Summary Basis for Approval

I. General Information

Licensed Product Name: Alpha-1-Proteinase Inhibitor (Human)

Proprietary Name: Zemaira™

Other Name: Alpha-1 Antitrypsin

Name and Address of Sponsor:

Aventis Behring L.L.C. (U.S. License No. 1281)
1020 First Avenue
P.O. Box 61501
King of Prussia, PA 19406-0901

Biologics License Application (BLA) Tracking Number: STN 125078/0

Date of Submission: January 6, 2003

Date of Filing: March 3, 2003

Review Designation: Fast Track

Date of Licensure: July 8, 2003

II. Indications and Usage

Zemaira™ is indicated for chronic intravenous augmentation and maintenance therapy in individuals with alpha$_1$-proteinase inhibitor (α_1-PI) deficiency and evidence of emphysema.

III. Dosage Form, Route of Administration, and Recommended Dosage

Zemaira™ is supplied in a single dosage size containing nominally 1 g (1,000 mg) of lyophilized α_1-PI. This product, purified α_1-PI in excipients, i.e., Alpha-1-Proteinase Inhibitor (Human) [A1PI], is to be reconstituted with 20 mL of Sterile Water for Injection (USP) [SWFI]. Zemaira™ is to be stored at temperatures not to exceed 25 °C (77 °F) and protected from freezing.

Zemaira™ is supplied as a sterile, white, lyophilized powder in a single use container to be administered by the intravenous route. Zemaira™ is packaged with: a single use

container containing 20 mL of SWFI for reconstitution; a sterile, double needled, vented transfer device for reconstitution; a sterile, 5 micron in-line filter; and a product information insert (package insert). The vial of SWFI is manufactured by Aventis Behring L.L.C. (AB), and the transfer needle and in-line filter are provided by another vendor. The transfer needle was previously approved by the Agency, and the in-line filter conforms with USP testing requirements and was qualified by AB.

Each single use container of Zemaira™ contains the labeled amount of functionally active α_1-PI in milligrams as stated on the vial label as determined by its capacity to neutralize human neutrophil elastase. The recommended dose of Zemaira™ is 60 mg/kg body weight administered once weekly.

When reconstituted as directed, Zemaira™ may be administered intravenously at a rate of approximately 0.08 mL/kg/min as determined by the response and comfort of the patient. Administration should be completed within 3 h of reconstitution. The recommended dosage of 60 mg/kg body weight will require approximately 15 min for infusion.

IV. Manufacturing, Chemistry, and Controls

Overview of Manufacturing Process

The Zemaira™ brand of A1PI is manufactured by AB, under U.S. License No. 1281, entirely at the Bradley, Illinois facility. The manufacture of A1PI starts with Fr. IV_1 + Fr. IV_4 (Fr. $IV_{1\text{-}4}$) precipitate, which is an intermediate by-product generated during the manufacture of licensed Fr. V products from pooled human plasma (Source Plasma) by a low temperature, cold ethanol fractionation procedure (a modified Cohn method). The Fr. $IV_{1\text{-}4}$ starting material contains high levels of α_1-PI although in crude form. Fr. $IV_{1\text{-}4}$ precipitate undergoes [redacted] extraction, dithiothreitol and silicon dioxide treatment to remove hydrophobic protein impurities, ion (i.e., [redacted]) exchange chromatography (IEC) to remove impurities with α_1-PI elution with [redacted] buffer, hydrophobic interaction chromatography (HIC) to remove additional hydrophobic impurities with recovery of α_1-PI from effluent, ultrafiltration/diafiltration, liquid heat treatment (at 60 °C for 10-11 h) of stabilized α_1-PI for viral inactivation, [redacted] filtration for viral removal, ultrafiltration/diafiltration into final formulation buffer, and sterile filtration. The sterile bulk solution is aseptically filled into previously sterilized vials, frozen, and lyophilized. The lyophilized vials are stoppered under vacuum then capped, inspected, labeled, and packaged.

After reconstitution with 20 mL of SWFI, each vial of Zemaira™ contains approximately 1.0 g of α_1-PI with a specific activity ≥0.7 mg of functional α_1-PI per milligram of total protein, 81 mM sodium, 38 mM chloride, 17 mM phosphate, and 144 mM mannitol. Hydrochloric acid and/or sodium hydroxide may have been added to adjust the pH. The purity is ≥90% α_1-PI. Zemaira™ contains no preservatives.

All final container lots meet the requirements of 21 CFR § 610 et seq. for potency, safety, sterility, purity, and identity and the established specifications for Zemaira™. 3 conformance lots were submitted to the Center for Biologics Evaluation and Research (CBER) in support of the BLA.

Validation of Assays Used for In-Process Intermediates and Final Container Product

The assays/tests used for in-process intermediates and/or final container product include: Appearance of Final Container Product; Solution Time of Final Container Product; Appearance of Reconstituted Product; Potency (functional α_1-PI); Total Protein ; Specific Activity; Hepatitis B Surface Antigen; Sterility; Moisture General Safety; Purity Mannitol; Phosphate; Chloride; Sodium; Vacuum; and Identification. Validation protocols and validation reports were provided for these assays. Where appropriate, assay data demonstrating linearity, precision, accuracy, limit of detection, limit of quantitation, interference, ruggedness, and robustness were provided.

The in-process intermediates include: Fr. IV_{1-4} Precipitate (starting material); and Sterile Bulk.

Validation of Assays Used to Analyze Patient Samples from Phase III Clinical Trial

Patient samples from the phase III clinical trial were analyzed at the The following assays were used for the testing of serum samples: α_1-PI Phenotype by Functional α_1-PI Level (Anti-Neutrophil Elastase Capacity [ANEC]); and α_1-PI Level by The following assays were used for the testing of bronchoalveolar lavage (BAL) samples, consisting of diluted endothelial lining fluid (ELF): ANEC; Antigenic Level of α_1-PI by Antigenic Level of Neutrophil Elastase (NE) by ; Antigenic Level of α_1-PI:NE Complex by and Urea Concentration. Abbreviated analytical method descriptions were provided. Validation data were provided for the ANEC assay and the α_1-PI Level by assay although validation protocols were not. Additional validation data were provided very late during the review period.

Validation of Manufacturing

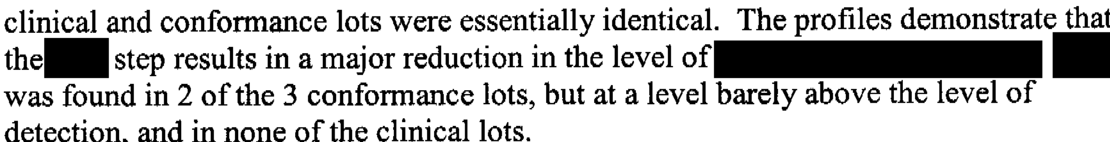
Clinical trials were carried out with A1PI product manufactured at pilot scale. The validation of manufacturing, including facilities, equipment, and utilities for the commercial manufacture of Zemaira™, was performed as defined in the Validation Project Plan and completed. The validation consisted of demonstrating that the product produced at commercial scale, i.e., the clinical lot size, is bioequivalent to that produced at clinical scale and used in clinical studies and demonstrating that the manufacturing process is consistent and results in product of requisite purity, potency,

efficacy, and safety. Analytical methods used for in-process and final product testing were developed and validated as well (See section above.).

4 lots were manufactured at commercial scale in support of this application; however, the first of these was manufactured with an improper closure and, thus, was not submitted to CBER in support of this BLA. Nevertheless, data from the manufacture of this lot as well as the other 3 lots, which were submitted as the official conformance lots, were used to validate the manufacturing process. The batch records and process validation protocols for commercial manufacture were based upon clinical trial manufacturing experience and development data from small scale studies as well as pre-clinical research carried out during the course of product development. Thus, for commercial scale manufacture, ranges and limits for various process control parameters, some of which were designated as critical, were set and acceptance criteria for critical quality attributes of in-process intermediates and specifications for the final container product were established. Ranges covered by the critical control parameters during conformance lot manufacture resulted in values of critical attributes that conformed with established acceptance criteria and the release specifications for the final container product thereby validating the ranges of the critical control parameters for commercial manufacture. Results for non-critical attributes were within the ranges/limits observed previously for clinical lots. The original release specification for Purity, i.e., ≥90%, was tightened to ▇▇▇, and other acceptance criteria were implemented after submission of the BLA, e.g., bacterial endotoxin limits.

To demonstrate consistency of manufacture of clinical lots and commercial lots and bioequivalence of these, values of certain attributes were compared for a number of clinical lots (approximately 16 lots) and the 4 conformance lots; all lots of conformance intermediates were considered. Functional α_1-PI, total protein, specific activity, purity, and ▇▇▇ data and protein and specific activity step yields for ▇▇▇ ▇▇▇ and final container product of clinical and conformance lots show lot-to-lot consistency for both clinical and commercial lots and comparable results between clinical and commercial in-process intermediates and final container products.

▇▇▇ data were also used to assess consistency of manufacture and bioequivalence of clinical and commercial products. Initially, for clinical lots ▇▇▇ were qualitatively identified in in-process intermediates by ▇▇▇ analysis and quantitated in final container product by ▇▇▇ All of the ▇▇▇ for which testing of clinical lots was carried out were detected in the ▇▇▇ extracted Fr. IV_{1-4} starting material whereas ▇▇▇ were detected in final container product. ▇▇▇, and ▇▇▇ were quantitated in final container product (from greatest to least amount); ▇▇▇ ▇▇▇ was detected but not quantitated due to lack of an appropriate standard. ▇▇▇ profiles of 6 clinical and the 3 conformance lots submitted to support the BLA were determined by monitoring by ▇▇▇ levels of ▇▇▇ ▇▇▇ in part, chosen as a result of the ▇▇▇ study, in in-process intermediates and final container products. A single lot of each intermediate giving rise to each of the final container lots was assayed. Overall the ▇▇▇ profiles for



clinical and conformance lots were essentially identical. The profiles demonstrate that the step results in a major reduction in the level of was found in 2 of the 3 conformance lots, but at a level barely above the level of detection, and in none of the clinical lots.

Viral Safety

The plasma used in the manufacture of this product has been tested and found to be non-reactive to HBsAg, negative for antibody to Hepatitis C Virus (Anti-HCV), and negative for antibody to Human Immunodeficiency Virus (Anti-HIV-1/HIV-2).

