CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125338

CHEMISTRY REVIEW(S)

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

Center for Drug Evaluation and Research
Office of Pharmaceutical Science
Office of Biotechnology Products
Division of Therapeutic Proteins
HFD-122

BLA: 125338

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FROM: Kathy Lee, M.S. and Ashutosh Rao, Ph.D. MK 11/23/09 AR 11/23/2009
THROUGH: Barry Cherney, Ph.D. Barry Cherney 11-23-09
SPONSOR: Auxilium Pharmaceuticals, Inc
PRODUCT: XIAFLEX (collagenase clostridium histolyticum) also known as AA4500
PROPOSED USE: For the treatment of advanced Dupuytren's Disease
CLINICAL DIVISION: Division of Anesthesia, Analgesia, and Rheumatology Products

GRMP TIMELINE:
Filing Meeting: March 26, 2009
60th Day: April 28, 2009
Mid-Cycle Meeting: May 29, 2009
Labeling Meeting: September 9, 17 and 28
Wrap-Up: September 1, 2009
Labeling Tcon with Applicant:
Primary Reviews/Draft CDTL Memo Due: September 4, 2009
Secondary Reviews/Draft CDTL Memo Due: September 11, 2009
CDTL Memo Due: October 23, 2009
PDUFA Action Date: November 2, 2009

REVIEW TEAM:
MO: Eric Brodsky, Sarah Okada
Product Team: Ashutosh Rao, V. Ravichandran, Baolin Zhang, Jee Chung, Fred Mills, Susan Kirschner, Kimberly Rains.
P/T: Dan Mellon, Ashok Mukherjee
Facilities: Anastasia Lolas
Clinical Pharmacology: Srikanth Nallani
Stats: Chris Wheeler
RPM: Margarita Tossa

TO: FILE

1. QUALITY SUMMARIES

I. EXECUTIVE SUMMARY

A. Description of the AA4500

AA4500 is a parenteral, lyophilized product comprised of two highly purified (b) (4) bacterial collagenases in an approximate (b) (4) mass ratio, collagenase AUX-I (Clostridial type I collagenase, and collagenase AUX-II (Clostridial type II collagenase). These collagenases are isolated and purified from the fermentation of *Clostridium histolyticum.* Collagenase AUX-I is a single polypeptide chain containing (b) (4) amino acids of known sequence and has a molecular weight of 114 kDa. AUX-II is (b) (4) amino acids long and has a molecular weight of 113 kDa. There is a 47% homology in the amino acid sequence of these two products. Both collagenases digest collagen by hydrolyzing the triple helical region of collagen under physiological conditions. Each collagenase has different specificity; Class I enzymes hydrolyze loci near the amino and carboxy termini of the triple helical domains of the collagen molecule and Class II collagenases make their initial cleavage near the interior of the molecule. The activity of type I collagenase is not limited to collagen but includes caseinase, clostripain and tryptic like activities, wherease type II is more non-specific and has higher levels of protease activity. While both collagenases are not immunologically cross-reactive and have different specificities, together they work synergistically, and provide a very broad hydrolyzing reactivity toward collagen[1].

Collagenases are also known as matrix metalloproteinases (MMPs, 23 members in the human family). Structural features shared among the MMPs include a catalytic zinc-binding domain with a conserved sequence motif of HEXXHXXGXXH. I*n vitro,* collagenolytic MMPs have been shown to attack a number of peptide substrates, other than collagens and gelatins[2]. Several examples are considered in the review by Nagase et al., and include osteonectin, IGF binding proteins, IL1ß and stromal cell-derived factor.[3] It has not been established, however, that there is proteolytic cleavage by MMPs of these non-collagenous substrates *in vivo*.

AA4500 is intended to treat patients with Dupuytren's disease. Dupuytren's disease has been characterized as a myofibroblast-mediated fibrosis due to excess collagen deposition and causes permanent flexion deformity that limits movement of one or more fingers. The lyophilized drug product will be diluted with a supplied sterile diluent composed of (b) (4) calcium chloride in 154 mM (0.9% w/v) sodium chloride. The proposed dosing regimen involves administration of a single dose of AA4500 0.58 mg into the Dupuytren's cord causing contracture of an affected joint. Injection and a finger extension procedure may be administered up to 3 times into each cord contracting a MP or PIP joint.

