CENTER FOR DRUG EVALUATION AND RESEARCH AND CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

APPLICATION NUMBER:

125117/0

ADMINISTRATIVE DOCUMENTS

MEMORANDUM
Re: Galsulfase/BLA STN 125117, Immunogenicity assays and Immunoassay review.
From: Ralph M Bernstein, DTP
To: Amy Rosenberg, Elizabeth Shores, Serge Beaucage, File (BLA STN 125117).
Date: 27 May 05

Maroteaux-Lamy Syndrome, or Mucopolysaccharidosis VI (MPS VI), is a metabolic (lysosomal) storage disorder caused by the insufficient activity of the lysosomal enzyme N-acetylgalactosamine-4-sulphatase (arylsulphatase B). This leads to the accumulation of dermatan sulphate intracellularly within multiple tissues. The disease manifests as growth retardation, facial dysmorphism, hirsutism, corneal opacification, organomegaly, upper airway obstruction, cardiomyopathy, and valvular heart disease (Mut, M, et. al., 2005). Arylsulphatase B and other lysosomal storage disorder related enzymes exhibit their highest activity in lysosomes, which, because of their key role in normal degradation of mucopolysaccharides as well as the targeting of extrinsic proteins to this compartment, present as good targets for therapeutics, i.e., enzyme replacement therapy (ERT). The therapeutic value of arylsulphatase B treatment for MPS VI (see Harmatz, et al, J Pediatr, 2004) has been shown in animal models, as well as in clinical trials. As is common in enzyme deficiency diseases, replacement with the therapeutic enzyme in a person whose endogenous enzyme is absent, due to gene deletions, or is mutated and thus non-functional, arylsulphatase B (herein referred to as Galsulfase) treatment had the unfortunate additional effect of eliciting anti-product IgG antibodies in all patients.

The assays reviewed in this document are ostensibly designed to detect the formation of anti product antibodies in patient sera. These assays, however, are poorly designed and implemented. The corrective actions suggested in this document could allow the sponsor to use these assays to monitor patient antibody titers in a reliable, reproducible, and credible manner. Accurate, reproducible, and sensitive quantitative assays are necessary for a number of reasons other than the most important, primary reason: monitoring patient safety. These assays can also be used by the sponsor at a later time for assessing tolerance induction over time, in the absence of additional treatment, as has been seen in the case of immune responses to glucocerebrosidase in the setting of Gaucher's disease, or for deliberate tolerance inducing regimens; and for comparability studies.

Summary:

Within BLA STN 125117, Biomarin has included 5 reports under BLA STN 125117/ 5.3.1.4 that are directly related to immunogenicity, immunogenicity assay concerns, and antibody assay quantification techniques. These include an IgE anti-product ELISA (see B, below under Assays and clinical analysis), an IgG anti-product ELISA (see E, below), an assay to detect rhASB in plasma (see D, below) and the development and use of a neutralizing antibody assay (see A and C, below).

The IgG anti product assay is most frequently utilized as a screening assay: e.g., in the pivotal phase 3 study, ASB-03-05, all patients' sera were analyzed using this assay at baseline and at weeks 1, 4, 6, 12, and 24. The IgE anti product assay was utilized to analyze only 2 patients' sera, the neutralization assay only one. There was no clinical

protocol requirement to assess either IgE anti product levels or neutralizing antibody levels in every patient.

The assays used lack adequate validation, including lacking adequate positive controls, improper cutpoint setting, and poor sensitivity levels (specifics are found below, in "assays and clinical analysis"). Multiple discussions with Biomarin have yielded a paper commitment (see below, attachment "informal commitment letter from Biomarin" dated 4Feb05, that will be reinforced with PMCs) that the Sponsor will properly validate the assays and reanalyze the patient samples with said assays. Consequently, I am unable to convey to the clinical review team that the results of the immunogenicity assays are valid. I can assure that the product is immunogenic in nearly 100% of patients, but as to how these levels relate in other than a qualititative measure is unknown at this time. Consequently, claims of decreasing immunogenicity are also hard to assess quantitatively.

