

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
125338

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

STN 125338
Collagenase for Dupytren's disease
Fred Mills DTP/OBP/CDER

Final 1
Immunogenicity review

Memo

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1-12-2010*
Amy Rosenberg 1-12-10

To: file
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Through: Amy Rosenberg, M.D., Director DTP, OBP, CDER, Susan Kirshner, Ph.D.,
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Subject: STN 125338 original licensing application
Immunogenicity of AA4500 (Aux I and AuxII -- Clostridial Collagenases) for treatment
of Dupytren's disease

Sponsor: Auxillium

Date: August 27, 2009

Revised December 8, 2009

Revised January 4, 2010 to include Auxillium versions of immunogenicity PMR and
PMC

Revised January 8, 2010 to include revisions by Amy Rosenberg

Revised January 11, 2010

Table of Contents

Section	page
Purpose of review	1
Table of Contents	2
Draft Immunogenicity PMCs	4
Executive Summary	8
Screening Assays for anti-Aux I and anti-Aux II antibodies	8
Potential for cross-reactivity with human proteins	8
Neutralizing antibody formation	9
Hypersensitivity	10
Incidence of injection site pruritis and erythema	10
Summary of discussion with the Clinical Division regarding hypersensitivity monitoring	12
Immunogenicity Results	14
Antibody Screening	14
Homology with Mammalian (Human) Matrix MetalloProteases (MMPs)	16
Neutralizing Antibody Results	21
Potential Hypersensitivity	22
Injection Site Pruritis and Erythema	23
Review of Assay Validation	27
Validation of the screening assay for anti-AUX-1 antibodies in human serum samples	27
Notable Reagents	27
Determination of cut-off points and constant K	29
Relative Titer Determination	30
Confirmatory assay-High dose Hook effect	32
Reviewer calculation of cut-off in terms of ng / ml	33

Table of Contents (continued)

Section	page
Reproducibility of high values for normal Serum samples 32 and 33.	33
Assessment of High Dose Hook Effect	34
Background Determination	35
Spike Recovery	35
Bench Top Stability	35
Freeze Thaw Stability	36
Long Term Stability	37
Sensitivity	37
Validation of the screening assay for anti-AUX-II antibodies in human serum samples	38
Notable Reagents	38
Determination of cut-off points and constant K	39
Relative Titer Determination	40
Confirmatory assay-High dose Hook effect	43
Reviewer calculation of cut-off in terms of ng / ml	43
Estimate of ng/ ml values of anti-Aux II cutoff	44
Assessment of High Dose Hook Effect	44
Background Determination	45
Spike Recovery	45
Bench Top Stability	45
Freeze Thaw Stability	46
Long Term Stability	46
Sensitivity	47
Cross-Reactivity with anti-Aux I antibodies	47
Long Term Stability of anti-Aux I and Anti-Aux II antibodies Frozen in human serum	49
Neutralizing Antibody Assays	49
Intra-Assay Precision	50
Inter-Assay Precision	50
Cutpoint Evaluation	51
Stability Studies	53
Freeze-Thaw Stability	53
Bench Top Stability	54
Sensitivity	54
Interference	54
Drug Tolerance	55

FDA draft PMRs

1. Please perform a study that will better define the reactivity of patient sera with human MMPs. As seen in Table 5 of the Integrated Section of Immunogenicity (Section 5.3.5.3), in the anti-Aux II antibody bridging assay for one out of 5 patient serum samples (b) (4) appears to be inhibited by MMP-2, MMP-3, and MMP-8. In addition, the bridging assay using the rabbit anti-Aux-II positive control also appears to be inhibited by MMP-3, MMP-8, and MMP-13.

It is recommended that your study contain the following elements:

- a. The frequency of occurrence for anti-Aux I and anti-Aux II antibodies that are inhibited by human MMPs should be determined by testing a larger set of patient sera (for instance, on robustly positive serum sample from each patient.)
- b. The ability of reactive sera to neutralize the enzymatic activity of human MMPs should be assessed.
- c. The study should determine if there is a correlation between anti-Aux I and anti-Aux II neutralizing antibodies and reactivity to human MMPs.
- d. Please investigate the possibility of cross reactivity of anti-product antibodies with polycystin I (b) (4) and KIAA0319 (b) (4) since these proteins contain sequences with similarity to segments in the Aux II pCK domains that are equivalent or greater than the similarities observed between Aux I, AuxII, and the human MMPs.

2. Assessment of neutralizing antibody persistence after multiple doses

You have provided data (Integrated Summary of Safety, Table 14.4.2) showing a decline in antibody titer with time after patients have received a single dose. However, patients who received multiple doses showed modest or no declines in titers when tested after the last injection. Furthermore, the data you have provided on neutralizing antibodies (from Study 857) include only one assessment of neutralizing antibodies at one visit, with the overwhelming majority (84%) of assessments made at Week 12 or 13.

The FDA requests that you assess the persistence of neutralizing antibodies in patients who have received multiple doses for the following reasons:

- a. There is significant potential for retreatment in Dupytren's disease.
- b. Literature for other protein therapeutics indicates that effects of neutralizing antibodies on clinical efficacy may occur only upon repeated administration, for instance: beta interferon for treatment of MS (Kappos et al. 2005, Neurology; 65, pp.40-47), alfa interferon-2 for treatment of Hairy Cell Leukemia (Steiss et al. 1991, Blood; 77, pp. 792-

798; Steiss et al. 1988, N Engl J Med ; 318 pp. 1409 -1413), and IL-2 for treatment of colorectal cancer (Skog et al. 2001, Clin. Cancer Res.; 7, pp. 1163-1170).

c. The data you have provided indicate that anti-AA4500 neutralizing antibodies correlate with increased titers, which in turn are correlated with multiple injections.

Auxillium draft of immunogenicity PMR and PPMC, after communication of FDA draft to Auxillium and telephone discussion between the FDA and Auxillium

PMR

1. Submit an *in vitro* study of human sera from patients who have received multiple Xiaflex injections to evaluate the potential for cross-reactivity of anti-product antibodies (i.e., anti-AUX-I and anti-AUX-II) with endogenous human MMPs including MMP-1, MMP-2, MMP-3, MMP-8, MMP-13) with similar homology and relevance to the protein components of Xiaflex. This study should assess the frequency of inhibition of the enzymatic activity of these human proteins by anti-product antibodies and by neutralizing anti-product antibodies. This study should also be designed to assess whether repeated treatment courses of Xiaflex injection result in anti-product antibodies that are more persistent and cross-reactive to endogenous proteins compared to initial anti-product antibody responses.

The timetable you submitted on [December 22, 2009], states that you will conduct this study according to the following timetable:

Final Protocol Submission: [March 2010]
Study Completion Date: [June 2010]
Final Report Submission: [December 2010]

PMC

To demonstrate the feasibility of an *in vitro* study of human sera from patients who have received multiple Xiaflex injections to evaluate the potential for cross-reactivity of anti-product antibodies (i.e., polycystin 1 and KIAA0319) and propose a path forward.

The timetable you submitted on [December 23, 2009], states that you will conduct this study according to the following timetable:

Final Report Submission: December 2010.

Reviewer Comment

The following information should be communicated to Auxillium in order to facilitate completion of the PMC to study potential for study of antibody crossreactivity with polycystin I and KIAA0319.

Collagenase for Dupuytren's disease
Fred Mills DTP/OBP/CDER

In a teleconference held December 23, 2009 between Auxillium and the FDA, there was discussion of Dr. Susan Kirshner's idea to test for cell surface reactivity of patient sera, since polycystin I (PKD1) and KIAA0319 are membrane proteins, and immune complexes by themselves might have a deleterious effect. A necessary control for binding specificity would involve competition with an anti-pKD1 or KIAA0319 antibody. Regarding anti-pKD1 antibodies, DTP Division Director Dr. Amy Rosenberg consulted (b) (6) at the Mayo clinic and learned that a high quality antibody (7e12) is now available commercially from several companies, including Santa Cruz (<http://www.scbt.com/datasheet-130554-polycystin-1-7e12-antibody.html>). In parallel, I performed a Google search for anti-KIAA0319 antibodies and found 3 anti-human KIAA0319 antibodies validated for ELISA (<http://www.biocompare.com/ProductListings/3194/KIAA0319.html?types=6-69945&sb=true>). It is likely that antibodies qualified for ELISA would work as competitors, the solid phase being similar to a cell surface.

The PMR, for study of patient antibody crossreactivity with MMP proteins includes studies assessing crossreactivity by competition with recombinant MMPs, an assay for which some results have already been provided in the Xiaflex BLA. Potential for MMP neutralization would also be assessed. In thinking about similar studies for the pKD1 and KIAA0319 crossreactivity PMC, Auxillium noted difficulty in finding commercial sources for recombinant pKD1 or KIAA0319. I have confirmed this, but I did find that recombinant expression clones are available from Origene for KIAA0319 (<http://www.origene.com/cdna/search-all.msp?term=KIAA0319&product=ALL&go.x=11&go.y=4>) and pKD1 (<http://www.origene.com/cdna/search-all.msp?term=polycystin+1&product=ALL&go.x=19&go.y=2>). As another avenue, Dr. Rosenberg was referred by (b) (6) as a source for pKD1 expression clones. Purification of recombinant protein from these expression clones is likely to require a reasonable amount of development work, and therefore the proposed Dec 2010 time for the pKD1/KIAA0319 PMC frame is reasonable.

The concerns giving rise to the following two PMCs proposed in my review have been integrated into the product labeling and Clinical Division REMS.

1. Monitoring of injection site pruritus.

Analysis of data provided in your May 6, 2009 safety update indicates that the incidence of injection site pruritus, when normalized to the number of doses received, increases with the number of injections. In the post-marketing setting, please collect data on injection site pruritus as a function of number of injections, in order to determine if there is a statistically significant correlation that would indicate sensitization and a potential immune basis for these reactions.

2. Continued hypersensitivity monitoring should be conducted.

Summary of Discussion with the Clinical Division regarding hypersensitivity monitoring

On December 10, 2009 I discussed the rationale for not having a hypersensitivity PMC with Dr Eric Brodsky, who is the clinical reviewer for this licensing application. This discussion is summarized as follows.

Collagenase for Dupytren's disease

Fred Mills DTP/OBP/CDER

The REMS for collagenase in Dupytren's requires a training program for physicians. This contains a specific warning about allergic reactions, and the company must distribute an assessment form for allergic reactions. The physicians are not compelled to fill this out, although Dr Brodsky believes that compliance is likely to be high.

There was a consult with CDER's Division of Pulmonary and Allergic products, with the bottom line that allergic reactions are likely, and the label should reflect this. Therefore, the label has a warning that there is a possibility of allergic reactions.