2 viral reduction steps are employed in the manufacture of Zemaira™: a liquid heat treatment step at 60 °C for 10- h of an aqueous solution of product in the presence of stabilizers and an ultrafiltration step involving the use of filters. These viral reduction steps have been validated in a series of *in vitro* experiments for their capacity to inactivate and/or remove Human Immunodeficiency Virus (HIV), Hepatitis A Virus (HAV), and the following model viruses: Bovine Viral Diarrhea Virus (BVDV) as a model virus for HCV, Canine Parvovirus (CPV) as a model virus for Parvovirus B19, and Pseudorabies Virus (PRV) as a model virus for large, enveloped DNA viruses (e.g., herpes virus). Total mean $\log_{10}$ reductions from the clearance steps are shown in Table 1.

Table 1: Individual and Cumulative Virus Reduction Factors

	Reduction Factor Heat Treatment [$\log_{10}$]	Reduction Factor Ultrafiltration Step [$\log_{10}$]	Cumulative Reduction Factor [$\log_{10}$]
HIV-1	≥ 6.7	≥ 5.5	≥ 12.2
BVDV	≥ 5.9	5.1	≥ 11.0
PRV	4.3	≥ 6.9	≥ 11.2
HAV	≥ 5.4	≥ 6.3	≥ 11.7
CPV	(0.9)	6.8	6.8

Batch Records

During the pre-licensing inspection, a number of suggestions were made, by both the FDA inspector and AB personnel, concerning the format of the batch records, and these changes were later implemented by AB in the revised batch records. Many of the modifications involved stating the required limits/ranges for the process control parameters next to the spaces used to record the actual values utilized and stating the acceptance criteria for quality attributes next to the spaces used to record the measured assay/test values of the attributes. In addition, the revised batch records included

tightened requirements for control parameters, which were implemented after submission of the original BLA.

Stability Studies of In-Process Intermediates

For in-process intermediates the following hold times and temperatures were validated by utilizing 3 lots of each intermediate:

Stability Studies of Final Container Product

Primary stability lots consisted of 4 clinical lots. Stability studies were carried out on these lots at 4 °C, 25 °C and 60% relative humidity (RH), and 40 °C and 75% RH for 30, 24, and 6 months, respectively. For the 30 and 24 month studies, the testing schedule was 0, 3, 6, 9, 12, 18, 24, 30, and 36 months, and for the 40 °C study, testing was carried out at 0, 1, 3, and 6 months. All lots met specifications at each time point studied, and linear regressions and statistical analyses of Potency (3 h after reconstitution), Molecular Weight Distribution (of monomer), Purity, Moisture, and pH were carried out. These results support a 24 month expiration dating period with storage at temperatures not to exceed 25 °C. Assays/tests included: Appearance (of unreconstituted product), Appearance of Reconstituted Solution, Solution Time, pH, Potency (immediately after reconstitution), Potency (3 h after reconstitution), Purity (SDS-PAGE), Molecular Weight Distribution, Moisture, Vacuum, and Sterility; however, not all testing was scheduled for each time point.

The 3 official conformance lots were placed in the long-term stability program (at 25 °C and 60% RH) and the accelerated program (40 °C and 75% RH). Up to the present, all test results meet the required specifications.

Shelf Life and Storage Conditions of Final Container Product

On the basis of the stability data provided for the final container product for the 4 primary stability lots and additional supporting data, a shelf life of 24 months from the date of manufacture was granted with storage at temperatures not to exceed 25 °C (77 °F). In order to avoid damaging the diluent container, freezing should be avoided.

Stability of Reconstituted Final Container Product

3 clinical lots were reconstituted and immediately assayed for Potency, stored at room temperature for 3 h, then re-assayed. For each lot, the Potency values determined 3 h post-reconstitution were >99% of those determined initially.

Labeling

The labeling for Zemaira™ is comprised of a product information insert (package insert), vial label, and carton label that have been found to be in compliance with prescription drug labeling requirements (21 CFR 201.57) and with requirements for Source Plasma. The trademark for this A1PI product, Zemaira™, is not known to be in conflict with the trademark of any product.

Pre-Licensing Inspection of Aventis Behring L.L.C.

A pre-licensing inspection of the Aventis Behring L.L.C. site in [redacted] IL was carried out by the Agency from March 24 through April 2, 2003; this site is the only production site for both the drug substance and drug product, the manufacturing of which was the subject of the inspection. At the end of the inspection, a Form 483 was issued to the responsible head. The Form 483 included observations concerning resolution of discrepancies, requalification of [redacted] and [redacted] resins and storage of these columns, environmental monitoring and trending data, aseptic media fill validation, pressure differentials, validation of equipment cleaning, assays for Potency and pH, and shipping validation. These observations were subsequently resolved by AB to the satisfaction of the Agency. Thus, the manufacturing of Zemaira™ is now considered to comply with current Good Manufacturing Practices.

Environmental Assessment

A request for a categorical exclusion according to 21 CFR Part 25 §25.31 (b) (c) Human drugs and biologics was granted.

V. Non-Clinical Pharmacology and Toxicology

Since efficacy testing of Zemaira™ in animal models reflecting the pathophysiology of emphysema secondary to alpha-1-proteinase inhibitor (α_1-PI) deficiency is hampered by the immune response against the human (heterologous) protein upon repeated administration of Zemaira™, no *in vivo* animal studies on the pharmacodynamic properties of Zemaira™ were conducted. No adsorption, distribution, metabolism, and excretion studies were performed with Zemaira™ in animals due to the interference of elimination kinetics with immune reactions directed against the human (heterologous) protein. Also, studies requiring longer-term administration were not carried out in animals (i.e., >5 days).

In vitro pharmacodynamic studies using a neutrophil elastase inhibition assay demonstrated at least equal specific activity of Zemaira™ as compared to the registered plasma-derived human Alpha-1-Proteinase Inhibitor (Human) [A1PI] product, Prolastin®.

Single dose toxicity studies in rats and mice given 60, 240, or 600 mg/kg demonstrated that IV administration of Zemaira™ was well tolerated at 60 and 240 mg/kg. Transient symptoms of distress were found at dosing with 600 mg/kg. The NOAEL (No Observed Adverse Effect Level) for a single Zemaira™ injection was 240 mg/kg.

Repeat dose toxicity studies in rats and rabbits administered 60 or 240 mg/kg /day for 5 days suggested a NOAEL of 60 mg/kg/day. A dose-related increase in absolute and relative spleen weights in Zemaira™-treated rats and rabbits was a common finding in all studies. In addition, apparent perivascular infiltration by granulocytes (heterophil cuffing) in the lungs of placebo and Zemaira™ animals indicated a potential vehicle mediated effect.

The occurrence of new antigenic determinants caused by the liquid heat-treatment step was studied in rabbits and gave no evidence for the existence of neoantigens in heat-treated Zemaira™ samples. Similarly, local tolerance studies in rabbits given 0.5 mL of a 43.25 mg/mL solution of the product demonstrated no difference in bruising, injection site reactions, or histopathology compared to the control group.

α_1-PI is a physiological constituent of human plasma and, thus, is not expected to have mutagenic, teratogenic, or carcinogenic effects. Consequently, genotoxicity, carcinogenicity, and reproductive toxicity studies were not conducted.

VI. Human Pharmacokinetics and Bioavailability

The pharmacokinetic properties of Alpha-1-Proteinase Inhibitor (Human) [A1PI], IV (Zemaira™) were consistent in all clinical studies in which Zemaira™ was administered and pharmacokinetic information was collected. Distribution of alpha-1-proteinase inhibitor (α_1-PI) was essentially confined to plasma, as shown by a volume of distribution at steady state of approximately 4 L for adult subjects across different studies. With a clearance of about 640 mL/day, elimination was fairly slow, resulting in an overall $t_{1/2\beta}$ of approximately 5 days. Steady state was reached by Week 5 of Zemaira™ treatment. In Study 201, trough serum concentrations from Week 7 to Week 26 showed a mean antigenic α_1-PI concentration of 16.9 μM (90% CI 15.9 to 17.9 μM) and a mean functional α_1-PI concentration of 15.7 μM (90% CI 14.5 to 17.0 μM). In Study 2002, the mean trough serum steady-state antigenic α_1-PI concentrations from Week 7 to Week 11 were 17.7 μM (90 % CI 16.9 to 18.5 μM) and 19.1 μM (90% CI 18.1 to 20.2 μM) in the 2 study groups for subjects receiving Zemaira™ and Prolastin®, respectively, and were above 11 μM for both study groups from Week 2 through Week 24. Mean trough functional α_1-PI concentrations, measured as anti-neutrophil elastase capacity (ANEC), remained constant throughout the study period. The increase from baseline of antigenic α_1-PI in epithelial lining fluid (ELF) as determined by analysis of specimens from bronchoalveolar lavage (BAL) was statistically significant in both treatment groups, with ELF α_1-PI concentrations increasing approximately 5-fold and ELF α_1-PI:neutrophil elastase complexes increasing approximately 25-fold in the

Zemaira™-treated subjects. These results demonstrated diffusion from serum into the lung and showed that the α_1-PI delivered to the lower lung was functionally active and able to bind its substrate, neutrophil elastase. Pre-treatment baseline ELF ANEC levels were anomalously high in the 10 subjects randomized to the Zemaira™ group who underwent BAL sampling. This was not the case for the 5 subjects randomized to Prolastin® who underwent similar sampling. Mean ELF ANEC actually fell, but non-significantly, from baseline to Week 11 during Zemaira™ administration. In contrast, the expected rise in mean ELF ANEC was demonstrated in the concurrent Prolastin® control group. Although the sponsor emphasized the potential for measuring activities due to other substances as well as that of α_1-PI in the ANEC assay, that would not explain the apparently disparate results between the randomized treatment subgroups that underwent BAL procedures. It should be noted, however, that the mean on-treatment Week 11 ANEC values were similar for both treatment subgroups.

Clinical Pharmacology Summary

Study 101, the first clinical trial, was a dose-ranging study that evaluated the safety, tolerability, and pharmacokinetics of Zemaira™ at single doses of 15, 30, 60, and 120 mg/kg in 19 patients. In this study, Zemaira™ demonstrated a dose-dependent increase in the area under the curve (AUC) and the maximum serum concentration of antigenic α_1-PI achieved. Median time from end of infusion to maximum concentration of α_1-PI in serum was approximately 15 min, with the dose-dependent maximum concentration (C_{max}) ranging from 16.1 to 67.4 μM (medians: 18.6, 34.1, and 56.3 μM for the dose groups 30, 60, and 120 mg/kg, respectively). The terminal half-life ($t_{1/2\beta}$) was approximately 5 days, independent of dose. The levels of antigenic α_1-PI in the ELF were about 10% of the serum α_1-PI levels and showed a dose-dependent increase. This is evidence of diffusion from serum into the lung.