The data generated from these partially flawed assays were submitted as part of the BLA, and reviewed to understand the effects of antibody on the safety and efficacy of Galsulfase. The IgG anti product assay indicated that 96% of 54 patients (52/54) developed antibodies to the product, all within 4-8 weeks of administration. Of these, 11 patients developed relatively high antibody levels, i.e., greater than 10 OD/ul (these OD values are generated by serial dilutions of patient sera; sera values in the linear range are back calculated to yield an OD/ul value). Patients from the phase I/II and II studies were studied up to 144 weeks, and the antibody titres were seen to gradually increase to about week 48, with a gradual decline beginning at week 72, to levels less than 2 OD/ul by week 144. The single patient that was tested for neutralizing antibodies was so tested because of a diminished reduction of GAG levels that correlated with a high IgG antibody titer. This patient's antibodies, in the unvalidated assay, neutralized rhASB by up to 31% by week 24. Of the 2 patients tested for IgE anti product antibodies, only one had demonstratable IgE anti product antibodies via the unvalidated assay; these antibodies seemed to increase 10 fold over a 24 week period. Anaphylactoid reactions occurred in 24% of treated patients, but there didn't appear to be a link with high antibody levels and these infusion reactions.

Biomarin has agreed to the following PMCs to address the significant flaws in the assays and in their testing scheme.

1. To develop and improve methods for immunogenicity testing including commitments:

a. To develop and validate an improved screening assay for detecting total antibodies to Galsulfase. The design and validation data for this improved antibody binding assay will be submitted by 11/31/05.

b. To develop and validate an improved immunogenicity assay for detecting neutralizing antibodies to Galsulfase. The design and validation data for this improved neutralization assay will be submitted by 11/31/05.

c. To develop and evaluate improved immunogenicity assays for detecting IgE antibodies to Galsulfase. The design and validation data for this improved IgE assay will be submitted by 11/31/05.

2. To analyze, using the improved and validated immunogenicity assays, archived serum samples from patients in the Phase 3 trials (ASB-03-05) for binding, neutralizing and IgE antibodies to Galsulfase. Analysis will evaluate immunogenicity
rates and individual patient titers to assess how antibody levels increase or decrease as a function of repeated exposure to better evaluate impact of repeated dosing on potential induction of immunological tolerance.
We commit to providing this data by 5/31/06.

3. To develop and improve methods for plasma levels of Galsulfase, including commitments:
a. To develop and validate an improved assay for detecting Galsulfase in human plasma. The design and validation data for this improved assay will be submitted by March 31, 2006.
b. Pending FDA approval of the revised plasma level assay, archived plasma samples from the Phase 3 and remaining plasma samples from the Phase I and II trials will be analyzed for levels of Galsulfase. This data will be submitted by July 31, 2006.

The draft FDA approval letter to Biomarin has the following listed as PMCs:

1 To develop and validate an improved screening assay for detecting total antibodies to Galsulfase. The design and validation data for this improved antibody binding assay will be submitted to FDA by November 30, 2005.

2 To develop and validate an improved immunogenicity assay for detecting neutralizing antibodies to Galsulfase. The design and validation data for this improved neutralization assay will be submitted to FDA by November 30, 2005.

3 To develop and evaluate improved immunogenicity assay for detecting IgE antibodies to Galsulfase. The design and validation data for this improved IgE assay will be submitted by November 30, 2005.

4 To analyze, using the improved and validated immunogenicity assays, archived serum samples from patients in the Phase 3 trials (ASB-03-05) for binding, neutralizing and IgE antibodies to Galsulfase. Analysis will evaluate immunogenicity rates and individual patient titers to assess how antibody levels increase or decrease as a function of repeated exposure to better evaluate impact of repeated dosing on potential induction of immunological tolerance. A final study report including these data will be submitted to FDA by May 31, 2006.