Dr. Brodsky dissuaded the idea of what power would be needed in a PMC epidemiological (no placebo) hypersensitivity study, given that there were no severe HS reactions in ~ 1000 patients in the pivotal trials. Applying the "Rule of 3", 3000 patients would give a 95% confidence interval for a given result. It seems unlikely the FDA could require this, and even then the study might be underpowered.

Reviewer comment

Given that hypersensitivity was infrequent in ~ 1000 Collagenase treated patients, and that the observed incidents were not severe adverse events, I agree with Dr. Brodsky that the product labeling and REMS adequately address concerns about hypersensitivity monitoring.

Executive Summary

Screening Assays for anti-Aux I and anti-Aux II antibodies

Screening for anti-product antibodies utilizes bridging antibody assays that are not dependent on the isotype of response and show high sensitivity. The sensitivity for the rabbit anti-Aux I polyclonal positive control is (b) (4), with an estimated cut-off of (b) (4). The sensitivity for the rabbit anti-Aux II polyclonal positive control is (b) (4), with an estimated cut-off (b) (4). The assays are appropriately validated. Positive screening results are confirmed by competition with Aux I or Aux II, which is also appropriately validated.

All patients became seropositive after 3 injections, and the average titers are very high ($\sim 10^5$). Auxillium provided data showing a decline in titer with time after patients received a single dose, but because of the potential for re-treatment in Dupytren's disease, a study(s) ought to be performed to assess the long term persistence of antibodies in patients who have received multiple doses of drug or who have received a second round of treatment.

Potential for cross-reactivity with human proteins

There is a potential for antibody cross-reactivity with limited stretches of sequence that are similar between Aux I, Aux II, and Matrix Metallo Proteases (MMPs). Homologies are limited to small segments at different positions in Aux I, Aux II, and MMPs, rather than either (1) extended homologies, or (2) small alignments between similar domains in the clostridial collagenases and the mammalian MMPs. Auxillium performed studies to investigate possible cross-reactivity between patient sera and human MMPs by adding recombinant MMPs to the bridging antibody assays for Aux I and Aux II. For the five patient sera tested, the MMPs did not appear to produce an appreciable inhibition of antibody binding to Aux I. However, one of the patients did show MMP inhibition of antibody binding to Aux II, indicating the presence of cross-reactive antibodies.

BLAST searching of the NCBI protein human protein database reveals that there are two other human proteins-polycystin I and KIAA0319, which have not been investigated by Auxillium, and which have as much or greater similarity to Aux II as do the MMPs. Polycystin I is involved in polycystic kidney disease (Chang and Ong, Nephron Physiol 2008; 108 pp. 1-7), and contains repeats of a pKC domain. Alignment with polycystin I is not unexpected, since Aux II has two pCK domains (Aux I has a single pKC domain), and in fact the Aux I/polycystin I alignment is in the Aux II pKCs.

The second protein, designated KIAA0319, is involved in neural migration, and may have a role in dyslexia (Human Molecular Genetics 2006, 15 pp. 1659-1666). Alignment between Aux II and KIAA0319 is also within the Aux II pKC domains. Therefore I recommend that in addition to their studies on MMPs, Auxillium should investigate the possibility of cross reactivity of anti-product antibodies with polycystin I and KIAA0319, since these proteins contain potential T cell epitopes with

similarity to segments in the Aux II pCK domains that is equivalent or greater than the similarities observed between Aux I, AuxII, and the MMPs.

Reviewer comments

I recommend that Auxilium perform a study that will better define the reactivity of patient sera with human MMPs. This should include:

- b. The frequency of occurrence for anti-Aux I and anti-Aux II antibodies that are inhibited by human MMPs should be determined by testing a larger set of patient sera.*
- c. The ability of reactive sera to neutralize the enzymatic activity of human MMPs should be assessed.*
- d. The study should determine if there is a correlation between anti-Aux I and anti-Aux II neutralizing antibodies and reactivity to human MMPs.*
- e. In addition to studies on MMPs, please investigate the possibility of cross reactivity of anti-product antibodies with polycystin I and KIAA0319, since these proteins contain potential T cell epitopes with similarity to segments in the Aux II pCK domains that is equivalent or greater than the similarities observed between Aux I, AuxII, and the MMPs.*

Neutralizing antibody formation

Neutralizing antibodies were assessed as inhibition of Aux-I or Aux-II enzyme activity by patient sera. The substrate for enzymatic activity is a Type I collagen that is labeled with fluorescein to such a high extent that the fluorescence of the intact substrate is quenched. Enzymatic cleavage releases labeled peptides that are then able to emit a fluorescent signal that is proportional to the degree of substrate turnover. The increase in fluorescence is determined by excitation at 485 nm and measurement of emission at 530 nm. Because collagenase must bind to its natural collagen substrate in order to produce a signal, this assay captures neutralization of the collagen binding domain, as well as neutralization of the active site. The positive control for the neutralization assays is a pool of patient sera from study AUX-CC-857. The validation reports for the neutralization assays state that the serum expires two years after collection.

There was a high rate of neutralizing Abs; i.e.
22 of 200 samples had anti-Aux-I neutralization
44 of 204 samples had anti-Aux II neutralization

This high neutralizing rate may in part be explained by the high titers observed, and the fact that there is a correlation between antibody titer and raising neutralizing antibodies. The current clinical trials did not show a correlation between neutralizing antibodies and

Collagenase for Dupytren's disease
Fred Mills DTP/OBP/CDER

product safety or efficacy, but post licensing, there is an expectation that patients will receive retreatment, and the potential effect of neutralizing antibodies in this situation is unclear.

Reviewer comment

Because of the potential in Dupytren's disease for re-treatment, a study should be performed to assess the long term persistence of neutralizing antibodies.

Hypersensitivity

At the request of the FDA, Auxilium provided justification for not performing IgE or skin prick testing, consisting of the following elements:

1. In early development, IgE testing was performed, and some subjects were positive for anti-product IgE. However, this did not correlate with hypersensitivity.
2. Auxilium was concerned that continued skin prick testing might result in sensitization, and that positive test results might result in un-blinding of the study. Therefore, with the approval of the FDA, IgE or skin prick testing was not used in the pivotal trials
3. Auxilium argues that in the absence of systemic hypersensitivity, there was no perceived clinical value for performing IgE testing in the clinical trial program, nor in the target subject population following approval.

In response, I would note that in the Phase III trials, there were in fact some observations of hypersensitivity. Of the 1028 subjects who received at least one injection 7 events were coded as hypersensitive. Auxilium considered three cases to be related on the basis of redness, itching, and swelling at injection site. Auxilium considered 4 cases to be unrelated to the study drug. These involved a rash behind the knee, nasal allergy, allergic cough, and/ or allergic symptoms. There was an additional 8th case in the May 6, 2009 safety update showing a swollen lip, although there was possibility this may have resulted from a bee sting. The "unrelated" events could actually have resulted from hypersensitivity to Aux I/ Aux II, involving a systemic IgE or non-IgE response.

Incidence of injection site pruritis and erythema

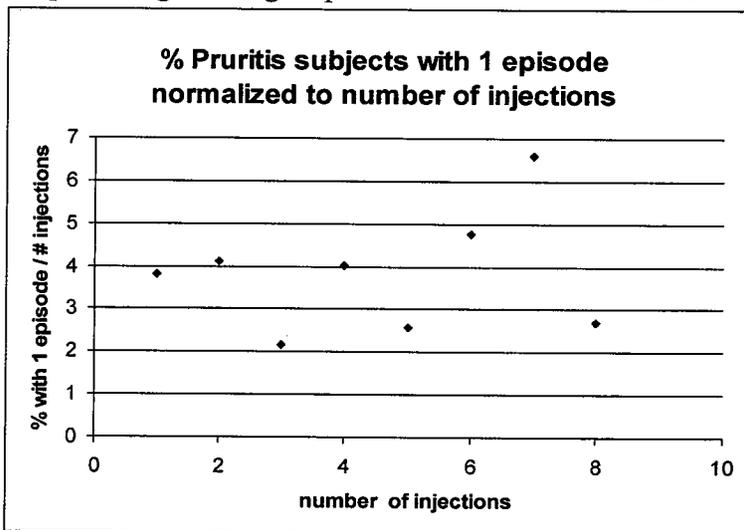
Data supplied for injections site pruritis is of interest, since itching could result from localized immune-mediated reactions.

Table 17: Pruritus by Injection Cohort

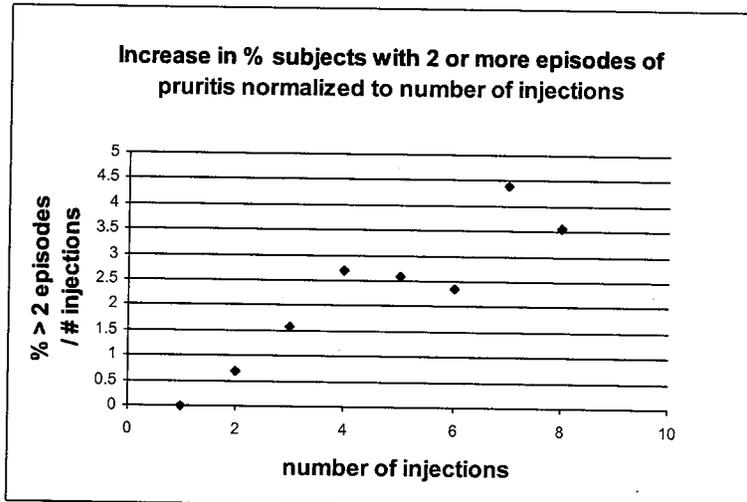
Subjects Who Received:	Percentage of Subject Who Experienced Pruritus:							
	1 Time	2 Times	3 Times	4 Times	5 Times	6 Times	7 Times	8 Times
1 Injection N=443	3.8%							
2 Injections N=219	8.2%	1.4%						
3 Injections N=170	6.5%	4.7%	0%					
4 Injections N=93	16.1%	8.6%	2.2%	0%				
5 Injections N=116	12.9%	9.5%	2.6%	0%	0.9%			
6 Injections N=14	28.6%	7.1%	0%	0%	7.1%	0%		
7 Injections N=13	46.2%	15.4%	0%	15.4%	0%	0%	0%	
8 Injections N=14	21.4%	7.1%	0%	7.1%	14.3%	0%	0%	0%

Data source: ISS Table 14.2.19.3

The percentage of single episodes of pruritus increases as a function of injections, but this appears to result simply from the fact that a single episode became more likely the more injections a subject received, as seen in the following, essentially level graph, in which the percentage of single episodes is normalized to the number of injections.



