Review Letter - CINRYZE

Our STN: BL 125267/0
Lev Pharmaceuticals, Inc.
Attention: Mr. Jason Bablak
675 Third Avenue
Suite 2200
New York , NY 10017
Dear Mr. Bablak :
We have completed the review of all submissions made relating to your biologics license application (BLA) for C1 Esterase Inhibitor (Human) for the treatment of patients with hereditary angioedema (HAE) submitted under section 351 of the Public Health Service Act.
In our review, we find that the information and data submitted are inadequate for final approval action based on the deficiencies outlined below.
Chemistry, Manufacturing, and Controls (CMC)
1. We have received your January 2, 2008 preliminary response to the pre-license inspections of --(b)(4)--- and Sanquin. As a number of items will be submitted at a later date, there are still outstanding issues related to the inspection.
2. Based on our December 20, 2007, telephone conversation with Lev Pharmaceuticals, Inc. (Lev), it is our understanding that there were no finalized standard operating procedures (SOPs) in place at Lev at that time.

a. Please submit a master list of all SOPs that have been finalized thus far or SOPs that you plan to finalize prior to approval.
b. Additionally, please submit all SOPs that are now finalized that pertain to quality assurance functions. Such quality assurance functions may include, but are not limited to, the following: handling of deviations and investigations; change control; release of lots for distribution; submission of biological product deviation reports (BPDRs); adverse event reporting; product recall procedures; approval of validation protocols; and, maintenance of regulatory records.

3. We understand that Parvovirus B-19 was detected in --(b)(4)-- manufacturing steps, and that material was processed into two clinical Cinryze™ lots that were administered to patients. Subsequently, these lots were quarantined. Please provide a detailed description and chronology of these events, including the corrective actions taken by Lev. Your description of the handling of these particular lots should include, but not be limited to: adherence to all SOPs (including quarantine SOPs); evaluation of lot acceptance criteria; evaluation of test results; quality assurance review and release of lots; investigations and deviations; documentation review and approval; and CBER notification of these events.

4. Please explain your procedures on retain samples, including the quantity of samples and where they are maintained.
5. We understand that your manufacturing process has been modified since the conformance lots were manufactured. In that regard:

a. Please provide complete testing data from the conformance lots manufactured with the current process (all data from pooling stage to the finished product).
b. If the batch record submitted to the BLA has been modified, please provide an English translation of the most current batch manufacturing record that will be used for routine production of this product.
c. Please clarify the breakdown of manufacturing and quality assurance responsibilities between Lev Pharmaceuticals, Inc., Sanquin, and --(b)(4)--. In cases where the responsibilities have changed since submission of the BLA, please indicate those changes. Please indicate which manufacturing facility will produce --(b)(4)--- from this point forward.
d. Please submit a listing of all major manufacturing equipment that will be used in the manufacture of this product according to the most current manufacturing batch record, with the exception of filters which have already been submitted. We suggest that the list is formatted in the same manner as the filter listing (Table 6a and 6b) in your November 23, 2007 amendment, minus the process parameters. This list should include all vessels used to store or process the product, all columns, and all major processing equipment (lyophilizer, filling machine, capping machine, etc.). This list does not need to include ancillary systems (i.e. CIP systems, WFI systems, autoclaves, etc.) as they are addressed in questions 6 and 7 below. Additionally, please indicate whether such equipment is dedicated or shared equipment. In cases where similar or identical equipment are used in the manufacture of both Cinryze™ and Cetor, please indicate how such equipment is segregated (including, but not limited to, the columns).

6. As requested in our November 7, 2007 information request, please provide a detailed summary of the performance qualification or validation summaries of the equipment and/or systems listed below for the Sanquin facility. This should include a description of the protocol, detailed summaries of the results, and a description of any deviation investigations performed. If these qualifications were performed long ago, routine monitoring data should be provided.

a. Heating, Ventilation, and Air Conditioning system (HVAC system)

b. Water for Injection (WFI).
c. Clean in Place (CIP).
d. Clean out of Place (COP).
e. Major computer systems that impact the manufacturing process.
f. Autoclaves for sterilizing equipment.
g. Dry Heat Oven.
h. Filling Line Equipment

7. For the -(b)(4)-- facility in ---(b)(4)----------, please provide a detailed summary of the performance qualification or validation summaries of the equipment and/or systems listed below. This should include a description of the protocol, detailed summaries of the results, and a description of any deviation investigations performed. If these qualifications were performed long ago, routine monitoring data will be sufficient.

a. Heating, Ventilation, and Air Conditioning system (HVAC system)
b. Water System.
c. Clean in Place (CIP).
d. Clean out of Place (COP).
e. Major computer systems that impact the manufacturing process.

