



## FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

MEMORANDUM

Date: 11.18.2011

From: Jennifer L. Reed, Ph.D.; CBER/OBRR/DH/LPD  
HFM-345; 301-496-0625

To: File for BLA 125402/0

Reference: IND 13840; STN BL 125105 (Immune Globulin Intravenous (Human), 10% Solution; Gammagard Liquid); NDA 21-859 (hyaluronidase human injection, Hylenex)

Through: Dorothy Scott, M.D.; CBER/OBRR/DH/LPD; HFM-345; 301-827-3016

Cc: Mark Shields; CBER/OBRR/DBA; HFM-380; 301-827-6173

Subject: Midcycle Product Review Memo  
Product: Immune Globulin Infusion (Human), 10% with Recombinant Human Hyaluronidase: HYQVIA  
Submission Date: June 30, 2011  
Manufacturer: Baxter Healthcare Corporation

### Product Information:

The proposed product is Immune Globulin Infusion (Human), 10% (IGI, 10%) with recombinant human hyaluronidase (rHuPH20), intended for the treatment of patients with primary immunodeficiency (PI) with defects in humoral immunity.

The combination product contains one vial of rHuPH20 and one vial of IGI, 10% for sequential administration. IGI, 10% is identical to the currently approved GammaGard Liquid (STN BL 125105) with no changes in manufacture of the final product. The rHuPH20 component is very similar to Hylenex hyaluronidase human injection drug product (NDA 21-859, approved 2005), -----(b)(4)-----.  
Cross-reference is made to the approved BLA and NDA submissions.

Hyaluronidase pretreatment of the injection site is intended to allow patients to receive a monthly dose of IGI, 10%, in a single subcutaneous injection. This could offer considerable convenience and quality of life benefits to some patients. IGI, 10% is typically administered at 300 to 600 mg/kg body weight in PI patients, while the proposed dose of permeation enhancer rHuPH20 is 80 U/g Ig. To facilitate a typical adult dose of 40 g subcutaneous IGI, 10% in a single site, the chosen administration site

would be pre-treated by injection of 20 ml rHuPH20 at 160 U/ml, followed by infusion of 400 ml IGI solution.

Hyaluronidase preparations from ovine and bovine sources have a six decades-long history of use as single-dose permeation enhancers in the US. Hyaluronidase permeation enhancers including rHuPH20 are frequently used off label to aid in administration of subcutaneous medications in patients with chronic disease, and such use is considered generally safe based on anecdotal experience. The proposed combination product, if approved, would be the first licensed chronic use of a hyaluronidase permeation enhancer in combination with a biologic product.

### **Issues for Continuing Discussion and Follow Up**

Status of assay development for anti-rHuPH20 antibody isotyping requires clarification. Additional tissue cross-reactivity data should be collected to clarify PH20 expression in male and especially in female tissue. The --(b)(4)-- GLP tissue cross-reactivity study should include a combined plasma sample from the 9 patients in clinical study 160603 who demonstrated boosted responses to rHuPH20. That same combined plasma sample should be used in the (b)(4)-based bridging assay to explore the possibility of binding to Hyal1 and Hyal2. In the event that these data do not raise additional safety concerns, we will need to discuss the specifics of a PMR to evaluate immunogenicity and potential adverse events related to immunogenicity.

### **Submission History**

6.30.11	Original BLA submitted
7.28.11	Amendment 1: Request for Proprietary Name Review: HyQvia
8.12.11	Amendment 2: Request for Pediatric Waiver
8.29.11	Amendment 3: Information request responses include 6 month stability data hyaluronidase; biosearch monitoring information; facility inspection information
9.1.11	Amendment 4: Labeling – updated carton and label mockups
9.2.11	Amendment 5: CMC section updated to include information inadvertently omitted: justification of specifications, drug product batch and fill sizes
9.27.11	Amendment 6: Response to immunogenicity IR
10.3.11	Amendment 7: Response to IR sterilization and aseptic processes, facility and equipment
10.4.11	Amendment 8: Response to clinical IR: mean and standard deviation data for studies 160601, 160602, 160603
11.8.11	Amendment 9: Proposed tissue cross-reactivity study and request for tcon

### **Review: Manufacturing process IGI, 10% and rHuPH20**

The Immune Globulin Infusion (Human), 10% component is not changed for this new combination product. Manufacturing process for rHuPH20 is identical to that in the approved NDA -----(b)(4)-----  
----- for this new combination product. The new elements of rHuPH20 formulation and product specifications are reviewed below in more detail.