However, if one performs the same normalized analysis for percentage having more than one pruritus episode, indicating sensitization, there does appear to be an increase with more injections.



These findings indicate that a subset of patients become sensitized with increasing doses, and thus more likely to develop pruritis at the injection site. These data would be needed to be strengthened with more observations, since for 6, 7, and 8 injections, there were only 14, 13, and 14 patients, respectively.

Auxillium has also provided data for injection site erythema, which could be immune-mediated. As is the case for pruritis, if one normalizes the incidence of single reactions to the number of injections, it is difficult to see a trend. Furthermore, only one patient had more than one episode of erythema (2 episodes). Thus it appears unlikely that there is sensitization that would result in an increased incidence of injection site erythema.

Summary of Discussion with the Clinical Division regarding hypersensitivity monitoring

On December 10, 2009 I discussed the rationale for not having a hypersensitivity PMC with Dr Eric Brodsky, who is the clinical reviewer for this licensing application. This discussion is summarized as follows.

The REMS for collagenase in Dupytren's requires a training program for physicians. This contains a specific warning about allergic reactions, and the company must distribute an assessment form for allergic reactions. The physicians are not compelled to fill this out, although Dr Brodsky believes that compliance is likely to be high.

There was a consult with CDER's Division of Pulmonary and Allergic products, with the bottom line that allergic reactions are likely, and the label should reflect this. Therefore, the label has a warning that there is a possibility of allergic reactions.

Dr. Brodsky dissuaded the idea of what power would be needed in a PMC epidemiological (no placebo) hypersensitivity study, given that there were no severe HS reactions in ~ 1000 patients in the pivotal trials. Applying the "Rule of 3", 3000 patients would give a 95% confidence interval for a given result. It seemed unlikely the FDA could require this, and even then it might be underpowered.

Reviewer comment

Given that hypersensitivity was infrequent in ~ 1000 Collagenase treated patients, and that the observed incidents were not serious adverse events, I agree with Dr. Brodsky that the product labeling and REMS adequately address concerns about hypersensitivity monitoring.

Immunogenicity Results

Antibody Screening

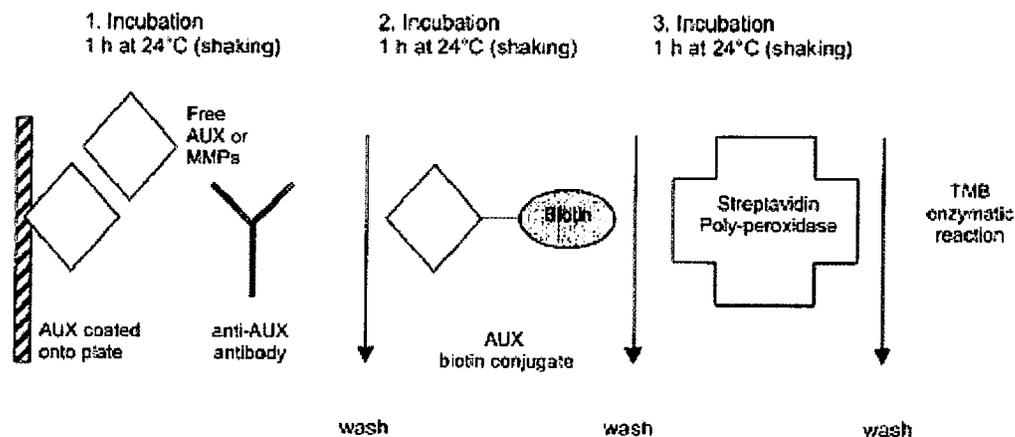
A screening assay for anti-Aux I and Aux II antibodies was performed using the following methodology

Integrated Summary of Immunogenicity, Module 5, Section 5.3.5.3

Page 12 of 37

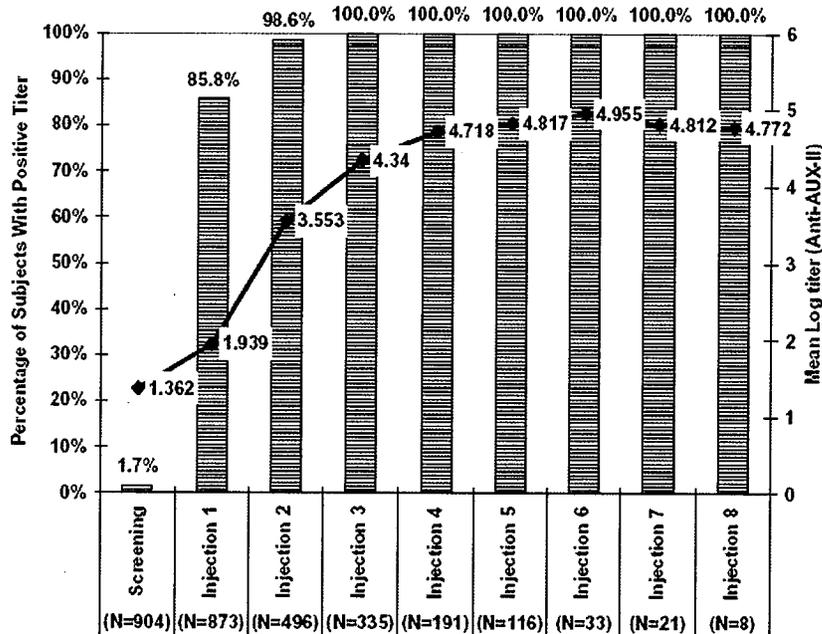
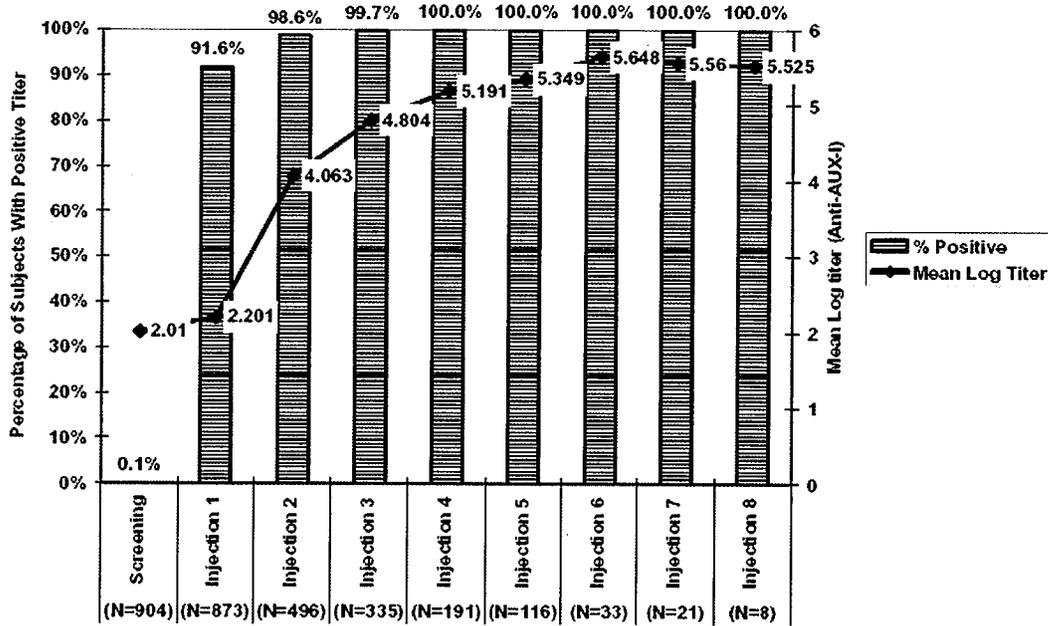
Figure 1: Bridging ELISA Format for Testing Cross-Reactivity of Human Anti-AUX-I or Anti-AUX-II Antibodies versus Different Human MMPs

Principle of the assay:



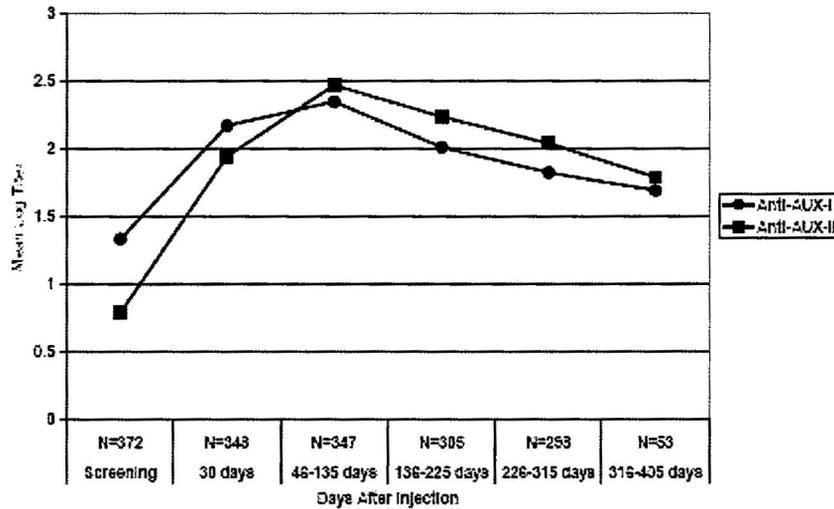
Sera positive in the screening assays were confirmed by competition of the assay with Aux I or Aux II.

As shown in the following graphs, all patients eventually became positive for anti-Aux-I and Aux II antibodies.



These are very high titers for both anti-Aux I and Aux II, and are likely to represent an appreciable percentage of the total serum IgG. Auxilium provided data indicating that for a single injection, there is a decline in titer with time.

Figure 3: Anti-AUX-I and Anti-AUX-II Titer: Over Time – Subjects Who Received a Single Injection of AA4500 0.58 mg



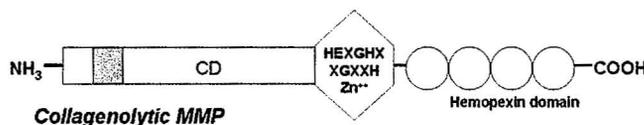
Reviewer comment

These data do not address the issue of potential persistence high antibody levels in patients who have received multiple injections. The development of anti-Aux I and Aux II antibodies in the majority of patients is expected because these are not endogenous human proteins. Therefore the immune system should react to the proteins.

Homology with Mammalian (Human) Matrix MetalloProteases (MMPs)

There is concern about possible immunological cross-reactivity between Aux-I and Aux II and human MMPs, since the clostridial collagenases have structural similarities to mammalian MMPs; i.e.