In the multiple-dose studies, Zemaira™ administered intravenously at a dose of 60 mg/kg once weekly resulted in mean steady-state trough serum antigenic concentrations of α_1-PI clearly above the historical target trough threshold of 11 μM in all treated subjects. In pivotal phase III Study 2002, no evidence was found that the biochemical surrogate of antigenic α_1-PI serum levels differed between any population subgroups. Because all subjects in these studies were Caucasian, a subgroup analysis by race was not applicable. Because all subjects were adults with only 3 subjects aged 65 years or older, an analysis by age group was not performed. Subgroup analyses were performed to evaluate whether any of the following potential prognostic factors had an impact on the magnitude of steady-state trough serum antigenic α_1-PI levels: center, gender, extent of previous treatment with A1PI, age, FEV_1 (% predicted), and baseline serum antigenic α_1-PI level. This was done by including these variables along with treatment group as main effects in an ANOVA model of the steady-state trough serum antigenic α_1-PI levels. None of the factors had a significant impact ($p < 0.05$) on steady-state trough serum antigenic α_1-PI levels except for the baseline α_1-PI level ($p = 0.0008$).

The pharmacokinetic and pharmacodynamic relationships also were evaluated. Time courses of functional α_1-PI serum concentrations (ANEC levels) were highly correlated with those of antigenic concentrations. The Spearman correlation coefficient within subjects ranged between 0.96 and 1.0, with a median value of 0.99 for Study 101; for Study 201, the mean value was 0.68 (range: 0.20 to 0.86). Considering all 156 post-infusion samples in Study 101, functional concentrations narrowly ranged between 89.6 and 105.5% of antigenic concentrations. These results were confirmed in single-dose Study 1002, in which the median ratio of functional to antigenic α_1-PI concentrations remained above 80% for up to 6 days after infusion and in which the overall correlation between the functional and antigenic concentrations was above 90%. In Study 2002, the median ratio of functional to antigenic serum α_1-PI was approximately 68% in both the Zemaira™ and the Prolastin® groups. In the blinded phase from Week 1 to Week 11, the mean Spearman correlation coefficients were 0.55 (±0.22) and 0.58 (±0.25) in the Zemaira™ -treated and Prolastin®-treated groups, respectively. In Study 2002, the ratio of functional to antigenic α_1-PI was lower than in the preceding studies. Since the functional α_1-PI levels in Study 2002 were lower at baseline and every subsequent time point, this probably represents a shift in the assay.

VII. Clinical Summary

A. Disease Overview

Alpha-1-proteinase inhibitor (α_1-PI, alpha-1 antitrypsin) deficiency is an autosomal co-dominant disorder in which there are below normal levels of α_1-PI in serum and in the epithelial lining fluid (ELF) of the lung. Patients with this disorder have a high risk for the development of emphysema in the third to fifth decades. Some patients also develop liver disease, which is not thought to be ameliorated by augmentation therapy with α_1-PI.

α_1-PI, which is synthesized in the liver, is a glycoprotein of M_r 52,000. It is a serine protease inhibitor that has the primary function of inhibiting neutrophil elastase. It is postulated that emphysema in severe α_1-PI deficiency results from the imbalance between the neutrophil elastase in the lung that has the capability to destroy elastin of the alveoli and the α_1-PI that is responsible for protecting the lung from the elastase.

The α_1-PI gene has been found to code for over 70 types of α_1-PI proteins, and the various subtypes of α_1-PI proteins are classified on the basis of their electrophoretic motilities. Subjects with various subtypes will have differing serum levels of α_1-PI.

Data from the NIH α_1-PI Registry (*Am. J. Respir. Crit. Care Med.* **158**, 49 (1998)), a non-randomized, epidemiologic prospective study, suggest that augmentation therapy *may* be efficacious in halting the progression of disease ***in the subgroup of patients who have an FEV$_1$ between 35-49% predicted***. However, it should be noted that the primary endpoint evaluating the slope of FEV_1 change in the subgroups of the entire study population who were (ever) on augmentation therapy vs. the slope in those who were

never on augmentation therapy with α_1-PI did ***not*** show a significant difference. Although one cannot draw definitive causal inferences from any epidemiologic study, it should also be noted that the NIH Registry did suggest a possible mortality benefit associated with augmentation therapy. Researchers in the field have voiced concern that baseline imbalances in socioeconomic and other factors not fully taken into account in the analyses of this study may have led to an overestimate of possible augmentation therapy effects on mortality.

B. Background to the Regulatory Approach to Alpha-1-Proteinase Inhibitor (Human) Intravenous Products

Prior to the licensure of Bayer Corporation's Alpha-1-Proteinase Inhibitor (Human) [A1PI], IV product, Prolastin®, a joint NIH-FDA Committee recommended criteria for clinical evidence of efficacy to permit licensure of A1PI intravenous products for augmentation therapy in congenital α_1-PI deficiency with demonstrable emphysema. The criteria consisted of a demonstration of the ability of a product, when administered according to its directions for use, to produce and maintain a stable trough level equivalent to those of MZ heterozygotes and also to demonstrate that administration of the product results in increases in antigenic and functional (anti-neutrophil elastase capacity [ANEC]) α_1-PI levels in ELF of the lungs, as determined by bronchoalveolar lavage (BAL). Although the average MZ heterozygote has antigenic α_1-PI levels significantly greater than 11 μM, FDA licensed Bayer's Prolastin® on the basis of a clinical demonstration of maintaining trough serum antigenic α_1-PI levels above the historical 11 μM target of augmentation therapy in conjunction with a demonstration of a rise in group mean ELF α_1-PI levels by BAL, both antigenic and functional (ANEC) levels. It should be noted that Kueppers and Black (*Am. Rev. Respir. Dis.* **110**, 176-194 (1974)) stated, "Heterozygotes with phenotypes MZ and MS and intermediate alpha-1antitrypsin levels also may be predisposed to obstructive pulmonary disease, but to a lesser degree." The authors cited numerous papers supporting the quoted statement. Thus, there is no assurance that boosting levels of α_1-PI in such severely affected individuals to levels seen in milder phenotypes will *necessarily* be adequate or optimal to achieve protease:anti-protease balance. Unfortunately, the *hypothesis* that maintaining a serum level of antigenic α_1-PI above any arbitrarily defined threshold, such as 11 μM, will restore protease-anti-protease balance and prevent further lung damage has never been tested in an adequately powered, controlled clinical trial.

The FDA CBER Blood Products Advisory Committee (BPAC) meeting held on June 19, 1998 considered the clinical requirements to support U.S. registration of A1PI intravenous augmentation products. The BPAC meeting was convened following release of the results of the NHLBI Registry study, a non-randomized epidemiologic study that failed to demonstrate an impact of treatment status with Bayer's α_1-PI augmentation therapy product in the overall treated population but did suggest the possibility of benefit in a subgroup of patients with moderate emphysema (baseline FEV_1 35-49% of predicted) as well as a possible mortality benefit. BPAC members voted 11 to 3 with 1 abstention to indicate that FDA should continue to accept maintenance of a plasma level of 11 μM in conjunction with the demonstration of an appropriately defined increment in

ELF α_1-PI/neutrophil elastase-related analyte levels, as sufficient for demonstrating clinical evidence of efficacy of intravenously administered A1PI products in phase III pivotal clinical studies. However, a majority of the committee members had reservations concerning the validity and scientific basis for the conventional target trough level. Committee members were concerned that studies to validate this target level would be difficult to conduct because of the sample size required to demonstrate clinical efficacy, either by decrease in FEV_1, CT changes, or death. It should be noted that there are no epidemiologic data that cleanly identify 11 μM as a "threshold" above which native endogenous α_1-PI levels will prevent progressive lung parenchymal destruction in congenital α_1-PI deficiency. In fact, it is not uncommon for SZ heterozygotes, who, it is generally agreed, are as a group at increased risk of emphysema, to have α_1-PI antigenic levels *above* 11 μM. It should also be noted that preliminary data from a BAL study conducted by the group of Dr. Mark Brantly at the University of Florida have demonstrated that a group of ZZ homozygotes with severe α_1-PI deficiency and antigenic α_1-PI levels <11 μM, but who lack significant pulmonary functional impairment (near normal to normal FEV_1), have significantly elevated polymorphonuclear leucocytes (PMN's) and elevated neutrophil elastase (NE) and elevations in various inflammatory markers in their ELF. Thus, there is no even theoretical assurance, much less clinical data, to support the notion that augmenting α_1-PI serum levels to levels of those genotypes of α_1-PI deficient heterozygotes not associated with markedly increased risk of emphysema will necessarily prevent progressive lung destruction and functional pulmonary impairment. These observations form the basis of a 2-step post-marketing approach to obtaining further clinical data beyond biochemical surrogates in order to address the lack of efficacy data involving hard clinically meaningful clinical endpoints with the Aventis Behring (or any other) IV A1PI product.

C. Summary of Clinical Studies Prior to Pivotal Phase III Study (Study 2002)

Clinical studies were conducted in which 89 subjects (59 males and 30 females) were treated with Zemaira™. 23 subjects were enrolled in more than 1 of the 4 studies. Therefore, 66 of those 89 subjects were unique. The subjects ranged in age from 29 to 68 years (median age 49 years). 97% of the treated subjects had the PiZZ phenotype of α_1-PI deficiency, and 3% had the M_{MALTON} phenotype. At screening, serum α_1-PI levels were required to be <11 μM and were between 3.2 and 10.1 μM (mean of 5.6 μM). The objectives of the clinical studies were to demonstrate that Zemaira™ augments and maintains antigenic serum levels of α_1-PI above 11 μM and increases antigenic and functional α_1-PI levels in ELF of the lower lung in addition to demonstrating short- and long-term safety and tolerability. Pharmacokinetic analysis was also performed.

4 studies were conducted by Aventis Behring (AB) to show that the Zemaira™ brand of A1PI, IV is effective for treating α_1-PI deficiency, in terms of its effects on biochemical surrogate endpoints, and is clinically equivalent to Prolastin®. There were 2 single-dose and 2 multiple-dose studies. The 2 single-dose studies were Study RPR 118635-101 (also referred to as Study 101) and Study CE1226/2-1002 (also referred to as

Study 1002). The 2 long-term multiple-dose studies were Study RPR 118635-201 (also referred to as Study 201) and Study CE1226/2-2002 (also referred to as Study 2002).