During the course of its development, the AA4500 drug substance has been manufactured at three sites, Biospecifics Technologies Corp. (BTC) Lynbrook, NY, (b) (4)

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[1] Seifter, et al., 1959; Bond and van Wart 1984; Steinbrink, et al., 1985; and Mookhtiar and vanWart, 1992
[2] Krane and Inada, Bone, 2008
[3] Nagase et al, Cardiovascular Res. 2006

(b) (4) and Auxilium's active pharmaceutical ingredient (API) manufacturing facility located in Horsham, PA.

AA4500 (Early BTC Process) was manufactured by BTC and used for early nonclinical and clinical studies conducted by BTC. AA4500 (BTC Process 1) was also manufactured by BTC and used by BTC for nonclinical and early clinical studies. Early BTC process was compared to Process 1 and the data for comparability was submitted to FDA. After review by FDA, comparability was accepted on Dec 21, 2001. The final Auxilium process (Process 3) was the final API manufacturing process used to manufacture non-clinical and clinical supplies at (b) (4) and Auxilium. Process 3 (b) (4), enhanced purification, improved the formulation and stability profile; and facilitated validation, scale-up and manufacture of AA4500 at commercial scale. FDA reviewed and accepted this comparability analysis on Sept 18, 2006.

Auxilium moved the manufacturing from (b) (4) to Horsham PA in 2006-07. The Horsham lots were produced using the same process as the (b) (4) lots. Auxilium generated reference standard, non-clinical and clinical lots using this process. A comparability exercise was conducted by Auxilium for the (b) (4) and Horsham manufactured lots (b) (4) lot (CTL2006#0809O) and Horsham lot (C80-2006-002)] in 2008. Auxilium conducted product quality and pre-clinical comparability studies. The analytical comparability data was reviewed by the FDA in October 30, 2008 and the (b) (4) and Horsham manufactured lots were found to be comparable.

AA4500 is a non-specified biologic and is subject to the CBER lot release program (21 CFR 610.2(a)). Given that Auxilium has a well controlled manufacturing process based on principles from ICH Q9 and has demonstrated through process validation and batch analysis that they can consistently produced high quality material, this product is not required to be on the CBER lot release program.

B. Complexity:

(b) (4)

Auxilium states that there is no evidence for any post-translational protein modifications, such as glycosylation, for either AUX-I or AUX-II.

Both calcium and zinc are required for the function of AA4500 drug product[4]. The calcium is supplied in the sterile diluent and the zinc (b) (4) No additional exogenous Zn is required for the activity of AA4500 drug product.

[4]Mookhtiar & van Wart, 1992; Matrix Supplement No.1, pp116-126

C. Mechanism of Action:

The mechanism of action for collagenase is digestion of collagen. Collagenases are secreted as zymogens (inactive precursors), which are then activated extracellularly[5]. The activation involves the cleavage of a conserved Cys bond, triggering the so-called cysteine switch activation mechanism, resulting in a loss of about 10-kDa molecular mass[6]. This cysteine residue in the propeptide is not freely accessible in the latent enzyme, but it is exposed or modified by the activation treatments (e.g. mercury) or other proteases[7]. It was proposed that a Cys of the latent enzyme is coordinated to the active-site zinc atom in a fashion that blocks the active site. All modes of activation lead to dissociation of Cys from the zinc atom with concomitant exposure of the active site. Accordingly, when the Cys is coordinated to the zinc, the activity of the enzyme is "off." Thus, the dissociation of a Cys from the zinc atom is viewed as the "switch" that leads to activation. For a number of MMPs including MMP-9, the activation process *in vivo* is mediated by other members of MMPs[8]. For example, MMP-2 has been determined to be an in vivo activator of MMP-9.
As a mixture, AUX-I and AUX-II, have approximately two fold activity greater than individual enzymes on collagen. Thus, the synergistic action of AA4500 is responsible for its activity.