***rHuPH20 Formulation***

The Sponsor notes that the excipients were chosen based on previous experience with Hylenex and known compatibility with hyaluronidase activity. The Sponsor states that albumin -----(b)(4)-----CaCl2 -----(b)(4)-----  
----- EDTA and -----(b)(4)-----.

**Table 2.3.P.1.2-1.  
rHuPH20 Drug Product Comp**

Strength (160 U/mL)			
Name of Ingredient	Unit and/or Formula 1-ml	Function	Reference to Standard
rHuPH20	160 U (~0.0013 mg)	Active ingredient	In house
Sodium Phosphate Dibasic Dihydrate	10 mM	pH buffer	(b) (4)
Sodium Hydroxide	4.25 mM	pH buffer	
Human Albumin (b)	1.0 mg/mL	Stabilizer	
Calcium Chloride Dihydrate	2.7 mM	Ionic modifier	
Sodium Chloride	145 mM	Tonicity modifier	
Edetate Disodium Dihydrate	2.7 mM	Stabilizer	

EP - European Pharmacopoeia  
NF - National Formulary  
USP - United States Pharmacopoeia

***Release Specifications for rHuPH20***

1) rHuPH20 activity and pH:

The rHuPH20 is formulated at neutral pH. -----  
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----- (b)(4) -----  
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2) ---(b)(4)--- testing:

An essential assay is the --(b)(4) test. In this test, hyaluronidase is -----  
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1 page determined to be not releaseable

----- (b)(4) -----:

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----- (b)(4) -----

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Proposed release specifications for rHuPH20 are listed below.

**Table 2.3.P.5.1-1.**  
**rHuPH20 Drug Product Release Specifications**

Specification Reference Number			MS-05-00092
Specification Version and/or Approval Date			Revision 3 20 May 2011
Test	Method Type	Source/Document No.	Acceptance Criteria
Appearance	Visual	(b) (4)	Clear, colorless solution, essentially free from foreign particles
pH	(b) (4)	(b) (4)	(b) (4)
Osmolality	(b) (4)	(b) (4)	290 – 350 mOsm/kg
Particulate Matter	(b) (4)	(b) (4)	(b) (4)
Bacterial Endotoxin	(b) (4)	(b) (4)	(b) (4)
Potency	(b) (4)	(b) (4)	160 U/mL (b) (4)
Purity	(b) (4)	(b) (4)	(b) (4)
Sterility	(b) (4)	(b) (4)	Sterile
Total Protein	(b) (4)	(b) (4)	(b) (4)

USP: United States Pharmacopoeia; EP: European Pharmacopoeia; NMT: Not More Than  
(b) (4)

1 page determined to be not releaseable

Release specifications. Three of the clinical batches and (b)(4) of the conformance batches were entered into stability monitoring.

#### STABILITY REPORT 99907-CMC-002

Report of stability of clinical lots rHuPH20, approved June 1, 2011

This report was intended to monitor the stability of Phase III clinical study materials. The study encompasses three rHuPH20 lots that were used in clinical studies. All 3 lots were formulated and filled on Line (b)(4) at the Sponsor's ---(b)(4)----- multi-product facility and shipped for stability monitoring to the Sponsor's ----(b)(4)----- facility. The decay of rHuPH20 activity ----(b)(4)---at 25°C storage, and is notably further --(b)(4)-- at 25°C. All three of the clinical lots were within specification with continuous 25°C storage for (b)(4) months, but not at (b)(4) months (the next time point investigated). The Sponsor also provides a --(b)(4)-- potency study, in which rHuPH20 vials were ---(b)(4)----- temperature storage for the last 3, ---(b)(4)--- months of shelf life. Again, all three of the clinical lots were within specification with 25°C storage for the last (b)(4) months of shelf life, but not with (b)(4) months of 25°C storage (the next time point investigated). (b)(4) --- (b)(4)--- data demonstrate that particles NLT (b)(4) and NLT (b)(4) are --(b)(4)- in samples stored at 25°C, although all particle counts remain within specification. The Sponsor's proposed shelf life of 24 months with (b)(4) months at NMT 25°C storage appears supportable based on potency, purity, and particulates values.