8. Please describe the cleaning process that will be used for all Cinryze™ manufacturing equipment (times, temperatures, cleaning reagents, etc.) on a routine basis. Equipment that undergoes the same cleaning cycle may be aggregated. In cases where a matrix approach is utilized, please provide rationale for such an approach. Please describe testing performed during the cleaning qualification and a rationale for the limits established.
9. Please describe the sterilization process that will be used for all Cinryze™ manufacturing equipment on a routine basis.
10. Please describe the sterilize-in-place (SIP) process that will be used for all Cinryze™ manufacturing equipment on a routine basis.
11. Regarding the Planova 15N Filtration, please explain why there was such variation in filtration times and flow rates for Batches USA-1 through USA-14 (Table 18 of the BLA). The total units of C1-Inhibitor ranges from -(b)(4)----, while the filtration times range from ---(b)(4)---------- and the flow rates range from -----(b)(4)----------. Please provide a correlation between units of product as compared to filtration time and flow rates.
12. In your January 2, 2008 submission, you indicated that new conformance lots were being manufactured with a modified process. Please note that the shelf-life determination of the final product will be based on the actual real-time stability data accumulated with these lots and submitted for review prior to approval of your product.
13. Regarding the --(b)(4)--- intermediate manufacturing at the ---(b)(4)------- facility:

a. Please provide critical operational and product parameters for the manufacturing process, including final acceptance and in-process

testing data for all ---(b)(4)--- batches that have been manufactured. In addition, please provide updated stability data to support the proposed shelf-life for the ---(b)(4)--- .

b. Please provide a head to head comparison of specifications, manufacturing and control parameters of ---(b)(4)--- produced at ---(b)(4)--- , and used in the manufacture of new conformance lots, with those obtained for the ---(b)(4)--- batches produced at the Amsterdam facility. Please explain the differences that may exist between the two intermediates, and their potential impact on the final product.

14. Regarding the release specifications of the final product:

a. The current specification for C1INH specific activity is ---(b)(4)---. Please provide an upper limit for this specification and the rationale for selection of this limit.

b. Please include the total protein concentration of C1INH as a final release specification, to allow verification of the product specific activity upon release. This should be reported in the release protocol as well.

c. Please establish tighter ranges for sodium, sucrose, and the amino acid additives in the finished product. These ranges should reflect expected assay variations for these measurements, as have been determined in your validation studies.

15. Sections 3.2.S.2.4-Amsterdam and 3.2.S.2.4----(b)(4)-- show that several limits/ranges for in-process/control parameters do not correspond to the specifications provided in the BSB036-Lev/1.4 document and other related Reports. Please update all relevant documents and provide a list of the amended documents in your response.

16. Please provide the process validation documentation relevant to -------(b)(4)---------- and ----------(b)(4)--- processes performed at ---(b)(4)--- .

17. The ---(b)(4)--- and ---(b)(4)---------- figures contained in Study Reports #036-068, 036-079, 036-082, 036-100, 036-084, 036-085, and 036-089 are of poor quality. Please improve the quality of these images and resubmit them to the BLA.

CLINICAL

Part A – Treatment of HAE Attacks

18. There are major problems in the conduct of Part A that jeopardize the integrity of the clinical study. Among these problems are the following:

a. Disregard of eligibility criteria through the granting of enrollment “waivers”.

b. Failure to use one central laboratory and one method of measurement of analytes using batch processing for all clinical samples (when these procedures were feasible).
c. The post hoc use of a “judiciary” to assist in the process of granting eligibility “waivers” when the results of C4 assays, and other assay-dependent eligibility criteria, were not met.
d. Inadequate vetting of submitted databases resulting in incorrect and/or illogical data entries in some databases.

We note that these major problems could have been avoided or mitigated if you had followed our advice given at the June 29, 2004, pre-IND meeting to conduct phase 2 studies prior to conducting a pivotal study.

19. Problems with the C4 Assay.

The C4 assay results were crucial to the successful completion of the Part A clinical study. The eligibility criteria strictly required that subjects demonstrate a decrease in the C4 level at the time of an HAE attack from the level measured at the time of screening. You state that the C4 assay underwent major changes at least 4 times during the Part A pivotal study. The 4 laboratory settings for the C4 assay are as follows:

a. -----(b)(4)--------- , 3/14/05 to 8/16/05, coded as “K229”.
b. ---------(b)(4)---------- , 8/16/05 to 11/14/05, coded as “I023” in your databases, and re-coded as “I023A” by FDA.
c. ------(b)(4)---------- , 11/23/05 to 11/02/06, coded as “J142”.
d. ---------(b)(4)---------- , 5/09/06 to 12/28/06, coded as “I023” in your databases, and re-coded as “I023B” by FDA.