D. Critical Product Quality Attributes:

	Critical product quality attribute	**Why**	**How controlled**
Biological	Collagenolytic activity of AUX-I	Mechanisms of action for drug is proteolytic activity on collagen	Two potency assays measure the activity of AUX-I or AUX-II on native collagen and synthetic substrate.
	Collagenolytic activity of AUX-II		
	Synergistic collagenolytic activity of AUX-I and AUX-II		
Physical and chemical	Identity of AUX-I and AUX-II	The two proteins are close in mol wt at 113 and 114 kDa.	RP-HPLC (b) SDS-PAGE
	Oxidation and deamidation	Both changes degrade product potency to unacceptable levels	(b) (4) analysis by RP-HPLC
	Moisture content		Specification of (b) (4) tested as per USP method
	Thermal stability	Low melting points of AUX-I and AUX-II (50-55°C)	Manufacture, storage and transport is below melting temperature.
	Zinc binding	Zinc binding is needed for enzymatic activity	Not required exogenously
	Calcium	Calcium is needed for enzymatic activity	Formulation buffer - (b) (4)
	Host cell protein	Immunogenicity	Silver staining of SDS-PAGE gel. Immune-based being developed.

[5] Morgunova et al., Science 1999
[6] van Wart et al., PNAS, 1990
[7] Krane and Inada, Bone, 2008
[8] Fridman et al., Cancer Res, 1995

		(b) (4)	
Impurities	Purity of drug substance		SEC-HPLC, RP-HPLC, and (b) (4) SDS-PAGE
	Particulate matter	Immunogenicity	DS/DP tested for (b) (4) particulates
Adventitious agents	Microbial contamination and sterility	Risk to patient	Bacterial endotoxin and sterility test by USP methods.

E. Biological Activity Assay:

(b) (4)

(b) (4)

II. Recommendation and Conclusion on Approvability

The Division of Therapeutic Proteins, Office of Biotechnology Products, OPS, CDER, recommends approval of 125338 for Clostridial Collagenase manufactured by Auxilium Pharmaceuticals, Inc. The data submitted in this application are adequate to support the conclusion that the manufacture of Clostridial Collagenase is well controlled, and leads to a product that is pure and potent. Although there are a number of improvements that can be made to better ensure product quality (see PMC section), the risk to product quality is relatively low especially as compared to the clinical benefit and should not hold up approval. It is recommended that this product be approved for human use (under conditions specified in the package insert).

III. Major Deficiency CMC Comments

IV. APPROVAL LETTER INFORMATION

Post Marketing Commitments (PMC):

1. Immune-based host cell protein assay: (b) (4) , the Sponsor should incorporate a validated ELISA as soon as feasible. This should be a post-marketing commitment or post-marketing requirement. While this is an important product quality issue it should not hold up approvability because of two main reasons. First, Auxilium has a crude but qualitative assay for detecting host cell proteins by SDS-PAGE gel and silver staining. Second, the data and discussion provided by them demonstrates the technical difficulty in developing an immune-based HCP assay for clostridial proteins. (b) (4)

2. Orthogonal immune-based identity assay: Auxilium has an RP-HPLC assay as the primary identity test. Auxilium was informed that SDS-PAGE is not a reliable identity test. They were asked to use an orthogonal immune-based assay. During PAI, Auxilium confirmed that they have antibodies for AUX-I and AUX-II. This should be a PMC. It is not an approvability issue because they currently have RP-HPLC based method to identify AUX-I and AUX-II peaks. They will also be revising their acceptance criteria to account for AUX-I and AUX-II peaks after mixing.

3. Orthogonal aggregate testing method: Auxilium currently uses SEC-HPLC as their aggregate test method. (b) (4) . While not as sensitive as AUC or FFF methods, these method will be acceptable as an orthogonal method and should be a PMC. The PMC should include information on showing equivalency between the SEC and (b) (4) or other proposed methods. The orthogonal method should be able to detect high molecular weight aggregates in a quantitative manner. (b) (4) Implementation of this testing into the specifications should be a PMC. When considered in the totality of their current testing scheme and proposal for including (b) (4) for aggregate testing, this deficiency is addressable after approval. This issue does not affect approvability because the Sponsor currently has two assays that are capable of detecting high-molecular weight species, namely SDS-PAGE (b) (4)% gel assay for product-related impurities and covalently linked (b) (4) species) and SEC-HPLC (for aggregates).