5 To develop and validate an improved assay for detecting Galsulfase in human plasma. The design and validation data for this improved assay will be submitted by March 31, 2006.

6 To analyze, using the improved and validated plasma level assay, archived plasma samples from the Phase 3 and remaining plasma samples from the Phase 1 and 2 trials for levels of Galsulfase. These data will be submitted to FDA by July 31, 2006.

Assays and clinical analysis:
Biomarin has developed several assays to 1) measure anti product antibodies and their relative levels 2) the effect of their presence on the activity of the product and 3) the presence of the product in human serum after administration. These include, and are listed in the BLA, under A) ASB-TR-IC-D-002: Development of an Assay for Inhibition of rhASB Enzyme Activity by Human Serum, B) ASB-TR-IC-D-003: Development of an Immunoassay for rhASB – Specific IgE Antibodies in Human Serum, C) CLO-BAR-002: Results for the Analysis of Serum Samples from Patient 024-003 (Protocol ASB-03-04) in a Neutralizing Antibody Assay for Recombinant Human N-Acetyl-Galactosamine 4-Sulfatase (rhASB), D) GAS-QR-01-011: Qualification Report: Measurement of rh-Arylsulfatase B (rhASB) in Human Plasma, and E) GAS-QR-03-001: Qualification Report: ELISA for Recombinant Human Arylsulfatase B - Specific Antibodies.

A) ASB-TR-IC-D-002: Development of an Assay for Inhibition of rhASB Enzyme Activity by Human Serum

It should be noted that Biomarin has tested only one patient serum sample for neutralization activity. This report describes the adaptation and validation of the "endpoint activity assay for Arylsulfase B" SOP: — for use in determining the inhibitory effect of antibodies developed against rhASB on the enzyme activity. In this assay, human serum samples, or — control sera — are —

Adam

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✓ § 552(b)(4) Trade Secret / Confidential

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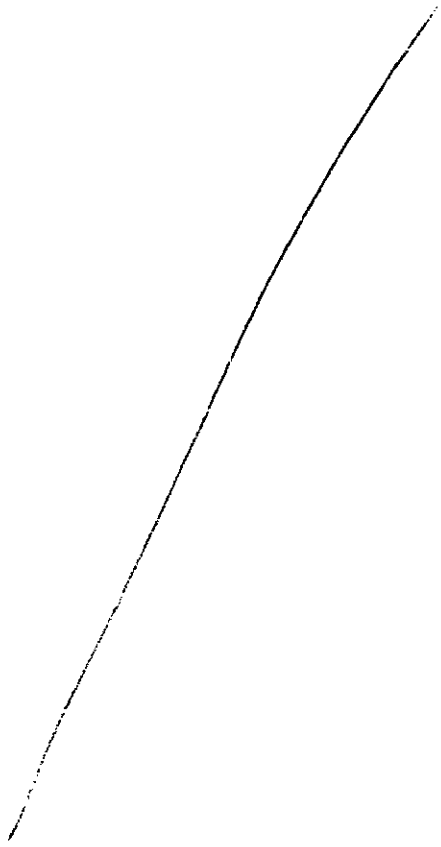

The sponsor has not yet demonstrated specificity of the positive control, by an [redacted], the sponsor has demonstrated that [redacted]. The sponsor has established a [redacted] egative control that give very low background [redacted] and estimated the LOQ at this time to be [redacted] It is my opinion, that this assay, once completely validated, should be able to provide a much lower LOQ.

The sponsor has used a single patient's serum in this assay. The patient, 024-003, exhibited less of a decrease in serum GAG levels, as well as higher IgG anti product antibodies and "an unusual pharmacokinetic profile" for the rhASB. The patient's results are shown in table 2, below.

