIVISION: Division of Anesthesia, Analgesia, and  
Rheumatology Products (HFD-170)

PHARM/TOX REVIEWER: Asoke Mukherjee, Ph.D.  
PHARM/TOX SUPERVISOR: R. Daniel Mellon, Ph.D. *R. Daniel Mellon*  
DIVISION DIRECTOR: Bob A. Rappaport, M.D. 9-18-2009  
PROJECT MANAGER: Chris Hilfiger

## Executive Summary

### I. Recommendations

#### A. Recommendation on approvability

I concur with Dr. Mukherjee's recommendation that from a nonclinical pharmacology toxicology perspective, BLA 125338 (XIAFLEX) may be approved pending agreement on the final labeling.

#### B. Recommendation for nonclinical studies

I concur with Dr. Mukherjee, no further nonclinical studies are necessary at this time.

#### C. Recommendations on labeling

NOTE: Labeling recommendations below are being made prior to the review team discussions and prior to discussion with the sponsor. The reader is referred to the final labeling for specific wording.

Sponsor's Proposed Labeling	Recommended Labeling	Rationale/Comment
(b) (4)		

## II. Summary of nonclinical development program and key findings

XIAFLEX (AA4500) is a (b) mixture of AUX-I and AUX-II collagenases isolated from the culture medium of *Clostridium histolyticum*. AUX-I and AUX-II demonstrate substrate specificity characteristic of class I and class II collagenase enzymes, respectively, and therefore are both functionally and antigenically distinct proteins. The Applicant maintains that the mixture of the two enzymes, which have differential collagen cleavage sites work synergistically to provide broad hydrolyzing activity toward collagen. Dupuytren's disease is manifested as a fixed flexion contracture deformity of the hand caused by increased collagen deposition resulting in decreased finger mobility and impairment of function. The disorder was named after Baron Guillaume Dupuytren, the surgeon who described the surgical procedure employed to correct the affliction. XIAFLEX is being proposed as a nonsurgical means to reduce the contracture by enzymatically breaking down the collagen surrounding the Dupuytren's cord thereby improving mobility and function. The proposed clinical dose is 0.58 mg per treatment (injected over three sites within the contracted cord).

The nonclinical development program for XIAFLEX included reliance upon information in the published literature regarding the enzymatic activity, mechanism of action and clearance of the enzymes from the body. In addition, the Applicant conducted several single and repeat dose GLP local toxicity studies in dogs, IV toxicology studies in the rat, genetic toxicology studies, and both fertility and early embryonic development and embryo-fetal development studies in the rat. All of these studies were completed with drug product manufactured by Process 1 and/or Process 3, the intended commercial product manufacturing process. As noted in the review by Dr. Mukherjee, and in consultation with the Product Review Team, the drug product manufactured by these different processes is deemed comparable. Prenatal and postnatal development studies were not required for this product due to the lack of systemic exposures in the clinical setting. Chronic toxicology studies and carcinogenicity assessment was also not required due to the lack of chronic use of the product.

As noted in Dr. Mukherjee's review, there was no clear NOAEL noted in the local toxicology studies, as the collagenase enzymes demonstrated predicted pharmacodynamic effects on the local tissues. These effects were also not associated with clinical efficacy, as there is no animal model of Dupuytren's disease. Information on local efficacy was therefore provided via in vitro studies with explanted Dupuytren's cords or Peyronie's plaques.

The Applicant conducted two 16-day repeat-dose intravenous toxicology studies in the rat to characterize the potential systemic toxicity that might result from inadvertent systemic exposure to the product. Dr. Mukherjee concludes that the NOAEL from the study was 0.029 mg protein/dose (~0.073-0.099 mg/kg). Given the clinical dose of 0.58 mg/treatment (0.0097

mg/kg assuming a 60 kg person), the animal NOAEL is approximately 10-times higher than the human dose if it were inadvertently injected intravenously. The toxicities at higher doses included injection site hemorrhage and inflammation, as well as significant liver changes including inflammation, necrosis, fibrosis, and bile duct hyperplasia. These findings are likely due to exaggerated pharmacodynamic effects of the collagenase enzymes acting in locations not intended due to the route of administration.

The Applicant conducted a standard battery of genetic toxicology studies. These studies were completed prior to international agreement that genetic toxicology studies are not necessary for large proteins which do not cross the cell membrane. Nonetheless, the data for the isolated enzymes exists and can be included in the label.

The Applicant conducted two intravenous reproductive and developmental toxicology studies using a rat model. There were no effects noted in the fertility and early embryonic development study or the embryo-fetal development study. The NOEL for these studies was the high dose of 0.13 mg/dose. This dose is over 40-times the human dose of 0.58 mg/dose, if inadvertently administered intravenously. Although a standard reproductive and developmental toxicology program would also include a second species embryo-fetal development study and a prenatal and postnatal development study, the lack of detectable systemic levels of collagenase enzyme following local administration indicates that such studies are not necessary.

Based on the lack of significant findings in the embryo-fetal development study conducted in the rat, and the lack of detectable systemic exposure in the clinical setting, the Applicant has proposed a Pregnancy Category B. Dr. Mukherjee has recommended a Pregnancy Category C due to the lack of a standard ICH battery of nonclinical reproductive and developmental toxicology studies and the concern that anti-product antibodies could theoretically cross-react with endogenous matrix metalloproteinases (MMPs) in the body. As the Division has concluded that the full battery of reproductive and developmental toxicology studies are not needed for this product due to a lack of detectable systemic exposure, and is not requiring the remaining studies from this battery, I do not think that a Pregnancy Category C is justified. Although virtually all individuals who have received XIAFLEX have developed anti-product antibodies, and there are some data that these antibodies may interfere with endogenous MMP activity (see product immunogenicity review completed by Dr. Mills), this is a theoretical concern and the current options listed in 21CFR§201.57 do not support the designation of a Pregnancy Category C for a theoretical concern. Nonetheless, I do think that it is reasonable to include some language to specifically raise the unknown clinical impact of the anti-product antibodies on the fetus. The exact language and location of this language will be discussed further with the entire review team. It is my understanding that Dr. Mills will be requiring additional studies to clarify the data suggesting potential for anti-product antibodies to interfere with activity of endogenous MMPs. Therefore, if the Applicant can provide adequate data to eliminate the concern that treatment with XIAFLEX could resulting anti-endogenous MMP proteins, the labeling could be adjusted accordingly.



FDA Center for Drug Evaluation and Research  
Division of Anesthesia, Analgesia, and Rheumatology Products  
10903 New Hampshire Avenue, Silver Spring, MD 20993

**Addendum to Supervisory Pharmacology Toxicology Secondary Review**

**Date:** October 21, 2009  
**To:** BLA 125338  
**From:** R. Daniel Mellon, Ph.D. *R. Daniel Mellon 10-21-2009*  
Supervisory Pharmacologist, DAARP  
**Subject:** **Addendum to Secondary Review**

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Dr. Mukherjee's primary review executive summary discussed a potential clinical implication of the nonclinical data as follows:

Although the applicant provided in vitro data to justify lack of interactions between anti-AA4500 and recombinant human matrix metalloproteinase, the role of antibody for future risks could not be determined in non-clinical models. Therefore, it is recommended that a user registry be developed to monitor the association of the antibodies to the development of systemic or local inflammatory diseases following the use of Xiaflex.

I concur with Dr. Mukherjee that the nonclinical data will not provide useful information regarding the potential clinical significance of anti-product antibodies, should they form in patients administered this product. The option of a user registry to monitor for a potential association of the antibodies to the development of systemic or local inflammatory diseases following use of Xiaflex should be based on the strength of the clinical data reviewed by product immunology reviewer and the existing clinical safety database. This suggestion has been discussed with the clinical review team.



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## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER: 125338  
SERIAL NUMBER: 000  
DATE RECEIVED BY CENTER: 2/27/2009  
PRODUCT: Xiaflex  
INTENDED CLINICAL POPULATION: Dupuytren's Disease  
SPONSOR: Auxilium Pharmaceuticals Inc.  
DOCUMENTS REVIEWED: M1, M2, M3, M4  
REVIEW DIVISION: Division of Anesthesia, Analgesia, and  
Rheumatology products (HFD-170)  
PHARM/TOX REVIEWER: Asoke Mukherjee Ph.D. *Asoke Mukherjee*  
PHARM/TOX SUPERVISOR: Dan Mellon Ph.D. *R. Dan Mellon 9-17-2009 9/17/2009*  
DIVISION DIRECTOR: Bob Rappaport, M.D.  
PROJECT MANAGER: Margarita Tossa

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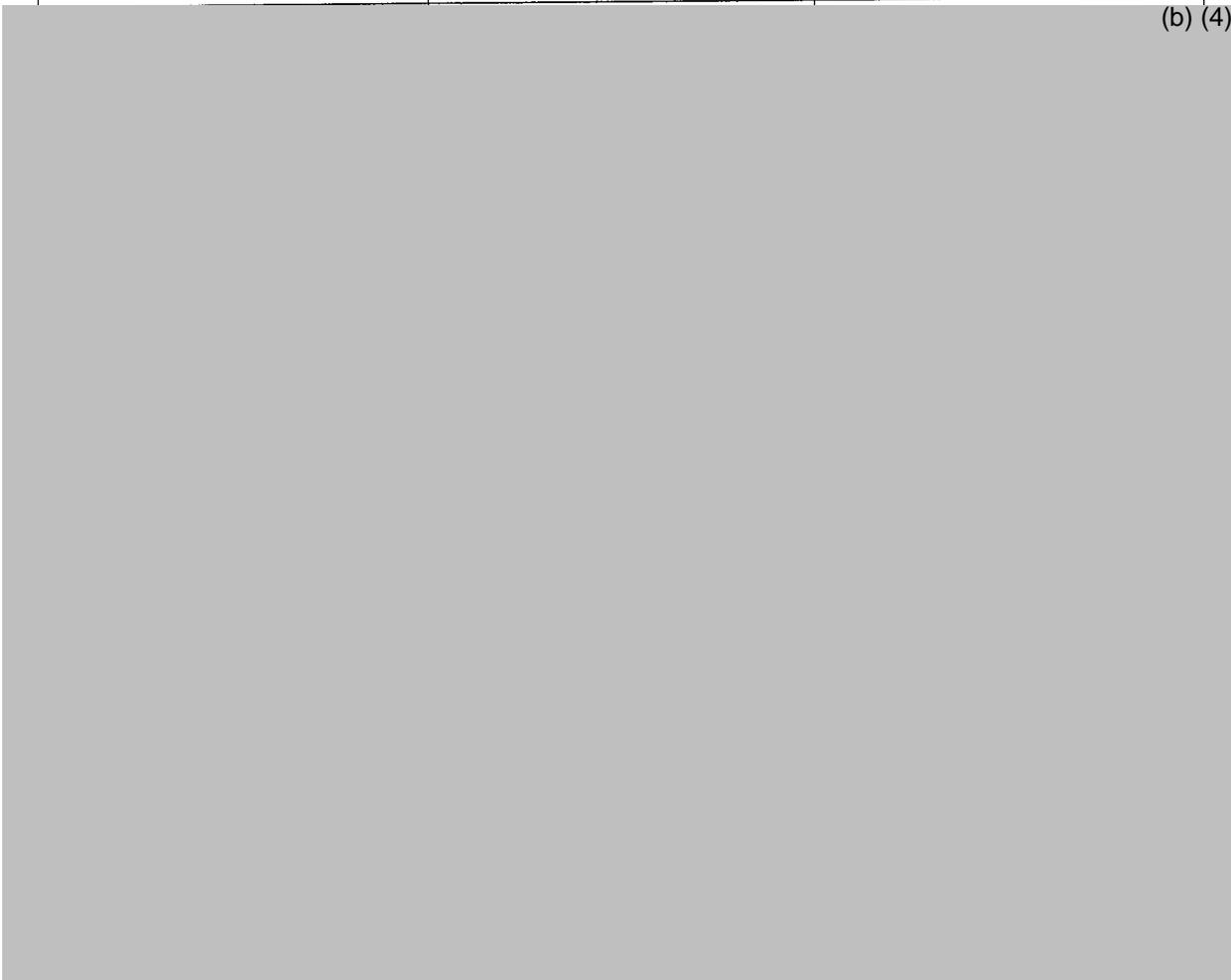
***EXECUTIVE SUMMARY***

**I. Recommendations**

- A. **Recommendation on approvability:** AA4500 (Xiaflex) can be approved based on non-clinical data and a lack of toxicity related to the interaction of anti-AA4500 antibodies with collagenase in non-clinical studies. Anti-AA4500 antibodies did not induce any systemic hypersensitivity reactions in the species tested following local and systemic administration.
  
- B. **Recommendation for nonclinical studies:** None
  
- C. **Recommendations on labeling:** NOTE: the labeling recommendations below are preliminary and have not been discussed with the full review team or the Applicant. For final labeling, the reader is referred to the action letter.

<b>Applicant Proposed</b>	<b>Reviewer's Recommendation</b>	<b>Comment</b>
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(b) (4)



(b) (4)

## II. Summary of nonclinical findings

### A. Brief overview of nonclinical findings:

AA4500 contains collagenase AUX-1 and AUX-2 at (b) proportion by mass. These collagenases have distinct catalytic activity and are expressed from different genes. (b) (4)

The collagenase activity was examined in surgically removed tissues in vitro up to 600 units as presented by the applicant from published literature. Two multidose toxicity studies were conducted in rats up to 16 days. The NOAEL was 0.029 mg protein/dose. Liver toxicity was noted at 0.13 and 0.29 mg protein/dose. Injection site perivascular inflammation and fibrosis were noted at all doses. Liver toxicity and injection site inflammation was non-reversible. Interestingly, inflammatory changes at the site of injection induces inflammatory cell migration. Theoretically, these cells also secrete collagenase that could extend the efficacy and safety of the drug.

Clostridial collagenase made by BTC, (b) (4) and Auxilium processes showed a similar toxicity profile and immunogenicity. Therefore, subsequent reproductive toxicity studies were conducted using the (b) (4) manufacturing process of the drug. AUX-1 and AUX-2 were transiently detected in the plasma. However, AUX-1 rapidly degraded in the plasma. Exposure to AUX-2 could be measured on days 1 and 7 of the study following IV injections in rats. Antibody formation to AUX-1 and AUX-2 was observed at all doses tested following IV injections (rats) and local injections (dogs).

A local toxicity study was conducted after injection of AA4500 in the dog penis. The study was conducted to support the safety of the drug in Peyronie's disease (b) (4) and the study report was submitted with this BLA submission. Data showed injection site inflammation in the treated groups at single and multiple doses. Doses ranged from 0.8 to 14.9 ug/kg. Both AUX-1 and AUX-2 in the plasma was detectable only within 60 min after dosing, suggesting minimal bioavailability of the drug in systemic circulation following local administration. However, antibodies to AUX-1 and AUX-2 were present in the serum even at recovery day 28. The study clearly showed antigenicity to AA4500 in animals following local injections.

The applicant conducted sensitization experiments in guinea pigs and did not observe immediate hypersensitivity reactions in guinea pigs when challenged by AA4500 by ip or intracardiac route.

The applicant conducted mutagenicity studies in Ames assay, in vitro chromosomal aberration in peripheral human lymphocytes and mouse micronucleus assay using clostridial collagenase prepared by BTC batches. The drug substance was not mutagenic in these assays. However, the batch used in the study was not intended for marketing under the BLA. In addition, mutagenicity study is generally not required for biologics because a large protein molecule does not have access into the cell. Mutagenicity studies to biologics may provide insights to process impurities that could be mutagenic. In terms of impurities, the genetic toxicology studies submitted in the BLA are not relevant to the proposed marketing batches.

The applicant provided data for fertility and early embryonic development and embryo-fetal development reproductive toxicology studies in rats. Male and female rats did not show any effect on fertility and early embryonic development up to 0.13 mg protein/dose by IV bolus injections of AA4500 manufactured by (b) (4) process 3. Pregnant rats also did not show developmental toxicity to pups when injected during organogenicity period. AUX-1 and AUX-2 antibodies were present in the fertility study. The applicant did not measure serum antibody titers in the embryo-fetal development study. However, antibodies to AUX-1 and AUX-2 were detected in a 16-day rat toxicity study and the fertility and early embryonic development study. Based on that, it is assumed that rats treated with AA4500 for developmental toxicity study would have developed antibodies to AUX-1 and AUX-2.

The applicant did not conduct prenatal and postnatal development study. The Agency's communication dated Sept 21, 2001 for end of Phase 2/Pre-Phase 3 Teleconference that stated, "Preclinical requirement for chronic toxicity, reproductive toxicity and carcinogenicity studies are waived due to the nature of the product, and its intended use in this specific clinical setting."

However, during the Pre-BLA meeting with the Division, the applicant was asked to provide justification why the segment 3 reproductive safety study would not be necessary to predict the effect of the drug on pre and post natal development. The applicant's response and reviewer's comments are provided in the review under the discussion section of the review. The applicant indicated that bioavailability of AA4500 following local injections was not measurable. The reviewer agreed with the applicant's position for not conducting the study based on the lack of systemic bioavailability of AA4500 in humans and results of the non-clinical toxicity studies. However, in the absence of segment 3 data, the reviewer recommends Pregnancy Category C

as opposed to Pregnancy Category B proposed by the applicant for the package insert.

The non-clinical data provided in the BLA clearly showed development of anti-product antibodies in the serum both after single and multiples injections irrespective of the route of administration. The immunogenicity to the product in the clinical setting has been reviewed by the product quality reviewer under a separate review. However, consequences of the anti-product antibody are unknown in the non-clinical studies. The clinical summary indicated that a similar anti-AA4500 antibody response was observed. The applicant stated, "Given the local mode of action of AA4500, it seems unlikely that neutralizing anti-AUX-1 or anti-AUX-2 antibodies would influence the clinical benefit-to-risk analysis." Based on the nonclinical data reviewed for the BLA, presence of an autoimmune response in animals was not evident. The accumulation of AUX-2 in the plasma also suggests that the AUX-2 perhaps was not cleared by its antibodies. In all cases injection site inflammation was noted that could be the result of collagenase activity as supported from the fact that rat tail collagen was used to assay for the potency of the drug substance. However, the role of the anti-product antibodies needs to be determined from the clinical observations because non-clinical toxicity studies did not indicate its relationship to the systemic toxicity and the lack of correlation between animal and human antigenicity data.

Based on above observations in the nonclinical studies, it is concluded that Xiaflex would contribute to injection site inflammation and antibody titers in humans. While the local inflammatory response and tolerance could be reduced in the patients because animal studies were conducted at higher multiples, the role of antibody to the long-term safety can only be determined after careful monitoring of clinical conditions of the patients. However, when Xiaflex was injected locally, nonclinical reports did not show any untoward systemic response that could be related to the antibody. The reviewer recommends approval of the product on the basis of nonclinical data.

#### **B. Pharmacologic activity:**

Dupuytren's disease is associated with collagen deposition between the skin and tendon in the hand that impairs extension of the finger. AA4500 contains clostridial collagenase 1 and 2 that would be injected locally to facilitate the extension of finger or other impaired conditions. There is no pharmacological model in experimental animals that could replicate the conditions of this disorder. The applicant conducted two in vitro studies using surgically removed tissues from human subjects diagnosed for Dupuytren's and Peyronie's diseases. The experimental efficacy was examined by tensile strength of the tissue to applied stress, release of amino acids and microscopic examination of thinning of the tissue. Up to 600 units of AA4500 was found effective for degrading collagen. No other secondary pharmacology and

safety pharmacology study was conducted due to lack of bioavailability of AA4500 in the systemic circulation. The product induces antibodies to AA4500 either by local or systemic administration. However, no pharmacodynamic effect related to the antibodies was reported in the animal testing.

**C. Nonclinical safety issues relevant to clinical use:**

Although the applicant provided in vitro data to justify lack of interactions between anti-AA4500 and recombinant human matrix metalloproteinase, the role of antibody for future risks could not be determined in non-clinical models. Therefore, it is recommended that a user registry be developed to monitor the association of the antibodies to the development of systemic or local inflammatory diseases following the use of Xiaflex. In addition, certain antibiotics like tetracycline possess collagenase inhibition. Therefore, uses of certain antibiotics with known effects on collagenase should be restricted following the treatment with AA4500.

**2.6 PHARMACOLOGY/TOXICOLOGY REVIEW**

**2.6.1 INTRODUCTION AND DRUG HISTORY**

**BLA number:** 125338

**Review number:** One

**Sequence number/date/type of submission:** 000 / Feb 27, 2009 / Original BLA

**Information to Applicant:** Yes ( ) No (x )

**Applicant:** Auxilium Pharmaceuticals, PA

**Manufacturer for drug substance:** Auxilium Pharmaceuticals Inc., 40 Valley Stream Parkway, Malvern, PA 19355

**Reviewer name:** Asoke Mukherjee, Ph.D.

**Division name:** Division of Anesthesia, Analgesia, and Rheumatology Products

**HFD #:** 170

**Review completion date:** July 14, 2009

**Drug:**

Trade name: Xiaflex

Generic name: Clostridial Collagenase for injection

Code name: AA4500, Nucleolysin

Chemical name: Biologics does not have chemical name

CAS registry number: Nil

Molecular formula/molecular weight: Mixtures of AUX-1 and AUX-2 for collagenase 1 and collagenase 2, respectively at (b) (4), each enzyme had approximately 1000 amino acids and molecular weight between 110 to 120 kDa.

Structure: The applicant provided amino acid compositions but the structure was not provided in the BLA.

**Relevant INDs/NDAs/DMFs:**

IND #	Drug	Status	Division	Indication	Stamp Date	Applicant
5780	Collagenase	Active	HFD-170	Dupuytren's disease	Sept 28, 1994	Auxilium Pharmaceuticals

(b) (4)

**Drug class:** Biologic, therapeutic protein, recombinant collagenase enzymes

**Intended clinical population:** Dupuytren’s Disease

**Clinical formulation:** Clinical formulations from the applicant’s table is shown below.

**Table 1: Composition of Drug Product Dosage Form**

Component	Amount per Vial (mg)	% w/w	Function	Quality Standard
Active Ingredient			(b) (4)	
AA4500	0.9			HDSD.001
Inactive Ingredients <sup>1</sup>				
Sucrose	18.5			USP/EP
Tromethamine	1.1			USP/EP
Hydrochloric acid	0.5			USP/EP

<sup>1</sup> Formulation excipients required for drug substance manufa

**Table 1: Composition of AA4500 Sterile Diluent**

Ingredient	Concentration	Quality Standard
Sodium Chloride	0.9%	USP/EP
Calcium Chloride, dihydrate	0.03%	USP/EP

(b) (4)

**Route of administration:** Locally into Dupuytren’s cord

**Disclaimer:** Tabular and graphical information are constructed by the reviewer unless cited otherwise.

**Studies reviewed within this submission:**

1. Collagenase in the treatment of Dupuytren’s disease: an in vitro study by Starkweather et al., J. Hand Surgery, 1996, 21A, 490-495.
2. Collagenase for Peyronie’s disease experimental studies, Gelbard et al. Urol. Res. 1982, 10, 135-140.
3. AA4500 and BTC collagenase: A 16-day intravenous comparative toxicity and toxicokinetic study followed by a 14-day recovery period in Sprague Dawley rats.

4. A 16-day, multiple-dose, intravenous toxicity study in rats comparing AA4500 with BTC collagenase and placebo.
5. Local Toxicity Study of AA4500 Injected into Dog Penis.
6. Micronucleus test, injectable collagenase ABC (Nucleolysin).
7. Reverse mutation in *Salmonella typhimurium*
8. Chromosome aberration in human lymphocytes cultured in vitro.
9. Intravenous fertility and general reproduction toxicity study of AA4500 in rats.
10. Intravenous developmental toxicity study of AA4500 in rats.
11. Guinea-pig sensitization study.
12. An exploratory study on the effect of injected Nucleolysin on the subcutaneous fat of female Zucker rats.

**Studies not reviewed within this submission:**

The reviewer gone through these reports. However, a written review of following study reports were not provided because full audited reports were not submitted in the BLA. These studies were also not conducted according to GLP.

(b) (4)



## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary:

Clostridial collagenase (AUX) 1 and 2 obtained from *Clostridium histolyticum* contains type 1 and type 2 collagenase at (b) (4) ratio in the drug substance, AA4500. The bacterial collagenase degrades type 1 and type 2 collagens. The enzymatic effect would reduce the contracture caused by the collagen deposition in Dupuytren's cord (disease). The

applicant provided two published reports to substantiate the pharmacodynamic effect of AA4500 in vitro as discussed below.

### **2.6.2.2 Primary pharmacodynamics**

Mechanism of action: The primary pharmacological effect of the drug is due to enzymatic cleavage of type 1 and type 2 collagen. The applicant provided published literature using BTC batch or commercially purified clostridial collagenase for in vitro efficacy of the enzyme using surgically removed Dupuytren's cord containing collagen. The in vitro study was conducted due to unavailability of suitable animal model for Dupuytren's disease.

#### **1. Collagenase in the treatment of Dupuytren's disease: an in vitro study by Starkweather et al., J. Hand Surgery, 1996, 21 A, 490-495.**

Dupuytren's cord were obtained at surgery from male volunteers and incubated with collagenase at 150, 300 and 600 units. The cord was tested by stress induced by mechanical load and stretching the cord up to the point it had ruptured. The force needed to rupture was the measurement of collagenase activity. Data suggested that 300 and 600 units of collagenase reduced the mechanical stress needed for the cord to rupture. The experiment indicated the efficacy of collagenase in the stretching the cord in vitro. Histopathology of the cord showed lysis of type 1 and type 2 collagen. Clostridial collagenase was obtained as Nucleolysin from Advance Biofactures, Lynbrook, NY.

#### **2. Collagenase for Peyronie's disease experimental studies, Gelbard et al. Urol. Res. 1982, 10, 135-140.**

Tissues from tunica albuginea from 3 patients with Peyronie's disease were treated with purified clostridial collagenase. Tissues were examined for the release of amino acids to probe collagenase activity in vitro. Collagenase activity was determined using a colorimetric method and thinning of tissues when examined microscopically at 200 and 400 units. The study was designed to investigate the role of collagenase treatment in Peyronie's disease and as an in vitro bioassay for collagenase activity.

Drug activity related to proposed indication: See above

**2.6.2.3 Secondary pharmacodynamics:** The applicant indicated that no systemic secondary pharmacological effect is expected due to the non-bioavailability of the enzyme in systemic circulation. No secondary pharmacology study was conducted. However, one issue raised in the literature as well as the BLA was the inhibitory effect of collagenase by certain antibiotics like tetracycline due to chelation of zinc. The sponsor did not provide data for the inhibition of clostridial collagenase in the presence of tetracycline. Therefore, the issue of treating patients with appropriate antibiotics that do not have collagenase inhibition needs to be addressed by the clinical review team as well as the package insert.

**2.6.2.4 Safety pharmacology:** No safety pharmacology study was conducted due to lack of systemic bioavailability of AA4500 following a local injection and lack of tissue distribution of the enzyme. AA4500 is also rapidly degraded upon systemic exposure due to the action of peptidase. It should be noted that dedicated safety pharmacology studies are not typically required for biologics due to their large molecular size, specificity, and the inability to transport across the biological membranes. A concern was raised for the development of anti-AA4500 antibodies that could contribute to the long-term safety of the drug. However, non-clinical studies reported in the BLA did not show any autoimmune response.

#### **2.6.2.5 Pharmacodynamic drug interactions**

The applicant did not conduct any pharmacodynamic drug interaction studies.