Study 101, the first clinical trial, was a dose-ranging study that evaluated the safety, tolerability, and pharmacokinetics of Zemaira™ at single doses of 15, 30, 60, and 120 mg/kg in 19 patients. The results of this study are discussed in Section VI.

Study 1002 compared the bioavailability of Zemaira™ with that of Prolastin® at a single dose of 60 mg/kg in 18 patients. Study 1002 demonstrated the comparability and statistical non-inferiority of Zemaira™ to Prolastin® in bioavailability as measured by the area under the curve (AUC) of functional (ANEC) serum α_1-PI level after a dose of 60 mg functionally active α_1-PI/kg body weight.

Study 201, an open-label, uncontrolled trial, was a multiple-dose study designed to evaluate steady-state serum trough levels and the safety of 60 mg/kg used once weekly A1PI, IV (AB), using a formulation different from that used for the commercial product. After 9 subjects had been enrolled, the study was discontinued and replaced with a randomized and controlled study design (Study 2002), using Zemaira™ manufactured by the process that was used to produce the commercial product. The results of Study 201 demonstrated that A1PI, IV (AB) at a weekly dose of 60 mg functionally active α_1-PI/kg consistently maintained antigenic and functional serum α_1-PI concentrations well above the historical target threshold of 11 μM and that there was no significant downward trend in the weekly steady-state trough serum α_1-PI concentrations.

D. Pivotal Phase III Study (Study 2002)

Study 2002 was the pivotal efficacy trial. In a double-blind, controlled clinical study to evaluate the safety and efficacy of Zemaira™, 44 subjects were randomized 2:1 to receive 60 mg/kg of either Zemaira™ or Prolastin® once weekly for 10 weeks. After 10 weeks, all subjects received Zemaira™ for an additional 14 weeks. All subjects were followed for a total of 24 weeks to complete the safety evaluation.

The AB-AB group in Study 2002 (those randomized to receive AB test product, Zemaira™, during the initial 10 weeks, followed by 14 weeks of open-label administration of Zemaira™) was comprised of more males compared with the Prol-AB group (those randomized to receive Prolastin® during the initial 10 weeks, followed by 14 weeks of open-label administration of Zemaira™), 70% vs. 50%, respectively. This difference was not statistically significant. The mean age of the subjects was 52 years (range: 37-72 years). The Prol-AB group was generally older than the AB-AB group (56 vs. 50 years, respectively). Although the difference is small, it is statistically significant ($p = 0.0095$)

Table 1. Demographic and baseline characteristics (All treated population)

	AB-AB (n = 30)	Prol-AB (n = 14)	Total (n = 44)
Sex			
Male	21 (70%)	7 (50%)	28 (64%)
Female	9 (30%)	7 (50%)	16 (36%)
Age (years)			
Mean	50	56	52
SD	7	9	8
Range	37-68	40-72	37-72
Race			
Caucasian	30 (100%)	14 (100%)	44 (100%)
Height (cm)			
Mean	177	175	176
SD	9	10	9
Range	163-196	155-191	155-196
Weight (kg)			
Mean	84.4	89.3	86.0
SD	18.3	17.6	18.0
Range	50.9-139.2	54.5-124.0	50.9-139.2
Body Mass Index (kg/m^2)			
Mean	26.9	29.2	27.6
SD	5.9	5.1	5.7
Range	18.1-49.9	22.7-40.5	18.1-49.9

The mean trough serum α_1-PI levels at steady state (Weeks 7-11) in the Zemaira™-treated subjects were clinically equivalent (non-inferior) to those in the Prolastin®-treated subjects. Both groups were maintained well above 11 μM (80 mg/dL). Despite a slight transient downward trend (mean slope –0.13 μM/week; 90% CI –0.18 to –0.09) in mean trough serum antigenic α_1-PI level from Week 7 to Week 24 in the Zemaira™-treated group, the mean trough serum antigenic α_1-PI level at steady-state remained above the historical target trough threshold of 11 μM, as did the mean trough levels in every subject.

Blinding in Study 2002

Each investigator was provided with emergency envelopes containing the treatment assignment of each subject enrolled at his or her site. No subject was unblinded for any reason.

Primary Efficacy Variables in Study 2002

Both primary objectives as described below were achieved. AB A1PI administered at a dose of 60 mg/kg/week over 24 weeks was demonstrated to be not inferior to Prolastin® by the pre-set criterion of maintenance of a mean serum antigenic trough level within 3

μM of Prolastin® and greater than the historical target threshold level of 11 μM. The results are described in the following subsections.

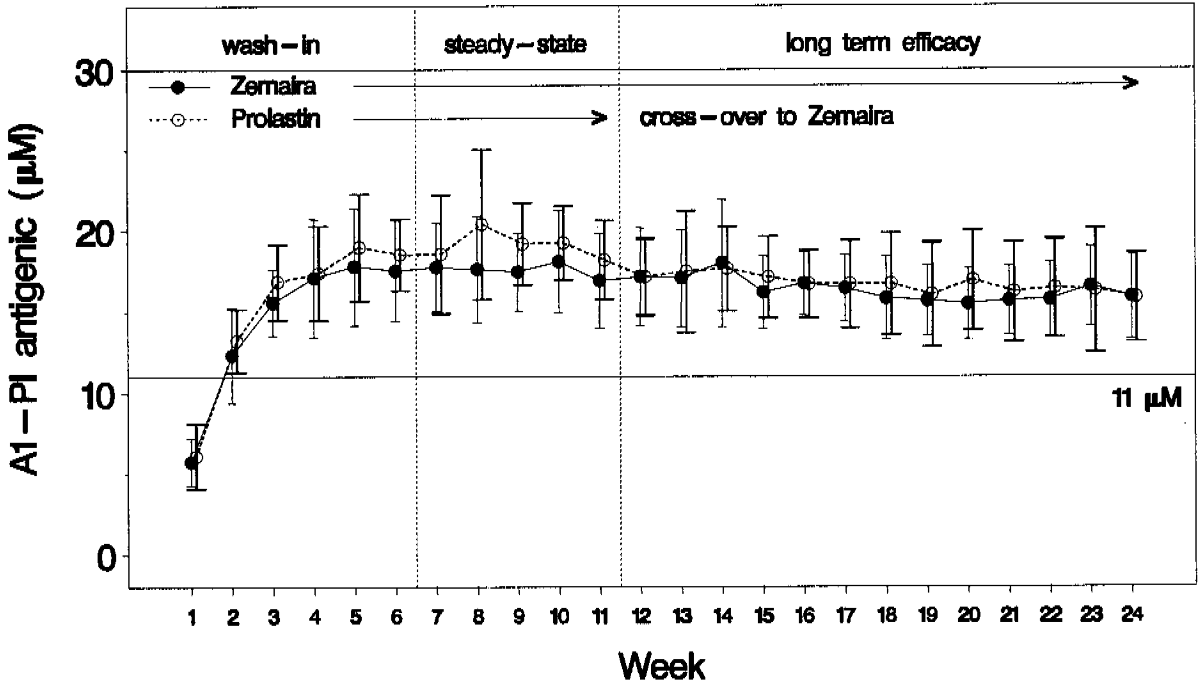
Primary objective 1:	The mean trough serum antigenic α_1-PI level from Week 7 to Week 11 in the AB-AB group treated with AB A1PI was within 3 μM of the corresponding mean value in the Prol-AB group treated with Prolastin® (lower limit of the 90% CI of the between-group difference was –2.77 μM).
Primary objective 2:	The mean trough serum antigenic α_1-PI level from Week 7 to Week 11 in the AB-AB group treated with AB A1PI was clearly greater than the "therapeutic" threshold of 11 μM (lower limit of the 90% CI was 16.88 μM).

The analyses were performed on both the per-protocol (PP) and intent-to-treat (ITT) data sets. The results of the ITT data set (n = 44) are consistent with the results of the PP data set (n = 43).

Comparison of AB A1PI to Prolastin® in Mean Trough Serum Antigenic α_1-PI levels

Although the trough serum antigenic α_1-PI levels in the AB A1PI group were not inferior to trough levels achieved after treatment with Prolastin®, thus fulfilling the primary objective, the difference was statistically significant ($p = 0.0722$) at a 2-sided alpha level of 10% (the upper limit of the 90% CI for the difference was –0.13, i.e., less than 0, see Table 23). To investigate this difference, AB assayed all lots of study drug available for antigenic and functionally active α_1-PI (Prolastin® Lot No. 601F041 was used as the control in Clinical Study CE1226/2-1002, but not in this study). The dose of study medication administered of 60 mg/kg was based on the labeled potency (i.e., functional) assay. However, the primary endpoint was the measurement of the serum antigenic level of α_1-PI that was achieved. A repeated assay confirmed that the labeled potencies of α_1-PI in both Prolastin® and AB A1PI were correct. However, an antigenic assay indicated a significantly higher level of antigenic α_1-PI per unit of functionally active α_1-PI in Prolastin® than in AB A1PI. The antigenic assay measures immunoreactive α_1-PI and is not necessarily an indicator of potency (ANEC). The potency of the products was not significantly different. The mean specific activity of AB A1PI was 1.07, clearly higher than that of Prolastin® at 0.77. Because the dosing was based on the functional α_1-PI content of the vial, the subjects received relatively larger amounts of antigenic α_1-PI in Prolastin® which may have contributed to the higher antigenic α_1-PI levels observed in the serum.

Figure 1.



Descriptive overview of trough serum antigenic α_1-PI levels over the entire study

Mean trough serum antigenic α_1-PI levels were above 11 µM from Week 2 through Week 24 in both the AB-AB and Prol-AB groups in both the PP and ITT data sets. Figure 2 below displays trough serum antigenic α_1-PI levels over the entire study for the PP data set.

A review of individual subject data reveals that from Week 3 through Week 24, all serum antigenic α_1-PI levels were above the historical target threshold of 11 µM with the exception of 2 measurements in a single subject. Subject No. 246 had levels of 9.8 µM and 10.4 µM at Weeks 13 and 23, respectively. Although a definitive cause for the lower levels has not been established, this subject's medical status during the study included anasarca, adrenal insufficiency, retroperitoneal abscess, retroperitoneal bleed, and urosepsis, and this may have altered her intravascular fluid protein balance and volume of distribution. This subject was hospitalized for much of the study.