[(b)(4)]

2 pages determined to be not releaseable

foreign particles in them to enter the stability monitoring program. The major CAPA arising from this result was additional training of ---(b)(4)----- inspectors on appearance testing, and replacement of coveralls to reduce particle shedding. All other processes were judged to be functioning normally. Surprisingly the finding of skin in a product vial did not trigger any specific CAPA.

## **Review: rHuPH20 Nonclinical Studies**

The Sponsor states that its non-clinical program results show that combined IGI, 10% and rHuPH20 have safety profiles similar to that obtained with the individual components alone. The rHuPh20 demonstrates little if any systemic absorption.

### Activity of rHuPH20

To demonstrate efficacy (spreading activity), the Sponsor presents R03002, a classic (b)(4) --- (b)(4)-- assay in (b)(4) mice with --- (b)(4)--- background. Intradermal injection of rHuPH20 facilitated --- (b)(4)----- in the skin of mice similar to a (b)(4) hyaluronidase (b)(4) standard. Similar studies of --- (b)(4)----- were performed in mouse studies R03003, R04002, R04013, R08104, and R08107. Enhanced --- (b)(4)----- was no longer seen 24 hours after rHuPH20 intradermal administration. IV injection of rHuPH20 also transiently enhanced ----- (b)(4)-----, and the effect was dependent on dose. The rHuPH20 infection enhanced -- (b)(4)-- of small molecules (e.g. (b)(4) in the skin better than large molecules (e.g. ----- (b)(4)-----). Study R08107 evaluated ----- (b)(4)----- forms of rHuPH20 in mice. (b)(4) -----  
----- (b)(4)-----.

Assessment of spreading activity in mice suggested that the -----  
(b)(4)----- . Spreading of (b)(4) was increased by rHuPH20 injection compared with control vehicle injection up to 6-18 hours after administration, suggesting that reconstitution of the hyaluronan in the dermal site was complete by 18 hours.

### Pharmacokinetics of rHuPH20

Clearance of single-dose, IV injected rHuPH20 ((b)(4) form) was evaluated in rabbits (R05107). Hyaluronidase activity tracked in plasma was detectable at 1 minute post-injection and was below level of detection (--- (b)(4)-- assay) by 5 and 30 minutes post injection, consistent with a high clearance rate. Similar results were observed with --- (b)(4)-- material administered in a single intravenous dose to mice (R09023), with no apparent differences in PK between --- (b)(4)----- of rHuPH20. In female --- (b)(4)--- monkeys (R07060), a single dose of rHuPH20 ((b)(4) form) administered IV was rapidly cleared from plasma with a half-life of 5 minutes. A single subcutaneous dose demonstrated a 10 to 16 hour half-life, suggesting that entrance into the plasma from the subcutaneous space was a rate limiting step. The Sponsor estimates a 1.5 to 4% bioavailability for subcutaneously administered rHuPH20.

### Toxicology Studies of rHuPH20

#### *Single Dose Studies:*

Low plasma levels of rHuPH20 after subcutaneous or intradermal dosing were observed in toxicokinetic studies in mice (R08127) and in ---(b)(4)----- monkey (R09050), confirming that rHuPH20 remains largely local after administration to the skin. Non-GLP, single dose studies of rHuPH20 conducted in 10 rats (R03005) demonstrated no clinical signs, symptoms, or behavioral abnormalities up to 2 weeks after receiving 10,500 U/kg rHuPH20 ((b)(4) form) by IV route. Male rats demonstrated hyaline casts after receiving rHuPH20 that were not observed in the 2 control rats.

#### *Repeat Dose Studies:*

A pilot study of ascending doses of rHuPH20 ((b)(4) form) demonstrated that 38 U/kg up to 12,000 U/kg rHuPH20 administered by 1 peribulbar and 1 SC injection were generally well tolerated. The no-effect level was 4,500 U / peribulbar injection, and 45,000 U/subcutaneous injection.