### **2.6.3 PHARMACOLOGY TABULATED SUMMARY**

Following tabulated summary was provided by the applicant:

**Table 3: Design and Endpoints of Tissue Explant Culture Studies Performed with AA4500 or Purified Commercial Collagenase**

Tissue(s) Evaluated	Collagenase and Dose/ Concentration Used	Route of Administration	Study Endpoints	Reference
Peyronie's plaque Tunica albuginea Corpus cavernosum Pericardium	Purified commercial collagenase (b) (4) 10-400 U	Incubation (collagen digestion rate) Injection (histomorphology)	Amino acid release by ninhydrin reaction (collagen digestion rate) Light microscopy (histomorphology)	Geibard et al. (1982)
Dupuytren's cord	AA4500 (early BTC process) 150-3600 U	Injection	Mechanical loading (tensile modulus and breaking force determination) Light microscopy (histomorphology) Picrosirius red staining (collagen subtype characterization)	Starkweather et al. (1996)

**2.6.4 PHARMACOKINETICS/TOXICOKINETICS**

**2.6.4.1 Brief summary:** Pharmacokinetic data from individual toxicity studies in rats are reviewed under the specific study. Local injections were made into tunica albuginea, corpus cavernosum or periurethral corpus spongiosum of testes in dogs at 140, 430 and 1430 units/dose, 3 times per week every 4 weeks for a total 9 doses (b) (4)520). The high dose was lowered to 1050 units/dose due to local inflammation. Additional animals were given 1430 or 2570 units as single doses. Plasma levels were determined on days 1 and 61 that represents first and last dose respectively. Plasma levels of AUX-1 and AUX-2 at 18-38 ng/mL were noted 5 minutes post injections, and in some occasions, within 5 minutes after the injections. Systemic exposure to AUX-1 and AUX-2 after the local injections was minimal in dogs. The applicant provided data for the individual plasma levels at specific time points indicated in the protocol. However, there was no summary table in the report that could be presented in the review. The applicant indicated that due to a short half-life of AUX-1 and AUX-2, PK parameters were not calculated.

PK data were presented for two toxicity studies in rats where AA4500 was injected intravenously. Systemic exposure to AUX-1 and AUX-2 was minimal on day one. However, on repeated administration of AA4500, AUX-2 exposure was increased. Data suggest that degradation of AUX-1 was faster than AUX-2.

Anti-AUX4500 data suggested negligible antibodies at pre-study samples. However, anti-AUX4500 (against AUX-1 and AUX-2) was present in the serum

in all animals treated with AUX4500 at the end of day 16 and longer and through the recovery period. One animal treated at 2.5 ug/kg also showed antibody after 8 days (two treatments). **Although the applicant did not mention about neutralization of AUX by its antibodies in the non-clinical studies, increased exposure to AUX-2 upon repeated dosing despite the presence of anti-AUX-2 when compared to the exposure on day 1 may indicate that AUX-2 was not neutralized by its antibody.**

#### 2.6.4.2 Methods of Analysis

[see under individual study reviews]

**2.6.4.3 Absorption:** the drug product was injected intravenously to determine the systemic toxicity. AA-4500 was injected locally in the dog penis to examine local toxicity. However, no systemic absorption following local injection was detected. Antibodies to AUX-1 and AUX-2 were present after the IV or local injections.

**2.6.4.4 Distribution:** The drug product is proteins and degraded following IV administration. In general, large molecular weight protein does not distribute to organs.

**2.6.4.5 Metabolism:** Metabolic fragments of AUX-1 and AUX-2 proteins were not determined.

**2.6.4.6 Excretion:** No excretion study was conducted.

**2.6.4.7 Pharmacokinetic drug interactions:** No pharmacokinetic drug interaction study was conducted.

**2.6.4.8 Other Pharmacokinetic Studies:** Nil

**2.6.4.9 Discussion and Conclusions:** AA4500 is comprised of AUX-1 and AUX-2 a (b) mass. These enzymes have collagenase type 1 and collagenase type 2 activities. Upon IV injections and local injections, AUX-1 plasma levels were minimal on day 1 and on day 16 in rats. Although plasma levels of AUX-2 was observed only within hrs after injections, exposure to AUX-2 was higher than AUX-1 after repeated IV administration. Antibodies to both AUX-1 and AUX-2 were present in rats and dogs. Data suggest that AA4500 is poorly bioavailable after the local injections.

**2.6.4.10 Tables and figures to include comparative TK summary:** see individual study review

#### 2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Following tabulated summaries were submitted by the applicant:

2.6.7.3. Toxicokinetics: Overview of Toxicokinetics Data

Test Article:		Overview of Toxicokinetics Data <sup>a</sup>				Study Duration	GLP Compliance	Study Number
Name <sup>b</sup>	Dose <sup>c</sup> U/dose (mg protein/dose)	Species: Rat						
		Male		Female				
		AUX-I	AUX-II	AUX-I	AUX-II			
AA4500	50 (0.0029)	NC <sup>d</sup>	C <sub>max</sub> : 1.67 T <sub>max</sub> : 0.25 AUC: 0.63	NC <sup>d</sup>	C <sub>max</sub> : 1.82 T <sub>max</sub> : 0.25 AUC: 0.68	16 d	GLP	(b) (4) 00006
AA4500	150 (0.0087)	NC <sup>d</sup>	C <sub>max</sub> : 22.47 T <sub>max</sub> : 0.25 AUC: 14.34	C <sub>max</sub> : T <sub>max</sub> :	C <sub>max</sub> : 33.37 T <sub>max</sub> : 0.25 AUC: 22.77	16 d	GLP	00006
AA4500	250 (0.0145)	NC <sup>d</sup>	C <sub>max</sub> : 24.3 T <sub>max</sub> : 0.25 AUC: 17.9	NC <sup>d</sup>	C <sub>max</sub> : 17.9 T <sub>max</sub> : 0.5 AUC: 13.4	M: ≤ 64 d F: ≤ 21 d	GLP	00012
		NA <sup>e</sup>	NA <sup>e</sup>	NC <sup>d</sup>	C <sub>max</sub> : 12.1 T <sub>max</sub> : 0.5	10 d	GLP	00009
AA4500	500 (0.029)	C <sub>max</sub> : 9.17 T <sub>max</sub> : 0.25	C <sub>max</sub> : 105.47 T <sub>max</sub> : 0.25 AUC: 76.86	C <sub>max</sub> : 9.72 T <sub>max</sub> : 0.25	C <sub>max</sub> : 135.27 T <sub>max</sub> : 0.25 AUC: 93.91	16 d	GLP	00006
		C <sub>max</sub> : 212 T <sub>max</sub> : 0.0	C <sub>max</sub> : 828 T <sub>max</sub> : 0.0 AUC: 250			16 d	GLP	1007-1671 <sup>f</sup>
AA4500 (Process 1)	500 (0.029)	C <sub>max</sub> : 2.97 T <sub>max</sub> : 0.25	C <sub>max</sub> : 25.41 T <sub>max</sub> : 0.25 AUC: 25.97	C <sub>max</sub> : 4.32 T <sub>max</sub> : 0.25	C <sub>max</sub> : 20.59 T <sub>max</sub> : 0.25 AUC: 18.28	16 d	GLP	00006
		C <sub>max</sub> : 87 T <sub>max</sub> : 0.0	C <sub>max</sub> : 446 T <sub>max</sub> : 0.0 AUC: 117			16 d	GLP	1007-1671 <sup>f</sup>

2.6.7.3. Toxicokinetics: Overview of Toxicokinetics Data (Continued)

Test Article:		Overview of Toxicokinetics Data <sup>a</sup>				Study Duration	GLP Compliance	Study Number
Name <sup>b</sup>	Dose <sup>c</sup> U/dose (mg protein/dose)	Species: Rat						
		Male		Female				
		AUX-I	AUX-II	AUX-I	AUX-II			
AA4500 (lor 7280)	500 (0.029)	C <sub>max</sub> : 164 T <sub>max</sub> : 0.0	C <sub>max</sub> : 613 T <sub>max</sub> : 0.0 AUC: 169			16 d	GLP	(b) (4) 1007-1671 <sup>f</sup>
AA4500	750 (0.0435)	C <sub>max</sub> : 11.0 T <sub>max</sub> : 0.25	C <sub>max</sub> : 160 T <sub>max</sub> : 0.25 AUC: 160	NC <sup>d</sup>	C <sub>max</sub> : 46.8 T <sub>max</sub> : 0.50 AUC: 173	M: ≤ 64 d F: ≤ 21 d	GLP	00012
		NA <sup>d</sup>	NA <sup>d</sup>	C <sub>max</sub> : 2.03 T <sub>max</sub> : 0.63	C <sub>max</sub> : 55 T <sub>max</sub> : 0.63 AUC: 133	10 d	GLP	00009
AA4500 (lor 7280)	2240 (0.13)	C <sub>max</sub> : 1454 T <sub>max</sub> : 0.0	C <sub>max</sub> : 4532 T <sub>max</sub> : 0.0 AUC: 1229			16 d	GLP	1007-1671 <sup>f</sup>
AA4500	2240 (0.13)	C <sub>max</sub> : 21.4 T <sub>max</sub> : 0.25 AUC: 16.4	C <sub>max</sub> : 308 T <sub>max</sub> : 0.25 AUC: 261	C <sub>max</sub> : 7.89 T <sub>max</sub> : 0.25	C <sub>max</sub> : 173 T <sub>max</sub> : 0.25 AUC: 186	M: ≤ 64 d F: ≤ 21 d	GLP	00012
		NA <sup>d</sup>	NA <sup>d</sup>	C <sub>max</sub> : 11.3 T <sub>max</sub> : 0.62	C <sub>max</sub> : 218 T <sub>max</sub> : 0.62 AUC: 446	10 d	GLP	00009
AA4500	5000 (0.29)	C <sub>max</sub> : 7075 T <sub>max</sub> : 0.0	C <sub>max</sub> : 11932 T <sub>max</sub> : 0.0 AUC: 4679			16 d	GLP	1007-1671 <sup>f</sup>
AA4500 (lor 7280)	5000 (0.29)	C <sub>max</sub> : 8250 T <sub>max</sub> : 0.0	C <sub>max</sub> : 11665 T <sub>max</sub> : 0.0 AUC: 4166			16 d	GLP	1007-1671 <sup>f</sup>

- <sup>a</sup> Due to limited exposure with repeated dosing, toxicokinetic parameters were generally only calculated for the first dosing day; these are the data represented in the table. AUX-1 exposure was generally too limited for calculation of any TK parameters other than  $C_{max}$ .  $C_{max}$  is in ng/mL, AUC is  $AUC_{0-24h}$  in ng·h/mL, and  $t_{max}$  is in h.
- <sup>b</sup> Unless otherwise specified, AA4500 was manufactured by Process 3 and derived from lot NFF-0035, manufactured by (b) (4) or 7280 was manufactured by Process 3 in the Sponsor's Hershman facility.
- <sup>c</sup> For all studies in which toxicokinetic parameters were determined, dosing was by IV bolus q48h (except study (b) (4) 0009, where dosing was daily).
- <sup>d</sup> NC = not calculated (no detectable plasma levels, or exposure inadequate to calculate parameter)
- <sup>e</sup> NA = not applicable; only pregnant females were included in this study.
- <sup>f</sup> Data were combined for toxicokinetic analysis and the results are presented in the "males" column.
- AUC = area under the curve

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

#### General toxicology:

The applicant conducted local and systemic toxicity to AA4500 in rats and dogs. Intravenous injections of AA4500 showed liver toxicity e.g. bile duct hyperplasia, hepatocellular necrosis and fibrosis at 0.29 mg protein/dose/IV. The local injections in dogs did not show any systemic toxicity up to 6.1 ug/kg. However, in all experimental studies, local inflammatory response and hemorrhage was noted. The NOEL for the injection site reactions could not be established due to the pharmacodynamic effect of the drug on collagen lysis. Guinea pig sensitization study did not show anaphylaxis like reaction and the repeat dose was tolerated without mortality. Injections of AA4500 either locally or systemically induced antibodies to collagenase-1 (AUX-1) and collagenase 2 (AUX-2). However, toxicity due to the presence of antibody was not evident.

Genetic toxicology: The applicant conducted an Ames assay, chromosome aberration in human lymphocytes and micronucleus tests in mice using collagenase made by BTC processes. However, the applicant changed the manufacturing process to Auxilium process and, in terms of potential impurities, the studies conducted for the BLA are not completely relevant. However, in general, mutagenicity studies for large proteins are not required for regulatory approval because these proteins do not transport across the cell membranes and induce mutagenicity.

Carcinogenicity: No carcinogenicity study was conducted due to a lack of systemic exposure and intermittent uses up to 3 injections in any Dupuytren's cord.

Reproductive toxicology: AA4500 does not have any effect on the fertility and early embryonic development or embryo-fetal development when administered up to 0.13 mg/dose/IV. The applicant did not conduct a pre- and postnatal development study. Pregnancy category C was recommended for the use of the drug in pregnant women.

Special toxicology: The applicant conducted a guinea pig sensitization test by IP injections of the protein. Guinea pigs did not show signs of hypersensitivity reactions following repeated dosing.

### 2.6.6.2 Single-dose toxicity

The applicant did not conduct single dose toxicity studies according to GLP and complete study reports were not submitted in the BLA. Study # (b) (4) 00014 showed death of a rat at 0.29 mg protein/dose after a single IV injection.

LD<sub>50</sub> in mice after IP injection was 57-80 units/mouse. In another report dated June 3 1976, deaths were reported at 80 and 104 units/mouse/IP. Autopsy showed hemorrhage in the pleural, peritoneal cavities, congestion in lungs, liver and kidneys. The LD<sub>50</sub> after IM injection in mice was 1280 units/mouse. Local tissue injury was reported after the single dose study.

Nucleolysin lot#N7903 showed transient erythema to guinea pigs at 300 units/kg and it was concluded that the protein is an irritant. However, the study was not relevant to the BLA because the batch used will not be used for the approval and marketing of the product.

### 2.6.6.3 Repeat-dose toxicity

**Study title: AA4500 and BTC collagenase: A 16-day intravenous comparative toxicity and toxicokinetic study followed by a 14-day recovery period in Sprague Dawley rats.**

**Key study findings:** NOAEL for toxicity was 0.029 mg protein/dose. At higher dose, liver toxicity and mortality was noted. Injection site inflammation was observed at all doses tested. From the toxicity standpoint, .Auxilium and (b) (4) batches of AA4500 were comparable

**Study no.:** 1007-1671

**Volume #M4, and page #:** 1

**Conducting laboratory and location:** (b) (4)  
(b) (4)

**Date of study initiation:** Aug 17, 2007

**GLP compliance:** Yes

**QA report:** yes (x) no ( )

**Drug:** AA4500 and BTC collagenase, lot #NFF-0035 ( (b) (4) and 7280 (Auxilium-Horsham process) for AA4500 and 992-7 for BTC batch

% purity:

0.9 mg protein/vial as lyophilized powder for AA4500 (Auxilium)-100% purity

0.7 mg/vial for BTC collagenase at 100% purity

0.9 mg/vial (b) (4) process collagenase at 110% purity

Diluent containing 0.9% sodium chloride and 0.03% calcium chloride, Lot # FIN-0265

Potency:

Lot	AUX-1	AUX-2
7280 (Auxilium)	0.93	0.95
NFF-0035 (b) (4)	0.91	0.81
992-7 (BTC)	0.86	1.02

**Methods**

Doses: See study design below.

Treatment Groups	Test Article (Lot number)*	Dose Level (mg protein/dose)	Dose Conc. (mg protein/mL)	Dose Volume (mL/animal)	Number of Animals					
					Main Study		Recovery		Toxicokinetics	
					Males	Females	Males	Females	Males	Females
1. Diluent*	N/A	0	0	0.5	10	10	5	5	3	3
2. Low Dose	BTC collagenase (992-7)	0.029	0.058	0.5	10	10	5	5	12	12
3. Low Dose	AA4500 (NFF-0035)	0.029	0.058	0.5	10	10	5	5	12	12
4. Low Dose	AA4500 (7280)	0.029	0.058	0.5	10	10	5	5	12	12
5. Mid Dose	AA4500 (7280)	0.13	0.26	0.5	10	10	-	-	12	12
6. High Dose	AA4500 (NFF-0035)	0.29	0.58	0.5	10	10	5	5	12	12
7. High Dose	AA4500 (7280)	0.29	0.58	0.5	10	10	5	5	12	12

\*Note: AA4500 Lot NFF-0035 was manufactured by (b) and AA4500 Lot 7280 was manufactured by Auxilium Pharmaceuticals' Hordsham facility, as noted under "Characterization of Test and Control Vehicle Articles" below.  
 \* Control Article (AA4500 Diluent).

Species/strain: Male and female Sprague Dawley rats

Number/sex/group or time point (main study): See study design above

Route, formulation, volume, and infusion rate: The test substance was administered by bolus intravenous injections, once per 48 hours over 16 days. A total of 8 injections were made. The dose volume was 0.5 mL for each injection. The applicant indicated that in one occasion, one male from group 7 was injected with 0.29 mg protein/dose from a lot #NFF-0035 instead of Lot # 7280.

Following animals could not be dosed as scheduled due to the injection site abnormality:

Dose	Group, Dose	Animal#
0.29 mg/dose	6	6502A, Main group, sacrificed on day 9
0.29 mg/dose	7	7511G, Recovery group, sacrificed on day 9; 7019K, TK group

The dosing solutions were analyzed to obtain the variability to the nominal dose. Data are shown below.

Group	Day	Batch	Conc, mg/mL	Recovery
2	1, 7, 15	BTC	0.058	77, 71, 70%

3	1, 7, 15	NFF-0035	0.058	96, 90, 97%
4	1, 7, 15	Auxilium	0.058	95, 87, 90%
5	1, 7, 15	Auxilium	0.26	98, 104, 104%
6	1, 7, 15	NFF-0035	0.58	117, 104, 111%
7	1, 7, 15	Auxilium	0.58	105, 106, 103%

Satellite groups used for toxicokinetics or recovery: As show on in the study design, 5 rats/sex except the mid dose, were allotted for a 14-day recovery. Another group of 3-12 animals/sex/group was allotted for toxicokinetics.

Age: 12 to 15 weeks

Weight: 350 to 475 g male, 232 to 329 g for female rats

Sampling times: Approximately 0.75 mL of the blood samples were collected from 3 rats/sex/group/time point from groups 2 to 7 on days 1, 7 and 15. Samples were collected at 0.5, 1, 2, 4, 8 and 24 hours after the injections. Blood samples were collected before and immediately after the injection from control animals in group 1 on day 1. Samples were collected from jugular vein puncture or from sublingual vein. The sponsor did not indicate if samples were collected under anesthesia. AUX-1 and AUX-2 levels were determined in the rat plasma by an ELISA method and toxicokinetic parameters were determined using a limit of detection 5 ng/mL. Toxicokinetics animals were euthanized under carbon dioxide inhalation and cervical dislocation.

Anti product antibody was determined from the blood samples collected from all main animals and recovery animals at predose, days 1, 7, 16 and 30 (recovery only). Serum antibody titers for anti-AUX-1 and anti-AUX-2 antibodies was determined

Unique study design or methodology (if any): Nil

**Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.**

Mortality: Mortality was checked once daily.

Clinical signs: Clinical signs were noted once a day. Detailed clinical examination was done once a week and on the day of necropsy.

Body weights: The body weight was recorded before dosing, once a week and at necropsy.

Food consumption: Food consumption was recorded on a weekly basis starting before dosing and during the study.

Ophthalmoscopy: Not conducted

EKG: Not recorded

Hematology: Standard hematology and coagulation parameters were determined at necropsy from the main study and recovery animals. Blood samples were collected from the abdominal aorta of fasted animals.

Clinical chemistry: Standard blood chemistry parameters were determined at necropsy from the main study group and recovery animals.

Urinalysis: Urine samples were collected for 16 hours before the necropsy and standard urine chemistry parameters were determined.

Gross pathology: Surviving animals were euthanized under isoflurane anesthesia on the study day 16 and recovery day 30. Any gross external and internal changes were recorded and the organs were fixed for histopathology.

Organ weights (specify organs weighed if not in histopath table): Organ weights were recorded for following organs: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid and uterus.

Histopathology: Protocol specified tissues were fixed in 10% formalin for histopathological examinations. Protocol specified tissues from control (group 1), groups 2, 3, 4, 6 and 7, dead or moribund animals, gross lesions from main or recovery animals were examined for histopathological changes.

In addition to tissues from above groups, histological changes in the injection site and liver from group 5 and recovery animals were examined.

Tissues were stained by hematoxylin and eosin-phloxin.

Adequate Battery: yes ( x ), no ( )

Peer review: yes ( x ), no ( )

## Results

Mortality: Following animals were reported dead or sacrificed in moribund conditions:

Group	Dose	Batch	Male	Female	Observation
6	0.29 mg/dose	NFF-0035, (b)	600C, Dead, day 10	6502A, Moribund, day 9	Moribund animal showed deteriorating condition of the tail, histology showed hemorrhage in thymus
6	0.29 mg/dose	NFF-0035, (b)	6003B, Dead, day 10	6504B, Dead, day 11	Blood clot in abdominal cavity
6	0.29 mg/dose	NFF-0035, (b)		6514I, Dead, day 9 (recovery)	Thymus congestion
7	0.29 mg/dose	7280, Auxilium		7509E, Dead, day 10	Hemorrhage and hematoma in liver
7	0.29 mg/dose	7280, Auxilium		7511G, Moribund, 10	Moribund animal showed deteriorating condition of the tail

No adverse clinical signs were reported for above animals. However, blood clots, change in the color of liver and spleen was noted in the gross necropsy.

Clinical signs: Most of the clinical signs at 0.29 mg/dose for (b) (4) and Auxilium batches in groups 6 and 7 were related to injection site changes e.g. redness, dark skin and wound after week one of the study. These changes could be related to collagenase activity also.

Male:

Thin and blue fur was noted in treated animals from most of the groups. Swelling of the skin was noted in groups 1, 2, 3, 4, 5, 6 and 7 rats.

Female:

Skin swelling was noted in the control, groups 2, 3, 4, 5, 6 and 7

Since control animals also showed swelling of the skin, its relationship to the treatment with AA4500 is not known.

Body weights: Average body weight (g) data are shown below.

Day	Gr 1		Gr 2		Gr 3		Gr4		Gr5		Gr6		Gr7	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
N=10-15														
0,	422	272	429	262	416	279	424	273	424	273	419	272	434	269
7	423	268	431	262	414	275	422	273	421	272	416	274	436	270
14	429	266	436	263	425	279	428	273	427	275	423	277	440	270
Gain 0-14	7	-6	7	1	9	0	4	0	3	2	4	5	6	1
N=5, Recovery														
21	444	257	442	275	432	268	423	272	NR	NR	424	276	447	266
28	471	266	460	285	451	276	442	281	NR	NR	448	283	470	274
Gain 0-28	49	-6	31	23	35	-3	18	8			29	11	36	5

NR= no recovery animal, M= Male, F= Female

Based on above data, there was no treatment related change in the body weight.

Food consumption:

The average food consumption in grams on a weekly basis is shown below.

Male:

Day	Gr 1	Gr 2	Gr 3	Gr 4	Gr 5	Gr 6	Gr 7
1-8	177	191	177	190	177	173	197
8-5	195	203	202	199	196	185	209
15-22	219	217	206	205	-	197	229
22-29	234	217	214	205	-	214	234

- = no recovery animal

Female:

Day	Gr 1	Gr 2	Gr 3	Gr 4	Gr 5	Gr 6	Gr 7
1-8	133	129	141	141	136	140	139
8-15	135	135	149	146	144	145	142
15-22	135	147	163	149	-	149	140
22-29	147	155	163	151	-	153	140

Based on above data, there was no treatment related change in the food consumption.

Ophthalmoscopy: No ophthalmological examination was conducted during the treatment to evaluate the effect of the drug in eyes.

EKG: EKG was not recorded.

Hematology: Male rats did not show treatment related change in the hematology parameters. Female rats showed a slight and statistically significant decrease in the RBC and hemoglobin levels in the plasma in groups 6 and 7 at the end of day 16. However, these changes are biologically not significant because a similar data are often observed in the untreated animals in the published reference. Recovery animals did not show any hematological change. Average data are shown below.

Female rats:

Group	RBC, x 10 <sup>12</sup> /L	Hb, g/L	HCT, L/L
1	7.24	147	0.38
2	7.14	142	0.38
3	7.16	143	0.38
4	7.08	142	0.37
5	7.12	141	0.37
6	6.70*	135*	0.35*
7	6.63*	135*	0.36*

Coagulation data did not show any treatment related effect.

Above data suggest that collagenase activity of AA4500 did not impact the circulating blood cell counts.

#### Clinical chemistry:

Clinical chemistry data did not show any treatment related change on day 16 and day 30 with the exception of changes in the transaminase activity in male rats was noted on day 16 in groups 6 and 7 as indicated below.

Highest AST and ALT activity in control male rats was 148 and 58 U/L, respectively. Male # 6010F, group 6 showed 303 and 103 AST and ALT, respectively. Male # 7009E and 7010F, group 7 had AST 225 and 235 U/L, respectively. ALT for these rats was 172 and 163, U/L, respectively.

#### Urinalysis:

Urine chemistry data did not show treatment related changes when compared to the control.

Gross pathology: Gross pathology changes were noted in the liver and injection sites mostly in groups 6 and 7 animals as shown in the table. However, these changes were not completely reversible within next two weeks.

<b>Male</b>							
Observation	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Liver, mass						3	1
Recovery						3	3
Liver, raised area						4	1
Recovery						3	1
Injection site, dark					1		1
Recovery		1				3	
<b>Female</b>							
Liver, mass						2	2
Recovery							2
Liver, raised area						2	2
Recovery						2	2
Injection site, dark		1			1	2	2
Recovery						1	1

Organ weights: Absolute organ weight of male rats showed a slight but statistically significant increase in the average weight of liver in groups 6 and 7 animals. The maximum weight of liver in group 1 male (control) was 11.3 g and 2.4% of the body weight. Male rats in groups 6 and 7 those showed higher than 11.3 g are shown below.

Group 6	Liver (g), % BW	Group 7	Liver (g), % BW
6002A	11.8, 2.4%	7002A	12.2, 2.8%
6008E	13.7, 3.2%	7003B	11.7, 2.9%
6009F	13.07, 2.7%	7004B	12.3, 2.7%
6010F	13.03, 3.6%	7007D	11.9, 2.7%

Male rats in the recovery groups did not show any change in the weight of the liver due to the treatment.

Female rats did not show any treatment related change.

Histopathology: Adequate Battery: yes (x), no ( )—explain

Peer review: yes (x), no ( )

Histological changes were noted in the liver and injection site. Data for histological changes in the liver and injection site are shown in the table below.

Male rats:

Lesion	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Number of animal	10	10	10	10	10	10	10
Liver, inflammation	0	0	0	0	5, min	8, 3=min, 2=mild, 2=mod	7, 4=min, 2=mild, 1=mod
Recovery	0	0	0	0	-	4, 1=min, 3=mild	3=min
Hepatocellular necrosis	0	0	0	0	0	3, 2=min, 1=mod	3, 1=mild, 2=mod
Liver, fibrosis	0	0	0	0	0	4, 1=min, 3=mod	2, 1=mild, 1=mod
Recovery	0	0	0	0	-	5, 3=mod, 2=marked	3, 1=mild, 2=mod
Liver, bile duct hyperplasia	0	0	0	0	0	8, 5=min, 3=mild	6, min=5, 1=mild
Injection site perivascular edema	1, min	7, 6=min 1=mild	1,min	3, min	3, 2=min, 1=mod	0	5, 4=min, 1=mild
Injection site, perivascular	1, min	4, min	3, min	1, min	5, 2=min,	0	2, min

Lesion	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
inflammation					3=mild		
Injection site fibrosis	0	4, min	0	0	2, 1=mild, 1=mod	0	5, min
Recovery	0	0	1, min	0	-	4, 1=min, 3=mild	1, min
Injection site ulcer	0	0	0	0	1, mod	0	1, min

Min= minimal, mod= moderate

#### Summary of histopathology in male rats:

No hepatic lesions were noted at 0.029 mg protein/dose in the BTC, (b) (4) and Horsham Auxilium batches. However, inflammatory changes were noted in the liver starting 0.13 mg protein/dose. The severity of inflammatory changes in the liver progressed to show necrosis, fibrosis and bile duct hyperplasia at 0.29 mg protein/dose in both (b) (4) and Horsham batches. Injection site inflammation due to collagenase activity led to fibrosis, necrosis and ulceration at the injection site at the high dose (0.29 mg protein/dose). Inflammatory activity at the injection site could be ranked as BTC>Auxilium> (b) (4) batches based on histopathology at 0.029 mg protein/dose.