<u>*ELF Endpoints*</u>

BAL was performed in Study 2002 at baseline and at Week 11 in a subgroup of subjects (10 Zemaira™-treated subjects and 5 Prolastin®-treated subjects). 4 α_1-PI related

analytes in ELF were measured: antigenic α_1-PI, α_1-PI:NE complexes, free NE, and ANEC. Within each treatment group, ELF levels of both antigenic α_1-PI and α_1-PI:NE complexes increased significantly from baseline to Week 11. This provides evidence that α_1-PI reaches the lower lung and has the capacity to bind its substrate, NE. The post-treatment ANEC values in ELF were not significantly different between the Zemaira™-treated and Prolastin®-treated subjects (mean 1,725 nM vs. 1,418 nM). However, no conclusions can be drawn about changes of ANEC values in ELF during the study period because the baseline values in the Zemaira™ -treated subjects were unexpectedly high. There was no evidence this anomalous finding was due to inadequate washout from prior pre-study α_1-PI augmentation therapy or from mis-timing of study drug administration on the day of baseline BAL testing. The Prolastin® subgroup that underwent BAL testing showed an approximately 16-fold increase from baseline to Week 11 in ANEC ($p < 0.05$ despite the very small sample size of $n = 5$). No free NE was detected in any sample. No α_1-PI analytes showed any clinically significant differences between the Zemaira™ and Prolastin® treatment groups.

Protocol Deviations in Pivotal Study 2002

The protocol stated that at least 30% of the subjects should be naïve to A1PI therapy and at least 50% of the subjects should not have received more than 2 doses of A1PI before entering the study. 11 subjects (25%) were completely naïve to any form of A1PI. In addition, 4 subjects (9%) had 1 to 2 previous doses of intravenous A1PI. Therefore, 15 subjects (34%) did not receive more than 2 previous doses of intravenous A1PI. Of the remaining 29 subjects (66%) who had received more than 2 doses of intravenous and/or aerosol A1PI, 10 subjects (23%) had no prior treatment with intravenous A1PI. Therefore, 21 subjects (48%) had not previously received intravenous A1PI and 25 subjects (57%) had received 2 doses or less of intravenous A1PI. 67% of the AB group and 64% of the Prolastin® group previously received more than 2 doses of A1PI while 33% and 36%, respectively, were considered naïve (as defined by 2 or fewer doses) (see Section 4.6.1 and Table 19 in Section 4.6.1).

Adverse event diaries maintained by the study subjects and the home infusion nurse's safety documentation were to be reviewed by the investigator at Weeks 11 and 24 for applicable adverse event reporting. In some cases, this documentation was not provided to the investigator for assessment. A follow-up safety audit was conducted by AB to confirm that all adverse events have been captured in the safety database regardless of the source (subject dairies or nurses notes). During the follow-up safety audit, it was learned that diaries were not maintained by all subjects, at all times.

- Subject No. 432 (AB-AB) was enrolled even though he had received a Prolastin® treatment 1 week prior to his screening visit (July 11, 2000). However, his first dose of study drug was not given until August 21, 2000 following a sufficient wash-out period. The subject's baseline α_1-PI serum level was taken just prior to the first study infusion. The inclusion criteria called for no treatment with α_1-PI concentrate or plasma-derived product (i.e., immunoglobulins, coagulation factors) for at least 4

weeks prior to the screening visit date, with the exception of subjects who had participated in a previous AB A1PI study.

- 2 subjects had FEV_1 values outside those required for study entry as described in Section 3.2. Subject No. 431 in the AB-AB group had an FEV_1 of 16% and Subject No. 433 in the Prol-AB group had an FEV_1 of 15% at screening.

Protocol violations involving dosing (selected)

- The dose of A1PI administered in this study was 60 mg/kg body weight, infused intravenously once per week for 24 weeks. Allowance of ±10% in dose level was allowed for inclusion in the per-protocol data set. Subjects Nos. 301 (Prol-AB), 303 (Prol-AB), and 201 (AB-AB), as noted below, had dose level excursions outside of the ±10% window during the blind phase of the study as a result of incorrect body weight measurements. A total of 10 doses exceeded 66 mg/kg, and 2 doses were less than 54 mg/kg. There was no evidence of adverse events related to the dose level excursions in these subjects.

    - 2 subjects assigned to the Prol-AB group had dose level excursions as follows.
        - No. 301: 53.1 mg/kg received Week 1
        - No. 303: 51.8 mg/kg received Week 1

      There was no impact for the analysis of Week 7-11 serum antigenic α_1-PI levels.

    - A single subject assigned to the AB-AB group had dose level excursions as follows.
        - No. 201: 70.3 mg/kg received Weeks 2, 3, 4, and 5; 70.4 mg/kg received Week 1; 70.6 mg/kg received Weeks 6, 7, 8, 9, 10

      Therefore, the trough serum α_1-PI data associated with these infusions were not included in the per protocol analysis of serum antigenic and functionally active α_1-PI levels.

Protocol violations involving BAL sampling

- Subjects No. 302, No. 304, and No. 305 (AB-AB) and Subjects No. 301 and No. 303 (Prol-AB) had their ELF samples taken on the same day as their first dose of the open-label treatment. The statistical analysis plan (SAP) stated that post-treatment ELF samples for the BAL analysis should be taken between 6 and 8 days after the last blinded dose and at least 24 h prior to the first dose of the open-label treatment. The 24-h rule was set for safety concerns. During the study, the investigators deviated from this rule for operational convenience based on their individual safety assessments. In addition, the post-treatment sample for Subject No. 301 was taken 5 days after the last blinded dose, which was outside the pre-defined window of 7 ±1 days. However, prior to unblinding, it was decided to include all ELF samples in the BAL subset for the analysis of secondary endpoints.

Protocol Violations Regarding Primary Endpoint Assay Validation and Related Issues/Remediation

Formally documented method for the primary endpoint assay

In May 2002, the [redacted] issued SOP No. [redacted] version 4, entitled "Determination of Alpha-1-Antitrypsin Levels — [redacted] (see Appendix VIII.1). This report formally documents the assay procedure as it was performed in the [redacted] for this study.

The serum antigenic α_1-PI level by [redacted] assay procedure existed as written instructions during the analysis. This method has been formally approved within the [redacted]. The lab journals were reviewed by AB to confirm that the controls defined in the method and equipment manual were adhered to, specifically that standard curves were run and that internal controls were within limits. ***Values that were generated in runs for which the necessary standard curves were not available or internal controls were not within the pre-specified range were marked as questionable and excluded from the robustness analysis.***

Documentation of the Assay Reference Standard

Dr. [redacted] written documentation on the existing in-house α_1-PI standard was misplaced during [redacted] AB's investigation of the standard indicated that there was no documented traceability for the standard used in the assay. There was no documented explanation of how the standard was developed or what experiments were done to assure the integrity or stability of the standard.

To confirm the method of preparation and the characterization of the standard, a comparator evaluation study was performed correlating the in-house standard to an industry-recognized standard purchased from [redacted] This study showed that a 10 to 15% difference existed between the 2 standards with the in-house standard testing being lower than the [redacted] standard. The impact of using the [redacted] standard for the clinical trial would result in an overall increase in all values over what was found by using the in-house standard. The method validation was conducted by using the [redacted]g standard. (See Appendix VIII.16)

Comparison of Results from Plasma to Those from Serum

The in-house standard that Dr. [redacted] used in the serum antigenic assay was a plasma sample. All of the samples run in the clinical trial were serum samples. Therefore, a study was carried out comparing both serum and plasma samples from the same donor to show that either could be run in the assay with the reagents and [redacted] used and that both would give comparable results. The conclusion of the study was that there was no significant difference in the measurements of α_1-PI levels in these matrices.

<u>*Performance of 100% Data Verification of AB Locked Database Against Source Documents*</u>

To confirm data traceability and that the use of unvalidated computer programs had not modified the data, a point-to-point verification from the locked AB database of the primary endpoint used to produce the statistical analysis was performed against the lab journals for analytical results. Additionally, where corrections to specimen numbers were changed by the laboratory without reasons documented, confirmation back to the primary source data was performed. Any questionable data points were excluded in the robustness analysis.

The remediation for the data associated with the assays of serum ANEC, ELF antigenic α_1-PI, and ELF α_1-PI:NE included:

1. *Formal documentation of assay procedures for ELF antigenic α_1-PI, ELF α_1-PI:NE complex, urea, and serum ANEC assays*

   The assay procedures for ELF antigenic α_1-PI, ELFα_1-PI:NE complexes, urea, and serum ANEC existed as written instructions during the study. All samples were run at the [redacted] during the Spring of 2001. The Laboratory documented changes to the procedure made in September 2001 and handwritten on the procedure. Discussions between the AB Remediation Task Force representatives and [redacted] staff indicate that the changes had been in practice during the study.

   AB reviewed the lab journals to confirm that the assay procedures defined in the written instructions were adhered to, specifically that standard curve criteria were met and that internal controls were within limits. Values that were generated in runs for which the necessary standard curve criteria were not met or for which internal controls were not within the limits were marked as questionable and excluded from the robustness analysis.

   The laboratory has documented the assay procedures "to their best recollection." The laboratory director has approved these documents.

2. *Improvement of documentation of assay standards*

   Dr. [redacted] written documentation on the existing in-house standard for the α_1-PI:NE Complexes assay was misplaced during [redacted] An existing commercial standard acquired from [redacted] was used for method validation and closely resembled the in house standard used during the study (see above).

3. *Performance of 100% data verification*

100% data verification was performed for the Serum ANEC, ELF Antigenic α_1-PI, ELFα_1-PI:NE Complexes, and ELF ANEC assay data. To confirm data traceability and that the use of unvalidated computer programs had not modified the data, the following procedure was performed: (a) data from the locked AB database were printed out; (b) the data were compared with those in the lab journals thereby documenting the results; (c) where corrections to specimen numbers were not documented appropriately, confirmation back to the primary source was performed; and (d) **questionable data points were excluded in the robustness analysis.**

4. *Validation of the following central laboratory assays:*

- ELF Antigenic α_1-PI,
- ELF α_1-PI:NE Complexes,
- ELF Urea, and
- Serum ANEC assays

Formal validation protocols were written and approved before initiation of the validation. The final validation reports were reviewed and approved and are included in Appendix VIII. In the validation of the assay for α_1-PI:NE complexes, it was found that there was a narrow linear range with insufficient data points.

5. *Prospective validation of free neutrophil elastase assay*

6. *Performance of free neutrophil elastase assay at AB's laboratory*

7. *Establishment of consistent reporting of re-tested data*

The investigation showed retesting without a documented rationale. A decision was made to exclude any retested data. See robustness analysis below.