The following table shows the results of the C4 assay at the time of screening:

C4 Laboratory Method Dates No. of Subjects	**Median**	**Average ± S.D.**
K229 = ---(b)(4)--- ---------- 3/14/05 to 8/16/05 N = 36	5	7 ± 4.6
I023A = ---(b)(4)--- ---------- 8/16/05 to 11/14/05 N = 18	5.5	8.9 ± 7.9
J142 = ---(b)(4)--- ----------	8	11.4 ± 8.7

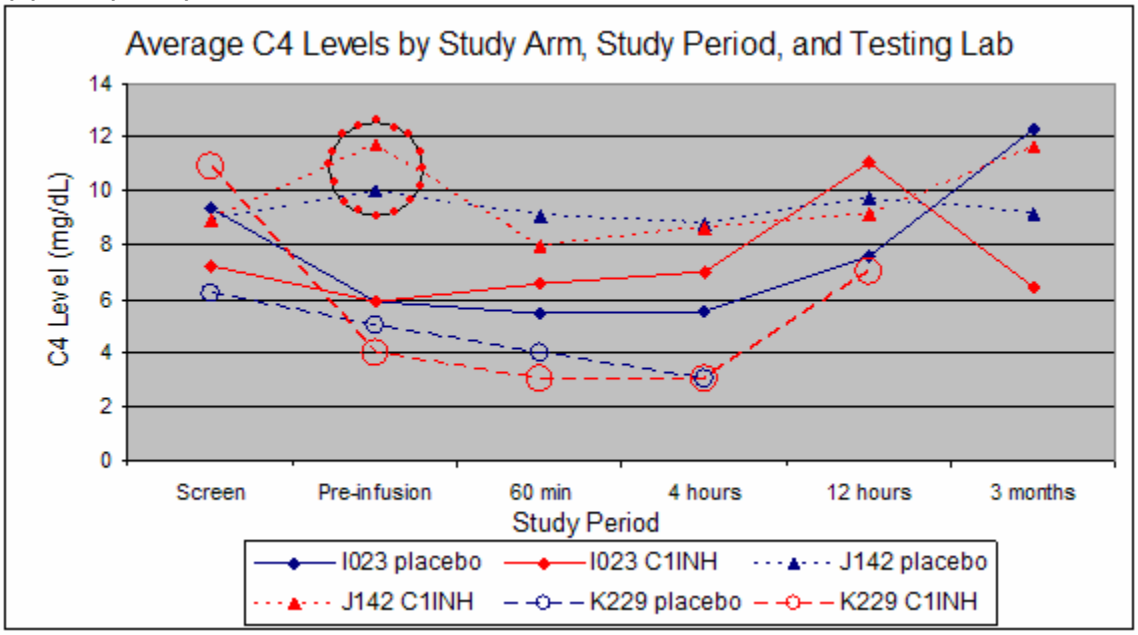
11/23/05 to 11/02/06 N = 150		
I023B = ---(b)(4)--- ---------- 5/09/06 to 12/28/06 N = 118	9.5	14.2 ± 15.6

It can be seen that the C4 measurements at screening became progressively higher over time, on average.

At the June 21, 2007, pre-BLA teleconference, FDA stressed that the BLA would need to contain complete validations for the assays that were used in the clinical study.

The following chart plots average C4 levels at different times from screening to attack and after treatment.

It can be seen that at the time of the attack the C4 levels decrease in samples measured at ---(b)(4)---------- but they do not decrease in samples measured at the --(b)(4)----- Laboratory, on average, indicating a sensitivity problem with the assay at the --(b)(4)----- (J142).



In fact, the --(b)(4)-----C4 assay values hardly changed at all, on average, during the course of the study.

Therefore, we conclude that the C4 assays have not been adequately validated across all laboratory settings.

20. Use of a post hoc "Judiciary."

The occurrence of a high percentage of subjects who failed to demonstrate a decrease in their C4 level at the time of the HAE attack, as strictly required by the eligibility criteria, caused you to ask an expert in hereditary angioedema to decide whether these subjects should be included into the analysis dataset, even though the protocol clearly stated that they should be excluded.

Some of these problems with the C4-related eligibility criterion may have arisen because you did not conduct phase 2 studies in which these problems might have been detected, and the need for validated assays using batch processing procedures might have been realized.