Liver toxicity and injection site inflammation was non-reversible.

Based on the toxicity profile, it was concluded that the Process 3 used for (b) (4) and Auxilium was compatible with respect to repeat dose toxicity in male rats.

#### Female rats:

Similar to the male rats, female rats also showed toxicity in the liver and injection site as shown in the table below.

Lesion	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Number of animal	10	10	10	10	10	11	11
Liver, inflammation	0	0	0	0	5, 3=min, 2=mild	7, 4=min, 3=mod	7, 4=min, 3=mild
Recovery	0	0	0	0	-	1, min	4, 3=min, 1=mild
Hepatocellular necrosis	0	0	0	0	0	4, 1=min, 2=mild, 1=mod	1, min
Liver, fibrosis	0	0	0	0	0	2, 2=mod	3, 2=mild, 1=mod
Recovery	0	0	0	0	-	2, mild	4, 1=min, 2=mod, 1=marked
Liver, bile duct hyperplasia	0	0	0	0	4, 3=min, 1=mild	7, 5=min, 2=mild	6, 4=min, 2=mild

Lesion	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Recovery	0	0	0	0	-	1, min	4, 3=min, 1=mild
Injection site perivascular edema	3, 2=min, 1=mild	7, 5=min, 2=mild	5, 5=min	1=min	6, 4=min, 2=mild,	7, 1=min, 3=mild, 2=mod, 1=marked	8, 2=min, 3=mild, 3=mod
Injection site, perivascular inflammation	4, min	9, min	1, min	2, min	5, 3=min, 2=mild	7, 3=min, 2=mild, 2=mod	9, 5=min, 4=mild
Recovery	1, min	1, min	1, min	0	-	1, min	1, min
Injection site fibrosis	0	0	0	1, min	2, min	3, 1=min, 2=mild	3, 1=mild, 2=mod
Recovery	3, min	3, 2= 2, 1=mild	3, 2=min, 1=mild	0	-	2, 1=min, 1=mod	3, 2=min, 1=mild
Injection site ulcer	0	0	0	0	1, mild	1, mild	1=min
Injection site, perivascular necrosis	0	0	0	0	0	3, 2=min, 1=mod	2, 2=mild

Min= minimal, mod= moderate

Above data suggest that minimal inflammation was present at the site of injections in the control and 0.029 mg protein/dose that could be related to the procedure. However, severity of inflammation at the injection site was increased at 0.13 to 0.29 mg protein/dose. With respect to ulcer, fibrosis, and necrosis of the injection site, both (b) (4) and Horsham batches were similar. BTC batch showed greater inflammatory changes than other two batches at 0.029 mg protein/dose.

Liver toxicity was also present at 0.13 and 0.29 mg protein/dose in female rats. Female rats showed greater toxicity in the liver than male rats.

Liver toxicity and injection site inflammation was non-reversible.

Based on the histopathology data, both (b) (4) and Horsham batches were comparable in male and female rats. The BTC batch showed higher incidences of inflammation at the site of injection at 0.029 mg protein/dose.

#### Toxicokinetics:

The average plasma levels of AUX-1 on day 1:

Group	T $\frac{1}{2}$ , hr	C <sub>max</sub> , ng/mL	AUC t <sub>0</sub> -t <sub>24</sub> , ng.hr/mL
1			
2		212	ND
3		147	ND
4		164	ND
5		1454	ND

6	0.10	7075	1831
7		8250	ND

ND, insufficient data to calculate, The quantitation limit was 5 ng/mL

Majority of samples were below the limit of detection by 30 min post dose. There was no gender difference in the kinetics and the pooled data were shown above. Due to a rapid disappearance of AUX-1 in the plasma, kinetic parameters could not be determined on days 7 and 15. Based on the kinetic data and undetectable levels for quantitation, AUX-1 could not be distinguished between BTC, (b) (4) and Auxilium processes when kinetic parameters were used.

#### AUX-2:

AUX-2 plasma levels could be detected between one to two hours due to slow degradation of the product compared to AUX-1 and the PK parameters could be estimated for AUC. The half-life for most of batches was between 0.3 to 0.55 hrs. The calculated exposure (AUC ng.hr/mL) on day 1 and 7 are shown below.

Group	Day 1	Day 7
1	ND	ND
2	119	ND
3	225	158
4	172	155
5	1238	1054
6	4710	2654
7	4175	2294

The applicant stated that there was insufficient data to calculate AUC on day 15.

Summary of PK data: AA4500 as AUX-1 was degraded within 30 min after the IV administration. Therefore, exposure could not be calculated.

BTC batch showed lower plasma exposure when AUC data at 0.029 mg/dose for AUX-2 was used. Both (b) (4) and Auxilium batches at 0.29 mg/dose were comparable. The high dose showed lower rate of degradation and higher exposure than the low dose.

#### Other:

##### Antigenicity:

Male and female rats developed anti-product antibodies to AUX-1 treatment. Since AUX-1 exposure in rats was limited, the reviewer attempted to find out if there was a relationship between liver toxicity and AUX-2 antibody.

Anti-product antibodies to AUX-2 were detected on days 7, 16 and on day 30 in recovery animals. Following table summarizes the relationship between the liver toxicity and anti-product antibody formation to AUX-2 in rats:

Male rats:

<b>Group</b>	<b>Animal#</b>	<b>Day sacrifice</b>	<b>Liver histology, major finding</b>	<b>AUX-2 antibody</b>
6	6008E	16	Fibrosis and necrosis	Positive on days 7, 16
6	6009F	16	Bile duct hyperplasia	Positive on day 16
6	6010F	16	Liver fibrosis and necrosis	Positive on days 7, 16
6	6007D	16	Liver fibrosis	Positive on days 7 and 16
6	6006C	10 (Found Dead)	No abnormal liver histology	Positive on day 7
6	6005C	16	Bile duct hyperplasia	Positive on day 16
6	6004B	16	Bile duct hyperplasia	Positive on days 7 and 16
6	6003B	10 (Found dead)	No abnormal liver histology	Negative on day 7
6	6002A	16	Bile duct hyperplasia	Positive on day 16
6	6001A	16	Fibrosis and necrosis of liver	Positive on days 7 and 16
7	7001A	16	Bile duct hyperplasia	Positive on day 16
7	7002A	16	Minimal mixed cell infiltrate in liver	Positive on days 7 and 16
7	7003B	16	Fibrosis and necrosis of liver	Positive on days 7 and 16
7	7004B	16	Min mixed cell infiltrate in liver	Positive on day 16
7	7005C	16	Bile duct hyperplasia	Positive on days 7 and 16
7	7006C	16	No serious liver finding	Positive on days 7 and 16
7	7007D	16	Fibrosis and necrosis	Positive on days 7 and 16
7	7008D		Bile duct hyperplasia	Positive on days 7 and 16
7	7009E	16	Hyperplasia bile duct	Positive on days 7 and 16
7	7010 F	16	necrosis	Positive on days 7 and 16

Above data in male rats in groups 6 and 7 suggest that most of rats with hepatotoxicity e.g. necrosis, fibrosis and bile duct hyperplasia had positive AUX-2 antibodies on days 7 and 16. However, few animals that died before the terminal sacrifice did not show above liver lesions despite the presence of antibodies. Necropsy data from these animals showed fragile liver conditions. These data suggest that the liver injury could have resulted from the exposure to AUX-2 or its antibodies towards the terminal phase of the treatment. However, animals in groups 2, 3 and 4 also showed positive titers in the absence of serious liver histology. Therefore, AA4500 induced liver damage could be due to the collagenase activity of the drug substance.

Female rats:

<b>Group</b>	<b>Animal #</b>	<b>Day sacrifice</b>	<b>Liver histology, major finding</b>	<b>AUX-2 antibody</b>
5	5501A	16	Hyperplasia of bile duct	Positive on days 7 and 16
5	5502A	16	No major finding	Positive on days 7 and 16
5	5503B	16	No major finding	Positive on days 7 and 16
5	5504B	16	No major finding	Positive on days 7 and 16
5	5505C	5505C	No major finding	Negative on day 7 and positive on day 16
5	5506D	16	Hyperplasia of bile duct	Positive on days 7 and 16
5	5507E	16	Hyperplasia of bile duct	Positive on days 7 and 16
5	5508E	16	No major finding	Positive on days 7 and 16
5	5509F	16	Bile duct hyperplasia	Negative on day 7 and positive on day 14
6	6501A	16	Bile duct hyperplasia	Positive on days 7 and 16
6	6502A	9 (moribund sacrifice)	No major finding	Negative on day 7
6	6503B	16	Hyperplasia of bile duct	Positive on days 7 and 16
6	6504B	11 (found dead)	No major finding	Negative on day 7
6	6505C	16	Fibrosis and necrosis of liver	Positive on days 7 and 16
6	6506C	16	Hyperplasia of bile duct and necrosis	Negative on day 7 and positive on day 16

6	6507D	16	Hepatocellular fibrosis and necrosis	Positive on days 7 and 16
6	6508E	16	Hyperplasia of bile duct and necrosis	Positive on days 7 and 16
6	6509F	16	Bile duct hyperplasia	Positive on days 7 and 16
6	6510F	16	No serious liver finding	Positive on days 7 and 16
7	7501A	16	Not serious liver finding	Positive on day 16
7	7502A	16	Fibrosis of liver	Positive on days 7 and 16
7	7503B	16	Necrosis of liver	Positive on days 1 and 16, no data for day 7
7	7504B	16	Bile duct hyperplasia	Day 7 positive, day 16 negative
7	7505C	16	Bile duct hyperplasia	Positive on days 7 and 16
7	7506C	16	No serious liver finding	Positive on day 16
7	7507D	16	Fibrosis of liver	Positive on day 16
7	7508D	16	Bile duct hyperplasia	Positive on days 7 and 16
7	7509E	10 (Found dead)	No serious liver finding	Positive on day 7
7	7510F	16	No serious liver finding	Negative on day 7 and positive on day 16

Animals in groups 2, 3 and 4 have also shown positive titers for anti-AUX-2 in the absence of liver lesions. Above data in the female rats were similar to that observed for male rats in relation to the role of AA4500 on liver toxicity.

Titers for AUX antibodies were increased to a highest level by day 30 and there was no separation on the antigenicity to AA4500 for BTC, (b) (4) and Auxilium batches of AA4500.

#### Summary of the study:

A toxicokinetic study was conducted in rats at once every 48 hour IV injections of BTC, (b) (4) and Auxilium batches of clostridial collagenase. The comparative study was conducted at 0.029 mg protein/dose. No systemic toxicity was noted at this dose. Therefore, comparability of 3 sources of collagenase could not be determined at this dose if toxicity to the drug product was compared as the end point. The study was extended using 0.29 mg protein/dose of (b) (4) and Auxilium batches of AA4500 for comparability in the toxicity study. Both batches are considered to be comparable based on toxicity data.

From the toxicity point of view, 0.29 mg protein/dose of AA4500 is toxic to liver. Several rats died at this dose. Both AUX-1 and AUX-2 antibodies in the serum were detected. Based on the TK data, it appeared that the liver toxicity was related to the collagenase-2 activity. Injection site reactions were noted that could be due to collagenase activity as well as the procedure.

NOAEL for systemic toxicity was 0.029 mg protein/dose. Liver was the target organ of toxicity. However, injection site inflammation is expected at all doses tested.

**Study title: A 16-day, multiple-dose, intravenous toxicity study in rats comparing AA4500 with BTC collagenase and placebo.**

This study is similar to one reviewed above. The findings of the present study are summarized below.

**Key study findings:** (b) (4) and BTC batches of Clostridial collagenase did not show systemic toxicity up to 0.029 mg protein/dose. However, injection site hemorrhage and inflammation was noted in the control and treated animals. AUX-1 and AUX-2 antibodies were detected at necropsy.

**Study no.:** (b) (4);00006

**Module # 1, and page #:** 1

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** July 3, 2006

**GLP compliance:** Yes

**QA report:** yes (x) no ( )

**Drug, lot #, and % purity:**

(b) (4) AA4500 (0.8 mg/vial), Lot # NFF-0035, potency AUX-1 was 0.91, AUX-2 was 0.81, Reconstituted batch # CTL2006#08500, potency for AUX-1 was 2884 units/mg protein and AUX-2 was 30674 units/mg.

BTC Batch, Lot #992-7, potency for AUX-1 was 0.86 and AUX-2 was 1.02

**Methods**

Doses: The study design is shown below.

## Experimental Design for the Toxicity and Toxicokinetic Phases

Group No.	No. of Animals				Dose Material	Dose Level (mg/dose) <sup>a</sup>	Dose Volume (mL/dose)	Dose Concentration (mg/mL)
	Toxicity		Toxicokinetics					
	Males	Females	Males	Females				
1	10	10	--	--	Placebo	0	0.5	0.0
2	10	10	9	9	AA4500	0.0029	0.5	0.0058
3	10	10	9	9	AA4500	0.0087	0.5	0.0174
4	10	10	9	9	AA4500	0.029	0.5	0.058
5	10	10	9	9	BTC Collagenase	0.029	0.5	0.058

<sup>a</sup>The dose levels selected for AA4500 are multiples (1x, 3x, and 10x) of the intended human dose by body weight. The dose level selected for BTC Collagenase is 10 times of the intended human dose by body weight.

Groups 4 and 5 represent the low dose of (b) (4) and BTC batches for the study # 1007-1671 already reviewed above. The applicant stated that the high dose of 0.029 mg/dose was considered to be 10x higher than the intended human dose (0.0029 mg/dose) as mg/kg. The applicant indicated that the high dose was intended for some toxic effects so that both (b) (4) and BTC batches could be compared.

Species/strain: Male and female Sprague Dawley rats

Number/sex/group or time point (main study): See study design above

Route, formulation, volume, and infusion rate: IV bolus injections Q48 hours for 16 days. A total of 8 injections were made into the lateral tail vein.

Satellite groups used for toxicokinetics or recovery: TK groups were allotted, recovery group was not added in the study.

Age: 8 Weeks at randomization (day -1)

Weight: 226-251 g (male), 177-201 g (female)

Sampling times: Blood samples for TK were collected from 3 animals/sex/group at each time point. Samples were collected up to 8 hours post dose on days 1 and 15 from jugular vein or orbital plexus under isoflurane anesthesia. Standard parameters of clinical pathology and urine chemistry were determined at necropsy.

Unique study design or methodology (if any): Serum antibody to the drug product was determined for immunogenicity before necropsy on days 17/18.

**Observations and times: The experimental procedures for the study were similar to that reviewed above under study # 1007-1671 and only results of the present study is summarized. The high dose of this study for both (b) (4) and BTC batches was also included in study # 1007-1671 reviewed above.**

## Results

The analysis of the dosing solutions showed degradation on storage. However, freshly prepared stocks and diluted solutions were used for the study and confirmed the required concentrations.

Mortality: No treatment related mortality was reported. Injection site discoloration was noted at 0.029 mg/dose (male and female) in groups 4 and 5. Some female rats in group 4 also showed injection site discoloration.

Body weight and food consumption:

There was no treatment-related change in the body weight and food consumption.

Clinical pathology: Hematology and coagulation parameters did not show any treatment related change. Clinical chemistry data showed a slight increase in the albumin and globulin levels in groups 4 and 5 female rats. However, biological significance of the change is not known. Data from the sponsor's table are shown below.

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TABLE 7

A 16-DAY, MULTIPLE-DOSE, INTRAVENOUS TOXICITY STUDY IN RATS

			SUMMARY OF CLINICAL CHEMISTRY DATA				FEMALES
GROUP:			1	2	3	4	5
LEVEL (MG/ECGSE):			0	0.0029	0.0067	0.029	0.029
ALBUMIN							
		G/DL					
DAY	17/18	MEAN	3.25 d	3.34	3.23	3.38	3.47*
		S.D.	0.183	0.176	0.186	0.146	0.147
		N	10	10	10	10	10
		% deviation vs. control		2.6	-0.7	3.8	6.6
GLOBULIN							
		G/DL					
DAY	17/18	MEAN	2.75 d	2.80	2.94	3.07**	3.08**
		S.D.	0.164	0.186	0.224	0.183	0.190
		N	10	10	10	10	10
		% deviation vs. control		4.6	6.2	11.0	11.3

Urinalysis: No treatment-related change in the urine chemistry was noted.

Gross pathology: No treatment-related change in the systemic organs was noted in male and female rats other than macroscopic changes in the injection site reported as clinical observations.

Organ weights (specify organs weighed if not in histopath table): Absolute organ weight data did not show any treatment related change.

Histopathology: Adequate Battery: yes (x), no ( )—explain  
 Peer review: yes ( ), no (x)

Injection site peri-vascular hemorrhage was noted in groups 4 and 5 animals at higher incidence than other groups. Injection site inflammation was also noted in the control and treated animals. Therefore, the injection site inflammation and hemorrhage was partly due to the procedure. No other treatment related histological change was noted up to 0.029 mg/dose in male and female animals.

#### Toxicokinetics:

The limit of detection was 2.5 ng/mL for AUX-1 and 4.08 for AUX-2, respectively. AUX-1 and AUX-2 levels were determined on days 1 and 15. AUX-1 was measured by an ELISA assay and AUX-2 was measured by a radio-immuno-assay. Both methods were validated.

Detectable levels of AUX-1 and AUX-2 were not achieved at 0.0029 mg protein/dose on day 1. However, detectable levels of AUX-1 and AUX-2 were present at 0.029 mg/dose only within one-hour sampling time on day 1.

On day 15 of the study, AUX-1 levels were undetectable or below the limit of detection at all doses as it was degraded or removed from the circulation. However, AUX-2 levels were detected at 0.0087 and 0.029 mg/dose. AUX-2 from AA4500 (b) (4) and BTC collagenase accumulated in the plasma after repeated administration. The applicant indicated that the degradation for AUX-2 could be slower than AUX-1. The AUX-2 exposure in male and female rats on days 1 and 15 is shown below from the applicant's table. From the PK point of view, it is difficult to compare (b) (4) and BTC bathes at 0.029 mg/dose due to a lower level of exposure of AUX-2 in male rats compared to female rats on day 15. However, the toxicity point of view both batches were relatively devoid of systemic toxicity.

Auxilium Pharmaceuticals, Inc.  
 AUX-CC-PC02  
 Table 4: AUX-II/ABC-II - AUC

Gender	DAY	Dose		AUC
		Group	Cavg	
Female	1	AUX0029	0.09	0.68
		AUX0087	2.94	22.77
		AUX0290	12.12	93.91
		BTC0290	2.36	18.28
Female	15	AUX0029	485.05	11641.14
		AUX0087	1248.36	29960.71
		AUX0290	955.34	22928.08
		BTC0290	1603.60	38486.48
Male	1	AUX0029	0.08	0.63
		AUX0087	1.85	14.34
		AUX0290	9.92	76.86
		BTC0290	3.35	25.97
Male	15	AUX0029	37.02	888.53
		AUX0087	440.90	10581.89
		AUX0290	593.52	14244.58
		BTC0290	216.58	5197.98

Other: Immunogenicity to AUX-1 and AUX-2 at the end of dosing period showed antibody formation as shown in a table and graphically from the applicant's submission below.

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Analysis Variable : TITER AUX-1/ABC-I Antibody Titer

Gender	Dose	Day	N		Mean	Median	Std Dev	Minimum	Maximum
			Normal	Obs					
Female	Placebo	17	5	5	0.0	0.0	0.0	(b) (4)	
		18	5	5	0.0	0.0	0.0		
	AUX0029	17	5	5	17951.1	4070.0	32266.5		
		18	5	5	5761.0	4100.0	4758.7		
	AUX0007	17	5	5	27940.0	17400.0	26999.2		
		18	5	5	18522.0	10800.0	15446.7		
	AUX0090	17	5	5	21474.0	22800.0	12143.2		
		18	5	5	40440.0	31100.0	23959.9		
	BTC0090	17	5	5	18960.0	14600.0	11038.0		
		18	5	5	29910.0	31100.0	19215.2		
	Male	Placebo	17	5	5	0.0	0.0		0.0
			18	5	5	0.0	0.0		0.0
AUX0029		17	5	5	1534.0	1080.0	1678.7		
		18	5	5	5133.0	786.0	8650.1		
AUX0087		17	5	5	16770.4	11700.0	15158.2		
		18	5	5	19566.0	6750.0	25100.6		

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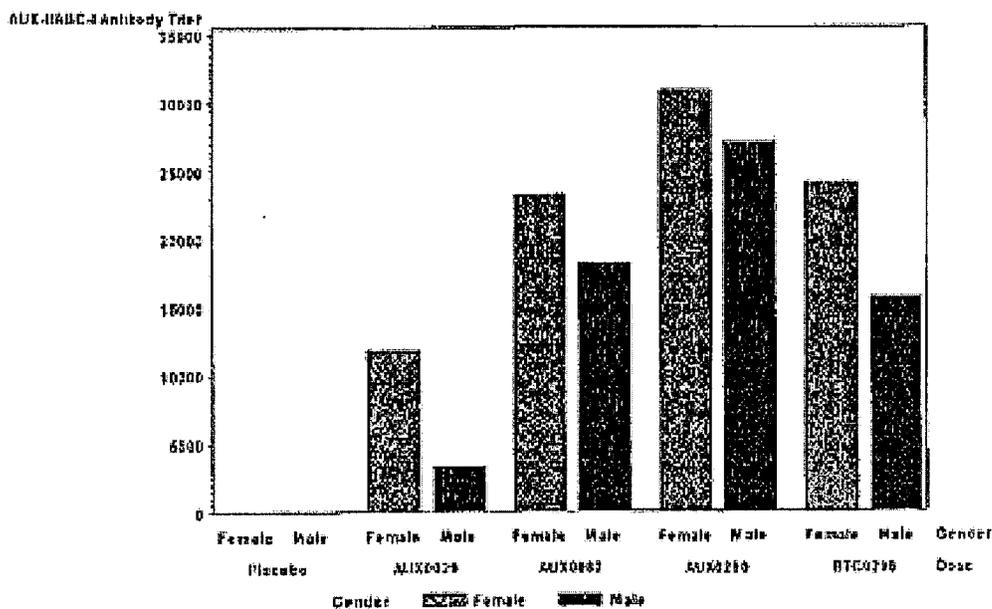
Analysis Variable : TITER AUX-1]/ABC-1] Antibody Titer

Gender	Dose	Day	N		Mean	Median	Std Dev	Minimum	Maximum
			Normal	Obs					
Male	AUX0290	17	5	5	23870.0	15800.0	21182.3	(b) (4)	
		10	5	5	30272.0	22500.0	34042.5		
	BTC0290	17	5	5	22072.0	30200.0	13266.2		
		10	5	5	9210.0	9700.0	6423.8		

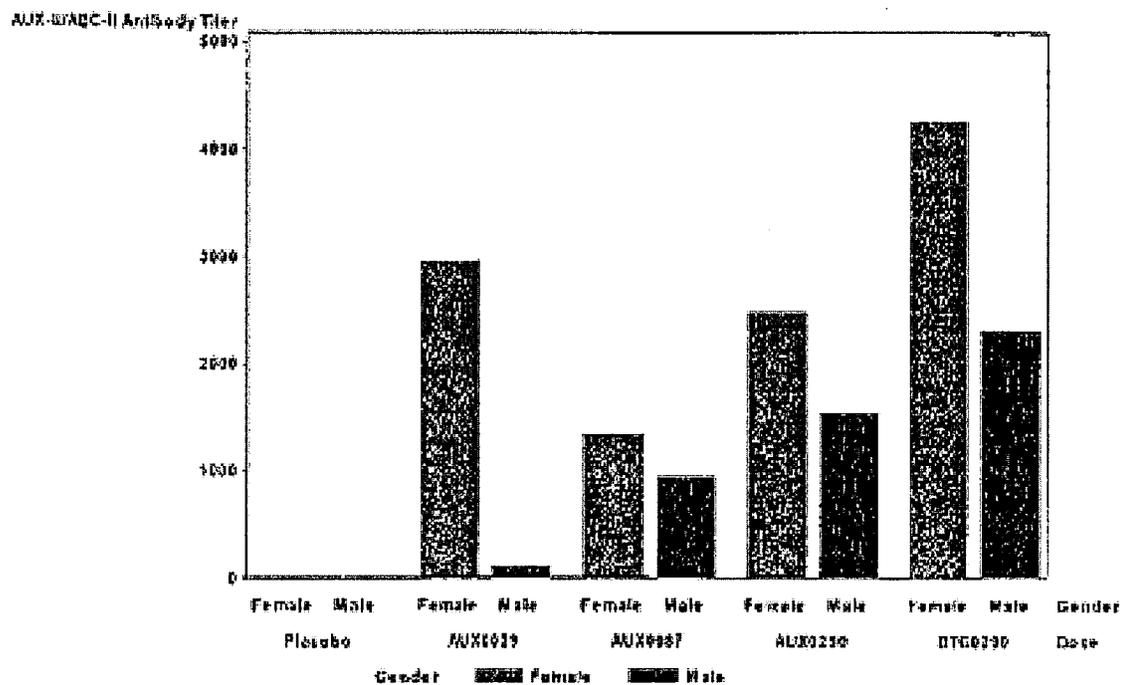
Analysis Variable : TITER AUX-1]/ABC-1] Antibody Titer

Gender	Dose	N		Mean	Median	Std Dev	Minimum	Maximum
		Obs	N					
Female	Placebo	10	10	0.0	0.0	0.0	(b) (4)	
	AUX0029	10	10	2959.3	489.6	7083.4		
	AUX0087	10	10	1347.8	1190.0	882.8		
	AUX0290	10	10	2486.0	2385.0	1045.5		
	BTC0290	10	10	4245.1	2340.0	5657.6		
Male	Placebo	10	10	0.0	0.0	0.0	(b) (4)	
	AUX0029	10	10	110.8	81.0	103.7		
	AUX0087	10	10	946.0	783.5	755.1		
	AUX0290	10	10	1541.6	1530.0	501.7		
	BTC0290	10	10	2313.7	1950.0	2057.6		

**Figure 1 Mean Titer Levels for Antibodies to AUX-I/ABC-I**



**Figure 2 Mean Titer Levels for Antibodies to AUX-II/ABC-II**



The table indicated that serum titer levels for AUX-1 antibodies was greater than AUX-2 antibodies although AUX-1 level was lower than AUX-2 in the plasma. The discrepancy of differences is not known.

Data indicated that antibodies to both AUX-1 and AUX-2 were present at the end of 16 days of treatment with AA4500 (b) (4) and BTC Clostridial collagenase.

### Summary of the study:

Both (b) (4) batch of AA4500 and BTC batch of clostridial collagenase did not show any systemic toxicity up to 0.029 mg protein/dose for 16 days. However, injection site hemorrhage and inflammation was present that could be related to the procedure and partly due to collagenase activity. No mortality and systemic toxicity was detected in the study. NOEL for systemic toxicity was 0.029 mg protein/dose. Pharmacokinetic data showed AUX-1 was transiently detectable on days 1 and 15. However, AUX-2 exposure data showed accumulation on day 15. Antibody titers were present for anti-AUX-1 and anti-AUX-2 antibody.

Study results were similar to that reviewed for study #1007-1671 except the high dose for this study was lower than the high dose used for #1007-1671.