A follow up study in 48 (b)(4) monkeys (R05014) examined single peribulbar injections in 1 or both eyes and once weekly subcutaneous injections up to two weeks. Doses of 0, 130, 3880, and 38880 U per injection were tolerated without adverse effects or anatomic pathological changes. No plasma hyaluronidase was detected in plasma 30 minutes post infusion, suggesting systemic bioavailability was low.

In ---(b)(4)--- monkeys, rHuPH20 ((b)(4) material) was dosed daily either via subcutaneous or intravenous route for 7 consecutive days (R08056). No hemolytic effect of rHuPH20 was observed in any of the animals across the study. Higher detection of plasma hyaluronidase activity was observed with IV dosing compared with subcutaneous. The no adverse event level was the highest dose used, 580,000 U/kg.

A GLP study in ---(b)(4)----- monkeys (R05108) evaluated intravesical administration of rHuPH20 ((b)(4) form) weekly for 6 weeks. Some clinical signs were associated with catheterization. No treatment-related macroscopic or microscopic observations were noted in this study. The Sponsor concluded that doses up to 200,000 U/animal were not associated with chronic toxicity.

A 39 week repeat dose study was performed in ---(b)(4)----- monkeys (R09050) to evaluate toxicity of rHuPH20 ((b)(4) form) administered subcutaneously at 0.02, 0.2, or 2 mg/kg once per week. There was no evidence of systemic toxicity and no changes in hematology in all dose groups.

Neutralizing antibody development, detected as loss of hyaluronidase activity in plasma, was observed in both rHuPH20 repeat dose studies in ---(b)(4)----- monkeys. The Sponsor indicates no adverse effects associated with the development of neutralizing antibodies over the course of the studies (2 weeks; 39 weeks).

#### *Local Tolerance Studies:*

The 39-week repeat dose study of (b)(4)-form rHuPH20 included assessment of the injection site at terminal necropsy (R09050). Subcutaneous immune cell infiltration (B cells and T cells) was observed in all animals at the highest dose group, which was

reduced after 4-week washout suggesting reversibility. The Sponsor did not list this finding as adverse.

In a non-GLP study performed in 21 rats, rHuPH20 ((b)(4) form) was administered intraperitoneally into rats on days 1, 3 and 6 (R05049). Hydrometra and cytologic changes in distal convoluted tubules of the kidney were found in animals receiving the higher doses of rHuPH20 (15,000 U/kg and some animals at the 1500 U/kg dose), and not in the lowest dose group or in controls. The Sponsor notes that these findings in the uterus and kidney were not reproduced in other preclinical studies. A no-effect level of 1500 U/kg is listed for this study.

#### *Combination Product Studies:*

The combination product was studied in rabbits which received bolus SC injections of 500 mg/kg IgI, 10% or saline, with or without rHuPH20 pretreatment (---(b)(4)-----). Injections were administered every seven days. No clinical signs were noted and no local irritation was apparent at the injection site, although microscopic evaluation revealed inflammation and lymph node enlargement in all groups receiving IGI, 10% either with or without rHuPH20, consistent with an adaptive immune response to human IgG. In these groups, local inflammation increased with repeated doses. Less inflammation was observed with rHuPH20 was administered alone. IGI, 10% at ----- (b)(4)----- elicited similar inflammatory responses with repeated SC injections in rabbits (---(b)(4)---) suggesting (b)(4)- did not contribute much to the inflammatory response.

In (b)(4) mice, SC administration of 0.1 ml IGI, 10% with 0.1 ml rHuPH20 (100 U) or 0.1 ml saline was performed on days 1, 8 and 15 (R09131). Similar skin changes including inflammation, epidermal necrosis, and degeneration / regeneration of subcutaneous skeletal muscle were found in all groups. The Sponsor interpreted no test article-related adverse effects were present in this setting of a severely immunocompromised host, incapable of adaptive immune responses to IGI, 10%.