(b) (4)



Moreover, there is evidence that the course of Dupytren's disease may be related to altered expression of MMPs, so cross-reactive antibodies against MMPs would have the potential to adversely affect the course of the disease (Johnston et al. J Hand Surg 2008;33A:1160–1167).

Auxilium has tabulated what appear to be impressive homologies among Aux I, Aux II, and the highest scoring human MMP as follows:

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

Auxilium states that these scores are based on analysis using the ExPasy suite of programs.

I performed the same analysis using the ExPasy SIM protein sequence alignment program, and found that the homologies are limited to small segments at different positions in Aux I, Aux II, and MMPs, rather than either (1) extended homologies, or (2) small alignments between similar domains in the clostridial collagenases and the mammalian MMPs

As an example, shown below are alignments for Aux I, and Aux II with MMP8. As tabulated above, these represent the highest homology scores provided by the sponsor.

Aux I vs. MMP 8

50.0% identity in ^(b)₍₄₎ residue overlap
in ^(b)₍₄₎

Aux I (b) (4)
MMP 8 (b) (4)

Aux II vs. MMP 8

52.6% identity in (b) (4) residue overlap
in (b) (4)

Aux II (b) (4)
MMP 8 (b) (4)

I performed a Protein BLAST search of the NCBI protein database and found Aux II alignments to two human proteins with higher similarities than those seen with MMPs. As a search sequence, I used the NCBI sequence corresponding to Aux II, designated by Accession number (b) (4).

One of the aligned proteins is, not unexpectedly, polycystin 1 (Accession (b) (4) since Aux II has two internal pCK domains (Aux I has a single pCK domain).

(b) (4)

Polycystin 1 is involved in polycystic kidney disease (Chang and Ong, Nephron Physiol 2008; 108 pp. 1-7)

A segment of alignment within the Aux II pCKs extends from Aux II amino acids (b) (4), with (b) (4) identities (43% homology), and includes a peptide with (b) (4) out of (b) (4) identical amino acids.

Aux = Aux II (b) (4)
pCK = polycystin 1 = (b) (4)

(b) (4)

The second protein, designated KIAA0319, is involved in neural migration, and may have a role in dyslexia (Human Molecular Genetics 2006, 15 pp. 1659-1666). The KIAA0319 Accession number is (b) (4) and this protein contains a sequence extending from Aux II amino acids (b) (4) (also in the pCK domain) that shows 30%

homology. This extended segment contains one (b) (4) amino acid segment with (b) (4) identities (56% homology) and a second (b) (4) amino acid segment with (b) (4) identities (64 % homology).

KI= KIAA0319 =Accession (b) (4)

(b) (4)

Reviewer comment

I recommend that in addition to their studies on MMPs, Auxillium should investigate the possibility of cross reactivity of anti-product antibodies with polycystin I and KIAA0319, since these proteins contain potential T cell epitopes with similarity to segments in Aux II pCK domains.

Auxilium did perform studies to investigate possible cross-reactivity between patient sera and human MMPs by adding recombinant MMPs to the bridging antibody assays for Aux I and Aux II.

For the five patient sera tested, the MMPs did not appear to produce an appreciable inhibition of antibody binding to Aux I, though 1 of 5 showed inhibition of antibody binding to Aux II by MMPs 2,3, and 8. .

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

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

^a Mean OD value in absence of added competing ligand.

^b Affinity purified rabbit polyclonal anti-AUX-I antibody.

However, one of the patients did show MMP inhibition of antibody binding to Aux II, indicating the presence of cross-reactive antibodies.

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

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

^a Mean OD value in absence of added competing ligand

^b Affinity purified rabbit polyclonal anti-AUX-II antibody.

Reviewer comments

I recommend that Auxilium perform a study that will better define the reactivity of patient sera with human MMPs. This should include:

- a. The frequency of occurrence for anti-Aux I and anti-Aux II antibodies that are inhibited by human MMPs should be determined by testing a larger set of patient sera, for instance, one robustly positive serum sample from each patient.*

b. The ability of reactive sera to neutralize the enzymatic activity of human MMPs should be assessed.

c. The study should determine if there is a correlation between anti-Aux I and anti-Aux II neutralizing antibodies and reactivity to human MMPs.

Neutralizing Antibody results

Neutralizing antibodies were assessed as inhibition of Aux-I or Aux-II enzyme activity by patient sera. The substrate for enzymatic activity is a Type I collagen that is labeled with fluorescein to such a high extent that the fluorescence of the intact substrate is quenched. Enzymatic cleavage releases labeled peptides that are then able to emit a fluorescent signal that is proportional to the degree of substrate turnover. The increase in fluorescence is determined using a 96 well fluorescent plate reader by excitation at 485 nm and measurement of emission at 530 nm, which is expressed as Relative Fluorescence Units (RFU).

The positive control for the neutralization assays is a pool of patient sera from study AUX-CC-857. The validation reports for the neutralization assays state that the serum expires two years after collection.

The cut-off for the neutralizing assays was defined as overall mean % inhibition + 1.645 x overall SD of 26 pre-dose patient sera
For Aux I the cut-off was 8.84% inhibition, and for Aux II , 11. 51% inhibition.

There was a relatively high rate of neutralizing Abs; i.e.

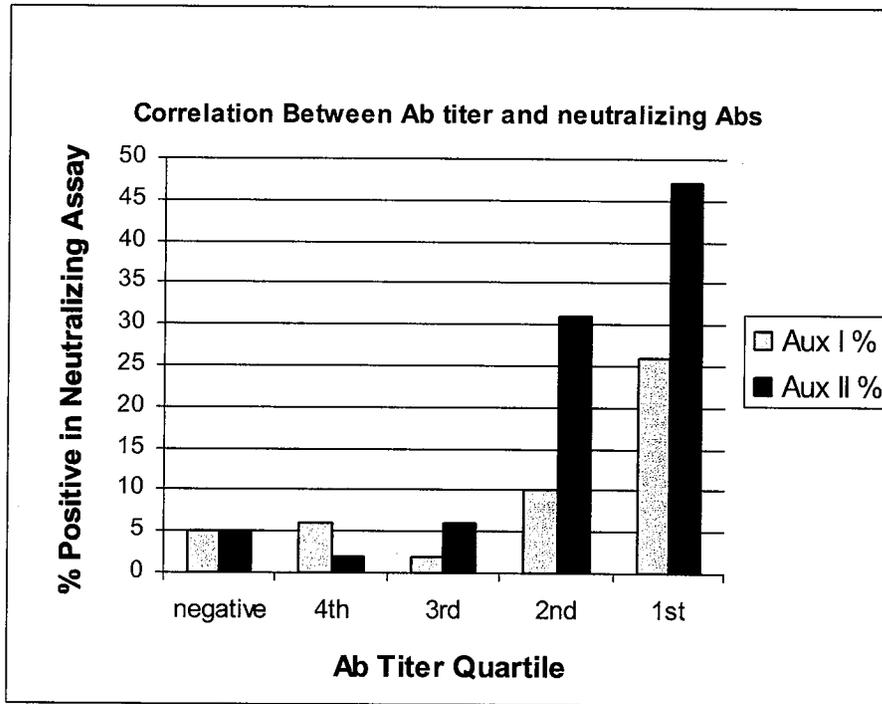
22 of 200 samples had anti-Aux-I neutralization
44 of 204 samples had anti-Aux II neutralization

This high neutralizing rate may in part be explained by the high titers observed, and the fact that there is a correlation between Ab titer and incidence of neutralizing Abs; i.e.

Table 16: Comparison of ADA Titer vs. Positive Result in Enzyme Activity Neutralization Assay by ADA Titer Quartile – Subjects Who Received AA4500 in Study AUX-CC-857

ADA Titer by Quartile	Proportion Positive in Neutralization Assay	
	AUX-I	AUX-II
Upper quartile	13/50 (26%)	24/51 (47%)
2 nd quartile	5/50 (10%)	16/51 (31%)
3 rd quartile	1/50 (2%)	3/51 (6%)
Lower quartile	3/50 (6%)	1/51 (2%)
Negative	8/175 (5%)	9/171 (5%)

For clarity, I have presented these results graphically as well.



Potential Hypersensitivity

Auxilium provided, at the request of the FDA, the following justification for not performing IgE testing. This justification is reproduced in summary as follows:

“In the earliest phase of the development program for the treatment of advanced Dupuytren’s disease with injectable purified clostridial collagenase, the immunogenicity and potential immune responses to the study drug were considered. While anti-collagenase IgE was demonstrated in a percentage of subjects, there was no evidence of systemic immune mediated hypersensitivity reactions or any findings of clinical significance suggestive of such a response. The potential sensitization to clostridial collagenase by continued skin prick testing and the potential un-blinding of the study by

evaluating these immunological parameters was considered. Subsequent clinical study design, with the endorsement of FDA, removed IgE measurements and skin prick testing requirements from the protocols and instead allowed the determination of total immunoglobulin levels from samples analyzed at the end of each study, as submitted in the BLA. This paradigm is consistent with clinical immunology practice in which it is reported that there may be no correlation between the presence or absence of serum IgE levels, positivity or negativity of a skin prick test result and anaphylactic responses. In clinical settings, these tests are utilized within the setting of demonstrated hypersensitivity responses only. In the absence of such systemic hypersensitivity events from the clinical studies of AA4500, there is no perceived clinical value for performing an IgE assay or skin prick assay prior to therapy with AA4500 in the clinical trial program or in the target subject population following approval.”

However, in the Phase III trials, there were in fact some observations of hypersensitivity. Of the 1028 subjects who received at least one injection, 7 events were coded as hypersensitive. Auxillium considered three cases to be related on the basis of redness, itching, and swelling at injection site. Auxillium considered 4 cases to be unrelated to the study drug. These involved a rash behind the knee, nasal allergy, allergic cough, and/ or allergic symptoms. There was an additional 8th case in the May 6, 2009 safety update showing a swollen lip, although there was possibility this may have resulted from a bee sting.

Reviewer comment

The “unrelated” events could actually have resulted from hypersensitivity to Aux I/ Aux II, involving a systemic IgE or non-IgE response. Auxillium should monitor hypersensitivity post-marketing, ideally via a patient registry.

Injection Site Pruritis and Erythema

Table 17: Pruritus by Injection Cohort

Subjects Who Received:	Percentage of Subject Who Experienced Pruritus:							
	1 Time	2 Times	3 Times	4 Times	5 Times	6 Times	7 Times	8 Times
1 Injection N=443	3.8%							
2 Injections N=219	8.2%	1.4%						
3 Injections N=170	6.5%	4.7%	0%					
4 Injections N=93	16.1%	8.6%	2.2%	0%				
5 Injections N=116	12.9%	9.5%	2.6%	0%	0.9%			
6 Injections N=14	28.6%	7.1%	0%	0%	7.1%	0%		
7 Injections N=13	46.2%	15.4%	0%	15.4%	0%	0%	0%	
8 Injections N=14	21.4%	7.1%	0%	7.1%	14.3%	0%	0%	0%

Data source: ISS Table 14.2.19.3

I have verbally expanded and graphed these data as follows:

1 injection 17/443 (3.8%) developed pruritis.