### Histopathology inventory (optional)

Study	16-day, #1007-1671
Species	Rat
Adrenals	X
Aorta	X
Bone Marrow smear	X
Bone (femur)	X
Brain	X
Cecum	X
Cervix	
Colon	X
Duodenum	X
Epididymis	X
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	
Gross lesions	
Harderian gland	
Heart	X
Ileum	X
Injection site	X
Jejunum	X
Kidneys	X
Lachrymal gland	
Larynx	

Liver	X
Lungs	X
Lymph nodes, cervical	
Lymph nodes mandibular	X
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	
Optic nerves	X
Ovaries	X
Pancreas	X
Parathyroid	
Peripheral nerve	
Pharynx	
Pituitary	X
Prostate	X
Rectum	X
Salivary gland	X
Sciatic nerve	X
Seminal vesicles	X
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X
Sternum	X
Stomach	X
Testes	X
Thymus	X
Thyroid	X
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X
Vagina	X
Zymbal gland	

X, histopathology performed

The local toxicity to AA4500 in dog penis was submitted under IND (b) (4) and reviewed by Dr. Yangmee Shin, Pharmacologist, HFD-580, or (b) (4). The review was signed off by Pharmacology supervisor on (b) (4). The copy of the review is presented below. The final study report was submitted to the BLA and there were no significant changes noted in the cover letter to the study.

**Study title: Local Toxicity Study of AA4500 Injected into Dog Penis (audited draft)**

Key study findings:

Study no.: #520

Conducting laboratory and location: (b) (4)

Date of study initiation: 8/18/07

GLP compliance: yes

QA report: yes (x) no ( )

Drug, lot #, and % purity: AA4500, #NFF-0035 (Process 3), 99.6-100%

Methods

Doses: 0, 0.8, 2.5, 8.3/6.1, 14.9 ug/kg

Species/strain: Male beagle dogs from (b) (4)

Number/sex/group or time point (main study): Groups 1, 5, 8, 11 and 14 were comprised of 3 dogs, which received only control article and served as a procedural control. Groups 1 through 4 were administered 3 injections over a week for 3 dosing cycles (with 3 weeks between treatment cycles, an acceleration of the proposed clinical schedule of six weeks between treatment cycles). Treatment days were thus Study Days 1, 3, 5, 29, 31, 33, 57, 59 and 61. Animals in groups 5-16 received only a single injection before termination. Two dogs from group 1 and four dogs from groups 2-4 were euthanized for necropsy approximately 24 hours following their last scheduled treatment. One dog from groups 5, 8, 11 and 14, and 3 dogs from groups 6, 7, 9, 10, 12, 13, 15 and 16 were euthanized for necropsy approximately 48 hours following their last scheduled treatment. Two dogs in groups 2-4, 6, 7, 9, 10, 12, 13, 15 and 16, and 1 dog in groups 1, 5, 8, 11 and 14 were retained for recovery assessment 28 days following their last scheduled treatment. The study design is outlined in the following table:

Group Number	Groups	Number of Animals	Treatment Site(s)	Dose (µg/kg)	Dose Volume (µL/kg)	No. of Injections	Number for Necropsy (Last Scheduled Dose)	Number for Necropsy (Recovery)
1	Control	3	Tunica Albuginea	0	3.6	3/week for 3 cycles	2	1
2	Low Dose	6		0.8	0.3	3/week for 3 cycles	4	2
3	Mid Dose	6		2.5	1.1	3/week for 3 cycles	4	2
4	High Dose	6		8.3/6.1*	3.6/2.6*	3/week for 3 cycles	4	2
5	Acute Control	3		0	6.4	1	2	1
6	Acute Low	5		8.3	3.6	1	3	2
7	Acute High	5		14.9	6.4	1	3	2
8	Acute Control	3	Corpus Cavernosum	0	6.4	1	2	1
9	Acute Low	5		8.3	3.6	1	3	2
10	Acute High	5		14.9	6.4	1	3	2
11	Acute Control	3	VAN Complex	0	6.4	1	2	1
12	Acute Low	5		8.3	3.6	1	3	2
13	Acute High	5		14.9	6.4	1	3	2
14	Acute Control	3	Urethra	0	6.4	1	2	1
15	Acute Low	5		8.3	3.6	1	3	2
16	Acute High	5		14.9	6.4	1	3	2

\* Dose level reduced because local effects precluded repeated dosing of the majority of the animals during the first dosing cycle.

Route, formulation, volume, and infusion rate: Intralesional, 0.03% CaCl<sub>2</sub> in 0.9% NaCl.

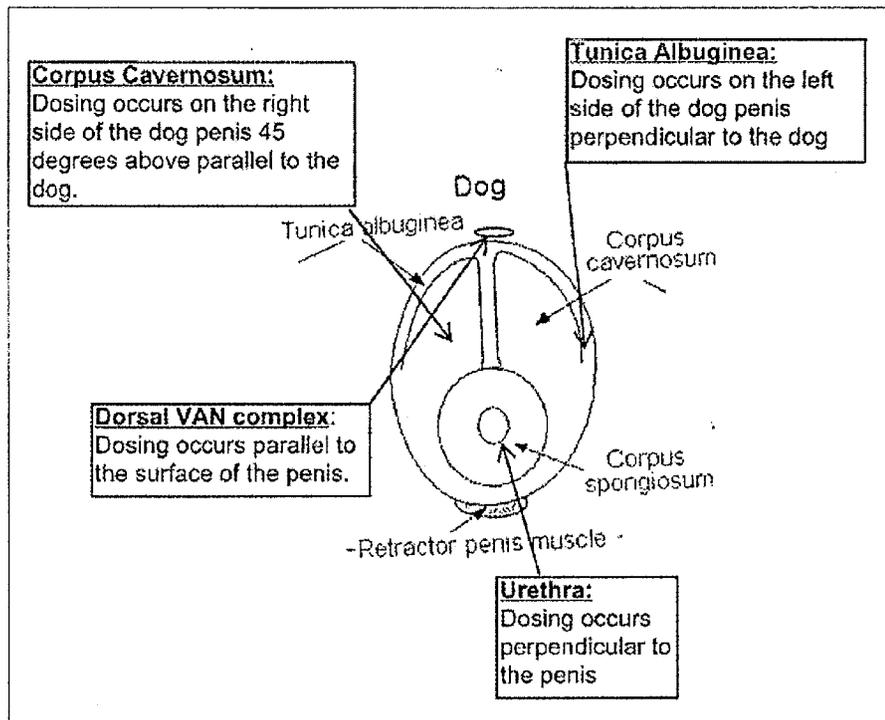
Satellite groups used for toxicokinetics or recovery: See Table above

Age: 8-14 months

Weight: 6.1-9.6 kg

Unique study design or methodology: Under general anesthesia (Domitor 0.03 mg/kg and ketamine 3 to 5 mg/kg given IV), the penis of each dog, proximal to the os penis, was exposed, measured with calipers, and a single dose of AA4500 was injected into or around the anatomic sites specified (tunica albuginea, corpus cavernosum, vein-artery-nerve complex and urethra). All injections were given using a 27 gauge, ½ inch needle and an appropriately sized microliter Hamilton syringe. The needle was held in place for approximately 30 seconds before it was removed. Following withdrawal of the needle from all dosing sites, manual pressure was applied to the penis for 30 seconds to ensure hemostasis and to prevent extravasation of AA4500 from the

injection site. Antisedin (5 mg/mL; equal v/v with Domitor) was administered intramuscularly to the dogs following the dosing procedure to reverse the effects of the Domitor anesthesia. Dosing procedures for each site are depicted in Figure below:



**Observation and times:**

Observations	Times
Mortality	Twice daily
Clinical signs	Once daily at post-dosing
Body weights/Food consumption	Pre-study & weekly thereafter
Water intake	Not conducted
Ophthalmology	Not conducted
Hemodynamics/ECG	Not conducted
Hematology/Clinical chemistry/Coagulation/Urinalysis	Pre-study, last dose & at end of recovery
Antibody formation (ECL method)	Pre-study, prior to last dose & at end of recovery for groups 1-4
Pathology (organ weights, gross/histopathology, injection site)	At 48 hrs post-dosing (acute) & end of recovery (acute/repeat)
Toxicokinetics	0, 0.08, 0.5, 1, 2, 4, 8 & 24 hrs post-dosing on Day 1 in Groups 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 & 16 or on Day 61 after 9 <sup>th</sup> dose in Groups 1, 2, 3 & 4

**Results:**

Mortality: one at 2.5 µg/kg euthanized prematurely on day 8 after exhibiting decreased activity, lethargy and significant weight loss due to excessive local reactions at the injection site following 2 doses (painful prepuce and surrounding area, full thickness wound with purulent pink-colored discharge in the area extended into musculature and subcutaneous tissue adjacent to left side of the penis/prepuce)

Clinical signs:**Repeated dosing:***Tunica albuginea*

- increased incidence and/or persistence of discoloration, bruising of the prepuce/penis/inguinal skin and thin appearance at  $\geq 2.5$   $\mu\text{g}/\text{kg}$
- increased incidence/severity and/or persistence of swelling of the penis at  $\geq 0.8$   $\mu\text{g}/\text{kg}$ , resulting in a decrease to 6.1  $\mu\text{g}/\text{kg}$  prior to the 2<sup>nd</sup> cycle (precluded dosing in 3/6 dogs on day 3 and 4/6 dogs on day 5 at  $\geq 8.3$   $\mu\text{g}/\text{kg}$ ); precluded dosing in 2/6 dogs on days 31 and 33 at 6.1  $\mu\text{g}/\text{kg}$  in the 2<sup>nd</sup> dosing week, but all receiving all doses in the 3<sup>rd</sup> week
- thin appearance and masses on the penis/prepuce/bulbus on days 15-23 and 29 for one dog (blood on the opening penis, scab on the penis, an underlying red spot and swelling), and on days 5, 8-13 and 15 for the other at 8.3/6.1  $\mu\text{g}/\text{kg}$

**Acute dosing:***Tunica albuginea*

- increased incidence/severity and/or persistence of discoloration of penis/prepuce and swelling of penis at  $\geq 8.3$   $\mu\text{g}/\text{kg}$
- increased severity of bruising at 14.9  $\mu\text{g}/\text{kg}$

*Corpus cavernosum*

- increased incidence/severity and persistence of discoloration of penis/prepuce at  $\geq 8.3$   $\mu\text{g}/\text{kg}$ , extending into scrotum at 14.9  $\mu\text{g}/\text{kg}$
- increased severity and persistence of swelling of penis at  $\geq 8.3$   $\mu\text{g}/\text{kg}$
- a mass in the bulbus area of penis in one animal starting on day 12 until the necropsy at 14.9  $\mu\text{g}/\text{kg}$

*VAN complex*

- increased incidence and persistence of discoloration of penis/prepuce and swelling of penis at  $\geq 8.3$   $\mu\text{g}/\text{kg}$
- increased incidence and/or severity of bruising at  $\geq 8.3$   $\mu\text{g}/\text{kg}$

*Urethra*

- increased severity and persistence of discoloration of penis/prepuce at  $\geq 8.3$   $\mu\text{g}/\text{kg}$ , affecting inguinal skin and scrotum in one animal at 14.9  $\mu\text{g}/\text{kg}$
- increased incidence/severity and persistence of swollen penis at  $\geq 8.3$   $\mu\text{g}/\text{kg}$
- increased incidence/severity of bruising at  $\geq 8.3$   $\mu\text{g}/\text{kg}$

Body weights: unremarkable

Food consumption: unremarkable

Ophthalmoscopy: not conducted

EKG: not conducted

Hematology: statistically significant increase in leukocytes, attributable to increased neutrophils and monocytes in the 14.9 µg/kg VAN complex group, which was reversible during recovery

Clinical chemistry: unremarkable

Urinalysis: unremarkable

Gross pathology:

- thick/red pigmentation correlated with subcutaneous hemorrhage in inguinal skin and lymph node
- red pigmentation correlated with sinus erythrocytosis in inguinal lymph node
- enlarged inguinal lymph node correlated with lymphoid hyperplasia
- small thymus correlated with atrophy

Organ weights (adrenal glands, brain, epididymis, heart, kidneys, liver, lungs, pituitary gland, prostate gland, salivary glands, spleen, testes, thymus, thyroid/parathyroid glands): unremarkable

Histopathology: Adequate Battery: yes (x), no ( )—explain

Peer review: yes (x), no ( )

The following tissues were processed for histologic evaluation:

Dose Groups	Injection Site	Tissues Examined	
		Main Study	Recovery
1, 2, 3, 4	Tunica albuginea (repeat dose)	All tissues	Injection site (penis) Inguinal lymph node All target tissues All gross lesions
5, 6, 7	Tunica albuginea (single dose)	Injection site (penis) Inguinal lymph node All target tissues All gross lesions	Injection site (penis) Inguinal lymph node All target tissues All gross lesions
8, 9, 10	Corpus cavernosum	Injection site (penis) Inguinal lymph node All target tissues All gross lesions	Injection site (penis) Inguinal lymph node All target tissues All gross lesions
11, 12, 13	VAN complex	Injection site (penis) Inguinal lymph node All target tissues All gross lesions	Injection site (penis) Inguinal lymph node All target tissues All gross lesions
14, 15, 16	Urethra	Injection site (penis) Inguinal lymph node All target tissues All gross lesions	Injection site (penis) Inguinal lymph node All target tissues All gross lesions

**Repeated dosing**: see the following tables for details

*Tunica albuginea*

Systemic lesions:

- chronic perivascular inflammation in brain/spinal cord and heart at 8.3/6.1 µg/kg
- eosinophilic casts in kidney at 8.3/6.1 µg/kg
- chronic inflammation in salivary gland, pancreas and prostate at 8.3/6.1 µg/kg
- testes degeneration at 8.3/6.1 µg/kg
- increased incidence of crypt dilatation of duodenum at  $\geq 2.5$  µg/kg

Local lesions:

- increased severity of adventitial hemorrhage and neovascular proliferation attributable to the injection procedure
- adventitial chronic-active inflammation and extension of hemorrhage and/or chronic-active inflammation into the adjacent preputial/inguinal subcutaneous tissues at  $\geq 0.8$  µg/kg (partially reversible)
- increased incidence and severity of sinus erythrocytosis in inguinal lymph node at  $\geq 2.5$  µg/kg secondary to hemorrhage at the injection site (partially reversible)
- pigmented macrophages in inguinal lymph node at 8.3/6.1 µg/kg secondary to hemorrhage at the injection site (partially reversible)

Acute dosing:

*Tunica albuginea*

Systemic lesions: unremarkable

Local lesions:

- increased incidence and severity of inguinal/adventitial hemorrhage (partially reversible at 14.9 µg/kg)
- collagen lysis consisting of focal disruption of dense collagen fibers of advential surface of tunica albuginea with decreased staining/fiber diameter present in one animal at 8.3 µg/kg (not extending through the full thickness of the tunica in trichrome sections)
- sinus erythrocytosis of inguinal lymph node in all treated groups, reflecting the presence of hemorrhage at the injection site

*Corpus cavernosum*

Systemic lesions: unremarkable

Local lesions:

- increased incidence and/or severity of subcutaneous hemorrhage in inguinal skin, stromal edema, stromal/adventitial necrosis and acute/subacute inflammation at  $\geq 8.3$  µg/kg
- replacement of chronic-active inflammation with chronic inflammation and accumulation of pigmented macrophages and hematoma formation, indicative of progression towards reversal at 14.9 µg/kg
- necrosis of venous wall, thrombosis and collagen lysis consisting of circumferential disruption of dense collagen fibers of tunica albuginea adjacent to corpus cavernosum with decreased staining/fiber diameter present in trichrome sections in one animal at 8.3 µg/kg
- increased incidence of sinus erythrocytosis of inguinal lymph node in all treated groups (partially reversible at 14.9 µg/kg)
- pigmented macrophages in inguinal lymph node at in one animal at 14.9 µg/kg

*VAN complex*

Systemic lesions: increased incidence and severity of sinus erythrocytosis of mediastinal lymph node

Local lesions:

- increased incidence and/or severity of adventitial/arterial wall/subcutaneous hemorrhage and neovascular proliferation in all treated groups with progression towards reversal evidenced by pigmented macrophage accumulation in the penis and inguinal lymph node, and hematoma formation (partially reversible)
- sinus erythrocytosis of inguinal lymph node accompanied by pigmented macrophage accumulation in all treated groups, reflecting hemorrhage and resolution of hemorrhage (partially reversible)
- increased incidence and/or severity of stromal/adventitial and venous wall necrosis in all treated groups

*Urethra*

Systemic lesions: thymus atrophy in treated groups only and interstitial hemorrhage in thymus at 14.9 µg/kg

Local lesions:

- increased incidence and severity of periurethral tissues
- increased incidence and severity of adventitial/arterial wall/subcutaneous hemorrhage and adventitial neovascular proliferation in all treated groups with partial recovery evidenced by pigmented macrophage accumulation in penis and inguinal lymph node, and hematoma formation
- increased incidence of acute/subacute inflammation of periurethral tissues in all treated groups with progression towards reversal evidenced by the change to chronic inflammation (partially reversible at 14.9 µg/kg)
- increased incidence of sinus erythrocytosis of inguinal lymph node accompanied by pigmented macrophage accumulation in all treated groups, reflecting hemorrhage and resolution of hemorrhage at the injection site (partial recovery)
- lymphoid hyperplasia in inguinal lymph node, stromal edema and venous wall necrosis at 14.9 µg/kg

Toxicokinetics: sporadic low plasma levels of AUX-I and AUX-II at ≤60 min following repeat-doses in tunica albuginea or acute injection highly in the vascular sites (corpus cavernosum or urethra)

Other: anti-AA4500 antibodies (see Tables below)

- high antibody titers against AUX-I and AUX-II in all repeat-dose treated animals at the end of the dosing and recovery period, anti-AUX-II antibodies being higher than anti-AUX-I antibodies
- detectable pre-study titers on day 1 (50 for anti-AUX-I and 250 for anti-AUX-II), and higher titers on day 8 (250 for anti-AUX-I and 1250 for anti-AUX-II) in the dog (#1635) prematurely euthanized on day 8

**Anti-AUX antibody titers:** The following tables are taken directly from the sponsor's submission.

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**Anti-AUX-I antibodies**

Sample No	Animal #	Day	Timepoint	Date	Misc.	Mean RLU Buffer (Run #)	Mean RLU with AUX-I (Run #)	Mean RLU with BSA (Run #)	% Immunocompetition with AUX-I	% Immunocompetition with BSA	Final Result	EPT
2	1629	61	Prior to last dose	8/24/07	Plasma for Antibody	4185 (8)	60 (8)	4483 (8)	98.6	-7.12	positive	156250
4	1630	61	Prior to last dose	8/17/07	Plasma for Antibody	183755 (3)	62696 (3)	182214 (3)	65.9	0.839	positive	>781250
8	1634	61	Prior to last dose	8/24/07	Plasma for Antibody	2683 (8)	73 (8)	2339 (8)	97.3	12.8	positive	>781250
9	1634	89	End of Recovery	9/21/07	Antibody Determination Plasma	5265 (8)	72 (8)	5180 (8)	98.6	1.61	positive	>781250
10	1635	1	Prior to dosing	6/18/07	Plasma for Antibody	11919 (3)	519 (3)	11706 (3)	95.6	1.79	positive	50.0
11	1635	8	Prior to moribund sacrifice	6/25/07	Plasma for Antibody	25307 (3)	622 (3)	23005 (3)	97.6	9.81	positive	250
12	1636	1	Prior to dosing	6/18/07	Plasma for Antibody	175 (3)	67 (3)	142 (3)	61.7	18.9	positive	<10.0
13	1636	61	Prior to last dose	8/17/07	Plasma for Antibody	32274 (8)	76 (8)	34014 (8)	99.8	-5.39	positive	156250
15	1637	61	Prior to last dose	8/24/07	Plasma for Antibody	2541 (8)	84 (8)	3599 (8)	96.7	-41.6	positive	>781250
17	1638	61	Prior to last dose	8/24/07	Plasma for Antibody	208 (3)	64 (3)	243 (3)	69.2	-16.8	positive	>781250
19	1639	61	Prior to last dose	8/17/07	Plasma for Antibody	2156 (8)	72 (8)	4346 (8)	96.7	-102	positive	>781250
20	1639	89	End of Recovery	9/14/07	Antibody Determination Plasma	5740 (8)	88 (8)	5327 (8)	98.5	7.20	positive	156250
22	1640	61	Prior to last dose	8/24/07	Plasma for Antibody	3102 (8)	81 (8)	3408 (8)	97.4	-9.86	positive	>781250
24	1641	61	Prior to last dose	8/17/07	Plasma for Antibody	46768 (8)	63 (8)	92051 (8)	99.9	-96.8	positive	31250
25	1641	89	End of Recovery	9/14/07	Antibody Determination Plasma	21406 (8)	73 (8)	32443 (8)	99.7	-51.6	positive	156250
27	1642	61	Prior to last dose	8/17/07	Plasma for Antibody	37064 (8)	69 (8)	47858 (8)	99.8	-29.1	positive	156250
29	1643	61	Prior to last dose	8/17/07	Plasma for Antibody	1835 (9)	103 (9)	1472 (9)	93.3	4.10	positive	>781250
31	1647	61	Prior to last dose	8/17/07	Plasma for Antibody	4312 (9)	79 (9)	5034 (9)	98.2	-16.7	positive	156250
32	1647	89	End of Recovery	9/15/07	Antibody Determination Plasma	2789 (9)	76 (9)	3535 (9)	97.3	-26.7	positive	>781250
34	1651	61	Prior to last dose	8/24/07	Plasma for Antibody	553 (9)	77 (9)	557 (9)	86.1	-0.723	positive	>781250
35	1652	1	0 minute	6/25/07	Antibody Determination	673 (4)	79 (4)	617 (4)	88.3	8.32	positive	10.0
36	1652	61	Prior to last dose	8/24/07	Plasma for Antibody	505 (9)	101 (9)	845 (9)	80.0	-67.3	positive	>781250
37	1652	89	End of Recovery	9/21/07	Antibody Determination Plasma	552 (9)	86 (9)	952 (9)	94.4	-72.5	positive	>781250
39	1655	61	Prior to last dose	8/17/07	Plasma for Antibody	3695 (9)	89 (9)	6276 (9)	97.6	-69.9	positive	>781250
41	1658	61	Prior to last dose	8/17/07	Plasma for Antibody	183929 (5)	67207 (5)	176948 (5)	63.5	3.80	positive	31250
46	1664	61	Prior to last dose	8/17/07	Plasma for Antibody	1870 (9)	73 (9)	2183 (9)	96.1	-16.7	positive	156250
47	1664	89	End of Recovery	9/14/07	Antibody Determination Plasma	1621 (9)	73 (9)	4214 (9)	95.5	-160	positive	156250
49	1665	61	Prior to last dose	8/17/07	Plasma for Antibody	12446 (9)	80 (9)	12768 (9)	99.4	-2.59	positive	156250

%Immunodepleted with Aux-I = [(Mean RLU at 0 µg/mL - Mean RLU at "x" µg/mL)/Mean RLU at 0 µg/mL]\*100.  
 %Immunodepleted with BSA = [(Mean RLU at 0 µg/mL - Mean RLU at "x" µg/mL)/Mean RLU at 0 µg/mL]\*100.  
 RLU = Relative Light Units  
 EPT = End point titer.

**Anti-AUX-II antibodies**

SAMPLE NO	Animal #	Day	Timepoint	Date	Misc.	Mean RLU Buffer (Run#)	Mean RLU with AUX-II (Run#)	Mean RLU with BSA (Run#)	% Immunocompetition with AUX-II	% Immunocompetition with BSA	Final Result	EPT
2	1629	61	Prior to last dose	8/24/07	Plasma for Antibody	4490 (6)	53 (6)	4742 (6)	98.8	-5.61	Positive	1310720
4	1630	61	Prior to last dose	8/17/07	Plasma for Antibody	94903 (6)	61 (6)	95395 (6)	99.9	-0.518	Positive	162840
5	1632	1	Prior to dosing	6/18/07	Plasma for Antibody	327 (11)	50 (11)	331 (11)	81.7	-1.22	Positive	50
6	1632	61	Prior to last dose	8/17/07	Plasma for Antibody	524 (11)	104 (11)	710 (11)	68.2	4.32	Positive	50
8	1634	61	Prior to last dose	8/24/07	Plasma for Antibody	6361 (11)	83 (11)	5962 (11)	98.7	6.27	Positive	>781250
9	1634	89	End of Recovery	9/21/07	Antibody Determination Plasma	15471 (11)	69 (11)	12944 (11)	95.6	16.3	Positive	>781250
10	1635	1	Prior to dosing	6/18/07	Plasma for Antibody	1120 (3)	138 (3)	971 (3)	87.7	13.3	Positive	250
11	1635	8	Prior to moribund sacrifice	6/25/07	Plasma for Antibody	5932 (3)	653 (3)	4956 (3)	89.0	16.5	Positive	1250
12	1636	1	Prior to dosing	6/18/07	Plasma for Antibody	219 (3)	62 (3)	72 (3)	43.6	34.5	Negative	-
13	1636	61	Prior to last dose	8/17/07	Plasma for Antibody	26058 (11)	68 (11)	27660 (11)	99.7	-6.15	Positive	>781250
15	1637	61	Prior to last dose	8/24/07	Plasma for Antibody	11697 (11)	83 (11)	9985 (11)	99.3	14.6	Positive	>781250
19	1639	61	Prior to last dose	8/17/07	Plasma for Antibody	1392 (11)	70 (11)	1358 (11)	95.0	-11.9	Positive	>781250
20	1639	89	End of Recovery	9/14/07	Antibody Determination Plasma	3299 (11)	67 (11)	3398 (11)	98.0	-3.28	Positive	>781250
22	1640	61	Prior to last dose	8/24/07	Plasma for Antibody	3065 (11)	75 (11)	3329 (11)	97.6	-8.61	Positive	>781250
24	1641	61	Prior to last dose	8/17/07	Plasma for Antibody	159671 (11)	84 (11)	168807 (11)	99.9	-5.72	Positive	>781250
25	1641	89	End of Recovery	9/14/07	Antibody Determination Plasma	71139 (11)	51 (11)	71842 (11)	99.9	-0.988	Positive	>781250
27	1642	61	Prior to last dose	8/17/07	Plasma for Antibody	40803 (12)	60 (12)	54950 (12)	99.9	-34.8	Positive	>781250
29	1643	61	Prior to last dose	8/17/07	Plasma for Antibody	4357 (12)	83 (12)	4057 (12)	98.1	6.89	Positive	>781250
31	1647	61	Prior to last dose	8/17/07	Plasma for Antibody	16205 (12)	66 (12)	16493 (12)	99.6	-14.1	Positive	>781250
32	1647	89	End of Recovery	9/14/07	Antibody Determination Plasma	14892 (12)	73 (12)	16483 (12)	99.5	-16.1	Positive	>781250
34	1651	61	Prior to last dose	8/24/07	Plasma for Antibody	2864 (12)	99 (12)	2383 (12)	96.5	16.8	Positive	>781250
35	1652	1	0 minute	6/25/07	Antibody Determination	139 (4)	69 (4)	126 (4)	50.4	9.35	Positive	>781250
36	1652	61	Prior to last dose	8/24/07	Plasma for Antibody	2868 (12)	90 (12)	3078 (12)	99.9	-6.58	Positive	>781250
37	1652	89	End of Recovery	9/21/07	Antibody Determination Plasma	4146 (12)	84 (12)	4383 (12)	98.0	-5.87	Positive	>781250
39	1655	61	Prior to last dose	8/17/07	Plasma for Antibody	12478 (12)	74 (12)	11610 (12)	99.4	6.93	Positive	>781250
41	1658	61	Prior to last dose	8/17/07	Plasma for Antibody	83590 (12)	59 (12)	9432 (12)	99.9	-12.8	Positive	>781250
46	1664	61	Prior to last dose	8/17/07	Plasma for Antibody	5353 (12)	80 (12)	5128 (12)	98.3	3.88	Positive	>781250
47	1664	89	End of Recovery	9/14/07	Antibody Determination Plasma	9438 (12)	80 (12)	10022 (12)	99.2	-6.61	Positive	>781250
49	1665	61	Prior to last dose	8/17/07	Plasma for Antibody	20875 (13)	59 (13)	24137 (13)	99.7	-15.6	Positive	>781250

%Immunodepleted with Aux-II = [(Mean RLU at 0 µg/mL - Mean RLU at "x" µg/mL)/Mean RLU at 0 µg/mL]\*100.  
 %Immunodepleted with BSA = [(Mean RLU at 0 µg/mL - Mean RLU at "x" µg/mL)/Mean RLU at 0 µg/mL]\*100.  
 RLU = Relative Light Units  
 EPT = End point titer.