Table 2: Neutralizing Antibody Results for Patient 024-003 with Antibody Titers

Sample	Timepoint	% Inhibition	Antibody Titer (O.D./µL)
L-0117041	Baseline	-	<0.2
L-0117220	Week 1	4.03%	<0.2
L-0117056	Week 4	2.29%	<0.2
L-0120034	Week 6	9.09%	4.7
L-0117058	Week 12	18.34%	6.2
L-0130655	Week 24	31.23%	27

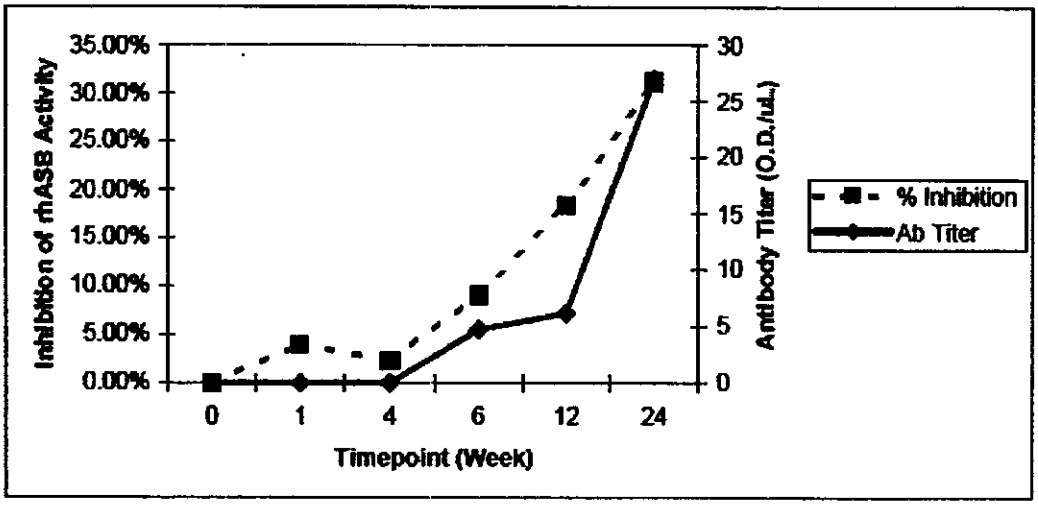

Figure 1: Graph of results for Patient 024-003's antibody titer and inhibition of rhASB enzymatic activity.

Note that as the patient's IgG anti product titer increased, the — — , indicating a reduction determined to be up to 31% inhibition in enzymatic activity. Figure 1, above, shows the correlation between AB titer and inhibition of rhASB activity.

Reviewer opinion: This neutralization assay, while not completely validated, appears to provide a semi quantitative measure of anti product antibody neutralization activity. The sponsor will have to complete validation, including demonstrating specificity of the positive control and demonstrating a more robust sensitivity.

VALIDATION	RESULTS	COMMENT
Sensitivity		
Specificity		
Cutpoint		

Precision and Reproducibility	
Robustness	

B) ASB-TR-IC-D-003: Development of an Immunoassay for rhASB – Specific IgE Antibodies in Human Serum

As what appear to be allergic reactions are seen while rhASB is administered, and IgE is often involved in allergic responses, Biomarin has developed an IgE anti product assay to attempt to detect baseline and elevated levels of that isotype. Normal levels of non specific IgE range from 20-400ng/mL in serum; a stark contrast when compared to IgG levels, which can range from 5-13mg/mL. Hence the need for a highly sensitive assay when attempting to analyze specific IgE responses. Biomarin has developed an assay that is similar to its IgG anti product assay in that it is also a — ELISA,

Two patients experienced multiple, manageable infusion reactions during rhASB administration. Using the IgE antiproduct assay, Biomarin determined that one of the patients had increasing levels of IgE anti product (slightly above the cutpoint (see table 2, below)) and that was confirmed when using an immunodepletion step prior to incubation on microtitre plates (see table 3, below). As of the finalization of this review, no further patients have been tested via the IgE anti product assay; the results from one patient are shown below.

Table 2: Results for patient samples.