8. *Conduction of a Robustness Analysis after elimination of questionable data points*

The statistical analysis of the ELF antigenic α_1-PI, ELF α_1-PI:NE complexes, and serum ANEC results was re-run, after excluding questionable data points as specified. The results of the robustness analysis of serum ANEC supported the original per-protocol analysis. Too few ELF samples were left in the robustness analysis datasets for meaningful robustness analyses of ELF analytes.

Robustness Analysis Database

Serum antigenic α_1-PI

A total of 1068 samples were collected for the determination of the primary endpoint, serum antigenic α_1-PI. Of the total, 198 samples were identified as questionable. A robustness analysis was performed to evaluate the impact of excluding these samples and comparing the results with those of the primary analysis. In addition, this robustness analysis was to apply a consistent handling of re-tested samples. 2 alternative approaches for the handling of multiple retests were applied leading to 2 robustness analyses as follows:

Robustness Analysis-1: Exclude all questionable values and take the first of multiple valid values per sample.

Robustness Analysis-2: Exclude all questionable values and take the average of multiple valid values per sample.

The rationale and the analysis plan of the robustness analysis are described in "Plan for an Additional Robustness Analysis of the Study Results Related to Serum Antigenic α_1-PI" (June 27, 2002).

The numbers of questionable samples identified for exclusion from the robustness analysis are summarized in table immediately below.

Table 2. Robustness Analysis – Excluded serum antigenic α_1-PI data points by reason for exclusion (all time points)

No. of data points	AB-AB	Prol-AB	Total
Total number of data points*	732	335	1067
Total number of exclusions#	135	63	198
Reason for exclusion			
Standard curve not available	63	29	92
Control out of range	52	29	81
Sample turbidity	13	6	19
Unclear sample traceability	9	1	10

* excluding 1 observation with missing value
# 4 data points were excluded for multiple reasons
Source: Appendix VII.1 (Table 8) of study report

Details on the numbers of samples excluded from the robustness analysis and the numbers of samples with multiple runs were provided.

As planned in the study protocol and in the SAP, the primary biochemical efficacy analysis was based on the mean antigenic serum α_1-PI levels obtained during Weeks 7-11

by using the per protocol (PP) dataset. The table immediately below summarizes the numbers of samples for serum antigenic α_1-PI obtained during the period between Week 7 and Week 11 and considered evaluable for the PP dataset. The numbers of samples excluded from the robustness analysis among this subset are also shown in this table.

Table 3. Robustness Analysis – Excluded serum antigenic α_1-PI data points from the per protocol dataset (Weeks 7–11)

No. of data points	AB-AB	Prol-AB	Total
Total number of data points in the complete per protocol dataset*	140	68	208
Total number of exclusions#	54	18	72
Total number of data points in the robustness analysis*	86	50	136

* excluding missing values
\# including exclusion of 3 data points because the correction to a Day 7 value could not be performed due to excluded baseline values
Source: Appendix VII.1 (Table 7) of study report

During the period between Week 7 and Week 11, which formed the basis for the primary efficacy analysis, 72 of 208 collected samples were excluded from the robustness analysis. However, all subjects in the PP population had at least 1 evaluable sample from this period.

ELF antigenic α_1-PI, ELFα_1-PI:NE Complexes, and Serum ANEC

ELF samples were collected from 15 of the 44 subjects. Each subject was to have 2 BAL procedures performed (Weeks 1 and 11) with sampling from 3 lobes during each procedure, resulting in a total of 45 baseline and 45 post-treatment ELF samples. Theoretically, this would lead to 90 samples for determination of the ELF antigenic α_1-PI, ELFα_1-PI:NE complexes, and ELF ANEC in the study. An actual total of 90 samples were collected and included in the clinical database. 1 of these samples did not have an assay result reported for either of the variables due to insufficient recovery of ELF during BAL (Subject No. 319, Visit No. 2).

Based on a review of the serum ANEC, ELF Antigenic α_1-PI, ELFα_1-PI:NE Complexes, and Urea assay results, data were recommended for exclusion from a robustness analysis by AB task force representatives when acceptance criteria or run conditions were not followed as described in the written instructions. The task force representatives were unaware of the treatment assignment at the time samples were being excluded. The following table summarizes samples that were excluded from the robustness analyses.

Data identified for exclusion from the robustness analysis for the secondary endpoint are detailed in the table immediately below.

Table 4. Robustness Analysis Study 2002– Excluded secondary endpoint data points

No. of data points	ELF Antigenic α_1-PI	ELF α_1-PI:NE Complex	Serum ANEC
Samples in primary database*	89	89	1068
Samples recommended for exclusion	66	84	323
Total samples available for robustness analysis	23	5	845

* excluding missing values

Source: Appendix VII.2 (Table 8) of study report

As can been seen in the table above, the number of BAL substudy subjects' samples remaining in the robustness analyses was too small to analyze in a meaningful way. This strengthens the basis for the recommendation that the sponsor conduct a BAL substudy as part of its phase IV commitment studies.

Table 5. Mean ± SD of trough serum antigenic α_1-PI, Study 2002
Robustness Analysis: Mean steady-state trough serum antigenic α_1-PI levels (Weeks 7-11), Primary Efficacy Analysis (within treatment group)

Robustness Analysis-1 (RA-1): Exclude all questionable data points and take the first of multiple valid values per sample.

Robustness Analysis-2 (RA-2): Exclude all questionable data points and take the average of multiple valid values per sample.

Group Treatment Mean steady-state trough antigenic α_1-PI levels (Weeks 7-11)	AB-AB AB A1PI			Prol-AB Prolastin®		
Dataset	**PP***	**RA-1**	**RA-2**	**PP**	**RA-1**	**RA-2**
N	29	29	29	14	14	14
No. Specimens Evaluable	140	86	86	68	50	50
Minimum (μM)	13.90	13.10	13.10	14.7	14.70	14.70
Median (μM)	17.48	17.30	17.30	19.09	19.18	19.18
Maximum (μM)	23.21	23.21	22.75	23.08	23.08	23.08
Mean (μM)	17.67	17.63	17.61	19.11	19.22	19.22
SD (μM)	2.50	2.69	2.64	2.20	2.31	2.31
Lower 90% CI[§,#] (μM)	16.88	16.78	16.77	18.07	18.12	18.13
Upper 90% CI[§,#] (μM)	18.45	18.48	18.44	20.15	20.31	20.31

[§]CI = confidence interval
[#]Based on a t-distribution with n-1 degrees of freedom
*PP = complete per-protocol dataset (primary efficacy analysis)
Source: Appendix IV.1 (Table 6.1.1.9) and Appendix VII.1 of study report.

The values obtained in the 2 robustness analyses of mean steady-state trough antigenic α_1-PI levels (Weeks 7-11) were remarkably close to and support the results of the original analysis.

E. Summary of Efficacy Across Studies

Serum Trough Antigenic α_1-PI Levels

Results of all 4 studies were consistent and indicated that Zemaira™ administered intravenously at a dose of 60 mg/kg once weekly can be expected to result in steady-state trough serum antigenic levels of α_1-PI well above the historical target threshold of 11 μM. In the pivotal efficacy study, Study 2002, Zemaira™ administered intravenously at a dose of 60 mg/kg once weekly was shown to result in mean steady-state trough serum antigenic levels of α_1-PI that were clearly above the historical target threshold of 11 μM and that were equivalent (statistically non-inferior) to mean steady-state trough serum antigenic levels of α_1-PI obtained with the same dosage of Prolastin® (see Table 1 and Figure 1). When assessing 762 steady-state levels of antigenic α_1-PI from all multiple-dose studies, the mean level obtained with Zemaira™ was 16.7 μM with a range of 10.4-29.1 μM. It should be noted, however, that Study 201 did not employ the to-be-marketed manufacturing method for Zemaira™. Thus, the above combined analyses should be considered purely exploratory.

Table 6. Antigenic α_1-PI serum trough levels at steady-state for subjects participating in multiple-dose studies (original analysis)

	Study 201#	Study 2002*		
Treatment Group Treatment	**Zemaira™**	**Zemaira™**	**Prolastin®**	**Difference Zemaira™ - Prolastin® (μM)****
Antigenic α_1-PI (μM)				
No. of Subjects (n)	9	29	14	
Baseline Value (Mean ± SD)	6.6 ± 1.5	5.8 ± 1.5	6.1 ± 2.0	
Steady-State Value: Mean	16.9	17.7	19.1	-1.45 90% CI
90% CI§	15.9-17.9	16.9-18.5	18.1-20.2	(-2.77, -0.13)***

#Weeks 7-26
*Weeks 7-11
**Within Study 2002
§CI=Confidence interval based on t-distribution with n-1 degrees of freedom
*** Based on a t-distribution with 41 degrees of freedom

Overall Evidence of Increases of α_1-PI Levels in ELF of the Lower Lung

ELF was obtained by BAL in Studies 101 and 2002 in a subset of subjects. Both studies showed increases in ELF antigenic α_1-PI levels; however, the results are not directly comparable. Study 101 was a single-dose study and BAL was performed 6 days after the dosing. The BAL in Study 2002 was performed 11 weeks after the start of the weekly dosing, 7 days after the 10th dose, at a time when serum α_1-PI levels had achieved steady state levels.

Based on the pivotal Study 2002 and supported by Study 101, the measurements of antigenic α_1-PI and α_1-PI:NE complexes in ELF support the hypothesis that intravenously administered α_1-PI reaches the lower lung in its active form and has the capacity to complex with its physiologic substrate, neutrophil elastase. However, evidence of a treatment-induced rise in subgroup mean functional α_1-PI levels in ELF after 11 weeks of augmentation therapy with Zemaira™ was lacking, unlike the case with the Prolastin® subgroup that underwent BAL testing (see discussion above). Also note that the number of BAL samples remaining in the robustness analysis was too few to analyze in a meaningful way as they represented well less than half of the original samples.

Serum Trough Functional α_1-PI Levels

The results from Studies 201 and 2002 were consistent and showed that Zemaira™ administered intravenously at a dose of 60 mg/kg once weekly can be expected to result in steady-state trough serum functional levels of α_1-PI above 11 μM. While this historical target trough threshold is defined for antigenic α_1-PI levels, it is a plus to find that the functional levels are also higher than 11 μM.