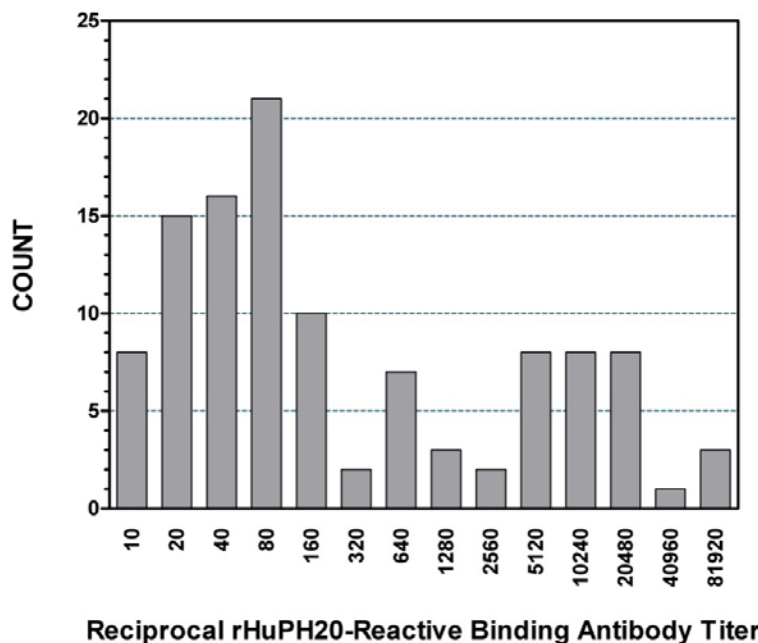
#### **Immunogenicity of rHuPH20**

Halozyme provides in the clinical review section an **integrated summary of immunogenicity** of IGI, 10% with rHuPH20 permeation enhancer, a report dated May 24, 2011.

1) The summary notes that plasma samples from normal volunteers and IGI, 10% manufactured from normal plasma contain hyaluronidase directed, non-neutralizing antibodies detectable in binding assays. Anti-hyaluronidase antibody titers of NMT 1:160 were established likely passively administered in PI patients receiving IGI, 10%. The antibody detection assay is modern, reproducible and sensitive. The cutoff of 1:160 is supported by the data and appears sound.

2) The summary asserts that no subjects developed neutralizing antibody responses to PH20. The neutralizing assay is based on the -----(b)(4)----- assay, which in the Sponsor's hands is sensitive and appears sound. The claim of no neutralizing assay development in patients receiving rHuPH20 during the clinical study is credible, as is the claim of no neutralizing PH20 directed antibodies in normal plasma and IGI, 10%.

- 3) The Sponsor claims no allergic reactions temporally associated with rHuPH20 administration, i.e. immediate-type hyperresponsiveness is low with the rHuPH20 product. This assertion appears supported by the data set.
- 4) The Sponsor’s analysis of AEs reveals no trend of adverse events in patients with neutralizing antibodies. CBER analysis of the data set agrees with this statement.
- 5) The Sponsor states that:  
 “Based on data available to date, the incidence of treatment emergent rHuPH20-reactive binding antibodies was low, and no cases of neutralizing antibodies have been observed in any subjects in this treatment population.” Anti-rHuPH20 antibodies have an incidence of 10%, which is rather high, and there is evidence of boosting.



**Table 5.**  
**Classification of rHuPH20-Binding Antibody Responses by Subject**  
 (Baxter Clinical Study 160603)

Negative	Single Positive Time Point	Weak Positive	Transient Positive	Persistent Positive
(b) (6)	(b) (6)	(b) (6)	(b) (6)	(b) (6)
41 subjects	24 subjects	6 subjects	3 subjects	9 subjects

- 6) The Sponsor states in relation to study 170901 part 4, that anti-PH20 antibodies were detectable in six patients, but that these were low and below the 1:160 threshold



expressed in females. The first (Beech et al 2002) is an mRNA only study which identified message for mRNA in normal and neoplastic breast tissue. No PH20 protein expression is confirmed. The second study (El Hajjaji et al, 2005) states that PH20 mRNA is present in chondrocytes and synoviocytes isolated from a patient or patients. No information on how many patients were involved is provided. Hyaluronan substrate gel electrophoresis demonstrates hyaluronidase activity in chondrocytes and synoviocytes, and rabbit antibodies raised against a PH20 peptide are reactive a 62 kDa band from chondrocyte extracts. There is no confirmation that the protein is PH20 and no demonstration whether the experimental rabbit antiserum recognizes other hyaluronidases. Other human hyaluronidases are of comparable size to PH20 so the identity of PH20 can't be assumed based on molecular mass. The third reference (Zhang et al 2003) demonstrates PH20 expression in the female reproductive tract of mice. While similar expression of PH20 may occur in reproductive tissue of humans, this hasn't been shown. Thus there isn't great confidence in PH20 antigen experience being similar in men and women based on the information provided here.