2 injections 18/ 219 (8.2%) had 1 episode,
3/ 219 (1.4%) had 2 episodes-meaning reactions for both injections

3 injections 11/170 (6.5%) had 1 episode
8/170 (4.7%) had 2 episodes-meaning reactions most of the time.

4 injections 15/93 (16.1%) had 1 episode
8/93 (8.6%) had 2 episodes-reactions half the time
2/93 (2.2%) had 3 episodes-reactions $\frac{3}{4}$ times

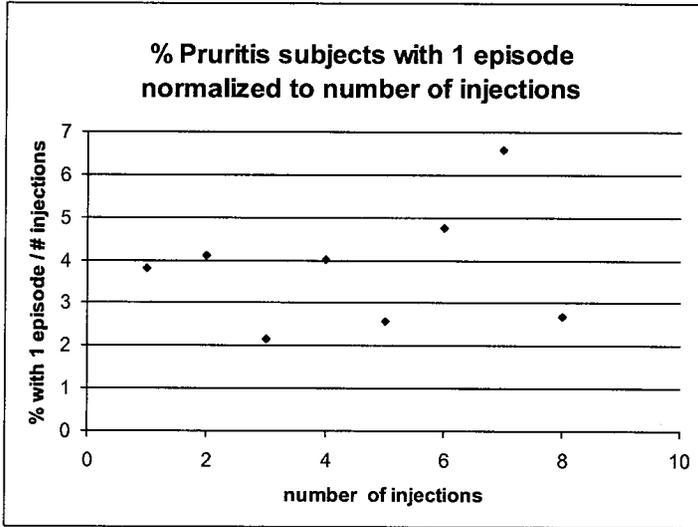
5 injections 15/116 (12.9%) had 1 episode
11/116 (9.5%) had 2 episodes
3/116 (2.6%) had 3 episodes-reactions $\frac{3}{5}$ of the time
1/116 (0.9%) had 5 episodes –reactions everytime

6 injections 4/14 (28.6%) had 1 episode
1/14 (7.1%) had 2 episodes
1/14 (7.1%) had 5 episodes –reactions $\frac{5}{6}$ of the time

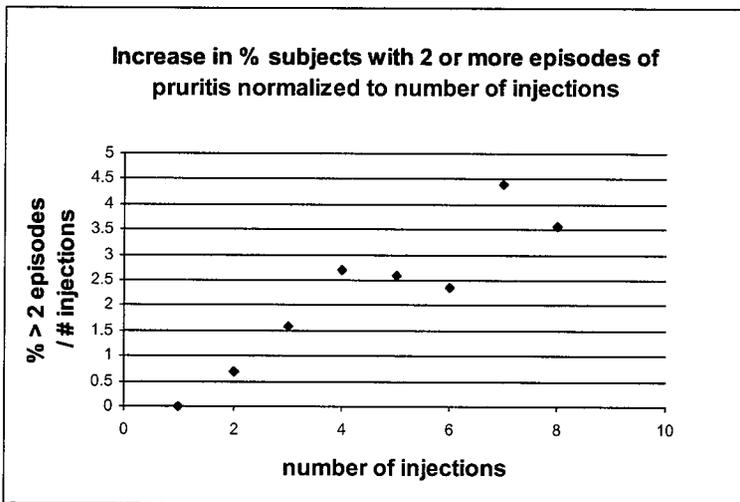
7 injections 6/7 (46.2%) had 1 episode
2/14 (15.4%) had 2 episodes
2/14 had 4 episodes-reactions $\frac{4}{7}$ of the time

8 injections 3/7 (21.4%) had 1 episode
1/14 (7.1%) had 2 episode
1/14 (7.1%) had 4 episodes –reactions $\frac{4}{8}$ of the time
2/17 (14.3%) had 5 episodes-reactions $\frac{5}{8}$ of the time

The percentage of single episodes of pruritis increases as a function of injections, but this appears to result simply from the fact that a single episode became more likely the more injections a subject received, as seen in the following, essentially level graph, in which the percentage of single episodes is normalized to the number of injections.



However, if one performs the same normalized analysis for percentage having more than one pruritis episode, there does appear to be an increase with more injections, indicating sensitization.



These findings indicate that a subset of patients became sensitized with increasing doses, and thus is more likely to develop pruritis at the injection site. These data would need to be strengthened with more observations, since for 6, 7, and 8 injections, there were only 14, 13, and 14 patients, respectively.

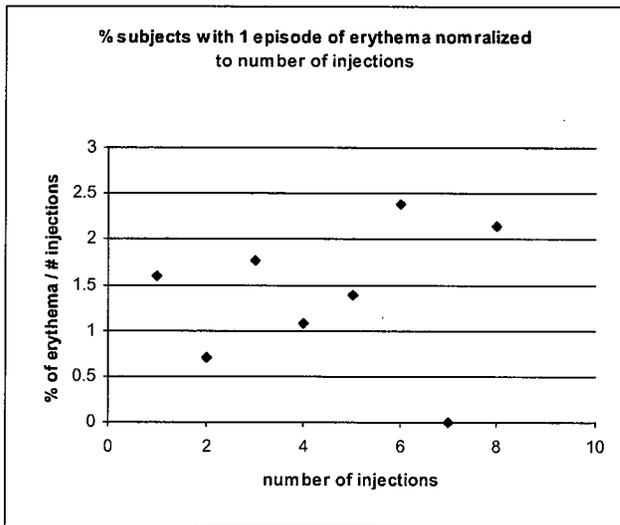
Auxillium has also tabulated data for injection site erythema, which could be immune-mediated.

Table 18: Erythema by Injection Cohort

Subjects Who Received:	Percentage of Subject Who Experienced Erythema:							
	1 Time	2 Times	3 Times	4 Times	5 Times	6 Times	7 Times	8 Times
1 Injection N=43	1.5%							
2 Injections N=28	1.4%	0.9%						
3 Injections N=170	5.3%	0.6%	0%					
4 Injections N=33	4.3%	0%	1.1%	0%				
5 Injections N=16	5.9%	0%	0%	0%	0%			
6 Injections N=14	14.3%	0%	0%	0%	0%	0%		
7 Injections N=13	0%	7.7%	0%	0%	0%	0%	0%	
8 Injections N=14	17.1%	0%	0%	0%	0%	0%	0%	0%

Data source: ISS Table 14.2.19.3

As is the case for pruritis, if one normalizes the incidence of single reactions to the number of injections, it is difficult to see a trend; i.e.



Furthermore, only one patient had more than one episode of erythema (2 episodes). Thus it appears unlikely that there is any sensitization that would result in an increased incidence of injection site erythema.

Reviewer comment

I recommend that in the post marketing setting, Auxillium collect data on injection site pruritis as a function of number of injections, in order to determine if there is a sensitization that would indicate an immune basis for these reactions. An immune basis for pruritis would in turn indicate enhanced susceptibility to additional hypersensitive reactions.

CLINICAL PHARMACOLOGY REVIEW

BLA: 125338	Submission Date(s): 2/27/2009
Brand Name	Xiaflex
Generic Name	Clostridial Collagenase
Clinical Pharmacology Reviewer	Srikanth C. Nallani, Ph.D.
Team Leader	Suresh Doddapaneni, Ph.D.
OCP Division	Division of Clinical Pharmacology II
OND Division	Anesthesia, Analgesia and Rheumatology Products
Sponsor	Auxilium Pharmaceuticals, Inc.
Relevant IND(s)	5780
Submission Type	Original BLA
Formulation; Strength(s)	Lyophilized powder for injection; 0.9 mg/vial
Indication	Treatment of advanced Dupuytren's disease
Proposed Dosage Regimen	Recommended dose is 0.58 mg per injection.

Table of Contents

1	Executive Summary	2
1.1	Recommendation	2
1.2	Phase IV Commitments.....	2
1.3	Summary of Clinical Pharmacology Findings	2
2	QBR	4
2.1	General Attributes	4
2.2	General Clinical Pharmacology.....	4
2.3	Intrinsic Factors.....	6
2.4	Extrinsic Factors	8
2.5	General Biopharmaceutics.....	8
2.6	Analytical Section	9
3	Labeling.....	12
4	Appendix	14
4.1	Proposed labeling.....	14
4.2	Individual Study Review	28
4.2.1	Study # 55 Review	28
4.2.2	Study # 02 Review	29
4.3	BLA Filing Memo	31

Signatures:

 9/2/09
Srikanth C. Nallani, Ph.D.

 9/3/09
Suresh Doddapaneni, Ph.D.

1 Executive Summary

1.1 Recommendation

The clinical pharmacology submission in BLA 125338 is acceptable.

1.2 Phase IV Commitments

None

1.3 Summary of Clinical Pharmacology Findings

Auxilium Pharmaceuticals, Inc., submitted BLA 125338 to support use of XIAFLEX (AA4500 or Clostridial Collagenase) for the treatment of advanced Dupuytren's disease or contracture, an orphan indication. Dupuytren's contracture is a condition of excessive collagen deposition resulting in the formation of a cord or an abnormal thickening of the tissue between the skin and the tendons in the palm. The BLA has been given priority review consideration due to the lack of safe and effective treatments for the disease.

AA4500 is a parenteral lyophilized product comprised of 2 collagenases in an approximate (b) mass ratio, Collagenase I (AUX-I, Clostridial type I collagenase, formerly known as ABC-I) and Collagenase II (AUX-II; Clostridial type II collagenase, formerly known as ABC-II). XIAFLEX should be reconstituted to the appropriate volume prior to use. For cords affecting metacarpophalangeal (MP) joints each dose is administered in an injection volume of 0.25 mL. For cords affecting proximal interphalangeal (PIP) joints, each dose is administered in an injection volume of 0.20 mL. One milligram (mg) of collagenase is equivalent to 17,000 units as determined by a potency assay by Biospecifics Technologies Corp.

In an early stage dose-escalation study, Dupuytren's patients received 600, 1200, 2400, 4800, 9600 and 10,000 U of collagenase into the cord that was causing contracture of the MP joints (Badalamante MA, Hurst LC, et. al. The Journal of Hand Surgery; 25A (4): 629 to 636). Based on the improvement noted at the 10,000 U (or 0.58 mg) of collagenase, the efficacy and safety of 0.58 mg of XIAFLEX was evaluated in 2 randomized, double-blind, placebo-controlled, multi-center trials in 374 adult patients with advanced Dupuytren's disease (Studies # 57 and # 59). In addition, studies # 02 and # 55 assessed the safety, tolerability and pharmacokinetics after single intra-cord injections of 0.58 mg of AA4500 in Dupuytren's disease patients.