	Patient I.D.					
	020-002			021-004		
Timepoint (weeks)	Mean Value (A450)	Stdev	CV%	Mean Value (A450)	Stdev	CV%
Baseline	-0.003	0.02		-0.012	0.01	
1	0.012	0.02		0.025	0.01	
4	-0.007	0.07		0.132	0.01	
6	-0.02	0.00		0.078	0.00	
12	NS			0.192	0.01	
24	-0.004	0.03		0.221	0.02	

NS - No Sample

Table 3: Repeat analysis of patient 021-004 and pre-incuation with rhASB.

	021-004 (1st Analysis)			021-004 (2nd Analysis)				021-004 pre-incubation with rhASB			
Time point (weeks)	Mean Value (A450)	std	CV%	Mean Value (A450)	std	CV%	% diff. from 1st analysis	Mean Value (A450)	std	CV%	% signal depletion
Baseline	-0.012	0.01		0.04	0.00		130.0	-0.021	0.01		152.5
1	0.025	0.01		0.023	0.01		-8.7	-0.019	0.03		182.6
4	0.132	0.01		0.026	0.0		-407.7	-0.024	0.02		192.3
6	0.078	0.00		0.066	0.00		-18.2	0.027	0.0		59.1
12	0.192	0.01		0.17	0.01		-12.9	0.04	0.01		76.5
24	0.221	0.02		0.21	0.0		-5.2	0.096	0.01		54.3

Reviewer opinion: The IgE anti product assay is marginally a better assay, in relative terms, than the IgG anti product assay (cutpoint development, immunodepletions to confirm true positives), but because of the sensitivity necessitated by the relative paucity of IgE in human blood, the assay should be properly validated, with an adequate positive control, etc, before it can be related to the clinical reviewers that the numbers are meaningful.

VALIDATION	RESULTS	COMMENT
Sensitivity		
Specificity		

Cutpoint
Precision and Reproducibility
Robustness

C) CLO-BAR-002: Results for the Analysis of Serum Samples from Patient 024-003 (Protocol ASB-03-04) in a Neutralizing Antibody Assay for Recombinant Human N-Acetyl-Galactosamine 4-Sulfatase (rhASB)

See A, above.

D) GAS-QR-01-011: Qualification Report: Measurement of rh-Arylsulfatase B (rhASB) in Human Plasma.

This report details a capture ELISA which has been qualified for quantification of rhASB, (and indeed detects all ASB) in patient sera. In this assay,

Biomarin refers to problems with certain analysis in the BLA as "potential antibody interference" in this assay; these items need to be addressed. *Biomarin has committed to redeveloping the assay and rescreening all available archived plasma samples (see PMCs).*

E) GAS-QR-03-001: Qualification Report: ELISA for Recombinant Human Arylsulfatase B - Specific Antibodies

This report details the "qualification" of Biomarin's h-IgG anti product antibody assay. This assay, a standard -ELISA

Biomarin has stated that they will commit, as a PMC, to developing and

validating a reliable assay, with a proper positive control, a determination of sensitivity, . and a properly determined cutpoint.

VALIDATION	RESULTS	COMMENT
Sensitivity		
Specificity		
Cut point		
Range/LOQ:		
Intra assay Precision and Reproducibility		
Robustness		

Using this assay, Biomarin has demonstrated that 52/54 patients, i.e., 96% of patients exposed to rhASB, develop IgG anti product directed antibodies. These antibodies develop within 4-8 weeks, and can vary greatly in their titer (via OD/ ul measurement). It is not clear if these antibodies are simply binding (i.e., do not affect activity), if they are neutralizing, or what their contribution is to, or from, bioavailability. Biomarin has developed a "qualified" neutralization assay, (see A, above) which has only been applied to one patient's sera sample. Long term studies, i.e., from the phase I/II and II trial, have demonstrated that there is a gradual increase in antibody titer through week 48 of treatment, with a decline beginning at week 72, to levels less than 2 OD/ul by week 144. Biomarin found that the "risk" of antibody development was greater in younger patients, and that developing higher OD levels correlated with "very low levels" of endogenous ASB.