The use of a "judiciary" to decide eligibility for 23 of 71 treated subjects was not pre-specified in the protocol. Furthermore, module 5, volume 1.7, section 16.1.14.1 "Determination of Evaluability", contains a discussion of the judiciary's recommendations, and includes statements such as "Therefore, we will accept the conclusion of Dr. Cicardi" (page 1003 of 1084). This raises a question about the finality of the judgment of the judiciary, and whether his "judgment" was taken by the sponsor as only a recommendation.

The effect of the use of the "judiciary" was to include all but 3 of the 23 subjects in question. Two of these 3 subjects did not achieve initial relief of symptoms within 4 hours, so they would have been censored by the SAS procedure PHREG that was used for the statistical analysis of the primary endpoint.

Therefore, the final effect of the use of the "judiciary' was to remove only a single subject from the analysis, subject 20-004 in the placebo arm who reported initial relief of symptoms at the first time point, 15 minutes.

In addition, it appears that the decision to refer a subject's data to the judiciary for review was handled inconsistently.

Please explain why the following 11 subjects were, or were not, referred to the "judiciary" for review:

a. Subjects 01-005, 13-009, 16-004, 16-005, and 18-004, all of whom showed the required decrease in C4 level at the time of the HAE attack, and yet they were referred to the "judiciary".

b. Subjects 03-001, 13-003, and 24-004, all of whom did not show the required decrease in C4 level at the time of the HAE attack, but they were not referred to the "judiciary".

c. Subjects 06-001, 14-003, and 20-001, all of whom were indeterminate for a decrease in C4 level at the time of the HAE attack because they lacked either a screening or a pre-infusion C4 level, yet only 2 were referred to the "judiciary".

The list of subjects whose data were referred to the judiciary is given in the "Evaluability Spreadsheet" (Module 5, Volume 1.7, p. 1007 of 1804). The data on C4 levels can be obtained from database "LEV1A". The following table gives the data for these 11 subjects:

Subjects Inconsistently Handled for Judiciary Review

Subject Group	Date Screen	C4 Result Screen (mg/dL)	Lab Screen	Date Pre-Infusion	C4 Result Pre-Infusion (mg/dL)	Lab Pre-Infusion	Time to Relief	Referred to Judiciary
C4 Decreased, yet Referred to Judiciary								
01-005	03/10/06	3	J142	10/25/06	2	J142	0.75	Yes

Placebo								
13-009 C1INH	09/13/06	15	J142	11/24/06	12	I023B	0.25	Yes
16-004 Placebo	06/16/06	4	J142	08/23/06	3	I023B	1.75	Yes
16-005 C1INH	06/15/06	7	J142	08/12/06	3	I023B	0.75	Yes
18-004 a.k.a. 01-016 C1INH	05/15/06	8	J142	10/24/06	2 (repeat after 31)	I023B	2	Yes
No C4 Decrease, but not Referred to Judiciary								
03-001 Placebo	04/11/05	<3	K229	04/06/06	15	J142	NA	No
13-003 Placebo	07/18/06	4	J142	12/11/06	4	I023B	NA	No
24-004 Placebo	10/30/06	11	J142	12/10/06	14	I023B	2.5	No
Missing C4 Levels Handled Inconsistently								
06-001 C1INH	03/14/05	18	K229	07/07/05	ND	K229	NA	Yes
14-003 a.k.a. 13-001 Placebo	07/06/06	7	J142	08/28/06	ND	ND	NA	Yes
20-001 C1INH	ND	ND	ND	12/07/06	8	I023B	2	No

ND = Not Done
NA = No relief of symptoms on-study
Lab Codes are as above in the description of C4 Measurement.

21. Errors in the Efficacy SAS Database.

The original submission of STN125267 failed to contain the SAS database that was used in the statistical calculations for the primary endpoint. On August 14, 2007, FDA requested that this database be submitted. In response, you submitted a database labeled “D_EFFI” which you stated is the database that you used to perform your statistical analysis.

We have examined database “D_EFFI” and we have determined that it contains errors that affect the statistical calculations. For example, subject 03-015 is listed in field “DUR” as having achieved the primary endpoint “initial relief of symptoms” in 6600 seconds (or 1.83 hours). This result is not possible based on the 15 minute monitoring intervals, and it does not agree with your listing of outcomes in listing 16.2.21 “Summary of Treatment Outcome – Randomized Subjects” (module 5, volume 1.9 p. 1798 of 1804). In addition, the field “DUR” appears to give incorrect data for subjects 06-005 and 07-002. There may be additional errors that you may find if you vet this database. Therefore, we cannot perform a final statistical analysis until the correct outcome databases are submitted.