In PK study # 55, an open label safety, tolerability and PK study, sixteen subjects were enrolled and treated with one injection of AA4500. Blood samples were collected for the determination of AUX-I and AUX-II plasma concentrations at the following time points relative to dosing: 15 minutes before, 5, 10, 20, 30 minutes after, 1, 2, 4, 8, 12, and 24 hours after (i.e., following the finger extension procedure to disrupt the cord), and seven and 30 days after (in 15 subjects). AUX-I and AUX-II levels were determined by validated double-sandwich enzyme-linked immunosorbent assays (ELISA). AUX-I and AUX-II levels were not detected in any subject at any time point through the first 24 hours, on Day 7, or on Day 30 following administration of a single 0.58 mg injection of AA4500 into a Dupuytren's cord. All samples were below the lower level of

quantification (i.e., ≤ 5 ng/mL for AUX I and ≤ 25 ng/mL for AUX II). There were no major adverse events when the AA4500 was administered in patients.

In several clinical studies # 54, #56, #57, #58, and #59, serum samples were collected at screening, before each injection, and at predetermined time points after each injection to determine if antibodies had formed against AUX-I and AUX-II. Most subjects ($\geq 85.8\%$) had positive antibodies to AUX-I and/or AUX-II 30 days after the first injection of AA4500. All subjects developed positive antibodies to both AUX-I and AUX-II after the third or fourth injection of AA4500. When titers levels were examined by the total number of AA4500 injections received, anti-AUX-I and anti-AUX-II titers decreased during the follow-up period after the last injection.

The neutralizing potential of ADAs on the activity of AA4500 was evaluated indirectly by an assay to determine the ability of ADAs present in human serum to inhibit the activity of AUX-I and AUX-II on intact (soluble) collagen. An analysis of the primary efficacy endpoint in study #57 (the proportion of primary joints that achieved a reduction of contracture to 5 degrees or less) that subset subjects' neutralizing antibody (Nab) status (positive or negative) after treatment with AA4500 has been conducted.

Results indicate that neutralizing antibody status for AUX-I and/or AUX-II does not have an apparent effect on efficacy outcome, irrespective of the severity of baseline contracture.

Over all, the clinical pharmacology submission in the BLA is acceptable.

2 QBR

2.1 General Attributes

Auxilium Pharmaceuticals, Inc., submitted BLA 125338 to support use of AA4500 or Clostridial Collagenase for the treatment of advanced Dupuytren's disease or contracture, an orphan indication. Dupuytren's contracture is a condition of excessive collagen deposition resulting in the formation of a cord or an abnormal thickening of the tissue between the skin and the tendons in the palm. The BLA has been given priority review consideration due to the lack of safe and effective treatments for the disease.

AA4500 is a parenteral lyophilized product comprised of 2 collagenases in an approximate (b) mass ratio, Collagenase I (AUX-I, Clostridial type I collagenase, formerly known as ABC-I) and Collagenase II (AUX-II; Clostridial type II collagenase, formerly known as ABC-II).

Mechanism of Action: Collagenases are proteinases with the unique ability to hydrolyze collagen in its native triple helical conformation under physiological conditions, resulting in lysis of collagen deposits. XIAFLEX for treatment of advanced Dupuytren's disease, a fixed flexion contracture deformity of the hand caused by increased collagen deposition, results in non-surgical, enzymatic disruption of the Dupuytren's cord.

2.2 General Clinical Pharmacology

In an early stage dose-escalation study, thirty-five Dupuytren's patients entered the study, 32 men and 3 women with a mean age of 64.8 ± 11.0 years. Five patients received 600, 1200, 2400, 4800, and 9600 U of collagenase, respectively, injected into the cord that was causing contracture of the MP joints. One patient was lost to follow up. The remaining 29 patients in this study had collagenase injections at a dose level of 10,000 U (or 0.58 mg) followed by a 10- to 12-hour period of hand immobilization in a soft bulky gauze dressing. After this period there was no further immobilization. The 10000 U collagenase was delivered in 0.25 mL for MP joints and 0.20 mL for PIP joints using a sodium/calcium diluent and an insulin syringe (Badalamante MA, Hurst LC, et. al. The Journal of Hand Surgery; 25A (4): 629 to 636). Twenty-eight of the 34 MP joint contractures corrected to normal extension (0°) and 2 of the 34 MP joint contractures corrected to 5° of normal extension, with full range of motion, within 1 to 14 days of injection. In the patients with PIP joint contractures, 4 of the 9 joints corrected to normal (0°). This observation prompted the use of 10,000 units or 0.58 mg collagenase in the clinical trials.

The efficacy and safety of 0.58 mg of XIAFLEX was evaluated in 2 randomized, double-blind, placebo-controlled, multi-center trials in 374 adult patients with advanced Dupuytren's disease (Studies 57 and 59). At study entry, patients must have had: (1) a fixed flexion deformity (contracture), caused by a palpable cord, of at least one finger (other than the thumb) of 20° to 100° in metacarpophalangeal (MP) joints or 20° to 80° in proximal interphalangeal (PIP) joints and (2) a positive "table top test" defined as the inability to simultaneously place the affected finger(s) and palm flat against a table top.

The cord affecting the selected primary joint received up to 3 injections of 0.58 mg of XIAFLEX or placebo on Days 0, 30, and 60. In Studies 57 and 59, the primary endpoint was the proportion of patients who achieved a reduction in contracture of the selected primary joint to within 0° to 5° of normal, 30 days after the last injection of that joint (on Days 30, 60, or 90).

Medical officer, Dr. Eric Brodsky, noted the following efficacy results in clinical trials: In Studies 57 and 59, a significantly greater percentage of XIAFLEX-treated patients (up to 3 injections) compared with placebo-treated patients, achieved a reduction in the contracture of the primary joint (MP or PIP) to 0° to 5°, 30 days after the last injection (see Table below).

Table Legend: Proportion of patients that achieved a reduction of the contracture of the primary joint to 0 to 5 degrees, 30 days after the last injection in the pivotal trials¹

	Primary Joint (MP & PIP)		
	N		p-value
Study 57 (up to 3 injections on Days 0, 30, and 60)			
Xiaflex 0.58 mg	203	64%	< 0.001
Placebo	103	7%	—
Study 59 (up to 3 injections on Days 0, 30, and 60)			
Xiaflex 0.58 mg	45	44%	< 0.001
Placebo	21	5%	—

¹ The pivotal trials were Studies 57 and 59 (patients received up to 3 injections of study medication (given on Days 0, 30, and 60).

² MITT population was the primary statistical population for the efficacy analyses in Study 57. The MITT population included all treated patients who had at least one post-treatment efficacy evaluation (contracture measurement) and had baseline contracture > 5 degrees. There was 1 patient in each of the Xiaflex and placebo groups who were included in the treated population (ITT) and excluded from the MITT population.

³ ITT population (all patients that received at least one injection of study medication) was the primary statistical population for the efficacy analyses in Study 59.

In PK study # 55, an open label safety, tolerability and PK study, sixteen subjects were enrolled and treated with one injection of AA4500. Blood samples were collected for the determination of AUX-I and AUX-II plasma concentrations at the following time points relative to dosing: 15 minutes before, 5, 10, 20, 30 minutes after, 1, 2, 4, 8, 12, and 24 hours after (i.e., following the finger extension procedure to disrupt the cord), and seven and 30 days after (in 15 subjects). AUX-I and AUX-II levels were determined by validated double-sandwich enzyme-linked immunosorbent assays (ELISA). AUX-I and AUX-II levels were not detected in any subject at any time point through the first 24

hours, on Day 7, or on Day 30 following administration of a single 0.58 mg injection of AA4500 into a Dupuytren's cord. All samples were below the lower level of quantification (i.e., ≤ 5 ng/mL for AUX I and ≤ 25 ng/mL for AUX II). There were no major adverse events when the AA4500 was administered in patients.

In an early development Phase 2 open-label study #02, four advanced Dupuytren's disease patients received a single injection of 0.58 mg AA4500 into the cord of the affected finger joint to investigate systemic absorption of AUX-I and AUX-II. However, the ELISA method used for detection of AUX-I did not use a purified anti-AUX-I antibody and hence the results are not reliable. There were no major adverse events when the AA4500 was administered in patients.

Because no systemic levels of AA4500 were noted, exposure vs. therapeutic response at the local injection could not be evaluated.

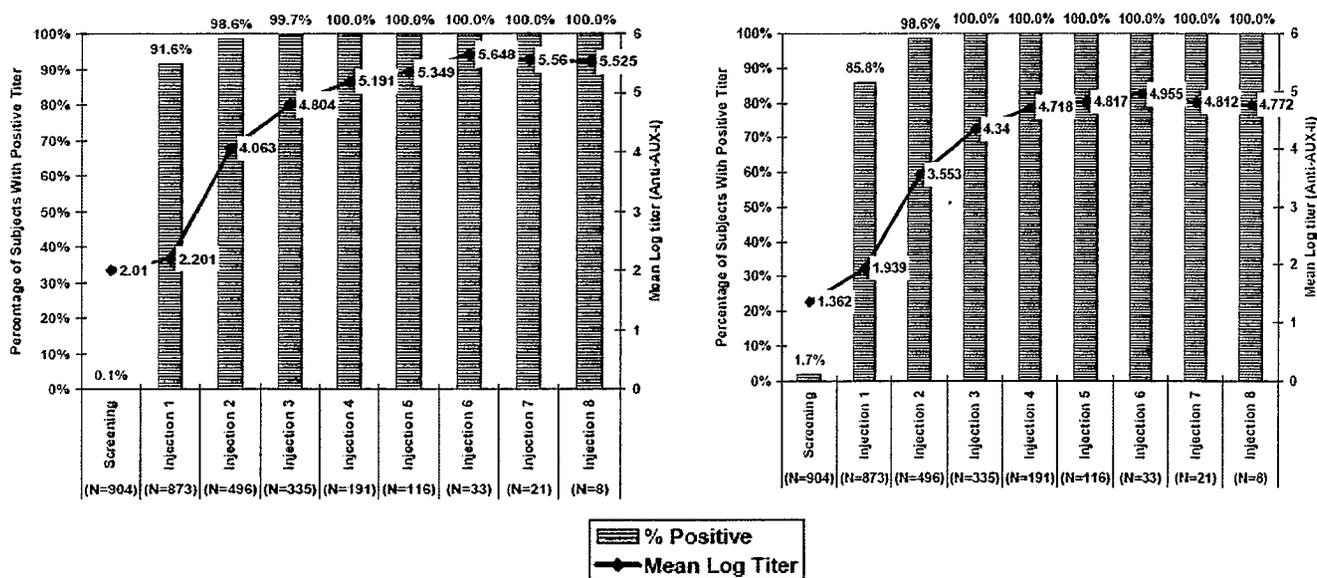
2.3 Intrinsic Factors

The current Phase 3 clinical studies conducted by Auxilium have evaluated the safety and efficacy of AA4500 in a subject population that is representative (i.e., in age, gender, and race) of the population targeted for commercialization. As systemic exposure to AA4500 after intralesional injection into Dupuytren's cords has not been detected, studies were not conducted to evaluate the metabolism of AA4500, PK in subjects with impaired hepatic or renal function.