Four patients with high antibody levels had differences in pharmacokinetic parameters relative to the overall population; one patient demonstrated low plasma concentrations of rhASB that correlated with high OD titers; three additional patients demonstrated an increase in clearance and "volume of distribution". Approximately 20% of patients experienced an "anaphylactoid-like" response during product administration; this could be contributed to by high IgG anti product titers.

Table 12-7: Antibody Development in Patients with Anaphylactoid Reactions

Patient ID	Weeks with Anaphylactoid Reaction	Maximum Antibody Level / Week that Maximum Level Occurred	Range of Antibody Levels during Period of Reactions
020–002	Weeks 21–24	1.8 OD/μL / Week 24	1.8 OD/μL[b]
020–007	Weeks 6[a], 7, 9, 15[a], 17, and 19	19.2 OD/μL / Week 24	4.4–19.2 OD/μL
024–003	Weeks 18[a], 19[a], and 23	27.0 OD/μL / Week 24	27.0 OD/μL[b]
024–005	Weeks 12, 14, and 22[a]	17. OD/μL / Week 24	8.7–17.1 OD/μL
026–005	Weeks 7, 8, 12, 13, 19-21, and 24	5.8 OD/μL / Week 24	2.8–5.8 OD/μL

Reference: Listings 16.2.7.1 and 16.2.10.

[a]Patient experienced intercurrent illness within 1 week prior to anaphylactoid reaction.

[b]Only 1 antibody determination was made during the period of anaphylactoid reactions.

Table 12-7, above, details the antibody levels of patients in the phase 3 trial, that experienced anaphylactoid-like responses during administration of product. While individual patient titers range greatly, there does appear to be some correlation with high antibody values, and the experience of an anaphylactoid-like response.

Biomarin has demonstrated that mean urinary GAG levels are clearly reduced, even in patients with antibody titers above 10 OD/ul serum (see Table 12-8, below).

Table 12-8: Mean Urinary GAG Levels in the Five Patients with the Highest Antibody Levels Compared to Mean Levels in All Patients

Group	Mean Urinary GAG Levels (μg/mg Creatinine) (Mean Percent Reduction from Baseline)	
	Baseline	Week 24
Patients with Antibody Levels Greater than 10 OD/μL Serum[1]	417[2]	112[2] (72%)
All rhASB Patients	346[3]	85[3] (73%)

[1] Patients 020-005, 020–007, 024–003, 024–005, 026-002.

[2] Baseline and Week 24 means and percent reductions are from Listing 16.2.8.1. Mean percent reductions were calculated using the mean of the individual patient percent reductions.

[3] Baseline and Week 24 means are from Table 14–28. Mean percent reductions were calculated using the mean of the individual patient percent reductions.

Reviewer opinion: This assay is poorly validated, and appears at best, a method to qualitatively compare relative levels between assay plates. Biomarin has agreed, through PMCs, to redevelop and validate this assay and to rescreen all archived Phase III patient samples, as listed below:

PMC2-To develop and validate an improved screening assay for detecting total antibodies to Galsulfase. The design and validation data for this improved antibody binding assay will be submitted to FDA by November 30, 2005.

PMC-5To analyze, using the improved and validated immunogenicity assays, archived serum samples from patients in the Phase 3 trials (ASB-03-05) for binding, neutralizing and IgE antibodies to Galsulfase. Analysis will evaluate immunogenicity rates and individual patient titers to assess how antibody levels increase or decrease as a function of repeated exposure to better evaluate impact of repeated dosing on potential induction of immunological tolerance. A final study report including these data will be submitted to FDA by May 31, 2006.

Labeling regarding immunogenicity.

Immunogenicity

Ninety-eight percent (53/54) of all patients treated with NAGLAZYME developed anti-galsulfase IgG antibodies. Initial evidence of antibody development typically appeared following 4 to 8 weeks of treatment. No association was observed between antibody development and urinary GAG levels.