Please submit the following to STN 125267/0:

a. A complete set of vetted SAS databases for all analyses submitted in STN 125267/0 (this should include the intent-to-treat database of 71 subjects, and the database for 68 subjects after input from the "judiciary").
b. 38 Modified intent-to-treat databases in which responding subjects have been censored one-by-one in each database (22 C1INH responders, 16 placebo responders),
c. Analyses of the primary endpoint time to relief of symptoms with and without using centers as a covariate.

Using these corrected databases, please submit the results of the following sensitivity analyses:

d. An analysis of time-from-attack to time-of-relief.
e. An analysis that removes all subjects who reported initial relief of symptoms at the earliest time point, 15 minutes.

The analyses should be both by treatment alone, and by treatment with centers as a covariate.

Until the discrepancies in the databases are resolved a definitive statement about trial outcome cannot be made. Nevertheless, your analysis of a treatment effect cannot be considered as robust since removal of 1 subject and an analysis of covariates taking into account a center effect are required in order for a statistical significant effect to be achieved. It is possible that a more robust outcome would have been observed if optimal dosing would have first been determined in a Phase 2 dosing study. Depending on the outcome of an analysis performed on the corrected database and your responses to the questions in this CR letter, you may need to perform a new study to show efficacy for Treatment.

Part B – Prophylaxis of HAE Attacks

22. At the June 19, 2004, pre-IND meeting for BB-IND (b)(4), you asked FDA the following question:

Question 5) Does FDA agree that the primary endpoint for Part B, the number of attacks of angioedema during each treatment phase, comparing each subject to himself/herself, is appropriate?

The statistical analysis plan in the original protocol for BB-IND (b)(4) contains similar language about using each subject as his/her own control, as does the final analysis plan for Part B.

Despite these questions and statements, you did not perform an analysis which uses each subject as his/her own control, rather you pooled HAE attack frequency data across subjects and compared treatment arms.

An analysis that used each subject as his/her own control would classify outcomes for individual subjects (success, failure). If you had taken your intended approach, you would
have seen that approximately half the subjects in Part B could be classified as "success" and half the subjects as "failure". A bimodal outcome such as this would not necessarily mean that the study had failed; it could mean that the product was effective in some subset of subjects that would remain to be defined.
We note that pooling the results across all subjects and reporting that there is an approximately 50% reduction in the frequency of HAE attacks misrepresents the expected outcomes for both groups, i.e. those in the "success" category and those in the "failure" category.
We recommend that you re-analyze the results of Part B according to your original intention to use each subject as his/her own control, and submit the analysis to STN 125267/0.
We note that an additional study designed to further define responding groups of subjects under appropriate dose schedules for prophylaxis may be required. Phase 2 studies, as proposed by FDA, may have resolved this.

STATISTICAL ANALYSIS

23. Regarding Part B.
You provided the SAS programs and the dataset which were used to estimate the mean number of attacks during treatment with Cinryze™ and during treatment with placebo. In this study, each subject was used as his or her own control. Since it was a crossover analysis, it would be expected that the repeated measure option "REPEATED=subject" in the PROC GENMOD would be used to reflect the crossover design. In your SAS code, this option was not used. Therefore, the estimation of the treatment effect was done as if it was a parallel group design. For the parallel group design, the sample size of 22 subjects may not be sufficient to provide necessary power. Please explain your approach.

PRODUCT LABELING

24. We reserve comment on the proposed labeling until the application is otherwise acceptable. We may have comments when we see the proposed final labeling.
You may request a meeting or teleconference with us to discuss the steps necessary for approval. For PDUFA products please submit your meeting request as described in the FDA Guidance for Industry: Formal Meetings With Sponsors and Applicants for PDUFA Products February, 2000 (http://www.fda.gov/cber/gdlns/mtpdufa.pdf). For Non PDUFA products, please contact the regulatory project manager. For details, please also follow the instructions described in CBER's SOPP 8101.1: Scheduling and Conduct of Regulatory Review Meetings with Sponsors and Applicants (http://www.fda.gov/cber/regsopp/81011.htm).
Within 10 days after the date of this letter, you should take one of the following actions: (1) amend the application; (2) notify us of your intent to file an amendment; or (3) withdraw the application.
We stopped the review clock with the issuance of this letter. We will reset and start the review clock when we receive your complete response.
If you have any questions, please contact the Regulatory Project Manager, Nannette Cagungun, at (301) 827-6174.

Sincerely yours,
Basil Golding, M.D.
Director
Division of Hematology
Office of Blood Research and Review
Center for Biologics Evaluation and Research