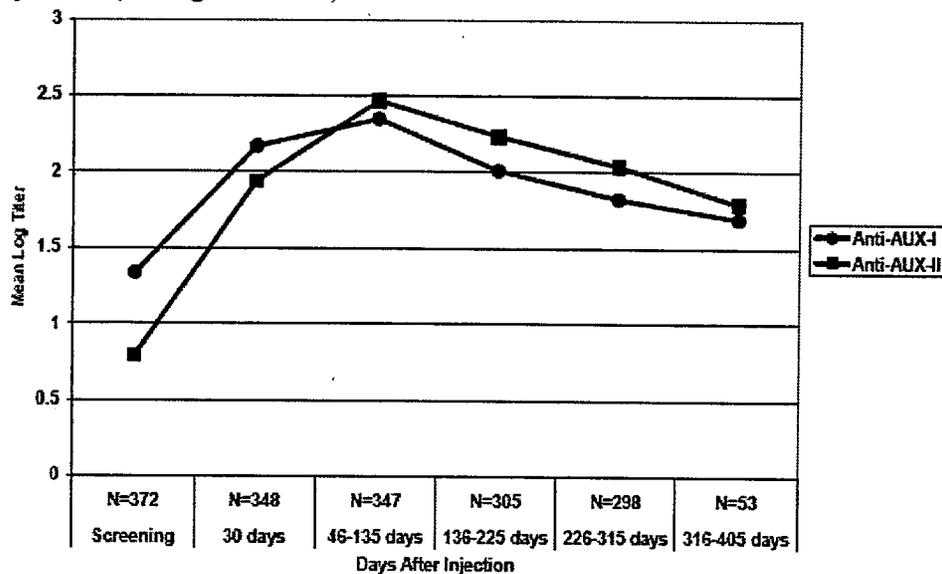
Immunogenicity:

What is the incidence (rate) of the formation of the anti-drug antibodies (ADA), including the rate of pre-existing antibodies, the rate of APA formation during and after the treatment, time profiles and adequacy of the sampling schedule?

In several clinical studies # 54, #56, #57, #58, and #59, serum samples were collected at screening, before each injection, and at predetermined time points after each injection to determine if antibodies had formed against AUX-I and AUX-II.



Most subjects ($\geq 85.8\%$) had positive antibodies to AUX-I and/or AUX-II 30 days after the first injection of AA4500 (see figures below). All subjects developed positive antibodies to both AUX-I and AUX-II after the third or fourth injection of AA4500. When titers levels were examined by the total number of AA4500 injections received, anti-AUX-I and anti-AUX-II titers decreased during the follow-up period after the last injection (see figure below).



Does the immunogenicity affect the PK and/or PD of AA4500?

As such systemic levels of AUX-I and AUX-II were below the limit of detection. Hence, impact of immunogenicity on PK and PD of AA4500 was not evaluated.

Do the anti-product antibodies have neutralizing activity?

The neutralizing potential of ADAs on the activity of AA4500 was evaluated indirectly by an assay to determine the ability of ADAs present in human serum to inhibit the activity of AUX-I and AUX-II on intact (soluble) collagen. Serum samples were collected from each subject treated with AA4500 0.58 mg in study #57 and assayed to determine the ability of ADAs present in human serum to inhibit the activity of AUX-I and AUX-II. Despite the high rate (100%) of ADA titers, only 22 of 200 samples among subjects who received AA4500 had neutralizing antibodies for AUX-I and 44 of 204 samples had neutralizing antibodies for AUX-II.

What is the impact of anti-product antibodies on clinical efficacy?

An analysis of the primary efficacy endpoint in study #57 (the proportion of primary joints that achieved a reduction of contracture to 5 degrees or less) that subset subjects' neutralizing antibody (Nab) status (positive or negative) after treatment with AA4500 has been conducted.

Results indicate that neutralizing antibody status for AUX-I and/or AUX-II does not have an apparent effect on efficacy outcome, irrespective of the severity of baseline contracture.

	Nab Positive	Nab Negative	Nab Status Unknown
Nab AUX-I			
MP Joints			
Low severity	4/5 (80.0%)	61/68 (89.7%)	7/8 (87.5%)
High severity	5/6 (83.3%)	21/42 (50.0%)	4/4 (100.0%)
PIP Joints			
Low severity	1/1 (100.0%)	15/19 (79.0%)	1/1 (100.0%)
High severity	2/8 (25.0%)	9/39 (23.1%)	0/2 (0.0%)
Nab AUX-II			
MP Joints			
Low severity	13/17 (76.5%)	52/56 (92.9%)	7/8 (87.5%)
High severity	5/10 (50.0%)	21/38 (55.3%)	4/4 (100.0%)
PIP Joints			
Low severity	0 ^a	16/20 (80.0%)	1/1 (100.0%)
High severity	3/15 (20.0%)	8/32 (25.0%)	0/2 (0.0%)

MP low severity= $\leq 50^\circ$; MP high severity= $>50^\circ$; PIP low severity= $\leq 40^\circ$; PIP high severity= $>40^\circ$

^a No subjects were in this category.

What is the impact of anti-product antibodies on clinical safety?

Majority of AA4500-associated adverse effects were local reactions. The most commonly reported were edema (mostly edema of the injected hand), contusion, hemorrhage, and pain involving the treated extremity and were likely related to AA4500 injection. As such the adverse events due to antibodies to AUX-I and AUX-II could not be differentiated from adverse events due to the product itself.

2.4 Extrinsic Factors

As systemic exposure to AA4500 after intralesional injection into Dupuytren's cords has not been detected, studies were not conducted to evaluate the potential for evaluation of drug interactions between AA4500 and coadministered drugs.

2.5 General Biopharmaceutics

AA4500 is a parenteral lyophilized product comprised of 2 collagenases in an approximate (b) mass ratio, Collagenase I (AUX-I, Clostridial type I collagenase, formerly known as ABC-I) and Collagenase II (AUX-II; Clostridial type II collagenase, formerly known as ABC-II).

The proposed commercial drug product, which was also utilized in clinical trials and nonclinical studies conducted by Auxilium, contains 0.9 mg AA4500 as a lyophilized presentation formulated in sucrose (18.5 mg/vial), trimethomine (1.1 mg/vial) and hydrochloric acid (0.5 mg/vial).

In study # 55, blood samples for the determination of AUX-I and AUX-II plasma concentrations were collected at the following time points relative to dosing (one 0.58 mg dose administered in the affected cord): 15 minutes before, 5, 10, 20, 30 minutes after, 1, 2, 4, 8, 12, and 24 hours after (i.e., following the finger extension procedure to disrupt the cord), and seven and 30 days after. AUX-I and AUX-II levels were determined by validated double-sandwich enzyme-linked immunosorbent assays (ELISA). AUX-I and AUX-II levels were not detected in any subject at any time point through the first 24

hours, on Day 7, or on Day 30 following administration of a single 0.58 mg injection of AA4500 into a Dupuytren's cord. All samples were below the lower level of quantification (i.e., ≤ 5 ng/mL for AUX I and ≤ 25 ng/mL for AUX II).

2.6 Analytical Section

What bioanalytical methods were used to assess therapeutic protein concentrations?

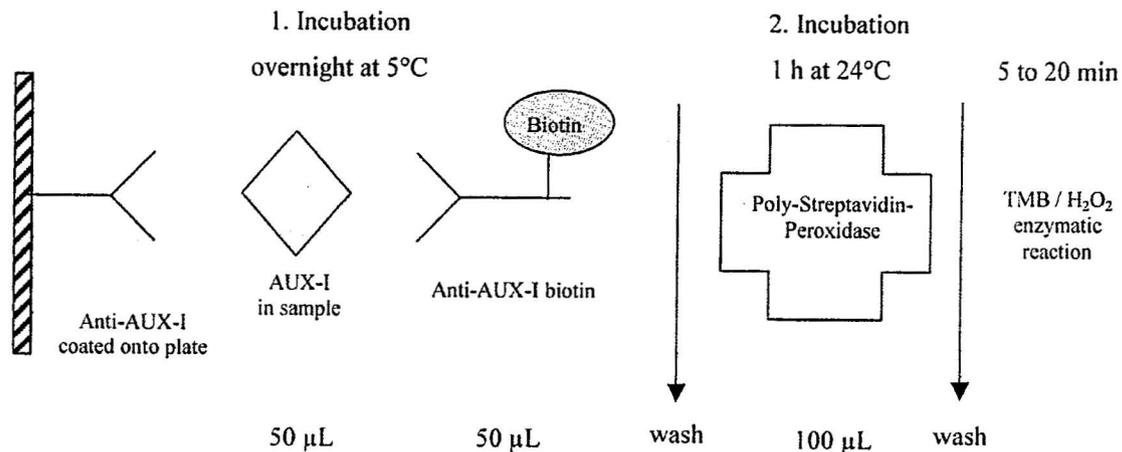
AUX-I and AUX-II levels were determined by validated double-sandwich enzyme-linked immunosorbent assays (ELISA). The format and sensitivity of the assays used to measure AUX-I or AUX-II in human plasma are summarized below.

AUX-I and AUX-II ELISA:

The amount of AUX-I and AUX-II was measured in human plasma by means of a one-step double-antibody sandwich ELISA developed by (b) (4)

(b) (4) Polyclonal anti-AUX-I or anti-AUX-II antibodies were pre-coated onto microplates. Samples are pipetted into the wells and anti-AUX-I or anti-AUX-II biotin is added. The minimum dilution for QC samples and study samples is (b) (4). Any AUX-I or AUX-II present in the samples is bound at the same time by anti-AUX-I biotin and the immobilized anti-AUX-I antibodies. After a further washing step, poly-streptavidine-peroxidase is added which binds to the anti-AUX-I biotin or anti-AUX-II biotin. After a further washing step, peroxidase bound in the complex is visualized by TMB substrate solution. After stopping the reaction with sulfuric acid, the intensity of the resulting color is determined at 450 nm. Three quality control samples were also included in the method validation for AUX-I (b) (4)

Inter assay precision was less than 7.3% and accuracy ranged between 96 – 99% for QC standards. Three quality control samples were also included in the method validation for AUX-II (b) (4). Inter assay precision was less than 7.9% and accuracy ranged between 93 – 99.4% for QC standards.