Five patients with high antibody levels had observable differences in pharmacokinetic parameters (see **CLINICAL PHARMACOLOGY: Pharmacokinetics**). Antibodies from one patient were analyzed for neutralizing effect and showed evidence of *in vitro* inhibition of galsulfase activity. Because only one patient sample was analyzed for neutralizing activity, the effects of neutralizing antibodies are unclear.

The data reflect the percentage of patients whose test results were considered positive for antibodies to galsulfase using an enzyme-linked immunosorbent assay (ELISA) for galsulfase-specific IgG- binding antibodies, and are highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibodies in an assay may be influenced by several factors including sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to galsulfase with the incidence of antibodies to other products may be misleading.

Commitment letter from Biomarin regarding assay development.

BIOMARIN

Memorandum

Date: February 4, 2005

To: Ralph Bernstein, PhD, FDA

From: Mary S. Newman, MS, and Gary Taniguchi, PhD, BioMarin Pharmaceutical

Subject: Response to Agency concerns on antibody assays for rhASB

Reference is made to a January 31, 2005 phone call with Ralph Bernstein PhD, FDA, Gary Taniguchi PhD, BioMarin and Mary Newman MS, BioMarin.

The purpose of this memorandum is to provide an overview of the general approach used in the development of the IgG, IgE and ASB inhibition assays for rhASB and to address the concerns expressed by Dr. Bernstein during the aforementioned telephone conversation.

BioMarin qualified the IgG, IgE and ASB inhibition (neutralizing antibody) assays prior to the initiation of the Phase 3 pivotal trial, ASB-03-05. As specified in the protocol, sample collectionfor IgG was obtained at baseline and prior to infusions at Weeks 1, 4, 6,12 and 24 (ASB-03-05 Protocol Section 9.5.1 Efficacy and Safety Measurements). There were no protocol-specified requirements to assess the presence of ASB neutralizing antibodies or IgE in Protocol ASB-03-05. However, these assays were qualified for use if there was a medical need to perform these assessments. An Allergic Reaction Review board (ARRB) was empanelled to review severe or serious infusion-related reactions that occurred during the study. The ARRB had the authority to require additional sampling for immune response. Other than an apnea case that was judged unrelated to rhASB infusion, there were no infusion reactions that fit the serious or severe criteria for ARRB to review. Consequently, no additional samples were requested for analysis.

The plans for assessing patient immune response were included in the Phase 3 protocol and this protocol was reviewed with FDA as part of the End of Phase 2 meeting held on June 24, 2003. Reports describing the qualification of the assays were provided in Section 5.3.1.4 in BLA 125117/0. Results from the IgG sample analyses were provided as part of the Phase 3 study report in BLA Section 5.3.5.1.

The majority of the assay development work was carried out prior to or during 2003 according to the standards the Assay Development and Validation groups were aware of at that time. BioMarin acknowledges that further efforts will be required to enhance the robustness of the assays to meet current 2005 standards in preparation for future assessment in the post-marketing setting.

The plans outlining further assay development and validation are described below. BioMarin seeks Agency input and recommendations for these efforts. The timeline for implementing enhanced assays will depend on the ultimate approaches taken. Our current target for implementing the approaches outlined below is Q4 2005. In general, further assay development will utilize the standards as described in the manuscript from June 2004, "Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products", Mire-Sluis, Anthony, et al., J Immunol Meth. Furthermore, all assays will meet validation criteria outlined in ICH guidelines.

IgG:

Several approaches will be taken to improve the IgG assay sensitivity to ——ng/mL or better. We will be investigating the use of a ——

IgE

To reduce the variability seen in inter-assay and intra-assay controls and normal human serum samples, ——

The attached report presents the results obtained for IgE analysis of serum samples from two patients in the Phase 3 study.

ASB Activity Inhibition

Several approaches to reduce the assay variability and increase sensitivity of the enzyme activity neutralization assay are under consideration. One approach would be to ——