4. Acceptance criteria for individual and combined AUX-I and AUX-II peaks should be developed for the RP-HPLC identity assay. For drug substance, the ratio of the two peaks (b) (4) should be developed as acceptance criteria. Sponsor should set specifications for each peak (b) (4) Additionally, the specification for the RP-HPLC assay will need to be modified to account for the (b) (4) and any other unexpected peak. If the peak is present the batch will need to be investigated and potentially discarded. The following comment was sent to the sponsor: AUX-I and AUX-II are intended to be at equal mass ratio (b) (4) in the final drug product for achieving a synergistic effect. However, there is no control for the levels of individual enzyme after mixing. Please establish individual acceptance criteria for AUX-I and AUX-II HPLC profile and their mass ratio. Establishment of these acceptance criteria should be a PMC item. It is not an approvability issue because control over individual enzymes is not the basis for the final product's biological activity. It is the synergistic action of the two enzymes on collagen. (b) (4)

5. Method validation, specifically robustness section are very lacking, the calculation for protein recovery for the HPLC assays were not performed using an orthogonal protein measurement assay. This is not an approvability issue because all other parameters for the method validations were acceptable and the assays are validated for their intended purpose.

6. Setting a appropriate specification for subvisible particle testing. Auxilium proposed to set a limit of (b) (4) per vial for subvisible particles. This is not an approvability issues because they are committed to performing subvisible particle testing. However, they did not provided data to support the proposed specification, as they have not performed the testing yet.

7. For the SDS-PAGE assay an addition of a staining/destaining control. The current method for the SDS-PAGE assay does not appropriately control for variability in staining and destaining times. Please establish a protein staining control (e.g., BSA or similar protein) for your SDS-PAGE assay. This is not an approvability issue because the current procedure for SDS-PAGE provides ranges of staining and destaining times.

8. The sponsor will submit real time stability data as an annual report for a request for extension of shelf-life. The purpose of an annual stability study is not to reevaluate the dating period but rather confirm that all the process changes (including personnel) made during the last year had no impact on product quality. Since a stability study performed at -70C is expected to have limited ability to detect significant changes that might affect product quality; the sponsor should include an accelerated or stress stability study that would be more sensitive to small but significant changes in product quality. Because this requirement involves the design of a study to be implemented next year, this study can be submitted following approval and can therefore be requested as a PMC (PMC # 9).

PMC LANGUAGE FOR SPONSOR WITH AGREED DATES:

1. To develop and validate an immune-based host cell protein assay and to add the validated assay to the release specifications for the drug substance. The validated assay and revised release specifications will be submitted in a supplement to the license by December, 2010.

2. To characterize the types and amounts of subvisible particles (b) (4) in the drug product under stress conditions, at release, throughout the dating period, and to propose an appropriate control strategy, based on the risk to product quality. The final study report and proposed control strategy will be submitted by June, 2010.

3. To establish individual acceptance criteria for AUX-I and AUX-II profile and their mass ratio for the RP-HPLC for release and stability testing of the drug substance and drug product. The updated assay and acceptance criteria will be submitted in a supplement to the license by September, 2010.

4. To calculate the protein recovery for each HPLC method validation (SEC and RP-HPLC) using an orthogonal protein measurement assay that provides added assurance that the method is suitable for its intended purpose. The final study reports will be submitted by December, 2010.

5. To develop and validate the RP-HPLC method to quantify potential impurities for AUX I intermediate, DS and DP. The updated assay and acceptance criteria will be submitted in a supplement to the license by December, 2010.

6. To establish and validate a staining and destaining control (e.g., BSA) for SDS-PAGE Coomassie and Silver Stain to ensure appropriate level of detection for product or process related impurities. The updated assay and acceptance criteria will be submitted in a supplement to the license by December, 2009.

7. To confirm the accuracy of the SEC-HPLC method for detecting aggregates using stress samples (e.g., light, heat, oxidation) using orthogonal test methods (e.g., AUC or FFF). The final study report will be submitted by June, 2010.

8. To develop and validate an immune-based identity assay and to add the validated assay to the release specifications for the drug substance and drug product. The validated assay and revised release specifications will be submitted in a supplement to the license by December, 2010.