On page 21 the Sponsor discusses SAFETY RISK CATEGORIES that might be applied to hyaluronidase. The Sponsor states that immunogenicity risk is high for PH20 itself, which is non-redundant with a unique function in fertility. The Sponsor argues that risk is medium for other hyaluronidases, on the basis that these are A) redundant to each other, and B) of limited sequence similarity ("distally related" to other hyaluronidases). Argument (A) does not make sense since there are disease states associated with the loss of HYAL1 and HYAL2. Argument (B) also is questionable. Data generated with guinea pig, mouse, and cynomolgus PH20 (Chan et al 1999; Life Science 64: 1989-2000) demonstrate partial cross-reactivity among PH20 preparations with divergent sequences. Peptide 3 (residues 94-119) was identified as highly immunogenic and contained a neutralizing epitope confirmed in guinea pigs with anti-PH20 associated infertility. Our own analysis shows Peptide 3 features higher sequence conservation (77-88% homology) among PH20 proteins of different species, and among different human hyaluronidases, than the 33-42% claimed by the Sponsor.

#### PEPTIDE 3 HOMOLOGIES

Species	protein	%identity	%homology
Guinea pig	PH-20	100	100
Domestic mouse	PH-20	88	88
Chimpanzee	PH-20 like	84	88
Rhesus macaque	PH-20	84	88
Domestic cow	PH-20	80	84
Domestic rabbit	PH-20	80	88
Human	PH20 isoform 1	80	84
Human	PH20 isoform 2	80	84
Human	Hyaluronidase 2	68	77
Human	Hyaluronidase 4	63	70

The Sponsor on p 23 recommends that the “unique nature of PH20” and its use in clinical settings should limit the evaluation of rHuPH20 immunogenicity to: neutralization of PH20 activity; binding to endogenous hyaluronidases resulting in impaired fertility or hyaluronic acid catabolism; eliciting an allergic response. PK and PD effects are not high priority based on the rapid, local action of PH20.

The Sponsor used an in (b)(4) model (b)(4) to evaluate potential MHC class II-binding T cell epitopes in rHuPH20. A total of (b)(4) in rHuPH20 were considered, and (b)(4) clusters of potential T cell epitopes were identified which were predicted to have some reactivity with at least 4 different class II alleles. Notably the (b)(4) hits did not match the peptides identified by Chan et al (1999) based on guinea pig anti-PH20 infertility studies.

The Sponsor acknowledges that process-related impurities (from CHO cells) may contribute to immunogenicity. The Sponsor states that host cell proteins are the major impurity in rHuPH20 and are limited to NMT (b)(4). (b)(4), (b)(4), (b)(4).

The Sponsor acknowledges that different (b)(4) of rHuPH20 are found in the (b)(4) process. The Sponsor states that because no analysis (b)(4),

Similarly the Sponsor acknowledges the C terminal sequences of (b)(4).

The Sponsor acknowledges that (b)(4) of rHuPH20 and (b)(4) of (b)(4) processes that can affect immunogenicity.

The Sponsor reviews several characteristics of the patient (immune competence, prior exposure to hyaluronidase, etcetera) which may affect immunogenicity of rHuPH20. The Sponsor suggests that the rHuPH20 is transiently active in a local site, thus limiting its immunogenic potential.

The Sponsor claims no hypersensitivity and no positive skin tests to rHuPH20 in clinical trials and none in the Hylenex post marketing pharmacovigilance safety system. The Sponsor has no evidence that coadministration of rHuPH20 with a large molecule protein therapeutic (LPMT) affects immunogenicity of either rHuPH20 or the LPMT.