In the pivotal efficacy Study 2002, the mean serum functionally active α_1-PI level during treatment with Prolastin® (Weeks 7-11) was lower than that during treatment with Zemaira™ (Weeks 17-24) with a difference of 0.61 μM. This difference, while statistically significant, was considered by the sponsor's clinical expert consultants and by the CBER medical reviewer not to be clinically relevant in the treatment of α_1-PI deficient patients. The mean steady-state levels (Weeks 7-11) in the Zemaira™ treatment group and in the Prolastin® treatment group were not statistically significantly different.

Evidence of Long-Term Augmentation Therapy on Biochemical Surrogates

In multiple-dose Studies 201 and 2002, the slopes of α_1-PI serum trough levels at steady state were evaluated with respect to time to assess whether there were declines over the long term.

In Study 201, the regression model for the α_1-PI trough serum levels from Week 7 to Week 26 showed a mean antigenic level (intercept) of 16.9 μM with a 90% confidence interval of 15.9 to 17.9 μM and a mean functional level (ANEC) of 15.7 μM with a 90% confidence interval of 14.5 to 17.0 μM. The respective mean slopes were −0.02 μM/week for the antigenic levels with a 90% confidence interval of −0.07 to 0.03 μM/week and −0.03 μM/week for the functional levels with a 90% confidence interval of −0.09 to 0.04 μM/week, i.e., there was no evidence of a downward trend. α_1-PI antibody assessments were performed in all of the 9 subjects at baseline and at Weeks 4, 12, and 26 during the study period. No evidence of antibodies to α_1-PI was found.

Although in Study 2002 there was a statistically significant downward trend

(–0.13 μM/week; 90% CI –0.18 to –0.09) in mean trough serum antigenic α_1-PI levels in subjects treated with Zemaira™ from Week 7 to Week 24, the mean trough serum antigenic α_1-PI level at steady-state remained above the historical target threshold of 11 μM, as did the mean trough levels in every subject. The negative slope in steady-state antigenic α_1-PI levels appeared to be transient with a plateau of about 16 μM being reached after about 20 weeks of treatment. In addition, during the period of direct comparison of steady-state levels from Weeks 7 to 11, the slope was not different between the Zemaira™ and Prolastin® treatment groups, with the Prolastin® group also having a negative slope. In contrast to the results observed for the trough serum antigenic α_1-PI levels from Week 7 to Week 24, trough serum functional α_1-PI levels remained constant, with mean values of 11.7 μM and 11.5 μM at Weeks 7 and 24, respectively. The mean slope was 0.00 μM/week (90% CI –0.03 to 0.03) from Week 7 to Week 24. There was no evidence of antibodies against α_1-PI at Week 11 or Week 24.

The long-term effects, if any, of Zemaira™ augmentation therapy on pulmonary exacerbation frequency and on the natural course of progression of emphysema, as measured by serial pulmonary function testing or by serial high resolution CT, are unknown as they have never been systematically studied in a controlled trial of long-term duration. The same uncertainty applies to Bayer's Prolastin® and to Alpha Therapeutics Corporation's A1PI IV product although inconclusive epidemiologic data are available for Prolastin®.

F. Safety Summary Across Studies

A total of 89 subjects were exposed to IV Zemaira™ in the 4 studies of the clinical development program; 66 of these 89 subjects were unique. A total of 76 subjects were exposed to IV Zemaira™ at the recommended dose of 60 mg/kg. 62 of these 76 subjects were unique. A total of 32 subjects were exposed to Prolastin®. 69 of a total 89 Zemaira™ subjects (78%) and a total of 20 of the 32 Prolastin® subjects (63%) reported at least 1 adverse experience (AE) in the 4 clinical studies. Most AE's were mild or moderate in severity.

Table 7 summarizes the adverse event data obtained with single and multiple doses during clinical trials with Zemaira™ and Prolastin®. No clinically significant differences were detected between the 2 treatment groups.

Table 7. Summary of Adverse Events

	Zemaira™	Prolastin®
No. of subjects treated	89	32
No. of subjects with adverse events regardless of causality (%)	69 (78%)	20 (63%)
No. of subjects with related adverse events (%)	5 (6%)	4 (13%)
No. of subjects with related serious adverse events	0	0
No. of infusions	1296	160
No. of adverse events regardless of causality (rate per infusion)	296 (0.228)	83 (0.519)
No. of related adverse events (rate per infusion)	6 (0.005)	5 (0.031)
No. of related serious adverse events	0	0

Table 8. Summary of adverse events (All treated population) in Study 2002

	Blind phase		Open-label phase		
Treatment Group Treatment	AB-AB AB	Prol-AB Prolastin®	AB-AB AB A1PI	Prol-AB AB A1PI	Total (open) AB A1PI
Subjects treated	30 (100%)	14 (100%)	30 (100%)	13 (100%)	43 (100%)
Subjects with AE's	27 (90%)	14 (100%)	22 (73%)	13 (100%)	35 (81%)
Subjects with related AEs	2 (7%)	3 (21%)	0	1 (8%)	1 (2%)
Subjects with serious AE's	3 (10%)	4 (29%)	2 (7%)	1 (8%)	3 (7%)
Subjects with related serious AE's	0	0	0	0	0

No subject treated with Zemaira™ died during any of the clinical studies nor did any Zemaira™ treated subject discontinue from any of the 4 clinical studies due to an AE. There was 1 case of a serious AE with death as the outcome in a patient treated with Prolastin®. This severe adverse event (SAE) was judged not to be related to the study medication. All SAE's were judged not to be related to the study medication. There was a somewhat higher incidence of SAE's in the group treated with Prolastin® vs. the group treated with Zemaira™. The total number of AE's divided by the number of infusions (not necessarily the same as the proportion of infusions associated with 1 or more adverse event) was 121/301 = 40.2% in the AB-AB group and 73/142 (51.4%) during the masked

phase of Study 2002. The trend toward somewhat fewer AE's in the AB-AB group compared to the Prolastin®-AB group is less likely due to true differences in product safety, in the view of this reviewer, because this between-treatment group trend persists even during the open-label period of the trial when both original randomization groups are receiving the AB A1PI test product.

The most frequently reported treatment-emergent AE's after Zemaira™ treatment regardless of causality were headache, upper respiratory infection, and sinusitis. Other relatively common AE's were sore throat, bronchitis, asthenia, fever, rhinitis, chest pain, injection site hemorrhage, increased cough, bronchospasm, and pain. Those that occurred somewhat more frequently in the AB test group in Study 2002 include:

Asthenia
Pain
Chest pain
Paresthesia
Bronchospasm
Rash

Table 9.
Incidence of most frequently reported (≥10% in either group) treatment-emergent AE's by body system during blind phase (All treated population) in Study 2002

Treatment Group Treatment		AB-AB (n = 30) AB A1PI		Prol-AB (n = 14) Prolastin®	
Body System	**Adverse event**	**n**	**%**	**n**	**%**
Body as a Whole	Abdominal pain	1	3%	2	14%
	Asthenia	5	17%	1	7%
	Fever	4	13%	2	14%
	Pain	4	13%	-	
Cardiovascular System	Chest pain	3	10%	-	
	Vasodilatation	4	13%	1	7%
Digestive System	Sore throat	4	13%	3	21%
Musculoskeletal System	Muscle cramps	1	3%	3	21%
Nervous System	Depression	-		2	14%
	Headache	11	37%	5	36%
	Paresthesia	3	10%	-	
Respiratory System	Bronchitis	3	10%	2	14%
	Bronchospasm	4	13%	1	7%
	Cough increased	3	10%	4	29%
	Pneumonia	-		2	14%
	Rhinitis	3	10%	1	7%
	Sinusitis	7	23%	2	14%
	Upper respiratory infection	5	17%	2	14%
Skin and Appendages	Rash	3	10%	-	

Note: A subject can be included in more than one category.
Source: Appendix VII.1 (Table 7.1.2.2) of study report

Note that 3 of 30 AB-AB subjects and 0 of 14 Prol-AB subjects reported paresthesia during the masked portion of Study 2002 and that paresthesia was 1 of the few drug-related AE's reported in the trial. The verbatim term referring to the latter was actually *cold arms, mild in intensity* that started in Subject No. 20 beginning 15 min after the start of the 5th infusion and lasted 2 h and resolved following administration of warm liquids by mouth. The incidence of AE's was fairly well balanced between the patients treated with Prolastin® vs. the group treated with Zemaira™.

The investigators rated treatment-emergent AE's as related to study medication in 5 subjects during the blind phase of Study 2002. The related treatment-emergent AE's in

the group treated only with Zemaira™ also included injection site pain and 1 case of asthenia (fatigue) that occurred in the open treatment phase with Zemaira™ in the group initially treated with Prolastin®. The related treatment-emergent AE's in the group treated with Prolastin® (before cross-over to Zemaira™) were a transient atypical seroconversion for viral hepatitis B, a parvovirus seroconversion, and an episode of vasodilatation. It should be noted, however, that community-acquired parvovirus infection could not be ruled out. [It is interesting to note, however, that a single case of parvovirus B19 seroconversion occurred in the Prolastin® treatment group in the pivotal trial supporting the recent licensure of Alpha Therapetic Corporation's A1PI IV product. No nucleic acid testing results from this lot of Prolastin® are available, insofar as one condition on which AB was provided control Prolastin® by Bayer for this trial was that AB would conduct no testing of any kind on the Prolastin® vial contents.]

Another related AE (joint pain) could not be assigned to a treatment due to an incomplete start date. There were no serious AE's considered related to Zemaira™ treatment.

The sponsor expected that the toxicity profile of the product would be similar to that of Bayer's Prolastin® brand of plasma-derived A1PI. This appears to be borne out by the data. The trend toward somewhat fewer AE's in the AB-AB group compared to the Prolastin®-AB group is less likely due to true differences in product safety, in the view of this reviewer, because this between-treatment group trend persists even during the open-label period of the trial when both original randomization groups are receiving the AB A1PI test product.

The listing of all SAE's throughout the trial follows in the table immediately below.