Tables below summarize the observations made for the repeat- and acute-dosing phases in dogs.

**Repeated dosing: Tunica albuginea**

Dose, µg/kg	0 3M	0.8 6M	2.5 6M	8.3/6.1 <sup>a</sup> 6M
<b>Observations</b>				
<b>Mortality, moribund sacrifice, day 8</b>			1 <sup>b</sup>	
<b>Clinical signs,</b>			1	
Discoloration, prepuce	1	1	3	6
penis		2		1
groin				2
Swollen, penis		1	1	6
Bruising, mild/severe			1	4
Nodule, penis, red				2
Mass, penis/prepuce/bulbus				2
Scab/red spot, penis				1
Swelling around red spot				1
Blood on opening of penis				1
Thin appearance			1	2
<b>Histopathology, main/recovery</b>				
<b>Systemic lesions</b>	N=2/1	N=4/2	N=3/2	N=4/2
Brain, inflammation, chronic, perivascular		1(+1)		2(+1)
Spinal cord, cervical, inflammation, chronic, perivascular				1(+1)
Kidney, eosinophilic casts		1(+1)		2(+1)
Heart, inflammation, chronic, perivascular				1(+1)
Salivary gland, inflammation, chronic		1(+1)		1(+1)
Small intestine, duodenum, dilatation, crypt		2(+1)	2(+1)	4(+1)
Pancreas, inflammation, chronic				1(+1)
Testes, degeneration				1(+1)
Prostate, inflammation, chronic				2(+1)
Lymph node, mandibular, sinus erythrocytosis				1(+1)
inguinal, sinus erythrocytosis	1(+1)	3(+1, +2)	3(+1, +2)	4(+1, +2, +3)
pigmented macrophages				3(+1, +2)
hyperplasia, lymphoid			2(+2)/1(+2)	2(+2)/1(+2)
inflammation, acute				1(+1)
Skin, inguinal, hemorrhage, subcutaneous		2(+2, +3)		1 (+3)
pigmented macrophages				1 (+3)
<b>Injection site, penis</b>	N=2/1	N=3/2	N=4/2	N=3/2
Neovascular proliferation, adventitial/tunica	2(+3)	1(+1)	2(+1, +2)/1(+1)	0/1(+1)
Inflammation, acute/chronic-active, adventitial/tunica		1(+1)	1(+2)	
<b>Toxicokinetics,</b>				
AUX-I				
C, ng/mL, 0 hr, Day 1		60.9 <sup>d</sup>		
0.5 hr, Day 1	54.9 <sup>c</sup>		-	-
AUX-II				
C, ng/mL, 0 hr, Day 1		141 <sup>d</sup>		
0.5 hr, Day 1	157 <sup>c</sup>	24.2 <sup>d</sup>		
1 hr, Day 1		25.5 <sup>d</sup>		

<sup>a</sup>reduced to 6.1 µg/kg prior to the 2<sup>nd</sup> dosing cycle due to significant irritation and swelling during the 1<sup>st</sup> cycle

<sup>b</sup>euthanized prematurely on day 8 after exhibiting decreased activity, lethargy and significant weight loss due to excessive local reactions at the injection site following 2 doses (painful prepuce and surrounding area, full thickness wound with purulent pink-colored discharge in the area extended into musculature and subcutaneous tissue adjacent to left side of the penis/prepuce)

<sup>c</sup>animal #1632

<sup>d</sup>animal #1658

BLQ= below the limit of quantitation at 0.25-16 ng/mL for AUX-I & 0.75-48 ng/mL for AUX-II in 2% plasma

Severity grade in parentheses; +1 minimal, +2 mild, +3 moderate, +4 marked

-; not available

**Acute dosing: Tunica albuginea**

Dose, µg/kg	0	8.3	14.9
Observations	3M	5M	5M
<b>Clinical signs,</b>			
Discoloration, prepuce		4	5
Swollen, penis		1	4
Bruising, mild/moderate	1(+2)		1(+3)
<b>Coagulation, day 2</b>			
Fibrinogen, mg/dL	224	258	267
<b>Histopathology, main/recovery</b>			
<b>Systemic lesions</b>	N=1/1	N=3/2	N=3/2
Lymph node, inguinal, sinus erythrocytosis		3(+3, +4)	3(+3, +4)
Skin, inguinal, hemorrhage, subcutaneous		2(+3, +4, N=2)	3(+3)
<b>Injection site, penis</b>	N=2/1	N=3/2	N=3/2
Hemorrhage, adventitial		3(+2)	2(+2, +3)/2(+1, +2)
subcutaneous		2(+3, +4)	3(+3, +4)
Inflammation, chronic-active, adventitial/tunica		1(+2)	
Collagen lysis, tunica albuginea		1(+3)	
Neovascular proliferation, corpus cavernosum			0/2(+1, +2)
<b>Toxicokinetics</b>	-	-	-

Severity grade in parentheses; +1 minimal, +2 mild, +3 moderate, +4 marked  
 BLQ= below the limit of quantitation at 0.25-16 ng/mL for AUX-I & 0.75-48 ng/mL for AUX-II in 2% plasma  
 -; not available

**Acute dosing: Corpus cavernosum**

Dose, µg/kg	0	8.3	14.9
Observations	3M	5M	5M
<b>Clinical signs,</b>			
Discoloration, prepuce		4	3
Swollen, penis		2	2
Scrotum, discolored			1
Bruising, mild/severe		1	2
Mass, bulbus base/penis, large			1
<b>Histopathology, main/recovery</b>	N=2/1	N=3/2	N=3/2
<b>Systemic lesions</b>			
Lymph node, inguinal, sinus erythrocytosis	1(+3)	2(+2, +3)	3(+3)/1(+3)
pigmented macrophages			0/1 (+3, +4)
Skin, inguinal, hemorrhage, subcutaneous		1(+4, N=1)/1(+4)	1(+4, N=1)/1(+4)
<b>Injection site, penis</b>			
Penis, hemorrhage, preputial, subcutaneous			1(+4, N=1)
Hemorrhage, stromal/adventitial		2(+2, +4)	3(+1, +2, +4)/2(+1, +2, +4)
subcutaneous		1(+4)	2(+4)
hematoma, subcutaneous			0/1 (+2, +4)
Edema, stromal		3(+1, +2, +3)	1(+2)
Inflammation, acute/subacute, stromal/adventitial		2(+1, +2, +3)	3(+1, +2, +3)
chronic, adventitial/tunica			1(+2)/1(+2)
Neovascular proliferation, adventitial		1(+2)	0/2(+1, +2)
Collagen lysis, tunica albuginea		1(+2, +4)	
Necrosis, venous wall		1(+4)	
stromal/adventitial		2(+3, +4)	3(+1, +2, +3)
Thrombosis		1(+4)	
Pigmented macrophages			0/1(+3, +4)
<b>Toxicokinetics,</b>			
AUX-I,			
C, ng/mL, 5 min, Day 1		-	27.7 <sup>b</sup>
AUX-II			
C, ng/mL, 5 min, Day 1			37.7 <sup>b</sup>

<sup>b</sup>animal #1653

BLQ= below the limit of quantitation at 0.25-16 ng/mL for AUX-I & 0.75-48 ng/mL for AUX-II in 2% plasma  
 Severity grade in parentheses; +1 minimal, +2 mild, +3 moderate, +4 marked  
 -; not available

**Acute dosing: VAN complex**

Dose, µg/kg	0	8.3	14.9
Observations	3M	5M	5M
<b>Clinical signs,</b>			
Discoloration, prepuce		4	5
Swollen, penis		1	4
Bruising, mild/moderate		2	1
<b>Hematology, day 2</b>			
WBC, 10 <sup>3</sup> /mm <sup>3</sup>	9.26	<b>10</b>	<b>13.52*</b>
Neutrophils, 10 <sup>3</sup> /mm <sup>3</sup>	6.0	<b>6.6</b>	<b>9.8*</b>
Monocytes, 10 <sup>3</sup> /mm <sup>3</sup>	0.44	<b>0.58</b>	<b>0.72*</b>
<b>Histopathology, main/recovery</b>	N=2/1	N=3/2	N=3/2
<b>Systemic lesions</b>			
Lymph node, inguinal, sinus erythrocytosis		3(+3)/1(+2)	3(+3, +4)/2(+1)
pigmented macrophages		0/1 (+3)	0/1(+3)
lymphoid hyperplasia	1 (+2)		1(+2)/1 (+2)
mediastinal, sinus erythrocytosis	1 (+2)	1(+4)	3(+3, +4)
<b>Injection site, penis</b>			
Hemorrhage, stromal/adventitial		3(+1, +2, +3, +4)	3(+2, +3, +4)
arterial wall		1(+3)	2(+2, +3)
subcutaneous		2(+3, +4)	3(+4)
Hemorrhage/hematoma, adventitial		0/1(+3)	
Inflammation, acute/subacute, stromal/adventitial		3(+1, +2, +3)	3(+1, +2, +3)
Neovascular proliferation, adventitial		1(+2)/1(+2)	3(+2)/2(+1, +2)
Necrosis, venous wall		1(+3)	2(+3, +4)
stromal/adventitial		3(+1, +2, +3)	3(+2, +3)
Pigmented macrophages		0/1(+2)	
<b>Toxicokinetics</b>		-	-

BLQ= below the limit of quantitation at 0.25-16 ng/mL for AUX-I & 0.75-48 ng/mL for AUX-II in 2% plasma  
 Severity grade in parentheses; +1 minimal, +2 mild, +3 moderate, +4 marked  
 Statistically significant from controls at p=0.05\*  
 -; not available

**Acute dosing: Urethra**

Dose, µg/kg	0	8.3	14.9
Observations	3M	5M	5M
<b>Clinical signs,</b>			
Discoloration, prepuce	1	3	4
scrotum/groin			1
Swollen, penis		2	4
Bruising, mild/severe		2	3
prepuce			2
<b>Coagulation, day 2</b>			
Fibrinogen, mg/dL	185	<b>254*</b>	<b>260*</b>
<b>Histopathology, main/recovery</b>	N=2/1	N=3/2	N=3/2
<b>Systemic lesions</b>			
Thymus, atrophy		1(+3, N=1)	2(+3, N=2)
hemorrhage, interstitial			1(+3, N=2)
Lymph node, inguinal, sinus erythrocytosis	1(+1)/0	2(+3, +4)/1(+2)	3/2(+1, +2)
hyperplasia, lymphoid			1(+1)
necrosis, focal			1(+3)
inflammation, acute			1(+3)
pigmented macrophages		0/1(+3)	0/2(+3)

Injection site, penis			
Hemorrhage/hematoma, stromal, adventitial	1(+2)	3(+1, +4)/1(+2, +4)	3(+2, +3, +4)/1(+4)
Hemorrhage, arterial wall subcutaneous		2(+2, +3) 2(+4)	2(+2, +3) 3(+2, +4)
Edema, stromal			2(+2)
Inflammation, acute/subacute, periurethral chronic, adventitial	1(+1)	3(+1, +2, +3)	3(+1, +2, +3) 0/1(+1)
Neovascular proliferation, adventitial		1(+1, +2)/1(+1, +3)	2(+2)/1(+2)
Necrosis, venous wall stromal/adventitial		1(+2, +3)	1(+2) 3(+2, +3)
Pigmented macrophages			0/1(+4)
<b>Toxicokinetics,</b> AUX-I C, ng /mL,                    5 min, Day 1		26.3 <sup>b</sup> 31.2 <sup>c</sup> 17.9 <sup>d</sup>	-

<sup>b</sup>animal #1841

<sup>c</sup>animal #1850

<sup>d</sup>animal #1851

BLQ= below the limit of quantitation at 0.25-16 ng/mL for AUX-I & 0.75-48 ng/mL for AUX-II in 2% plasma

Severity grade in parentheses; +1 minimal, +2 mild, +3 moderate, +4 marked

Statistically significant from controls at p=0.05\*

-; not available

**Summary and Conclusions:** AA4500 was injected into the dog penis including the tunica albuginea, corpus cavernosum, urethra and subcutaneous tissue adjacent to the main vein, artery and nerve of the penis (VAN complex) either on a single- or a repeat-dosing schedule. The intended site of administration under clinical use, tunica albuginea was administered 3 injections over a week for 3 dosing cycles with 3 weeks between treatment cycles. The potential inadvertent sites (i.e., corpus cavernosum, urethra and VAN complex) were administered a single-dose design to allow for an evaluation of the effects of higher doses than could be reliably repeated. This approach was considered more relevant to the clinical situation since repeated inadvertent injection is not anticipated in patients.

The dosing regimen of 3 injections over a week for 3 dosing cycles with 3 weeks between treatment cycles is an acceleration of the proposed clinical schedule of 6 weeks between treatment cycles. However, it cannot be determined if the dosing frequency in dogs is relevant to the intended clinical dosing regimen in the absence of an appropriate PK measure. The administered doses of 0.8, 2.5, 8.3/6.1 and 14.9 µg/kg correspond to the human doses of 0.1-, 0.4-, 1.5/1.1- and 2.7-fold on a mg/kg basis or 0.04-, 0.14-, 0.46/0.34- and 0.83-fold on a mg/m<sup>2</sup> basis, respectively. The doses were selected based on the severity and/or persistence of the local effects noted in all treated animals in pilot studies (see previous review).

In the repeat-dose portion of the study, dogs initially dosed at 8.3 µg/kg were unable to receive all of the scheduled doses due to excessive swelling that prevented access to the dosing site; 4 received 2 doses and 2 received only 1 dose during the 1<sup>st</sup> cycle. During the 2<sup>nd</sup> cycle after lowering the dose to 6.1 µg/kg, 4 out of 6 dogs received all 3 doses, and the remaining 2 doses received only 1 dose. All dogs at 6.1 µg/kg received all 3 doses in the 3<sup>rd</sup> week.

One premature euthanasia occurred in the repeat-dosing study. One animal at 2.5 µg/kg had significant weight loss resulting from severe local reactions at the injection site (severe bruising, swelling, pain on manipulation and a full-thickness ulcer involving the prepuce) following the 2<sup>nd</sup> dose. The dog was euthanized on Day 8, and had detectable anti-AA4500 titers on day 1 at pre-study and day 8 prior to sacrifice.

Clinical signs related to AA4500 treatment consisted of discoloration/bruising of the penis and/or adjacent skin and swelling of the penis. Most of the findings were seen in treated groups, but not in controls and all injection sites with the increased incidence/severity and persistence with increasing dose levels, reflecting the treatment-related findings rather than the procedure-related phenomena.

Injection-site findings following the repeated injection of AA4500 into the tunica albuginea were manifested as hemorrhage and inflammation (affecting both into the adventitial tissue of the penis and the surrounding subcutaneous tissue of the prepuce or inguinal skin), and neovascular proliferation (granulation tissue) in the penile adventitial tissue, being more severe or extensive in treated animals. The primary findings at all dose levels and all injection sites in the single-dose phase of the study were hemorrhage, edema, necrosis, inflammation and neovascular proliferation with the increased severity and extent. Histopathology measures indicated that resolution was generally in progress, but incomplete at the end of the treatment-free period of 28 days. Gross necropsy observations corresponding to the discoloration of the inguinal skin and/or inguinal lymph node correlated to the histologic findings of subcutaneous hemorrhage and sinus erythrocytosis and/or pigmented macrophages.

There was no significant systemic toxicity (i.e., changes in body weight, food consumption, hematology, clinical chemistry, urinalysis or organ weights) following intrapenile administration of single- or repeat-doses of AA4500. Inflammatory signs, however, were noted in multiple systemic organs including the brain, kidney, small intestine and prostate in treated groups upon repeat-dosing into the tunica albuginea, although the severity is minimal. The findings in draining lymph nodes (e.g., lymphoid hyperplasia, pigmented macrophages, sinus erythrocytosis) indicate the extension of the local inflammation to the systemic inflammatory reaction. Considering the large size of collagenase, AA4500 and/or its degraded products may have also entered the systemic circulation through the lymphatic system, which drains into the regional lymph nodes. It is unknown if the systemic findings are due to an immune-mediated response consequent to an immune complex-reaction or a non-specific systemic inflammatory response secondary to the injection site reaction.

Necrosis was noted in stromal/adventitial and venous wall at the injection sites. It was stated that necrosis did not affect the non-collagenous structures within or adjacent to the injected tissue, nor was there any evidence of residual effects on these structures in recovery animals in all phases of the study. In particular, arteries, nerves and large veins were unaffected, with lysis (necrosis) seen only in smaller veins comprised mostly of collagen and minimal smooth muscle. Collagen lysis quantified using trichrome stain was detected in one each animal injected into the tunica albuginea at 8.3 and 14.9  $\mu\text{g}/\text{kg}$  dose, and in one animal injected into the corpus cavernosum at 8.3  $\mu\text{g}/\text{kg}/\text{dose}$  on day 3, and was detected in a subset of tissues (1-3 sections) from the injected areas, not extending to the full thickness of the tunica, and was reversible by the study day 62.

Low levels of AUX-I and/or AUX-II ( $\leq 40$  ng/mL) were seen at 5 minutes post-dosing on day 1 in a few dogs in which AA4500 was injected into highly vascular tissue (corpus cavernosum or urethra, with extravasation into the corpus spongiosum) following a single dose. Upon repeat-dosing, low levels of AUX-I at pre-dose (60.9 ng/mL) and AUX-II (LLOQ of  $\leq 22.5$  ng/mL) at 30 minutes (24.2 ng/mL) and 60 minutes (25.5 ng/mL) were also reported on Day 1 for one dog injected with 0.8  $\mu\text{g}/\text{kg}/\text{dose}$  into the tunica albuginea, but no detectable exposure was noted for this dog on day 62. No further PK analysis could be performed on the plasma sample data due to lack of sufficient drug levels over time in the plasma at any dose level, injection site or treatment

schedule. The reason for the detectable levels of AUX-I (54.9 ng/mL) and AUX-II (157 ng/mL) for one vehicle animal in the 30 minute sample is unknown. The sponsor stated that the lack of stability of AUX-I (up to 24 days) and AUX-II (up to 132 days) in plasma samples stored at -80°C precluded analysis of samples from additional time points taken from the animal to determine the source of these values.

Antibodies to AA4500 components were detected in all repeat-dose dogs following their last dose on Day 61. Titers were high (ranging from 31,250 to >781,250 for anti-AUX-I and 131,720 to >781,250 for anti-AUX-II), and either persisted or increased in most dogs until the end of drug-free period of 28 days after the last dose, suggesting that the accumulation of antibodies on prolonged therapy. Plasma samples from vehicle and pre-study animals were mostly negative for anti-AUX-I and anti-AUX-II antibodies. One vehicle (prior to dosing and prior to last dose) and one from at 8.3/6.2 µg/kg dose group (on day 1) had detectable levels of anti-AUX-I and/or anti-AUX-II antibody titers, suggesting some cross-reactivity between the clostridial species. The consequences of the antibody formation with respect to PK, PD and/or toxicity are unknown. The sponsor stated that the presence of antibodies did not result in any apparent adverse systemic effects. Nor did production of antibodies appear to alter the toxicokinetics or pharmacodynamic effects of AA4500; plasma levels on day 1 were comparable to those on day 62 and clinical signs related to AA4500 administration occurred with approximately equal frequency and severity during all dosing weeks in the study. The higher tolerance of the high dose group to the dosing regimen in the 3<sup>rd</sup> cycle versus the 2<sup>nd</sup> cycle was attributed to the result of an improvement in technique or additional recovery from lesions developed following the first dosing cycle that had not completely resolved. However, it is unknown if the interpretation of the data is compromised by immune responses consequent to the deposition of anti-AA4500 antibodies.

The NOAEL for the repeated administration of AA4500 into the tunica albuginea would be <0.8 µg/kg rather than the sponsor's NOAEL of 0.8 µg/kg due to the increased incidence and severity of sinus erythrocytosis in inguinal lymph node noted in all treated groups. No NOAEL was identified in the single dose study phase due to the injection-site toxicity. The LOAEL of 0.8 µg/kg (HED=1.44 µg/kg, 2.66 mg/mL) is approximately 0.1-fold the proposed human dose of 0.58 mg (9.6 µg/kg, 2.32 mg/mL) on a mg/kg basis.

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

The intralesional administration of the Auxilium's collagenase, AA4500 into the tunica albuginea, corpus cavernosum, urethra and subcutaneous tissue adjacent to the main vein, artery and nerve of the penis in the dog produced local toxicity with a primary inflammatory response (hemorrhage, edema, necrosis, inflammation, neovascular proliferation, lymphoid hyperplasia) in treated groups following single- or repeat-dosing. The local inflammatory response was dose-related with respect to the incidence, severity and/or persistence, and was of an extended duration following dosing cessation. One treated dog was euthanized *in extremis* due to excessive local reactions at the injection site of tunica albuginea following 2 doses. The systemic inflammatory signs observed following repeated dosing of AA4500 probably result from the regional and disseminated inflammatory process originating at the injection sites. The injection-site reactions may have been consequent to the anti-AA4500 antibody formation that may lead to the systemic reactions. In addition, the potential to trigger subsequent effects for degraded fragments from the fibrotic plaque due to the enzymatic breakdown of collagenase cannot be dismissed, considering the involvement of collagen in inflammatory process and wound repair. The persistence of the local response could result from collagen remodeling in chronic inflammatory process.

The low systemic bioavailability of AA4500 via the intrapenile route could be due to rapid renal clearance and degradation to small peptides and amino acids by proteolytic enzymes. The low levels of AA4500 were seen in a few dogs, in which AA4500 was injected into highly vascular tissue (i.e., corpus cavernosum or urethra with extravasation into the corpus spongiosum) may have been due to the systemic circulation of its degradation products that were not detected by the current method. The potential exposure to the systemic circulation of the intralesional collagenase still exists, given that the affected tissue (tunica albuginea) is surrounding the corpora carvenosa, in which sinusoidal spaces are rich in vascular supply.

Collagen lysis was noted in some animals injected into the adjacent tunica albuginea at higher doses. Necrosis was noted in stromal/adventitial and venous wall at the injection sites, suggesting that a rupture of the tunica albuginea wall may occur with AA4500. Although a direct extrapolation of the animal findings to humans would be difficult, the potential risk of the collagenase leakage into the adjacent normal structures cannot be dismissed depending on the size and the location of the plaque within the penis that may lead to collagen digestion of healthy tissues.

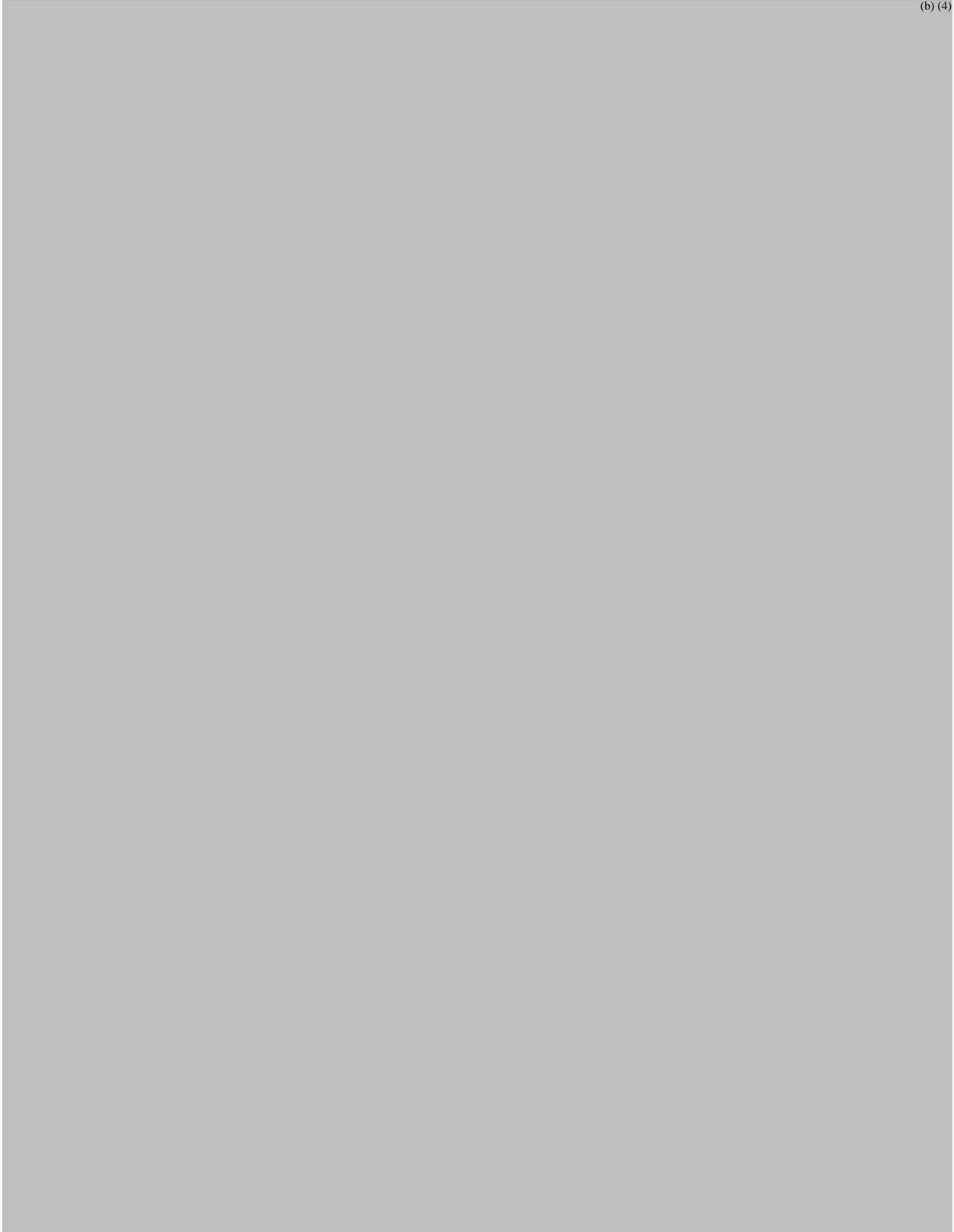
Antibodies against AUX-I and AUX-II were detected in all treated animals following repeat doses on day 61 and at the end of recovery. The antibody responses have not been characterized. The higher serum antibody titers could be due to the increased immunogenicity of protein aggregates and/or precipitates at the injection sites. Considering the high incidence and the persistent nature of antibodies seen in the study, repeated injection of AA4500 may have immunological consequences if a booster dosing is given in the presence of substantial antibody titers following a repetitive dosing schedule in humans. Auxilium collagenase would be expected to be immunogenic in man, given the large size and the nature of the product derived from non-human origin. Inadvertent injection of collagenase into the surrounding highly vascular tissues may also release into the blood/lymphatic vessels, and trigger immune responses.

Overall, the results from the local toxicity study in dogs suggest that intrapenile administration of AA4500 produces inflammatory responses at all injection sites with incomplete recovery following single- and/or repeated doses at doses comparable to or lower than the clinical dose. The repeat-dose of intrapenile injection of AA4500 appears to activate the local inflammatory process that may lead to the stimulation and release of inflammatory mediators responsible for the systemic inflammatory effects, probably due to the increased immunogenicity at the injection site. Anti-product antibodies were formed in all repeated-dosing dogs with unknown consequences. Collagen lysis/necrosis affecting the adjacent tunica albuginea and stromal/adventitial/venous wall at the injection sites was observed in some animals, implying the potential damage of normal structures by enzymatic digestion of collagenase.