	AUX-I	AUX-II
Assay format	Double Antibody Sandwich ELISA	
Capture antibody	Rabbit affinity-purified anti-AUX-I polyclonal	Rabbit affinity-purified anti-AUX-II polyclonal
Detection antibody	Biotinylated version of capture antibody	
Test matrix	Human plasma (lithium-heparin)	
Dilution of test matrix	1 in 50	1 in 50
Quantitation range	0.1 – 0.8 ng/ml	0.5 – 10 ng/ml
LOQ in undiluted matrix	5 ng/ml	25 ng/ml
Validation study number	AA41767CH-EB-03	AA41768CH-EB-01
Validation study number for long-term stability	AA41767CH-EB-04	AA41768CH-EB-02

What bioanalytical methods were used to assess the formation of the anti-product antibodies?

To determine if antibodies had formed against AUX-I and AUX-II, the following methods were applied to human studies:

- Bridging ELISA assays to detect either anti-AUX-I or anti-AUX-II, including long-term stability.
- Bridging ELISA assays performed in a competitive format to detect the capacity of a panel of recombinant human matrix metalloproteinases (MMPs) to serve as competitive ligands for AUX-I or AUX-II (evaluation of cross-reactivity of human antibody positive samples with endogenous collagenase).

Screening Assay:

The screening assay is used to pre-select "reactive" samples, which are positive for anti-AUX-I or anti-AUX-II antibodies ($OD > OD_{cut-off}$) from non-reactive samples, which are negative for anti-AUX-I or anti-AUX-II antibodies ($OD \leq OD_{cut-off}$).

Confirmatory Assay:

A true positive signal due to specific binding to AUX-I or AUX-II is suppressed by adding free AUX-I or AUX-II to the assay. Controls and samples are assayed in the absence and presence of unlabeled AUX-I or AUX-II (1 mcg/mL added to dilution buffer). A sample is confirmed positive for anti-AUX-I or anti-AUX-II antibodies, when the added AUX-I or AUX-II inhibits the signal by more than 50%.

After confirmation of reactive samples for presence of anti-AUX-I or AUX-II antibodies, the screening assay format was also be used for titer determination. For titer determination, anti-AUX-I or AUX-II antibody positive samples were serially diluted in at least four ^(b)₍₄₎ dilution steps and analyzed.

What is the performance of the binding assay(s)?

The characteristics and performance of these different assays as applied to human samples are summarized in the table below.

Assay	Validation Study Report ^a	Performance Characteristics
Anti-AUX-I Bridging ELISA (ADA titer)	AA33061-01	Dilution of test matrix (human serum) = 1/10 LOD in undiluted matrix ≈ 8 ng/mL ^b In-study assay cut-point
Anti-AUX-I Bridging ELISA (ADA titer) – Long term stability	AA33061-02	Same as above. Stability confirmed up to 26 months at -20° and -80° C
Anti-AUX-II Bridging ELISA (ADA titer)	AA33062-01	Dilution of test matrix (human serum) = 1/10 LOD in undiluted matrix ≈ 19 ng/mL ^b In-study assay cut-point
Anti-AUX-II Bridging ELISA (ADA titer) – Long term stability	AA33062-02	Same as above. Stability confirmed up to 23 months at -20° C, and up to 26 months at -80° C.
Bridging ELISA, competitive format (MMP cross-reactivity)	AA74233CH-EB	IC ₅₀ of AUX-I or AUX-II < 62.5 ng. Inhibition of signal ≤ 75-80% by AUX-I or AUX-II at 1000 ng/mL. No significant inhibition of signal by MMP-1, -2, -3, -8 or -13 at 1000 ng/mL.

^a Validation reports are included in Module 5, Section 5.3.1.4.

^b Positive control = Affinity-purified rabbit anti-AUX-I or anti-AUX-II polyclonal IgG

What is the performance of the neutralizing assay(s)?

The neutralizing potential of ADAs on the activity of AA4500 was evaluated indirectly by an assay to determine the ability of ADAs present in human serum to inhibit the activity of AUX-I and AUX-II on intact (soluble) collagen. The limited volume of the human positive control serum precluded estimation of the sensitivity of the neutralizing antibody assays in terms of mass units of IgG. Thus, at the present time, this assay can be used only in the qualitative sense to investigate whether samples detected as positive in the ADA bridging ELISA have capacity to inhibit the enzyme activity of AUX-I or AUX-II.

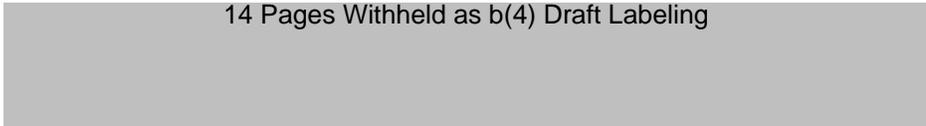
3 Labeling

(b) (4)





14 Pages Withheld as b(4) Draft Labeling



4.2 Individual Study Review

4.2.1 Study # 55 Review

Study Design: Study # 55 was a Phase 1, open-label pharmacokinetic study in subjects with advanced Dupuytren's disease.

Objective: To determine if there was systemic exposure following a single injection of AA4500 0.58 mg directly into the cord affecting either the metacarpophalangeal (MP) joint or proximal interphalangeal (PIP) joint and to determine the safety of AA4500.

Blood Sampling: Blood samples for the determination of AUX-I and AUX-II plasma concentrations were collected at the following time points relative to dosing: 15 minutes before, 5, 10, 20, 30 minutes after, 1, 2, 4, 8, 12, and 24 hours after (i.e., following the finger extension procedure to disrupt the cord), and seven and 30 days after. AUX-I and AUX-II levels were determined by validated double-sandwich enzyme-linked immunosorbent assays (ELISA). The format and sensitivity of the assays used to measure AUX-I or AUX-II in human plasma are summarized in the tablet below.

	AUX-I	AUX-II
Assay format	Double Antibody Sandwich ELISA	
Capture antibody	Rabbit affinity-purified anti-AUX-I polyclonal	Rabbit affinity-purified anti-AUX-II polyclonal
Detection antibody	Biotinylated version of capture antibody	
Test matrix	Human plasma (lithium-heparin)	
Dilution of test matrix	1 in 50	1 in 50
Quantitation range	0.1 – 0.8 ng/ml	0.5 – 10 ng/ml
LOQ in undiluted matrix	5 ng/ml	25 ng/ml
Validation study number	AA41767CH-EB-03	AA41768CH-EB-01
Validation study number for long-term stability	AA41767CH-EB-04	AA41768CH-EB-02

Safety was monitored during the inpatient and outpatient follow-up periods through the recording of adverse events, vital sign measurements, clinical safety laboratory testing, and grip strength in the affected hand.

Results: Sixteen subjects were enrolled and treated with one injection of AA4500. Fifteen (93.8%) of the 16 subjects completed the study. One subject was lost-to-follow after being in the study for eight days. This subject had a reduction in contracture from 60° at baseline to 10° on Day 7. AUX-I and AUX-II levels were not detected in any subject at any time point through the first 24 hours, on Day 7, or on Day 30 following administration of a single 0.58 mg injection of AA4500 into a Dupuytren’s cord. All samples were below the lower level of quantification (i.e., ≤ 5 ng/mL for AUX I and ≤ 25 ng/mL for AUX II).

In conclusion, there was no quantifiable systemic exposure following a single injection of AA4500 0.58 mg into the cord of the affected finger in subjects with Dupuytren’s contracture.

4.2.2 Study # 02 Review

Study Design and Objective: Open-label study of DUPY-202, four previously unexposed subjects with advanced Dupuytren’s disease received a single injection of AA4500 10,000 units (equivalent to 0.58 mg) into the cord of the affected finger joint to investigate the absorption and excretion of AA4500.

Blood sampling: Blood samples for the determination of AUX-I and AUXII in serum were collected before injection (time 0) and at 10, 20, 30 minutes, 1 hour, 4 hours and one day after the injection. In addition, urine was collected at time 0, 30 minutes, 1 hour,

4 hours and one day after the injection. Safety was monitored through the recording of adverse events, vital sign measurements, and grip strength.

Results and Conclusion: AA4500 was not detected in any serum sample at any time point through 20 to 24 hours following administration of a single injection of AA4500 10,000 unit (equivalent to 0.58 mg) into a Dupuytren's cord. All samples were below the lower level of quantification (i.e., < 4 ng/mL). However, the ELISA assay employed for the sample analysis was not adequately validated.

4.3 BLA Filing Memo

Office of Clinical Pharmacology New Drug Application Filing and Review Form				
General Information About the Submission				
	Information			Information
BLA Number	125338	Brand Name		Xiaflex
OCP Division	DCP2	Generic Name		Clostridial collagenase
Medical Division	DAARP	Drug Class		Enzyme
OCP Reviewer	Srikanth C. Nallani, Ph.D.	Indication(s)		Treatment of Dupuytren's Disease
OCP Team Leader	Suresh Doddapaneni, Ph.D.	Dosage Form		Lyophilized powder for Injection
		Dosing Regimen		Once every 30 days
Date of Submission	2/27/09	Route of Administration		Injection into affected cord
Estimated Due Date of OCP Review		Sponsor		Auxilium Pharmaceuticals Inc.
PDUFA Due Date	8/27/09	Priority Classification		Priority
Division Due Date	9/3/09			
Clin. Pharm. and Biopharm. Information				
	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.				
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling				
Reference Bioanalytical and Analytical Methods				
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:				
multiple dose:				
Patients-				
single dose:	X	2	2	
multiple dose:				
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:				
hepatic impairment:				
PD:				
Phase 2:				
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:				

Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				
2II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other Clin Pharm Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		2	2	
Filability and QBR comments				
	"X" if yes	Comments		
Application filable ?	X	Reasons if the application is <u>not</u> filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?		
Comments sent to firm ?	-	Comments have been sent to firm (or attachment included). FDA letter date if applicable.		
QBR questions (key issues to be considered)				
Other comments or information not included above				
Primary reviewer Signature and Date				
Secondary reviewer Signature and Date				