Table 10. Listing of serious adverse events (All treated population) in Study 2002

Phase	Group/ Treat-ment	Subject	Sex	Age	Adverse Event (preferred term)	Onset study day	Severity	Study drug related
Blinded	AB-AB/ AB A1PI	217	Male	50	Chest pain	14	Severe	No
		309	Male	52	Skin melanoma	9	Mild	No
		432	Male	43	Bronchitis	8	Severe	No
	Prol-AB/ Prolastin®	220	Female	45	Pneumonia	71	Severe	No
		246	Female	65	Sepsis	15	Severe	No
					Respiratory disorder	15	Severe	No
					Myocardial infarction	17	Severe	No
					Supraventricular tachycardia	17	Severe	No
					Retroperitoneal hemorrhage	21	Severe	No
					Abscess	41	Severe	No
		301	Male	47	Abdominal pain	54	Severe	No
		403	Female	72	Lung disorder	18	Moderate	No
					Apnea	69	Severe	No
Open	AB-AB/ AB A1PI	309	Male	52	Chest pain	168	Severe	No
					Peripheral vascular disorder	172	Severe	No
		320	Female	42	Electrolyte abnormality	83	Severe	No
	Prol-AB/ AB A1PI	308	Male	64	Tendon rupture	126	Severe	No

Except for the events listed as AE's, there were no clinically relevant changes in clinical laboratory values, pulmonary function tests, vital signs, physical examinations, EKG's, and chest X-rays for any of the subjects. There were no apparent clinically relevant differences between Zemaira™ and Prolastin® in these test results.

The investigators identified 6 clinically relevant laboratory abnormalities among 6 subjects during the masked portion of the trial. Of these, only 4 are noteworthy. A single subject (AB-AB) experienced a rise in serum creatinine from 1.3 mg/dL at baseline to 1.9 mg/dL at Week 4, which fell to 1.6 mg/dL at Week 6. [There was no difference in the slight rise in the median serum creatinine between treatment groups, and AB-AB group serum BUN was essentially unchanged from baseline to Week 11. However, 3 of 30 AB-AB subjects had changes exceeding predefined change limits (PC) for creatinine and only A single subject in the Prolastin®-AB group had such a change in creatinine. The other creatinine change that resulted in an abnormal value of 0.8 mg/dL at baseline to 1.6 mg/dL was in the case of Subject No. 306.] Prolastin®-AB Subject No. 308 had a blood sugar of 126 mg/dL at baseline, which was increased to 184 mg/dL at Week 11. The elevated blood sugar resolved within 7 days. [Changes from baseline to Week 11 in median blood glucose were trivial in both treatment groups.] Subject No. 402, a diabetic, had marked deterioration of blood glucose during the trial, rising progressively from 115 mg/dL at baseline to 573 mg/dL at Week 24. The fact that the rise was progressive suggests either inadequate medical care or poor compliance or both.

3 subjects (Nos. 247, 403, and 432) lacked viral serological follow-up at 6 months following final study drug exposure due to withdrawal of consent or death.

Prolastin®-AB Subject No. 433 had an increase in serum potassium from 4.3 to 5.7 mg/dL. The group median potassium did not change from baseline to Week 11.

Prolastin®-AB Subject No. 246 developed marked treatment-emergent hypoalbuminemia. Baseline albumin was normal at 4.3 g/dL. Serum albumin fell to 2.8 g/dL at Week 6, 1.9 g/dL at Week 8, and rose to 2.5 g/dL at Week 11. Bilirubin rose from 0.4 mg/dL at baseline to 2.0 mg/dL at Week 6 and to 2.4 mg/dL at Week 11, and ALT rose markedly from 13 IU/L at baseline to 578 IU/L at Week 4. Total protein fell from 7.4 g/dL at baseline to 5.3 g/dL at Week 6, to 4.6 g/dL at Week 8, and to 5.2 g/dL at Week 11. "None of these changes was considered to be clinically relevant by the investigator[!]." These abnormalities are clearly clinically significant and indicate hepatitis with significant hepatic synthetic dysfunction or hepatitis or myopathy or myocardial infarction (MI) or severe hemolysis and are another cause of concomitant severe hypoalbuminemia and hyperbilirubinemia.

Inspection of the table of SAE's reveals that this subject, a 65-year-old female, had sepsis and a respiratory disorder on Day 15, an MI complicated by superventricular tachycardia on Day 17, retroperitoneal hemorrhage on Day 21 (which could explain the hyperbilirubinemia independent of liver disease), and an abscess on Day 41. The sepsis can explain the hypoalbuminemia and could contribute to moderate aminotransferase elevation. However, the ALT is elevated to a surprising level too many days following the MI to implicate the identified MI as the cause of the elevated ALT. Note also that this subject had a markedly abnormal total WBC count at baseline, $19.5\times10^3/\mu L$, which could have disqualified the subject from participation in the study. The WBC rose to $30.7\times10^3/\mu L$ at Week 4 and to $67.1\times10^3/\mu L$ at Week 8. None of these events was attributed to the study drug by the investigator.

A single subject had a rise in WBC count from 5.8×10^3 to $16.5\times10^3/\mu L$.

7 subjects had treatment-emergent serology to hepatitis A virus that was attributed to active vaccination.

No evidence of blood-transmitted infectious diseases was noted in any Zemaira™-treated subject during the clinical trials or after 6 months of follow up. Testing for HBV, HCV, HIV 1 and 2, and Parvovirus B19 was included in the study design. It should be noted that studies of the size performed in this BLA have extremely low power to detect viral seroconversions.

Clearly, Study 2002 was too short and too small to expect meaningful changes in pulmonary function tests to emerge in terms of between-treatment-group differences. The mean FEV_1's in AB-AB and Prolastin®-AB groups at baseline were 48.0% and 45.23%, respectively, at Week 11 were 49.1% and 47.5%, respectively, and at Week 24 were 48.9% and 42.5%, respectively.

A single subject in the AB-AB group of Study 2002 had treatment-emergent *diffuse interstitial lung disease*, noted on the Week 24 chest x-ray but not present at baseline.

Based on the method of manufacture of the product and the results of the clinical studies, Zemaira™ intravenously administered at a dose of 60 mg/kg once weekly appears to have adequate safety for use in the treatment of α_1-PI deficiency and emphysema.

Special Populations

Safety and effectiveness in pediatric and geriatric (age >65 years) patients have not been established. The pivotal study involved only Caucasians.

VIII. Bioresearch Monitoring Inspections

For the pivotal phase III trial (Study 2002), James Stocks, M.D., of the University of Texas Health Center in Tyler, TX was the lead clinical investigator and had 9 subjects in the AB-AB group and 3 subjects in the Prol-AB group; in addition, he was responsible for 9 of the 15 bronchoalveolar lavage (BAL) procedures carried out. Mark Brantly, M.D., (Gainesville, FL) had 8 subjects in the AB-AB group and 4 in the Prol-AB group; he was responsible for the other 6 BAL procedures that were performed. There were 5 additional study sites, and the following indicates the investigator, site location, and number of subjects in the AB-AB group/number of subjects in the Prol-AB group: Alan Barker, M.D., Portland, OR, 4/3; Friedrich Kueppers, M.D., Philadelphia, PA, 2/1; Charles Strange, M.D., Charleston, SC, 3/2; James Donohue, M.D., Chapel Hill, NC, 2/1; and Robert Sandhaus, M.D., Denver, CO, 2/0. The Agency conducted Good Clinical Practice inspections of 3 of the clinical investigator sites during 2003, i.e., those of Drs. Barker, Brantly, and Strange. No Form 483 was issued for Dr. Strange (Medical University of South Carolina). However, a Form 483 was issued for both Dr. Barker (Oregon Health Sciences University) and for Dr. Brantly (University of Florida Department of Medicine) although none of the findings significantly impacted the safety and efficacy data in the BLA. Drs. Barker and Brantly submitted responses in April and May 2003, respectively, which were found to adequately address the inspectional observations at both sites. In addition to the clinical site inspections, the Agency inspected the [redacted] the facility responsible for the analyses of patient samples from the phase III clinical trial, and issued a Form 483 with observations concerning assay validation, methodology, and retesting. AB addressed these issues by submitting a remediation plan and robustness analysis of the primary and secondary endpoint data, which were discussed and presented to CBER during an end-of-phase III meeting in August 2002 prior to submission of the BLA (also see Section VII of this SBA).

IX. Post-Licensure Commitments

The following post-licensure commitments were made by AB.

1. AB commits to conduct a clinical trial, which shall be comprised of two stages as described below. The conduct of the second stage will be contingent on the outcome and results of the first stage.

   Stage 1

   Pilot trial of clinically meaningful endpoint(s). Examples of acceptable endpoints include pulmonary exacerbations, serial pulmonary functions, and serial quantitative computerized axial tomographic (CT) lung scans.

   Details include:
   - A randomized, controlled, parallel, masked design.
   - A minimum enrollment of 60 subjects (30 subjects per treatment group) in the pilot study.
   - The control group may be a different dose of the test product (i.e., higher, such as 120 mg/kg/week or 240 mg/kg/2 weeks) in comparison to the labeled dosing regimen of the test product or placebo.
   - The trial will be a minimum of one-year's duration to avoid seasonal bias in pulmonary exacerbations.
   - The trial design will include measurement of baseline and steady-state antigenic and functional α_1-PI blood levels.
   - The trial may include a post-trial follow-up assessment.
   - A final protocol will be filed to the IND and BLA 6-12 months after product approval.
   - The trial will be initiated within 6-12 months after protocol acceptance by the FDA.
   - Alternate study designs and features may be discussed with the Agency following feedback from experts.
   - The final study report will be submitted in a timely fashion to the IND and BLA.

   Stage 2

   Adequately powered study of clinically meaningful endpoints(s).

   - Based on the results of the pilot study and the available scientific data at the time that this study is being designed, AB will work with entities maintaining registries of α_1-PI deficient patients and with the National Institutes of Health (NIH) to

design and conduct an adequately powered study of a clinically meaningful endpoint(s). The study design could involve a single product or could potentially involve a cooperative simultaneous study of multiple products in parallel arms, using a factorial design. In the event that the study involves more than one product, AB commits to provide sufficient product to administer to an equal proportion of subjects as are being provided any of the other products. The design/conduct of the study may be contingent upon:
  - The amount of product available.
  - The number of available subjects.
  - The number of subject-years necessary to attain an adequately powered study based on the results of the previous study and current scientific data.
  - The participation of other manufacturer(s) of this product class.
- The results of the pilot study will be taken into account in the design of the follow-up study. A strong positive outcome in the pilot study may obviate the need for a follow-up study.
- The trial may include one or more post-trial follow-up assessment(s).
- The final protocol for this study will be filed to the IND and BLA within one year of the filing of the final report of the pilot study.
- The final study report will be submitted in a timely fashion to the IND and BLA.

2. Validation studies will be performed to demonstrate the capacity of the Zemaira™ manufacturing process to inactivate and remove West Nile Virus. These studies will be complete within six months after product approval.

4. The first three lots of Zemaira™ manufactured post-licensure will be placed into the long-term stability program at °C. Every year thereafter during which Zemaira™ is manufactured, one lot will be placed on long-term stability. Data will be provided in the product Annual Report.