**Conclusions:** The submitted local toxicity data do not ensure the safe use of the intralesional collagenase in humans. The localized inflammatory response induced by collagenase injection that may lead to the systemic reaction is of concern, considering the possible consequence of antibody formation. The potential to rupture the injection site wall and/or damage normal structures surrounding the plaque exists following the intrapenile collagenase. The fate of breakdown fragments from the fibrotic plaque that may trigger subsequent effects is unknown.

**Recommendations:** Pharmacology/Toxicology defers to the medical team for the safe use of intrapenile collagenase in humans. The submitted dog data suggest that the intralesional injection of collagenase have a potential to induce systemic as well as local inflammatory reactions

possibly consequent to immune responses, and lack assurance that the intrapenile collagenase is confined to the plaque with no extension to adjacent/attached sites.



(b) (4)



#### **2.6.6.4 Genetic toxicology**

Genetic toxicity of biologics is not generally required unless it is deemed necessary to study the potential toxicity of an impurity in the drug product formulation or unless there is a specific cause for concern related to the specific protein in question. The Applicant conducted genetic toxicology studies for the collagenase enzymes using batches that do not represent the commercial batches. These studies were conducted prior to the general acceptance that such studies are not necessary. Therefore, only summary and major findings of genotoxicity studies are presented in the review.

**Study title: Micronucleus test, injectable collagenase ABC (Nucleolysin)****Key findings:** Collagenase ABC was not mutagenic in the mouse micronucleus assay.**Study no.:** 200002-M-00491**Volume # M4, and page #: 1****Conducting laboratory and location** (b) (4)**Date of study initiation:** Jan 31, 1991**GLP compliance:** Yes**QA reports:** yes (x) no ( )**Drug: Collagenase 3, Nucleolysin, lot #587/88/89/90, and % purity:** 2.1 mg/mL protein

The concentration of the test material was prepared immediately before the use and it contained 17000 ABC units/mL. The applicant stated that the stability and homogeneity of the test substance was not determined.

**Methods**

Micronucleus test was conducted in bone marrow erythrocytes from CD-1 mice. The dose levels are shown from the sponsor's table below.

Group code (Label)	Dose-level (U/kg)	Animal numbers		Sampling time	
		Males	Females		
1 (WHITE)	Vehicle  0.00	2-10	1-9	24	hrs
		42-50	41-49	48	hrs
		82-90	81-89	72	hrs
2 (YELLOW)	NUCLEOLYSIN <sup>R</sup>  1070	12-20	11-19	24	hrs
		52-60	51-59	48	hrs
		92-100	91-99	72	hrs
3 (BLUE)	NUCLEOLYSIN <sup>R</sup>  2140	22-30	21-29	24	hrs
		62-70	61-69	48	hrs
		102-110	101-109	72	hrs
4 (RED)	Mitomycin-C  2.00 mg/Kg	32-40	31-39	24	hrs
		72-80	71-79	48	hrs
		112-120	111-119	72	hrs

The treatment was given once by ip injection and animals were sacrificed at 24, 48 and 72 hours post dose. The dose was selected on the basis of LD 50 at 80.2 units/mouse/ip. The procedure of the assay was standard. The summary of results is shown from the

applicant's table below. Incidences of micronucleated cells per 1000 erythrocytes were scored.

TEST SUBSTANCE : NUCLEOLYSIN<sup>A</sup>  
 ROUTE OF ADMINISTRATION: Intraperitoneal  
 VEHICLE : 2 mM Calcium chloride in physiological solution

Treatment	Dose-level (U/kg)		Incidence of Micronucleated PCE's		NCE/PCE Mean Ratio
	M	F	Mean ± SE	Range	
<u>24 hr sampling time</u>					
Vehicle	10 ml/kg		0.9 ± 0.3	(b) (4)	1.13
Test Substance	1070 U/kg		0.3 ± 0.1	(b) (4)	0.93
Test Substance	2140 U/kg		0.8 ± 0.2	(b) (4)	1.08
Mitomycin-C	2.00 mg/kg		46.2***± 4.6	(b) (4)	1.86
<u>48 hr sampling time</u>					
Vehicle	10 ml/kg		0.5 ± 0.2	(b) (4)	1.07
Test Substance	1070 U/kg		0.8 ± 0.5	(b) (4)	1.17
Test Substance	2140 U/kg		0.2 ± 0.1	(b) (4)	1.09
Mitomycin-C	2.00 mg/kg		Insufficient cells recovered		
<u>72 hr sampling time</u>					
Vehicle	10 ml/kg		0.7 ± 0.2	(b) (4)	0.90
Test Substance	1070 U/kg		1.2 ± 0.2	(b) (4)	1.01
Test Substance	2140 U/kg		0.6 ± 0.2	(U) (+)	0.90
Mitomycin-C	2.00 mg/kg		Insufficient cells recovered		

Key:

PCE: Polychromatic erythrocyte  
 NCE: Normochromatic erythrocyte  
 \* : Incidence significantly greater than control value at p<0.05  
 \*\* : Incidence significantly greater than control value at p<0.01  
 \*\*\*: Incidence significantly greater than control value at p<0.001  
 U : ABC units

Based on the data, Clostridial collagenase (Nucleolysin) given by ip route was considered to be non-genotoxic. However, the process and the batch used in the study are different from the intended clinical batch manufactured under Auxilium batches. Therefore, relevance of the study is unknown.

**Study title: Reverse mutation in *Salmonella typhimurium***

**Key findings:** Collagenase 3 is not mutagenic in Ames assay in the absence and presence of S-9 mixtures

**Study no.:** 200003-M-00591

**Volume #M4, and page #: 1**

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** Jan 18, 1991

**GLP compliance:** Yes

**QA reports:** yes (x) no ( )

**Drug: Collagenase 3, Collagenase ABC, Nucleolysin, lot # 587/88/89/90, and % purity:** 2.1 mg/mL, 17000 ABC units/mL

## Methods

Strains/species/cell line: *Salmonella typhimurium* strains TA1535, TA1537, TA 98, and TA 100 were used with or without rat S-9 liver homogenates.

Doses used in definitive study: 213, 425, 850, 1700 and 3400 units/plate, the test substance was made fresh for the assay and the applicant did not assay for the test substance for its stability.

Basis of dose selection: Preliminary cytotoxicity was conducted. However, no cytotoxicity was detected in the assay. The applicant chose to use the maximum concentration of the protein possible.

Negative controls: Distilled water, DMSO

Positive controls: Sodium azide, 9-aminoacridine, 2-nitrofluorene and 2-aminoanthracene

Incubation and sampling times: Plates were incubated for the growth of revertant colony for 72 hours at 37C.

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Two fold or greater increase in the revertant colonies at two consecutive doses was considered for a positive response.

Study outcome: Data did not show any increase of the revertant colonies in the absence and presence of S-9 mixtures. Based on the study criteria, the test material was considered negative for the Ames assay.

**Study title: Chromosome aberration in human lymphocytes cultured in vitro**

**Key findings:** Collagenase 3 was not mutagenic in this assay.

**Study no.:** 200001-M-00391

**Volume #M4, and page #:** 1

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** March 6, 1991

**GLP compliance:** Yes

**QA reports:** yes (x) no ( )

**Drug:** Collagenase 3, lot # 587/88/89/90, and % purity: 2.1 mg/mL (17000 units/mL)

## Methods

Strains/species/cell line: S-9 liver homogenates were prepared from Sprague Dawley rats for the induction of metabolism. The study was conducted with or without metabolic activation systems. Peripheral blood from a 31-year-old male volunteer was collected and lymphocyte cultures were prepared for the chromosomal aberration study. Cells were stimulated with PHA for replication and cells were treated after 48 hours after the addition of PHA to maximize replications.

Doses used in definitive study: 210, 97.4, 45.2, 21.0, 9.74, 4.52, 2.10, and 0.97 ug/mL in the absence and presence of S-9 mixtures. Cells were score at 210, 97.4 and 45.2 ug/mL in the absence and presence of S-9 mixtures.

Basis of dose selection: Maximum feasible dose

Negative controls: Culture medium

Positive controls: Mitomycin C and cyclophosphamide (for metabolic activation system) were dissolved in distilled water of injectable grade for the use as positive controls.

Incubation and sampling times: Cells were incubated for 24 hours in the absence of metabolic activation systems. Cells were treated with the test substance for 2 hours in the presence of S-9 mixtures and harvested after 12 or 24 hours. Cell mitosis was arrested by the addition of Colcemid two hours before harvesting cells. The cells were prepared for aberration following staining with Giemsa stain. Mitotic index was determined as a measure of cytotoxicity. The sponsor stated that they did not conduct any test to determine the stability of the test substance during the incubation. Duplicate samples were tested at each concentration.

## Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The cytotoxicity was determined by mitotic index. The sponsor intended to choose the highest concentration corresponding to 50% inhibition of mitotic index. If cytotoxicity was not observed, the highest feasible concentration was used for the assay. Validity of the study was based on statistically significant increase in the aberration

compared to the control and aberration at above the historical control. Also, the increase in aberration could be duplicated in the study. Cells were scored without gaps.

Study outcome: One hundred metaphase cells per culture were scored. The mitotic index at 210 ug/mL was 71% and 65% in the absence of S-9 and presence of S-9 mixtures, respectively. Results of the aberration study in the absence and presence of S-9 is shown below from the sponsor's table.

SCHEDULE NO.: 200-001

Table 10

SUMMARY TABLE

TEST SUBSTANCE: NUCLEOLYSIN<sup>R</sup>  
SOLVENT : CULTURE MEDIUM

Treatment	Dose-level (ug/ml)	Absence of S9		Presence of S9 Metabolism			
		24 hr Harvest %CA (Rel.MI)		12 hr Harvest %CA (Rel.MI)		24 hr Harvest %CA (Rel.MI)	
Untreated	/	0.0	(100)	0.0	(100)	0.5	(100)
Test Substance	45.2	0.5	(98)	0.0	(69)	0.5	(121)
Test Substance	97.4	0.0	(105)	0.0	(64)	0.5	(108)
Test Substance	210	0.0	(72)	0.5	(65)	0.5	(120)
Cyclo-phosphamide	20.0	-	-	9.0***	(22)	18.5***	(22)
Mitomycin-C	0.30	18.5***	(21)	-	-	-	-

Key:

% CA : Percentage of cells bearing aberrations (excluding gaps)  
Rel.MI : Mitotic index relative to negative controls (percent).  
- : Not tested or not selected for the scoring of aberrations  
\* : Stat. sig. at P<0.05 after correction for multiple comparisons  
\*\* : Stat. sig. at P<0.01 after correction for multiple comparisons  
\*\*\* : Stat. sig. at P<0.001 after correction for multiple comparisons

Above data suggest that the test substance did not cause aberrations in the peripheral blood lymphocytes from a human donor.

**Summary of mutagenicity:** Mutagenicity was conducted according to ICH guidelines for Nucleolysin (Collagenase 3) that was made by a BTC process. The genotoxicity studies for biologics are not a regulatory requirement because the large proteins do not enter the cell. However, results of tests could reflect process impurities. Since the proposed marketing batches would be made by a process different from that used for Nucleolysin, the relevance of these studies is not known.

**2.6.6.5 Carcinogenicity:** No carcinogenicity study was conducted.

#### 2.6.6.6 Reproductive and developmental toxicology

##### Fertility and early embryonic development

**Study title:** Intravenous fertility and general reproduction toxicity study of AA4500 in rats

**Key study findings:** AA4500 did not show any abnormality in the sperm counts, motility, fertility and early embryonic development in rats up to 0.13 mg/dose. NOEL was 0.13 mg/dose for fertility and early embryonic development.

**Study no.:** (b) (4) 00012

**Volume #M4, and page #:** 1

**Conducting laboratory and location** (b) (4)

**Date of study initiation:** Nov 28, 2008

**GLP compliance:** Yes

**QA reports:** yes (x) no ( )

**Drug: AA4500, lot #NFF-0035, and % purity:** 100% by HPLC assay for AUX-1 and AUX-2 based on the certificate of analysis from (b) (4) for process 3

Vehicle Lot #J6H560, J6K462, J6K548, C711127, Placebo lot#FIN0251, Diluent containing 2 mM calcium chloride in 0.9% saline Lot# FIN-0206, FIN-0265

##### Methods

Doses: The study design is shown below from the applicant's table.

Male rats for spermatogenesis and mating:

Dose Group	Dose (mg protein/dose)	Concentration (mg protein/mL)	Dose Volume (mL/rat)	Injection Rate (mL/min)	Number of Rats	Assigned Numbers	
						Main Study	Toxicokinetic Study
I	0 (Placebo)	0.0	0.5 mL	Bolus	25	29801 - 29825	N/A
II	0.0145	0.029	0.5 mL	Bolus	25 + 6 <sup>a</sup> + 6 <sup>b</sup>	29826 - 29850	29281 - 29286 <sup>a</sup> 6281-6286 <sup>b</sup>
III	0.0435	0.087	0.5 mL	Bolus	25 + 6 <sup>a</sup> + 6 <sup>b</sup>	29851 - 29875	29287 - 29292 <sup>a</sup> 6287-6292 <sup>b</sup>
IV	0.13	0.26	0.5 mL	Bolus	25 + 6 <sup>a</sup> + 6 <sup>b</sup>	29876 - 29900	29293 - 29298 <sup>a</sup> 6293-6298 <sup>b</sup>

The test article was considered 100% active/pure for the purpose of dose calculations. All dose calculations were based on the vial content of AA4500 or AA4500 Placebo provided in each Certificate of Analysis.

- Six rats assigned to the initial study for use in toxicokinetic sample collection.
  - Six rats assigned to the study extension for use in toxicokinetic sample collection.
- N/A - Not Applicable

Female rats for fertility and early gestational effect:

Dose Group	Dose (mg protein/dose)	Concentration (mg protein/mL)	Dose Volume (mL/rat)	Injection Rate (mL/min)	Number of Rats Per Sex	Assigned Numbers	
						Main Study	Toxicokinetic Study
I	0 (Placebo)	0.0	0.5 mL	Bolus	25	21801 - 21825	N/A
II	0.0145	0.029	0.5 mL	Bolus	25 + 6 <sup>a</sup> + 6 <sup>b</sup>	21826 - 21850	21951 - 21956 <sup>a</sup> 6381-6386 <sup>b</sup>
III	0.0435	0.087	0.5 mL	Bolus	25 + 6 <sup>a</sup> + 6 <sup>b</sup>	21851 - 21875	21957 - 21962 <sup>a</sup> 6387-6392 <sup>b</sup>
IV	0.13	0.26	0.5 mL	Bolus	25 + 6 <sup>a</sup> + 6 <sup>b</sup>	21876 - 21900	21963 - 21968 <sup>a</sup> 6393-6398 <sup>b</sup>

The test article was considered 100% active/pure for the purpose of dose calculations. All dose calculations were based on the vial content of AA4500 or AA4500 Placebo provided in each Certificate of Analysis.

- Six rats assigned to the initial study for use in toxicokinetic sample collection.
- Six rats assigned to the study extension for use in toxicokinetic sample collection.

The high dose was selected on the basis of the pilot study and based on the tolerability of the drug. The rationale was discussed for the segment 2 study reviewed below.

Species/strain: Male and female Crl:CD (SD) rats.

Number/sex/group: See the study design above

Route, formulation, volume, and infusion rate: Solutions of the drug product were made on each day of the administration of dose. The stability of the dose solution was tested. The solution containing 0.029 mg/mL AA4500 showed 39-76% of the nominal concentration. The solution containing 0.087 mg/mL AA4500 showed 56-101% of the nominal concentration and the solution containing 0.26 mg/mL AA4500 showed 84-110% of the nominal concentration. Stability data for the solution at 0.025 mg/mL on ice for 8 hours and -80°C were within 94 to 100%. Similarly, stability data for 0.3 mg/mL solution stored in the ice for 8 hours and -80°C showed 109-110% recovery. Based on the high recovery data for 0.26 mg/mL solution in the analytical study and presence of antibodies to the drug, it was concluded that the study was conducted up to a maximum feasible exposure in rats.

As shown in the study design, the treatment was given by IV bolus injections to maximize the exposure. The applicant also wanted to examine immunogenicity to the treatment.

Male rats were treated with AA4500 once every other day beginning 28 days before the mating, during the mating period and up to euthanasia days 61-64 by IV route. Female rats were treated once every other day beginning 15 days before mating, during mating, gestation days 0, 3, 5 and 7 by IV route. Injections were made into the lateral caudal vein.

Satellite groups used for toxicokinetics: Six rats/sex/drug treatment group were assigned for the toxicokinetics and 6 rats/sex/drug treatment groups were assigned as extension groups to replace the toxicokinetic animals if needed. Blood samples were collected at several time points from 3 rats/sex/time point on dosing day 1 from treated rats, study day 55 in male treated rats and gestation day 7 in female treated rats for the determination of plasma AUX-1 and AUX-2 levels by a validated enzyme linked immunosorbent Assay (ELISA assay). Blood samples were collected from the lateral tail vein or jugular vein. Satellite animals were sacrificed by carbon dioxide inhalation. The applicant stated that TK study was repeated because the original samples were not stable. Serum anti-AUX-1 and AUX-2 antibody was determined by ELISA assay at necropsy in which antigen-antibody reactive samples were quantitated by the displacement with unlabelled AUX-1 and AUX-2.

Study design: Male and female rats were cohabitated at 1:1 ratio from the same group for a total of 21 days. Female rats that were not mated during 14 days were cohabitated with a male with proven fertility from the same group for another 7 days. Unmated female rats were considered to be gestation day 0 also. Estrus cycle was monitored during pre-mating and mating period. Copulation was confirmed by the presence of spermatozoa in the vaginal smears and it was considered as gestation day 0.

Animals were observed twice daily for viability and clinical signs. Animals were also examined once a week for any gross changes on the body surface. The body weight of male rats was recorded daily during the treatment period up to the day of necropsy. The body weight of female rats was recorded daily during the treatment, gestation day 0, 7, daily during the post dose period and on the day of euthanasia. The food consumption was recorded weekly for male rats except during the cohabitation period. The food consumption was recorded weekly until cohabitation, gestation day 0, 7, 8 and 13. The food consumption was calculated as g/day.

Male rats were euthanized after cohabitation between study days 64 to 67. Gross visceral changes were noted. Reproductive organs were weighed and fixed for histopathology, sperm counts, motility and sperm/gm of tissue were recorded. Testes were fixed in Bouin's solution and other reproductive tissues were fixed in 10% formalin.

Female rats were euthanized on the gestation day 13 by carbon dioxide asphyxiation.

Gross changes in the visceral organs were recorded. Following caesarean sections on gestation day 13, uteri were examined for the pregnancy status. Both uteri and ovaries from each rat were fixed in formalin for histopathology if needed.

Parameters and endpoints evaluated: Number of corpora lutea, pregnancy, implantation sites, viable and non-viable embryos and placentas were examined.

## Results

Mortality: Two male rats died on the study day 15 at 0.0435 (#29865) and 0.13 mg/dose (#29880) groups, respectively. No female rats were found dead due to the treatment. Histological data showed acute congestion in the liver, kidneys and lungs in animal# 29865. A similar finding was noted in rat # 29880. Relationship of the treatment to these acute changes is unknown due to a small number of rats that showed above changes and lack of a similar finding at a higher dose.

Clinical signs: Soft feces, purple color at the injection sites and swollen injection sites were observed at mid and high doses in male rats. Swollen injection sites were noted at low, mid and high doses in female rats. Purple coloration at the injection site was evident with significantly higher incidences at the high dose compared to the control in female rats.

### Body weight:

Male rats (g):

Day	Group 1	Group 2	Group 3	Group 4
1	361	359	357	358
28	457	457	463	452
43	493	492	500	485
63	536	533	543	527
Weight gain	175	174	186	169

Based on above data, the treatment related effect on the body weight gain was not biologically significant.

Female rats (g):

Day	Group 1	Group 2	Group 3	Group 4
1 (pre mating)	255	254	257	255
15 (pre mating)	267	269	270	268
Gestation day 1	269	267	267	267
Gestation day 7	296	295	296	288
Gestation day 13	332	331	329	328
Weight gain day 1 to gestation	77	77	72	73

Day	Group 1	Group 2	Group 3	Group 4
day 13				

Overall decrease in the body weight gain was reduced by about 6% in mid and high dose animals. Based on these data, female rats showed a slight decrease in the body weight gain compared to the control in groups 3 and 4 animals that could be due to lower food consumption as discussed below.

Food consumption:

Male rats (g/day):

Day	Group 1	Group 2	Group 3	Group 4
1-7	27.2	26.6	27.0	27.1
21-28	26.8	26.6	27.1	26.6
37-45	28.2	27.8	28.7	27.6
57-64	28.3	27.4	28.6	27.3
1-64	27.7	27.4	28.4	27.4

Based on above data, there was no treatment related effect on the food consumption.

Female rats (g/day)

Day	Group 1	Group 2	Group 3	Group 4
1-7	17.1	17.7	17.6	16.9
7-15	17.8	17.4	17.8	17.7
Gestation days 0-8	22.2	22.0	21.8	20.0*
Gestation days 0-13	22.4	22.6	22.2	21.3

\* Statistically significant compared to control.

When compared to the control, food consumption was slightly (5%) but significantly reduced during gestation in group 4 animals on week 1. However, the food consumption was similar at the end of gestation day 15.

Toxicokinetics:

PK information for AUX-1 and AUX-2 is shown below from the applicant's table.

AUX-I TK Parameters (DS 1)							
Dose Level (mg protein/dose)	Parameter						
Males	AUC <sub>last</sub> ± SE (ng·hr/mL)	CL (mL/h)	C <sub>0</sub> (ng/mL)	C <sub>max</sub> ± SE (ng/mL)	t <sub>1/2</sub> (h)	t <sub>max</sub> (h)	V <sub>ss</sub> (mL)
0.0145	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>
0.0435	NC <sup>a</sup>	NC <sup>a</sup>	11.0	11.0 ± 0.68	NC <sup>a</sup>	0.25	NC <sup>a</sup>
0.13	16.4 ± 1.85	NC <sup>a</sup>	69.1	21.4 ± 2.37	NC <sup>a</sup>	0.25	NC <sup>a</sup>
Females							
0.0145	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>
0.0435	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>	NC <sup>a</sup>
0.13	NC <sup>a</sup>	NC <sup>a</sup>	7.99	7.99 ± 4.06	NC <sup>a</sup>	0.25	NC <sup>a</sup>
AUX-II TK Parameters (DS 1)							
Dose Level (mg protein/dose)	Parameter						
Males	AUC <sub>last</sub> ± SE (ng·hr/mL)	CL (mL/h)	C <sub>0</sub> (ng/mL)	C <sub>max</sub> ± SE (ng/mL)	t <sub>1/2</sub> (h)	t <sub>max</sub> (h)	V <sub>ss</sub> (mL)
0.0145	17.9 ± 4.45	NC <sup>a</sup>	46.6	24.3 ± 12.8	NC <sup>a</sup>	0.25	NC <sup>a</sup>
0.0435	160 ± 6.24	130	588	160 ± 5.96	0.59	0.25	55.9
0.13	261 ± 16.9	NC <sup>a</sup>	430	308 ± 46.4	NC <sup>a</sup>	0.25	NC <sup>a</sup>
Females							
0.0145	13.4 ± 2.08	NC <sup>a</sup>	13.5	17.9 ± 4.11	NC <sup>a</sup>	0.50	NC <sup>a</sup>
0.0435	42.1 ± 8.46	NC <sup>a</sup>	35.3	46.8 ± 9.51	NC <sup>a</sup>	0.50	NC <sup>a</sup>
0.13	166 ± 24.7	378	559	173 ± 87.3	0.52	0.25	161

a. NC = not calculated

Above data suggest that AUX-1 degrades or neutralizes faster than AUX-2 on the first day of dosing and there was no accumulation. Biological half-lives of AUX-1 and AUX-2 were very short. No AUX-1 and AUX-2 was detected at predose indicating there was no measurable collagenase from bacterial sources was present in the rats. Elimination of AUX-1 and AUX-2 in the plasma was rapid.

The sponsor stated that clostridial collagenase was detected only in one animal at 0.13 mg/dose at one time point of each sex in samples collected on subsequent study days.

Above kinetic data indicate that AUX-1 and AUX-2 from AX4500 degrades in vivo spontaneously and the drug had minimal exposure after IV administration.

Data for antibody titers at necropsy are shown from the applicant's table below.

Dose Level (mg protein/ dose)	Sex	Anti-AUX-I Responses			Anti-AUX-II Responses			AUX-I/ AUX-II <sup>a</sup>
		Titer Range	Median Titer	Percent Positive	Titer Range	Median Titer	Percent Positive	
0.00	M	(b) (4)	0.00	28%	(b) (4)	65.8	72%	NC <sup>b</sup>
	F	(b) (4)	0.00	28%	(b) (4)	0.00	8%	1.00
0.0145	M	(b) (4) - (b) (4)	197000	100%	(b) (4) (b) (4)	38400	100%	5.13
	F	(b) (4) - (b) (4)	73500	100%	(b) (4) - (b) (4)	61400	100%	1.20
0.0435	M	(b) (4) - (b) (4)	235000	100%	(b) (4) (b) (4)	27050	100%	8.69
	F	(b) (4) - (b) (4)	120000	100%	(b) (4) - 1(b) (4)	32300	100%	3.72
0.13	M	(b) (4) - (b) (4)	252500	100%	(b) (4) - (b) (4)	24400	100%	10.35
	F	(b) (4) - (b) (4)	237000	100%	(b) (4)	22300	100%	10.63

- a. AUX-I/AUX-II = ratio of group median anti-AUX-I titer to group median anti-AUX-II titer
- b. NC = not calculated (denominator = 0)

Anti-AA4500 antibodies were detected at the end of the treatment in all rats. However, a slight positive response for the presence of antibody was also reported in the control.

Necropsy: Necropsy of female rats did not show any abnormality.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Mating and fertility summary for male rats are shown below.

Parameter	Group 1	Group 2	Group 3	Group 4
# Cohabitated	25	25	24	24
#Rats mated	25	25	24	24
# rats with confirmed mating	25	24	24	24
#Rats Pregnant/# in cohabitation	25	25	24	24
Necropsy findings normal	24	25	25	24
% sperm motility	93	93	93	93
Sperm count in ten fields	136	138	153	155

One male in the control and one male at high dose showed small epididymides. However, average organ weight data did not show any treatment related change in the reproductive organs.

Based on above data, it was concluded that the treatment had no effect on fertility, sperm counts and sperm motility in rats.

#### Female mating and fertility:

Female rats in the control showed 3.3 to 3.4 days of average estrus cycle that was not significantly altered in the AA4500 treated rats. Female rats co-habited with the first male from the same group, mated within two and half days. Fertility index, percent pregnant, corpora lutea, implantations, live embryos for the control and treated rats were comparable and did not change significantly as shown in the table below.

Parameter	Group 1	Group 2	Group 3	Group 4
# Rats in cohabitation	25	25	25	25
# rats mated	25	25	25	25
Fertility index (%)	96	88	88	92
Pregnant (%)	96	88	88	92
Average corpora lutea	16.4	16.3	15.6	16.5
Implantations	15.4	15.1	14.8	15.7
Litter sizes	15.4	15.1	14.8	15.7
Live embryos	340	315	294	335
Dams with viable embryos	24	22	22	23

Fertility Index= # rats pregnant / # rats mated

Conclusion: Male and female rats treated up to 0.13 mg/dose did not show any abnormal effect on fertility and early embryonic development. AA4500 degraded rapidly after the IV injection and anti-AUX antibodies were detected at the terminal sacrifice. Injection site swelling was present at 0.0435 and 0.13 mg/dose.

### Embryofetal development

**Study title:** Intravenous developmental toxicity study of AA4500 in rats

**Key study findings:** No developmental toxicity was noted in the study. NOEL for the developmental toxicity was 0.13 mg protein/day.