9. To include an accelerated or stress stability condition as part of the annual stability program for the drug substance and drug product. The final approved protocol will be submitted and implemented by June, 2010.

10. To evaluate the minimal fill volume required for appropriate dosage withdrawal and to adjust the final fill volume for the drug product to reduce the likelihood that a patient could be overdosed with the excess reconstituted drug product. The final study report and new fill volume will be submitted in a supplement to the license by March 2010.

<u>Other comments to Sponsor (as of 10/27/2009):</u>

1. Please submit your revised and finalized SOP for RP-HPLC. The revision must include the change in acceptance criteria to account for the absence of any unexpected peaks. Sponsor responded on 09/25 with revised SOP 30-2-0011. However, the language is inadequate since it only accounts for (b) (4) peaks greater than (b) (4) (in section 6.8) and not any

'unexpected peaks' including but not limited to clostripain. This will be communicated to them during the teleconference regarding the PMCs.

2. Please provide the revised and finalized SOP for the (b) (4), SDS-PAGE methods. They must include the (b) (4)' as an acceptance criteria and staining/destaining controls. Sponsor responded on 09/23 stating that this would be implemented as a PMC since they would have to validate the assay after change in controls. See PMC #6.
3. Please revise your pH specification and submit new specifications with supporting data and justification to the BLA file. The acceptance criteria should reflect the stability profile and formulation buffering capacity. In their response on 09/23, they have revised their specification to (b) (4)
4. The storage condition for the DS should be ≤ -70°C instead of ≤ -60°C because the stability studies were performed at ≤ -70°C. Sponsor responded on 9/25. The studies to support -70°C were conducted at a range of -90 to -60°C by (b) (4) The storage temperature of DS is within the tested range. Additionally, according to Auxilium, the glass transition temperature of the DS is -39°C (no data provided). They also conducted a shipping qualification study at -60°C on dry ice. Hence, storage conditions of ≤-60°C should not present a risk.
5. Please provide a justification for the wide acceptance criteria for sub-visible particles. Sponsor responded on 09/23 saying there is no guidance on setting the spec at this size. Also they do not have sufficient historical data to set a tighter spec at this time. They set this spec based on batch analysis of 6 lots of DP. They will revise, if justified, after 30 lots. I am concerned how they came up with such a high number even with just 6 lots of DP. See PMC #2.
6. In regards to the excess volume after reconstitution of drug product Auxilium stated that "While this overfill appears to be a substantial percentage of the total volume after reconstitution, it is a reasonable amount that ensures that the dose required for administration can be removed from the vial". We asked them to provide the details of any studies that have conducted to support this statement. We noted that the excess volume is significantly greater than the recommended excess volume and not consistent with the USP <1151> cited. Sponsor responded on 09/25 with study data from BTC and Auxilium. The BTC study was with (b) (4) mg powder (instead of 0.9 mg) and resulted in complaints from clinicians that the product was very difficult to remove from vials. BTC conducted a study that showed (b) (4) mg was required for ease of removal of 0.58 mg. This change was communicated to FDA in Dec 2005. Auxilium conducted a lab test with the current vials and showed that less than the theoretical excess volume is actually recovered from the vials after removal of 0.58 mg containing liquid. Some liquid remains in the stopper after inversion of the vial for withdrawal. The level of reconstituted liquid is too low in the vial to be withdrawn without inversion of the vial. On the basis of BTC and Auxilium's study, there is now less concern for excess fill volumes. However, the risk of overdosage with the entire contents of the reconstituted vial still remains. See PMC #10.
7. Since Auxilium has used (b) (4) for the release testing of drug product intended for clinical use upon licensure, (b) (4) needed to undergo FDA inspection. This firm has never been inspected by FDA. The Office of Compliance and the Atlanta Field Office of the FDA organized the inspection. Mr. Ibad Khan of the Atlanta Field Office conducted the inspection of (b) (4) between October 5-9 2009. No 483 observations were reported and the district recommended approval. Anastasia Lolas of the Office of Compliance (DMPQ) also recommends approval (with separate PMCs from their side) as of 10/20/2009.

V. SUMMARY OF QUALITY ASSESSMENTS

61 Pages Withheld as b(4) Trade Secret/Confidential