The Sponsor makes reference to routine pharmacovigilance activities for ongoing clinical trials. Activities include real-time monitoring of serious adverse events, adverse events resulting in patient withdrawal from a trial, and post-marketing adverse drug experience reports from various sources. The Sponsor makes reference to “periodic” assessments of safety data, and quarterly reviews of published medical and scientific literature. The Sponsor states that anti-rHuPH20 antibody testing as was performed in study 160603 will be conducted “in all studies in which sufficient dose exposure warrants such testing.” Sampling will occur pre-dose, and post-dose sampling will be specified within each study protocol. Review of all immunogenicity data will be performed by Clinical Development and Medical Affairs at Halozyme ---(b)(4)---, or more frequently “as needed”. Review will include anti-rHuPH20 binding and neutralizing antibody data, available adverse events, serious adverse events, and post-marketing safety reports. Results of each review will be presented internally with representation from relevant Halozyme department representatives.

### ***Immunogenicity IR***

In light of immunogenicity information provided by the Sponsor, the following IR was sent on September 8, 2011:

*1. Please cross-reference or provide to CBER any tissue cross-reactivity studies you have performed related to the binding of anti-rHuPH20 antibodies in a normal human tissue panel.*

*Rationale:*

*Your immunogenicity risk analysis presents an argument that anti-PH20 antibodies are unlikely to bind to other human hyaluronidases, on the basis of low overall sequence homology (33-42%). However Chan et. al. (1999) demonstrated that a 25 residue PH20 peptide bearing epitopes associated with fertility suppression in guinea pigs was 84-88% homologous in PH20s of various species, and was 70 to 77% conserved in HYAL2 and HYAL4 of humans based on our own analysis. Thus the likelihood of anti-PH20 binding to other human hyaluronidases might be higher than has been previously considered. An evaluation of tissue deposition of anti-PH20 antibodies in a normal human tissue panel (including both male and female tissues) would help to clarify PH20 expression particularly in tissues from females, and suggest whether anti-PH20 has the potential to bind other tissue hyaluronidases, for example, those found in bone and joint. We feel this information could refine post-marketing surveillance activities and ensure that potentially debilitating consequences of PH20 immunogenicity are identified promptly.*

The Sponsor replied to these comments in Amendment 6 on September 27, 2011.

a) The Sponsor acknowledged that a tissue cross-reactivity study has not been performed previously, and agreed to perform such a study. The Sponsor indicated that a proposed protocol would be forwarded to CBER.

b) The Sponsor repeats the assertions from the immunogenicity risk assessment, that rHuPH20 is expressed in tissues outside of the testes, citing the same references (Beech et al 2002; El Hajjaji et al, 2005; Zhang et al 2003) discussed on page 15 of this review.

These data are not sufficient to make a statement about the range of PH20 expression in human tissue from men and particularly from women.

c) The Sponsor alluded to a study in which rHuPH20-directed antibodies were affinity purified from ----(b)(4)-----, and used to detect binding using the Sponsor's bridging assay format, which is ----(b)(4)----- based and probably of good sensitivity although we haven't seen the raw data. The Sponsor indicates that rHuPH20 directed antibodies from ---(b)(4)----- did not react with HYAL1 or HYAL2. Similarly, -----(b)(4)-----  
-----in the same assay.

[(b)(4)]

d) The only other data the Sponsor relates is information available online through ----- (b)(4)-----, which sells antibodies from a commercial source called ---(b)(4)----- for IHC applications. Purportedly representative IHC data using the ---(b)(4)----- antibodies is available on (b)(4) website. The Sponsor makes reference to ---(b)(4)-- polyclonal antibody ---(b)(4)-----, which was raised in rabbits against a recombinant fragment of HuPH20. This antibody reacts strongly with testes tissue but also recognizes some GI and prostate tissue, bronchial epithelium, and some other epidermal sites. The information posted by ---(b)(4)----- is intended for advertising and has no particular assurance of quality.

Amendment 9 received on November 8<sup>th</sup> 2011 contains a proposed tissue cross reactivity protocol, developed jointly between Halozyme Therapeutics and ---(b)(4)-----.  
---(b)(4)-- is a commercial source of human tissues and performs GLP tissue cross reactivity studies by contract. The proposed protocol would examine the binding of a ---(b)(4)----- mouse anti-rHuPH20 monoclonal, versus an isotype matched ---(b)(4)---- control monoclonal antibody. After optimization of binding to testis tissue in study Phase 1, ---(b)(4)-- would proceed to extend the IHC analysis to a panel of 36 tissues acquired from 3 males and 3 females without known disease. The larger study would be GLP.