**Study no.:** (b) (4)0009

**Module #4, and page #:** 1

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** Dec 17, 2006

**GLP compliance:** Yes

**QA reports:** yes (x) no ( )

**Drug: AA4500** (b) (4) batch), lot # NFF0035, and % purity: 100% by HPLC system; Placebo lot # FIN-0251, Auxilium Pharmaceuticals; the drug product solution was made once daily. The analytical determinations of the sample for its stability and concentrations are shown below from the applicant's appendix 4.

Concentration	Recovered concentration	% Claim
0.029 mg/mL	0.023- 0.025 mg/mL	78-85%
0.087 mg/mL	0.076-0.88 mg/mL	87-99%
0.26 mg/mL	0.25-0.29 mg/mL	98-114%

Although some of the solutions at 0.029 and 0.087 mg/mL concentrations showed lower than the target concentration, most of the high dose solutions confirmed the target concentration and the study is acceptable to determine the reproductive risk under the given conditions.

### Methods

Doses: The study design is shown below from the applicant's table.

Dose Group	Dose (mg protein/day)	Concentration (mg/mL)	Dose Volume (mL/rat)	Number of Rats	Injection Rate	Assigned Rat Numbers
I	0 (Placebo)	0.0	0.5	25	Bolus	23701 - 23725
II	0.0145	0.029	0.5	25 + 6 <sup>a</sup> + 6 <sup>b</sup>	Bolus	23726 - 23750; <b>1676 - 1681<sup>a</sup>; 2181-2186<sup>b</sup></b>
III	0.0435	0.087	0.5	25 + 6 <sup>a</sup> + 6 <sup>b</sup>	Bolus	23751 - 23775; <b>1682 - 1687<sup>a</sup>; 2187-2192<sup>b</sup></b>
IV	0.13	0.26	0.5	25 + 6 <sup>a</sup> + 6 <sup>b</sup>	Bolus	23776 - 23800; <b>1688 - 1693<sup>a</sup>; 28800, 2194-2198<sup>b</sup></b>

The test article was considered 100% active/pure for the purpose of dose calculations. All dose calculations were based on the vial content of AA4500 or AA4500 Placebo provided in each Certificate of Analysis.

**Bolded numbers** represent rats assigned to the toxicokinetic portions of the study.

- Six rats assigned for use in toxicokinetic sample collection for the initial study
- Six rats assigned for use in toxicokinetic sample collection for the study extension.

The high dose were selected on the basis of pilot study (b) (4);00014) in which 0.29 mg/day showed sloughing of tail. Mortality was noted at higher dose. However, a dose of 0.13 mg protein/day was tolerated (b) (4)0018).

Species/strain: Rats, CrI:CD(SD) from (b) (4)

Number/sex/group: See study design above

Route, formulation, volume, and infusion rate: The drug product or placebo was injected intravenously as bolus injections once a day between gestation days 7 and 17. AA4500 was reconstituted with sterile 0.9% sodium chloride and 0.03% calcium chloride solutions. The route of administration was chosen to maximize systemic exposure and to induce immunogenicity to the treatment.

Satellite groups used for toxicokinetics: 6/group, see study design above. Initial TK samples were not appropriate to measure the drug substance due to degradation of the

enzyme. Further samples were collected from animals assigned as extension of the TK study as indicated in the study design.

**Study design:** Male and female rats were mated. Pregnant rats were used for the organogenicity study. The body weight was recorded daily and food consumption was recorded on days 0, 7, 10, 12, 15, 18 and 21. Blood samples (0.2 mL) were collected from the tail or jugular vein at predose, 30 min, 2, 4, 8, 24, 48, 72 hours post dose on gestation days 7 and 17 for the determination of plasma levels of AUC-1 and AUX-2 using validated ELISA assay. Blood samples were taken from three rats at each time point.

Any gross lesions, heart, lungs, liver, kidneys, stomach and spleen were fixed in 10% formalin and retained for histological evaluation if needed. Surviving TK animals were euthanized on gestation days 19 and 20 via carbon dioxide inhalation. Surviving animals from the main study group were euthanized by carbon dioxide inhalation on gestation day 21. Caesarean section and gross necropsy was conducted on each rat.

**Parameters and endpoints evaluated:** Following parameters were recorded from pregnant rats:

Pregnancy status, corpora lutea, implantation sites, early (pre-organogenesis) and late resorption, live and dead fetuses, placental size and color will be recorded. Fetal weights, sex, gross lesions were recorded. Live fetuses were euthanized by sodium pentobarbital. Half of fetuses were fixed in Bouin's solution for determination of soft tissue alterations and variations. Remaining half was digested with alcohol and stained with Alizarin red for the examination of skeletal alterations.

## Results

**Mortality (dams):** One dam at 0.0435 (satellite) and one dam at 0.13 mg/day (#23786) were sacrificed due to decreased motor activity and injection site swelling, respectively.

**Clinical signs (dams):** Swelling at the injection site was noted dose dependently at 0.0435 and 0.13 mg/day. The applicant stated that most of the swelling were in the base of the tail and hind leg related to the injection in groups 3 and 4 animals. The applicant did not mention in the method section where the injections were given. Assuming that the injections were given into the caudal vein, swelling in the hind leg was noted following the first injection at 0.13 mg/day. In the absence of further characterization of hind leg swelling, it is not possible to conclude that the swelling was due to an autoimmune response. However, a similar change was not observed in the toxicity studies.

**Body weight (dams):** The treatment did not have any effect on the overall body weight gain of animals through out the gestation. The body weight data (g) on selected days of gestation are shown below.

Gestation Day	Group 1	Group 2	Group 3	Group 4
0	233	233	233	233

Gestation Day	Group 1	Group 2	Group 3	Group 4
7	270	270	270	270
17	334	328	335	338
21	414	404	411	411
BW change	181	171	178	178

**Food consumption (dams):** The average food consumption (g/day) between days 0 to 21 was 25 g/day across the groups. Therefore, the treatment had no effect on the food consumption.

**Toxicokinetics:** Most of the plasma samples showed below the quantitation limit of 5 ng/mL for AUX-1 or AUX-2 with some exceptions. Calculated AUX-1 and AUX-2 data for PK parameters on day 7 showed very short half-life of the protein. The plasma levels were measurable transiently in few samples. Examples of AUX-2 levels in animal # 28800, #2194, # 2195 from group 4 at 2- hour post dose on gestation day 7 showed 32.13, 17.73, 13.68 ng/mL, respectively. Animal # 2190, #2191, from group 3 at 3, 0.5 hour post dose on gestation day 7 showed AUX-2 levels of 76.55, 10.25 ng/mL, respectively. Overall, the PK data suggest minimal systemic exposure in pregnant rats up to 0.5 hour post dose. The applicant's summary table is shown below.

Plasma AUX-I (DG 7)							
Group	AUC <sub>0-24</sub> ± SE (ng·h/mL)	CL (mL/h)	C <sub>0</sub> (ng/mL)	C <sub>max</sub> ± SE (ng/mL)	t <sub>1/2</sub> (h)	t <sub>max</sub> (h)	V <sub>ss</sub> (mL)
AA4500 0.0145 mg protein/day	NC ± NC	NC	NC	NC ± NC	NC	NC	NC
AA4500 0.0435 mg protein/day	NC ± NC	NC	2.03	2.03 ± 2.03	NC	0.63	NC
AA4500 0.13mg protein/day	NC ± NC	NC	11.8	11.8 ± 1.88	NC	0.62	NC
Plasma AUX-II (DG 7)							
Group	AUC <sub>0-24</sub> ± SE (ng·h/mL)	CL (mL/h)	C <sub>0</sub> (ng/mL)	C <sub>max</sub> ± SE (ng/mL)	t <sub>1/2</sub> (h)	t <sub>max</sub> (h)	V <sub>ss</sub> (mL)
AA4500 0.0145 mg protein/day	NC ± NC	NC	12.1	12.1 ± 6.51	NC	0.50	NC
AA4500 0.0435 mg protein/day	133 ± 22.4	NC	241	55.0 ± 22.4	NC	0.63	NC
AA4500 0.13mg protein/day	446 ± 30.9	291*	618	218 ± 30.0	0.528*	0.62	142*

NC = Not calculated

\* = calculated using two timepoints over the terminal phase

Clearance of AUX-1 was faster than AUX-2. The sponsor stated that AUX-1 and AUX-2 were only detected on gestation day 7.

**Terminal and necropsic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):** At necropsy, swelling at the injection site was noted. No other gross change in the dams was noted. Caesarean data are shown below.

Parameter	Control	0.0145	0.0435	0.13 mg/day
Pregnant	24 (96%)	25 (100%)	24 (96%)	25 (100%)

# Caesarean	24	25	24	24
Live fetuses	346	346	338	348
Dams with viable fetuses (%)	100	100	100	100
% Male	49.6	49.5	49.2	49.4
Dead fetuses	0	0	0	0
Early resorption	10	15	14	16
Late resorption	0	0	0	0
% fetuses with any alteration/litter	3.8	2.9	2.3	1.7

Offspring (malformations, variations, etc.): Variation data in fetuses are shown below.

Parameter	Control	0.0145	0.0435	0.13 mg/day
Cleft palate, % fetuses	0.6	0	0.6	0
Folded retina, % fetuses	0	0.6	0	0
Skull, incomplete ossification, % fetuses	0	0	0.6	0

Above data showed no variations or abnormalities in fetuses up to 0.13 mg/day IV dose. PK data showed AUX-1 and AUX-2 degraded shortly after the IV injections. Injection site reactions were noted at mid and high doses. The NOEL for developmental toxicity was 0.13 mg/kg. The sponsor did not determine anti-AUX-1 and anti-AUX-2 antibodies in this study. Although no teratogenicity or maternal toxicity (except local reactions) at 0.13 mg/day (equivalent to 1.1 mg in a 60 kg humans) was noted in the study, it is necessary to verify if IV injections developed antibodies in rats from other studies so as to justify that the developmental effect or organogenicity in rats was also not impaired by the presence of antiAUX-1 and AUX-2 antibodies. The reviewer compared data for antibody formation in other toxicity studies in rats and concluded that the AA4500 antibodies could present in the plasma in the present study.

### **Prenatal and postnatal development**

The applicant did not conduct a prenatal and postnatal reproductive safety study because the Division agreed that such a study would not be necessary due to lack of plasma bioavailability of the drug after a local injection. However, the Division asked the applicant to discuss the issue with respect to necessity of the study if the antibody

neutralized AA4500. See discussion and conclusion section of the review below for a response to Pre BLA issues and the rationale for not conducting the study.

#### 2.6.6.7 Local tolerance:

##### **Study Title: An exploratory study on the effect of injected Nucleolysin on the subcutaneous fat of female Zucker rats, # 95-2384.**

The study was conducted in May 9, 1995. The lot # was 42901 for lyophilized collagenase. The drug substance was reconstituted with 0.9% NaCl and 2 mM calcium chloride solutions.

The study was conducted to determine the effect of local injection of collagenase into inguinal fat pad using obese Zucker rats. Injections were given into each inguinal fat pad. The method indicated that 5 injections were given in each fat pad spreading over several edges of the fat pad. The average body weight of rats was 395 g. Nucleolysin was injected at 500 and 1000 units/dose as a single dose and control animals received the vehicle. Animals were sacrificed forty-eight hours after the injection and histopathology of left and right fat pads was conducted in all animals. The study design is shown from the applicant's table below.

Group	Test Material	Compound Administration			Number of Females		
		Dose	Concentration	Volume <sup>a</sup>	Initial	Necropsy	Microscopic Pathology <sup>b</sup>
		units fat pad	units/mL	mL fat pad			
I	diluent for Nucleolysin <sup>®</sup>	0	0	1	7	7	7
II	Nucleolysin <sup>®</sup>	500	500	1	7	7	7
III	Nucleolysin <sup>®</sup>	1000	1000	1	7	7	7

<sup>a</sup>The 1 mL volume was divided into 5 injections of approximately 0.2 mL/injection per fat pad. Only the inguinal fat pads were injected.

<sup>b</sup>Microscopic pathology of the inguinal fat pads only were performed for all animals.

All animals survived following the treatment. Hemorrhage and inflammation was noted in all drug treated animals. Data also suggest fat cell disruption associated with red blood cells, inflammatory cells and fibrin accumulation around fat cells at 1000 units/dose.

The study indicates presence of inflammatory response following subcutaneous injection in rats and no NOEL was established.

#### 2.6.6.8 Special toxicology studies

##### **Study title: Guinea-pig sensitization study**

**Key study findings:** Guinea pigs did not show evidence of sensitization to Nucleolysin when given by ip or intra-cardiac route within 2 weeks.

**Study no.:** Not provided

**Volume # M4, and page #:** 1

**Conducting laboratory and location:** Not provided

**Date of study initiation:** Not provided

**GLP compliance:** No

**QA reports:** yes ( ) no (x)

**Drug: Nucleolysin, lot # N7903, and % purity:** 590 units/mL

**Formulation/vehicle:** Physiological saline containing 2 mM CaCl<sub>2</sub>

## Methods

### Study design and results:

Doses: Four ip injections of 0.1 mL (300 U/kg) of the test substance were given to guinea pigs. Animals were challenged 2 weeks after the first sensitizing injection by 300 U/kg dose of Nucleolysin by ip route. No clinical sign was reported subsequent to the challenging dose given by ip route.

Another study was conducted in guinea pigs at (b) (4) New York. Animals were immunized by 4 ip injections of collagenase form 3. Three injections were given on alternate days and the fourth injection was given on day 7. Seven days after the last injection, animals were challenged with intracardial injection of the test material. Animals were observed for next one hour for symptoms of antigen challenge. Each dose was 300 units/kg that was about LD<sub>10</sub> for mice. The LD<sub>50</sub> in mice was 3000 units/kg/ip.

No mortality or other treatment related clinical sign was observed following the antigen challenged.

Results of two studies showed that repeated doses for two weeks given by parenteral routes did not cause hypersensitivity reactions in the guinea-pig model.

## 2.6.6.9 Discussion and Conclusions:

AA4500 is a fixed combination of collagenase 1 and collagenase 2 at (b) (4) on weight basis. Collagenase was obtained from *Clostridium histolyticum*. Several toxicity studies were conducted in rats and dogs to determine its systemic toxicity, local toxicity and reproductive toxicities. AA4500 was manufactured using BTC, (b) (4) and Auxilium processes. Comparability studies were also conducted in rats for toxicity, analytical purity and potency. However, potency and impurity profiles of several batches of the drug substance, as well as immunogenicity to AA4500, will be reviewed by the product quality reviewer in a separate review.

AA4500 showed local toxicities at the site of injection in dogs and rats at equal or lower than human doses on mg/kg basis. NOEL for the effect was difficult to establish due to the procedural effects and due to collagen-lytic effect of the drug. Repeat dose toxicity in rats showed liver toxicity at 0.29 mg/dose/IV that is about 100 times higher than 0.58 mg/dose injections in humans at mg/kg basis. The applicant conducted segment 1 and segment 2 toxicity studies in rats following IV injections. However, the treatment did not show any deleterious effect for fertility and embryofetal development. The applicant conducted a battery of mutagenicity studies that showed no mutagenicity to AA4500 manufactured by the BTC process. Since the drug product for the clinical use would be made by Auxilium process and large molecular proteins do not transport across the cell membrane, mutagenicity study for the product is not a regulatory requirement. The applicant addressed several issues discussed in the Pre-BLA meetings and these items are referenced below. One of the issues was related to the role of anti-product antibodies formed after the systemic or local injections in animals. Data submitted in the BLA did not show any neutralization of the drug substance by its antibodies based on the toxicity data. The antibodies also did not cross-react with the recombinant human collagenase in vitro. However, these data would be further reviewed by the immunologist to comment on the applicant's position. Based on the non-clinical data and response of the applicant for the Pre-BLA issues, the reviewer does not recommend that any additional non-clinical study for the approval of this biologic. The BLA can be approved for the treatment of Dupuytren's disease from the non-clinical point of view. The Pharm/Tox recommendations for the package insert are provided in the executive summary.

#### **The response of the applicant on issues discussed in Pre-BLA meeting:**

During the Pre-BLA meeting dated Sept 15, 2008, the Division responded with following remarks:

A. "The Division notes that your GLP toxicology study in the dog does not demonstrate a clear NOAEL. In the absence of a clear NOAEL, your BLA submission should include rationale for why the histological findings in the dog do not raise safety concerns for your drug product. Final determination of the adequacy of the existing nonclinical toxicology studies can only be provided once a final determination of the comparability of the nonclinical batches to the clinical formulation is made"

Applicant's response:

The applicant provided the response in M1 as shown below:

1. The GLP toxicology study in the dog combined two different study designs. A single dose local tolerance study and repeat dose general toxicology study.
2. A clear NOAEL was demonstrated for local, systemic, and antibody-mediated effects in the repeat dose toxicology study.

3. The absence of a clear NOAEL (based on injection site findings) in the single dose (local tolerance) study is considered neither adverse nor of concern for the safe clinical use of AA4500 for the following reasons:

It was designed to characterize the effects and reversibility of maximum tolerable dose, tissue responses at the injection site with no clear NOEL/NOAEL were expected. No systemic toxicity or damage to tissue elements critical to the structural or functional integrity of the injected tissue sites resulted from AA4500 injection at any dose level. The injection site reactions were expected secondary manifestations of the primary pharmacologic activity of AA4500 (degradation of collagen into biologically active peptide fragments), and all injection site changes reversed partially to completely following cessation of dosing.

Reviewer's response: The reviewer agreed with the applicant's position that the local changes at the injection site were combination of the procedure and pharmacodynamic effects (collagen lysis). Additionally, collagen from the tail (injection sites in many studies) was also used for determination of the potency of the drug. Therefore, a clear NOEL would be difficult to establish. The injection site reactions were not fully reversible within next 28 days as stated by the applicant. Based on the pharmacodynamic properties and absence of systemic pharmacological effects and toxicities following the local injections in dogs, the response of the applicant is acceptable to the reviewer. However, the reviewer fails to understand the statement by the applicant as "role of pharmacological activity of AA4500 (degradation of collagen into biologically active peptide fragments)". Antibodies to AUX-1 and AUX-2 were also observed in human and non-clinical studies. The toxicity to the antibodies is not predictable based on the results of local and systemic injections to AA4500. From the regulatory point of view, the reviewer does not recommend further actions on this issue.

B. "Your BLA submission should contain a summary table outlining the impurity specifications in all nonclinical and clinical batches in order to document the applicability of the nonclinical studies conducted to support your proposed clinical studies".

Applicant's response: The sponsor provided specifications of the proposed drug product in Module 2 in a tabular form. The batches used for the IV toxicity studies, local toxicity study and reproductive toxicity studies met the specifications.

Reviewer's Response: The reviewer agreed on the applicant's response that AA4500 used for the non-clinical studies met the proposed specifications for the drug product. No regulatory action is needed for the non-clinical studies with this respect.

C. "If comparability is not demonstrated, your BLA submission must include a 3-month repeat dose toxicology study with the clinical formulation that demonstrates a clear NOAEL and evidence of complete reversibility of the histopathological changes."

Applicant's response: The applicant provided analytical and toxicity comparability for AA4500 manufactured by different processes. The toxicity study in rats showed comparability to several manufacturing processes of the drug. In addition, the applicant indicated analytical comparability data submitted on Feb 11, 2009 was acceptable to the FDA.

The reviewer's response: Non-clinical toxicity studies used for comparing BTC, (b) (4), and Auxilium batches of AA4500 showed comparable toxicity. From the regulatory point of view, further non-clinical bridging toxicity studies are not required.

D. "Your BLA submission should address the potential for general and reproductive and developmental toxicity, if anti-product antibodies form which cross react with self-antigens. This must include a discussion of the potential for anti-product antibodies to bind to and neutralize endogenous collagenase."

Applicant's response: The response from the applicant's submission is shown below.

1. The potential for anti-product antibodies to cross-react with/neutralize endogenous collagenase is considered highly unlikely based on the following:
  - Mammalian endogenous collagenases, matrix metalloproteinases (MMPs), are substantially different from clostridial collagenase in terms of size, amino acid homology, structure and organization of the catalytic and collagen binding domains.
  - Recombinant human MMPs were unable to act as effective competing ligands in the human anti-AUX-I and anti-AUX-II antibody assays, indicating a lack of antigenic cross reactivity.
2. Anti-AUX-I and anti-AUX-II antibody responses were evaluated in all repeat dose general and reproductive toxicity studies (except (b) (4)00009). The responses had the following characteristics:
  - The onset, percent of animals responding and magnitude of titers were comparable between rats and dogs, and between IV and local administration.
  - Positive titers occurred rapidly, following as few as two doses of AA4500.
  - Positive responses/high titers occurred in the majority (70- 100%) of the animals at the end of dosing and in 100% of the animals at the end of recovery.
  - Titers were persistent and also increased in magnitude following cessation of dosing (recovery periods of up to 30 days).
3. The presence of early onset, sustained high titer responses in the majority of the animals allowed them to be useful in evaluating responses attributable to either AA4500 or anti-product antibodies in both the general and reproduction toxicity studies and to discriminate between the cause of any effects seen as follows:
  - No effects on fertility, mating, implantation, placentation or embryo-fetal development were seen in the reproduction toxicity studies, in the face of high anti-product antibody titers.

Systemic MMP inhibition in rodents results in characteristic adverse effects on implantation and placental development and embryo-fetal anomalies; the absence of these effects in the reproduction toxicity studies with AA4500 supports a lack of physiologically relevant cross-reactivity with or neutralization of endogenous MMPs by anti-product antibodies.

Effects directly attributable to AA4500 were expected to show clear dose responses and to diminish in incidence and/or severity at the end of the recovery period.

Effects attributable to anti-product antibody formation were expected to show less clear dose responses and were not expected to reverse, but instead were expected to persist and/or increase in incidence/severity at the end of the recovery period.

Effects attributable to physiologically relevant cross-reactivity with or neutralization of endogenous collagenase (MMPs) were expected to resemble those resulting from pharmacologically-mediated MMP inhibition, as described in the literature.

4. None of the effects seen in either the general or reproductive toxicity studies could be attributed to anti-product antibody formation and/or cross-reactivity with or neutralization of endogenous collagenase:

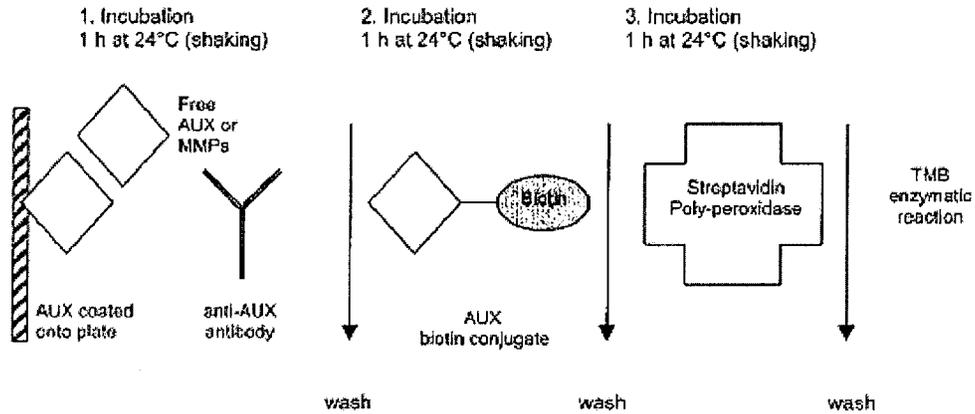
All of the SYSTEMIC and LOCAL effects seen following AA4500 administration showed dose responses and reversed partially to completely following cessation of treatment.

The systemic effects noted in the general toxicity studies were not consistent with the well-characterized effects of broad spectrum systemic MMP inhibition in rodents, further supporting a lack of physiologically relevant cross-reactivity with or neutralization of endogenous MMPs by anti-product antibodies.

Reviewer's response: Anti-AUX-1 and anti-AUX-2 antibodies were detected in the local and systemic toxicity studies. The summary of clinical safety data also showed the presence of antibodies. In several instances, the antibody was present in the absence of the drug substance in the nonclinical studies. However, there is no clear evidence in the toxicity data that systemic toxicity resulted from the antigen-antibody reactions. An analysis of data related to the liver toxicity, the reviewer analyzed the association of liver toxicity to the antibodies. However, data failed to show any relationship in rats. The applicant further characterized the recombinant stromelysin's ability to become a catalyst for the degradation of AUX-1 and AUX-2 antibodies. Data showed minimal affinity of the antibodies to several recombinant human stromelysin. A schematic of the MMPs or free AUX competition with anti-AUX-1 or anti-AUX-2 antibodies are shown from the applicant's submission below.

**Figure 1: Bridging ELISA Format for Testing Cross-Reactivity of Human Anti-AUX-I or Anti-AUX-II Antibodies versus Different Human MMPs**

**Principle of the assay:**



Similarly, data for the AUX antibodies and collagenase interactions are shown from the applicant's submission below.

**Table 4: Percent Inhibition of Binding of Human Anti-AUX-I by Competing Antigen**

Sample ID	Reference OD <sup>a</sup>	Percent Inhibition by 1 µg/mL of:					
		AUX-I	MMP-1	MMP-2	MMP-3	MMP-8	MMP-13
(b) (4) 345	1.001	86.71%	3.80%	-3.30%	-1.80%	0.60%	0.00%
552	0.848	83.02%	-0.71%	0.83%	0.94%	2.83%	2.83%
535	0.796	83.29%	1.51%	2.51%	3.89%	1.51%	-0.25%
544	0.783	82.12%	2.43%	2.30%	3.32%	-2.17%	-0.13%
493	1.056	87.03%	5.40%	-0.57%	2.46%	-0.38%	-1.89%
Positive control <sup>b</sup>	0.783	80.20%	0.38%	2.43%	5.62%	-4.21%	-1.28%

Data source: Section 5.3.1.4; Report AA74233CH-EB, Table 4a

<sup>a</sup> Mean OD value in absence of added competing ligand.

<sup>b</sup> Affinity purified rabbit polyclonal anti-AUX-I antibody.

**Table 5: Percent Inhibition of Binding of Human Anti-AUX-II by Competing Antigen**

Sample ID	Reference OD <sup>a</sup>	Percent Inhibition by 1 µg/mL of:					
		AUX-II	MMP-1	MMP-2	MMP-3	MMP-8	MMP-13
(b) (4) 345	0.709	74.47%	-4.09%	-14.25%	-7.76%	-21.16%	0.00%
552	0.676	75.00%	-2.81%	-6.66%	-0.74%	-4.14%	-8.88%
535	1.102	83.67%	8.89%	11.16%	6.81%	13.43%	10.62%
544	0.914	81.95%	-3.06%	-13.68%	-10.94%	0.77%	2.63%
493	0.743	79.95%	-3.77%	-8.34%	-13.73%	-7.40%	-17.90%
Positive control <sup>b</sup>	0.859	70.43%	-2.10%	1.05%	10.13%	11.41%	8.03%

Data source: Section 5.3.1.4; Report AA74233CH-EB, Table 4b

<sup>a</sup> Mean OD value in absence of added competing ligand

<sup>b</sup> Affinity purified rabbit polyclonal anti-AUX-II antibody.

Above in vitro data suggest that several recombinant MMPs had poor affinity to the AUX antibodies, whereas, the drug substance AUX-1 and AUX-2 competed with the positive control. The applicant provided amino acid sequences for AUX-1, AUX-2 and several MMPs to support that there was no cross-reactivity between human MMPs and anti-AUX antibodies.

**Table 1: Amino Acid Sequence Homology of AUX-I and AUX-II Versus Human MMPs**

Major Collagenolytic Human MMPs		Primary Substrate(s)	% Sequence Homology vs.	
Protein name	Alternative Name		AUX-I	AUX-II
MMP-1	Collagenase-1	Fibrillar collagen	37	35
MMP-2	Gelatinase-A	Gelatin	28	42
MMP-3	Stromelysin-1	Non-fibrillar collagen MMP proenzymes	24	24
MMP- 8	Collagenase-2	Fibrillar collagen	50	53
MMP- 9	Gelatinase-B	Gelatin	39	39
MMP-13	Collagenase-3	Fibrillar collagen	34	29
MMP-14	MT1-MMP	Fibrillar collagen MMP proenzymes	44	32

Based on the in vitro data and non-clinical toxicity data, the reviewer concluded that anti-product antibodies were present but there was no evidence of neutralization and other deleterious effect related to anti-AUX-1 and anti-AUX-2 in animals tested. However, the immunogenicity data presented in Module 5 needs to be reviewed by the immunologist from product quality to reaffirm that the antibody was non-neutralizing in humans.

D. “Based on the information submitted to date documenting the lack of systemic exposure to AA4500, further reproduction and developmental toxicology studies will not be required.”

Applicant’s response: No action needed

Reviewer’s response: The reviewer agreed with the sponsor that no systemic toxicity was noted in dogs after local injections. Clinical summary also indicated a lack of systemic exposure to AA4500. The other issue is related to neutralizing anti-product antibodies. As discussed above for item # C, due to a lack of evidence of neutralizing antibodies in animal studies, the reviewer does not recommend further reproduction studies. The product reviewer opinion on neutralization on the neutralization of AA4500 by its antibodies would be further discussed for the product label. See further recommendation for reproduction information for the package insert.

**2.6.6.10 Tables and Figures: see below**

The comparative toxicity table for human and animals is shown below.

Species	Dose, mg protein/dose	Average body weight	Dose,	Animal dose : Human dose	Toxicity	Presence of antibody
Human	0.58 local injection	60 kg	0.0096, mg/kg (9.6 ug/kg)		Peripheral edema, contusion, injection site pain and hemorrhage, pain in extremity	Yes
Rat	0.0029/IV	0.3 kg	0.0096 mg/kg	1	Injection site inflammation	Yes
Rat	0.029/IV	0.3 kg	0.096 mg/kg	10	Injection site inflammation	Yes
Rat	0.29/IV	0.3 kg	0.96 mg/kg	100	Injection site Inflammation and liver toxicity	Yes
Rat, seg 1	0.13/IV	0.3	0.43 mg/kg	45	Injection site swelling	Yes
Rat, seg 2	0.13/IV	0.3	0.43 mg/kg	45	Injection site swelling	Not determined
Dog	Local injection at Tunica Albuginea in penis	8 kg	0.8 ug/kg, 9 doses	0.08	Local inflammation at tunica albuginea; minimal perivascular inflammation in brain and inflammation in salivary gland	Yes
Dog	Local injection at Tunica Albuginea in penis	8 kg	2.5 ug/kg, 9 doses	0.26	Local inflammation At tunica albuginea;	Yes
Dog	Local injection at Tunica Albuginea in penis	8 kg	6.1 ug/kg, maximum 9 doses	0.63	Local inflammation at tunica albuginea; minimal inflammation in brain, spinal cord, heart, salivary gland, pancreas and prostate	Yes
Dog	Local injections at at Tunica Albuginea, corpus cavernosum, van complex in penis and urethra	8 kg	8.3 and 14.9 ug/kg single dose	0.86 and 1.5	Hemorrhage, edema, inflammation of injection site. Thymus atrophy was observed when injected into urethra	Antibodies were not determined

**2.6.7 TOXICOLOGY TABULATED SUMMARY**

The applicant presented following tabulated toxicity summaries in the BLA:

Drug Product Specifications:

2.6.7.4. Toxicology

		Drug Substance <sup>1</sup>	Test Article: AA4500		
<i>Proposed Drug Product Specification</i>					
Strength	Purity (%) (Method)	Specified Impurities	Potency Rel. to Ref (Method/Component)		
(b) (4)					
<i>Drug Product Specifications for Batches Used in Toxicology Studies</i>					
Batch No. (Strength)	Purity (%) (Method)	Specified Impurities	Potency Rel. to Ref (Method/Component)	Study Number	Type of Study
(b) (4)					Rat Local Tolerance
(b) (4)					Rat IV repeat dose toxicity Rat IV repeat dose toxicity

2.6.7.4. Toxicology (Continued)

<i>Drug Product Specifications for Batches Used in Toxicology Studies</i>					
Batch No. (Strength)	Purity (%) (Method)	Specified Impurities	Potency Rel. to Ref (Method/Component)	Study Number	Type of Study
[Redacted]				(b) (4)	Rat IV repeat dose toxicity
					Rat IV embryonic development
					Rat IV general fertility/early embryonic development
[Redacted]					Rat IV repeat dose toxicity
					Dog (b) (4) repeat dose toxicity/local tolerance study
[Redacted]					Rat IV repeat dose toxicity

<sup>a</sup> Drug Product was used for all toxicology studies

<sup>b</sup> ND=not determined

<sup>c</sup> NA=not applicable (methods in proposed specification not used)

(b) (4)

[Redacted]

2.6.7.7A Repeat-Dose Toxicity: Pivotal Studies

Report Title: A 16-Day, Multiple-Dose, Intravenous Toxicity Study in Rats Comparing AA4500 with BTC Collagenase and Placebo				Test Articles: AA4500 (lot NFF-0035) and AA4500 (Process 1) (Lot 992-7) Note: AA4500 (Process 1) = BTC collagenase							
Species/Strain: Rats/Sprague-Dawley		Duration of Dosing: 16 days			Study No. (b)30006						
Initial Age: 8 wks at study start		Duration of Postdose: 0 days									
Date of First Dose: 03Jul2006		Method of Administration: IV bolus (0.5 mL/animal)									
		Vehicle/Formulation: 2 mM CaCl <sub>2</sub> in 0.9% NaCl (used to make 17,241 U/mL stock solution; final concentrations made by further dilution with Sterile Water for Injection)/ 0, 100, 300 and 500 U/mL			GLP Compliance: GLP						
Special Features: Animals were dosed q48h (every other day). TK samples (serum) collected on Days 1 and 15 from satellite dose groups (9/sex/dose from AA4500 treated groups only). Control article was placebo used in clinical studies (10 mM TRIS, 60 mM sucrose, diluted in 2 mM CaCl <sub>2</sub> in 0.9% NaCl).											
No Observed Adverse Effect Level: 500 U/dose (0.029 mg protein/dose) for systemic toxicity (150 U/dose (0.0087 mg protein/dose) for injection site findings)											
Dose Level (U/dose) (mg protein/dose)		0 (0)		50 (0.0029)		150 (0.0087)		500 (0.029) (NFF-0035)		500 (0.029) (992-7)	
Number of Animals <sup>a</sup>		M:10	F:10	M: 10 + 9	F: 10 + 9	M: 10 + 9	F: 10 + 9	M: 10 + 9	F: 10 + 9	M: 10 + 9	F: 10 + 9
Toxicokinetics: AUC (ng·h/mL)											
AUX-I (Day 1)				0.00	0.00	1.26	1.71	3.44	3.65	1.11	1.62
AUX-I (Day 15)				0.00	0.00	9.32	0.00	0.00	0.00	0.00	0.00
AUX-II (Day 1)				0.63	0.68	14.3	22.7	76.9	93.9	26.0	18.3
AUX-II (Day 15)				888.5	11641	10582	29961	14245	22928	5198	38486

**2.6.7.7A Repeat-Dose Toxicity: Pivotal Studies (Continued)**

Dose Level (U/dose) (mg protein/dose)	0 (0)		50 (0.0029)		150 (0.0087)		500 (0.029) (NIF-0035)		500 (0.029) (992-7)	
Number of Animals <sup>2</sup>	M:10	F:10	M: 10 + 9	F: 10 + 9	M: 10 + 9	F: 10 + 9	M: 10 + 9	F: 10 + 9	M: 10 + 9	F: 10 + 9
Noteworthy Findings:										
Clinical Observations (frequency/incidence) <sup>3</sup>										
Tail discoloration	0/0	0/0	0/0	0/0	0/0	2/1	6/4	8/6	6/5	14/8
Gross Pathology										
Injection site discoloration	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	2/10
Histopathology										
Injection site:										
Hemorrhage, perivascular										
Minimal	1/10	1/10	1/10	1/10	1/10	2/10	5/10	1/10	3/10	2/10
Mild	1/10	0/10	1/10	0/10	1/10	0/10	0/10	1/10	1/10	4/10
Inflammation, chronic, perivascular										
Minimal	3/10	3/10	2/10	0/10	3/10	2/10	5/10	2/10	3/10	1/10
Mild	0/10	1/10	0/10	0/10	0/10	0/10	4/10	6/10	3/10	9/10
Median antibody titers (Day 15):										
Anti-AUX-I (% response)	0 (0%)	0 (0%)	903 (100%)	4085 (100%)	9225 (100%)	15200 (100%)	19050 (100%)	28650 (100%)	11950 (100%)	13984 (100%)
Anti AUX-II (% response)	0 (0%)	0 (0%)	111 (100%)	489 (100%)	946 (100%)	1190 (100%)	1542 (100%)	2325 (100%)	1950 (100%)	2340 (100%)

<sup>2</sup> M= males, F= females

<sup>3</sup> Frequency = number of study days on which the observation was made; incidence = number in dose group with the finding

BTC = early BTC process

AUC = area under the curve

2.6.7.7B Repeat-Dose Toxicity: Pivotal Studies

Report Title: AA4500 and BTC Collagenase: A 16-Day Intravenous Comparative Toxicity and Toxicokinetic Study Followed by a 14-Day Recovery Period in Sprague-Dawley Rats		Test Article: AA4500 (Lots NFF-0035 and 7280) and AA4500 (Process 1) (Lot 992-7) Note: AA4500 (Process 1) = BTC collagenase; AA4500 Lot NFF-0035 was manufactured by (b) (4) and AA4500 Lot 7280 was manufactured by the Sponsor's Horsham facility													
Species/Strain: Rats/Sprague-Dawley		Duration of Dosing: 16 days				Study N (b) (4) 1007-1671									
Initial Age: 12 to 15 weeks		Duration of Postdose: 14 days													
Date of First Dose: 04Sep2007		Method of Administration: IV bolus (0.5 mL/animal)													
		Vehicle/Formulation: 2 mM CaCl <sub>2</sub> in 0.9% NaCl/ 0, 1000, 4480 and 10,000 U/mL				GLP Compliance: GLP									
Special Features: Animals were dosed q48h (every other day). TK samples (plasma) collected on Days 1, 7 and 15 from satellite dose groups (3/sex/dose from vehicle control, 12/sex/dose from AA4500 treated, groups). 5/sex/group at 0, 500 and 500 U/dose for recovery animals															
No Observed Effect Level: 500 U/dose for systemic toxicity; No Observed Adverse Effect Level: 2240 U/dose for local effects															
Dose : U/dose (mg protein/dose) (AA4500 Lot Number)		0 (0)		500 (0.29) (992-7)		500 (0.29) (NFF-0035)		500 (0.29) (7280)		2240 (0.13) (7280)		5000 (0.29) (NFF-0035)		5000 (0.29) (7280)	
Number of Animals <sup>a</sup>		M:	F:	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:	M:	F:
		18	18	27	27	27	27	27	27	22	22	27	27	27	27
Toxicokinetics: AUC (ng·h/mL) <sup>b</sup>															
AUX-II (Day 1)				117		250		169		1229		4679		4166	
AUX-II (Day 7)				57.7		152		152		1039		2624		2289	
AUX-II (Day 15)				< 120		< 123		< 121		< 126		< 771		< 258	

2.6.7.7B Repeat-Dose Toxicity: Pivotal Studies (Continued)

Dose : U/dose (ng protein/dose) (AA4500 Lot Number)	0 (0)		500 (0.29) (992-7)		500 (0.29) (NFF-0035)		500 (0.29) (7280)		2240 (0.13) (7280)		5000 (0.29) (NFF-0035)		5000 (0.29) (7280)	
Number of Animals <sup>d</sup>	M: 18	F: 18	M: 27	F: 27	M: 27	F: 27	M: 27	F: 27	M: 22	F: 22	M: 27	F: 27	M: 27	F: 27
Noteworthy Findings:														
Died or Premature Euthanasia	0/18	0/18	0/27	0/27	0/27	0/27	0/27	0/27	0/22	0/22	2/27	4/27	1/27	3/27
Clinical Observations (frequency/incidence) <sup>e</sup>														
Inj. Site discoloration	11/6	14/9	14/7	33/8	21/8	19/7	14/6	16/7	36/6	28/7	20/6	45/13	26/10	66/12
Hematology														
RBC count (X10 <sup>12</sup> /L)	8.10	7.24	7.79	7.14	8.11	7.16	8.12	7.08	7.79	7.12	7.71	6.70 <sup>*</sup>	7.99	6.63 <sup>*</sup>
Hemoglobin (g/L)	152	147	151	142	154	143	155	142	151	141	148	135 <sup>*</sup>	153	135 <sup>*</sup>
Hematocrit (L/L)	0.40	0.38	0.40	0.38	0.41	0.38	0.41	0.37	0.40	0.37	0.39	0.35 <sup>*</sup>	0.40	0.36 <sup>*</sup>
Reticulocyte (X10 <sup>6</sup> /L)	222.0	196.8	243.2	229.7	244.1	194.6	246.2	211.3	253.0	198.2	372.7	270.3	226.9	353.4
Reticulocyte (%)	2.7	2.7	3.1	3.2	3.0	2.7	3.1	3.0	3.3	2.8	5.1	4.1 <sup>*</sup>	2.9	5.7 <sup>*</sup>
RDW (%)	13.3	12.6	13.2	12.9	13.0	12.6	13.2	12.7	13.8	12.6	15.1	13.1	12.9	14.8
RBC Morphology:														
Anisocytosis -1	0/10	0/10	0/10	0/10	0/10	0/10	1/01	0/10	1/10	0/10	1/10	0/10	0/10	1/10
Anisocytosis +2	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
Polychromasia +1	0/10	1/10	0/10	1/10	0/10	0/10	2/10	0/10	1/10	0/10	1/10	2/10	2/10	3/10
Polychromasia +2	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10	0/10	0/10	1/10
NRBC	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	1/10

2.6.7.7B Repeat-Dose Toxicity: Pivotal Studies (Continued)

Dose : U/dose (mg protein/dose) (AA4500 Lot Number)	0 (0)		500 (0.29) (992-7)		500 (0.29) (NFF-0035)		500 (0.29) (7280)		2240 (0.13) (7280)		5000 (0.29) (NFF-0035)		5000 (0.29) (7280)	
Number of Animals <sup>a</sup>	M: 18	F: 18	M: 27	F: 27	M: 27	F: 27	M: 27	F: 27	M: 22	F: 22	M: 27	F: 27	M: 27	F: 27
Serum Chemistry														
ALT (U/L)	41	30	39	30	38	28	37	45	47	30	63	37	88	37
AST (U/L)	118	105	111	97	98	92	110	168	140	95	157	119	153	102
Organ Weights (%)														
Absolute liver wt.(g)	9.64	6.50	10.2	6.12	9.79	6.79	10.1	6.59	9.71	6.98	11.4*	7.23	11.2*	7.34
Relative liver wt. (%BW)	2.34	2.50	2.40	2.50	2.39	2.50	2.41	2.50	2.38	2.70	2.77*	2.73	2.60*	2.80
Absolute spleen wt (g)	0.74	0.54	0.79	0.51	0.81	0.56	0.81	0.53	0.90	0.56	0.90	0.57	0.86	0.69*
Rel. spleen wt (%BW)	0.18	0.21	0.19	0.21	0.20	0.20	0.19	0.20	0.22	0.22	0.22	0.22	0.20	0.26*
Gross Pathology														
Liver - mass	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	3/10	2/11	1/10	2/11
Liver - raised/dark/pale focus/area	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	5/10	4/11	4/10	2/11
lnj. Site - discoloration	0/10	0/10	1/10	1/10	0/10	0/10	0/10	0/10	2/10	1/10	0/10	2/10	1/10	2/10
Histopathology														
Liver:														
Hemorrhage/hematoma	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	5/10	3/11	4/10	4/11
Necrosis, hepatocellular	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	3/10	4/11	3/10	1/11
- Inflamm., chron.-active	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	5/10	5/10	8/10	7/11	7/10	7/11
Fibrosis	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	4/10	2/11	2/10	3/11

2.6.7.7B Repeat-Dose Toxicity: Pivotal Studies (Continued)

Dose : U/dose (mg protein/dose) (AA4500 Lot Number)	0 (0)		500 (0.29) (992-7)		500 (0.29) (NFF-0035)		500 (0.29) (7280)		2240 (0.13) (7280)		5000 (0.29) (NFF-0035)		5000 (0.29) (7280)	
	M: 18	F: 18	M: 27	F: 27	M: 27	F: 27	M: 27	F: 27	M: 22	F: 22	M: 27	F: 27	M: 27	F: 27
Hyperplasia, bile duct	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	4/10	8/10	7/11	6/10	6/11
Injection site: (Severity range) <sup>6</sup>														
Edema/hemorrhage	1/10 (1)	3/10 (1-2)	7/10 (1-2)	7/10 (1-2)	1/10 (1)	5/10 (1)	3/10 (1)	1/10 (1)	3/10 (1-3)	6/10 (1-2)	0/10	7/11 (1-4)	5/10 (1-2)	8/11 (1-3)
Inflammation	2/10 (1)	5/10 (1)	5/10 (1)	9/10 (1)	3/10 (1)	1/10 (1)	2/10 (1)	3/10 (1-2)	5/10 (1-3)	5/10 (1-2)	0/10	7/11 (1-3)	2/10 (1-3)	9/11 (1-4)
Fibrosis, perivascular	0/10	0/10	4/10 (1)	0/10	0/10	0/10	0/10	1/10 (1)	2/10 (2-3)	2/10 (1)	0/10	3/11 (1-2)	5/10 (1)	3/11 (2-3)
Necrosis, perivascular	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	3/11 (1-3)	0/10	2/11 (2)
Median antibody titers														
Anti-AUX-I (Day 7) (% response)	0 (0%)	0 (0%)	4.6 (40%)	19.1 (53%)	74.7 (6.7%)	53.4 (13%)	20.2 (20%)	12.4 (40%)	17.2 (100%)	48.0 (100%)	110 (20%)	460.5 (40%)	190 (46%)	24.6 (20%)
Anti-AUX-I (Day 16) (% response)	0 (0%)	0 (0%)	15100 (93%)	48600 (93%)	9980 (100%)	11700 (100%)	11350 (93%)	14800 (80%)	13500 (100%)	12800 (100%)	13950 (100%)	13350 (100%)	14700 (100%)	14200 (100%)
Anti-AUX-II (Day 7) (% response)	0 (0%)	0 (0%)	0 (0%)	4.6 (47%)	11.9 (33%)	4.3 (67%)	22.6 (33%)	4.6 (47%)	18.0 (62%)	25.0 (80%)	121.5 (73%)	92.0 (73%)	22.3 (91%)	99.8 (50%)
Anti-AUX-II (Day 16) (% response)	0 (0%)	0 (0%)	3420 (93%)	2940 (87%)	2430 (100%)	2825 (93%)	2430 (100%)	2410 (100%)	2000 (100%)	6505 (100%)	1625 (92%)	1150 (100%)	573 (100%)	1042 (92%)
Postdose Evaluation: Number Evaluated	5	5	5	5	5	5	5	5	0	0	5	5	5	5

2.6.7.7B Repeat-Dose Toxicity: Pivotal Studies (Continued)

Dose : U/dose (mg protein/dose) (AA4500 Lot Number)	0 (0)		500 (0.29) (992-7)		500 (0.29) (NFF-0035)		500 (0.29) (7280)		2240 (0.13) (7280)		5000 (0.29) (NFF-0035)		5000 (0.29) (7280)	
Number of Animals <sup>a</sup>	M: 18	F: 18	M: 27	F: 27	M: 27	F: 27	M: 27	F: 27	M: 22	F: 22	M: 27	F: 27	M: 27	F: 27
Clinical Observations (frequency/incidence) <sup>c</sup>														
Inj. Site discoloration	0/0	0/0	0/0	0/0	13/2	2/1	0/0	1/1	0/0	0/0	22/2	25/1	1/1	16/2
Gross Pathology														
Liver – mass	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/0	0/0	3/5	0/4	3/5	2/4
Liver – raised/dark/pale focus/area	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/0	0/0	4/5	2/4	3/5	3/4
Liver – malformation	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/0	0/0	2/5	0/4	1/5	2/4
Liver – adhesion	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/0	0/0	2/5	0/4		1/4
Inj. Site – discoloration	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/0	0/0	3/5	1/4	0/5	1/4
Histopathology														
Liver:														
Hemorrhage/hematoma	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	NA*	NA*	4/5	2/4	3/5	3/4
Inflammation, chronic	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	NA*	NA*	4/5	1/4	3/5	4/4
Fibrosis	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	NA*	NA*	5/5	2/4	3/5	4/4
Pigment, brown/green	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	NA*	NA*	5/5	2/4	3/5	4/4
Mineralization	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	NA*	NA*	4/5	0/4	2/5	2/4
Hyperplasia, bile duct	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	NA*	NA*	0/5	1/4	0/5	4/4
Injection site:														
Inflammation, chronic	0/5	1/5	0/5	1/5	1/5	1/5	0/5	0/5	NA*	NA*	1/5	1/4	0/5	1/4

2.6.7.7B Repeat-Dose Toxicity: Pivotal Studies (Continued)

Dose : U/dose (mg protein/dose) (AA4500 Lot Number)	0 (0)		500 (0.29) (992-7)		500 (0.29) (NEF-0035)		500 (0.29) (7280)		2240 (0.13) (7280)		5000 (0.29) (NEF-0035)		5000 (0.29) (7280)	
Number of Animals <sup>3</sup>	M: 18	F: 18	M: 27	F: 27	M: 27	F: 27	M: 27	F: 27	M: 22	F: 22	M: 27	F: 27	M: 27	F: 27
Fibrosis	0/5	3/5	0/5	3/5	1/5	3/5	0/5	0/5	NA <sup>e</sup>	NA <sup>e</sup>	4/5	2/4	1/5	3/4
Median antibody titers														
Anti-AUX-I (Day 30) (% response)	0 (0%)	0 (0%)	77500 (100%)	301000 (100%)	337000 (100%)	31150 (100%)	77200 (100%)	265000 (100%)	NA <sup>e</sup>	NA <sup>e</sup>	94200 (100%)	61800 (100%)	303000 (100%)	27200 (100%)
Anti-AUX-II (Day 30) (% response)	0 (0%)	0 (0%)	36200 (100%)	60200 (100%)	51700 (100%)	60200 (100%)	26000 (100%)	57150 (100%)	NA <sup>e</sup>	NA <sup>e</sup>	19700 (100%)	13150 (100%)	21900 (100%)	43350 (100%)

<sup>3</sup> M= males, F= females

<sup>b</sup> AUC was not calculated for AUX-I due to limited exposure. Parameters for AUX-II were calculated using the data combined for both sexes (since exposure was not different between the sexes, this ensured adequate data points for calculation of TK parameters). When value is preceded by “<”, the AUC represents an estimated possible AUC<sub>0-24h</sub>

<sup>c</sup> Frequency = number of study days on which the observation was made; incidence = total number in dose group affected

<sup>d</sup> Severity scores; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

<sup>e</sup> NA = Not applicable (no recovery animals at this dose level)

<sup>f</sup> Significantly (p ≤ 0.05) different from 0 U/dose mean value

BTC = early BTC process

RBC = Red Blood Count

RDW = red cell distribution width

AUC = area under the curve

2.6.7.7C Repeat-Dose Toxicity: Pivotal Studies

Report Title: Local Toxicity Study of AA4500 Injected into Dog Penis		Test Article: AA4500 (NFF-0035)		
Species/Strain: Dog/Beagle (Males only)	Duration of Dosing: 62 days (3X/week q 4 weeks)	Study No. (b) (4)		
Initial Age: 10-14 months	Duration of Postdose: 30 days			
Date of First Dose: 18Jun2007	Method of Administration (b) (4) injection			
	Vehicle/Formulation: 2 mM CaCl <sub>2</sub> in 0.9% NaCl/ 40,000 U/mL	GLP Compliance: GLP		
Special Features: Repeated injections into the tunica albuginea, 3X (q48h) per week, with three weeks between treatment cycles, for three cycles (62 days total dosing)				
No Observed Adverse Effect Level: ~140 U/dose (0.8 mg protein/kg/dose)				
~U/Dose (mg protein/kg/dose)	0 (0)	~140 (0.8)	~430 (2.5)	~1430/~1050 (8.3/6.1)
Number of Animals	M: 2	M: 4	M: 4	M: 4
Noteworthy Findings:				
Died or Premature Euthanasia	0/2	0/4	1/4	0/4
Clinical Observations (frequency/incidence) <sup>2</sup>				
Discoloration/bruising (penis/prepuce/skin)	3/1	3/2	26/3	87/6
Swelling (penis)	0/0	2/1	3/1	47/ 6
Mass/nodule/red spot on penis	0/0	0/0	0/0	37/3
Gross Pathology				
Discoloration, prepuce/inguinal skin	0/0	2/4	0/4	1/4

2.6.7.7C Repeat-Dose Toxicity: Pivotal Studies (Continued)

~U/Dose (mg protein/kg/dose)	0 (0)	~140 (0.8)	~430 (2.5)	~1430/~1050 (8.3/6.1)
Number of Animals	M: 2	M: 4	M: 4	M: 4
Discoloration/enlargement, inguinal lymph node	0/2	1/4	0/4	2/4
Histopathology				
Injection site (severity range) <sup>b</sup>				
Hemorrhage, adventitial	2/2 (+1,+2)	2/4 (+1)	1/4 (+1)	3/4 (+1,+2)
Neovascular proliferation	2/2 (+1)	1/4 (+2)	2/4 (+1,+2)	0/4
Inflammation	0/4	2/4 (+2)	1/4 (+2,+3)	0/4
Median antibody titers (% response)				
Anti-AUX-I (Day 62)	0 (33%)	156250 (100%)	468750 (100%)	718250 (100%)
Anti AUX-II (Day 62)	0 (33%)	781250 (100%)	718250 (100%)	718250 (100%)
Postdose Evaluation: Number Evaluated	1	2	2	2
Histopathology:				
Hemorrhage, adventitial	0/1	1/2 (+1)	0/2	0/2
Neovascular proliferation	0/1	1/2 (+1)	1/2 (+1)	1/2 (+1)
Median antibody titers (% response)				
Anti-AUX-I (Day 90)	0 (0%)	468250 (100%)	156250 (100%)	718250 (100%)
Anti AUX-II (Day 90)	0 (0%)	781250 (100%)	718250 (100%)	718250 (100%)

<sup>a</sup> Frequency = number of study days on which observation made; incidence = number of animals in dose group affected

<sup>b</sup> Numerical severity scores (scale of 1-4) were used in this study. Score range indicates severity range both between animals and across the length of the examined tissue within each animal.

**OVERALL CONCLUSIONS AND RECOMMENDATIONS**

**Conclusions:** AA4500 is a mixture of collagenase from bacterial sources. AA4500 showed collagenolytic effect in the in vitro pharmacodynamic studies in human tissues and nonclinical toxicity studies. These effects were related to the injection site inflammation and hemorrhage, and liver toxicity when injected intravenously. AA4500 also showed local inflammatory effect in dogs. Reproductive data did not show any effect on fertility and early embryonic development. Pregnancy category C was recommended for Xiaflex. Bioavailability and exposure to AA4500 in the systemic circulation was minimal. However, antibodies to AUX-1 and AUX-2 were detected in nonclinical studies. The role of the antibodies is not known. However, non-clinical data in the presence of the antibody did not show any toxicity concerns.

**Unresolved toxicology issues (if any):** Nil

**Recommendations:** The reviewer recommends approval of Xiaflex for the treatment of Dupuytren’s disease on the basis of the non-clinical data and recommendations for the package insert.

**Suggested labeling:** See labeling recommendations in the executive summary

Signatures (optional):

Reviewer Signature \_\_\_\_\_

Supervisor Signature \_\_\_\_\_ Concurrence Yes \_\_\_ No \_\_\_

**APPENDIX/ATTACHMENTS: NIL**