



**Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Biologics Evaluation and Research**

BLA: 125402

Cross references: IND 13840
STN BL 125105 (GAMMAGARD LIQUID Immune Globulin Intravenous (Human), 10% Solution)
NDA 21-859 (Hylenex, (hyaluronidase human injection))

From: Evi Struble, Ph.D., Pharmacologist, CBER, DH, LPD

Through: Dorothy Scott, M.D., Laboratory Chief, CBER, DH, LPD

CC: Jennifer Reed, PhD
Mark Shields, RPM

Applicant: Baxter Healthcare Corporation

Product: Immune Globulin Infusion (Human), 10% with Recombinant Human Hyaluronidase; Proposed proprietary Name: HYQVIA

Subject: Preclinical Pharm-Tox Review

1. Recommendations

1.1 Recommendation on approvability

There are safety concerns that prevent this application from being approved.

1.2 Recommendation for non-clinical studies (Letter Ready)

Sponsor needs to perform preclinical studies in relevant species to assess:

1. Possible toxicity of life-time exposure to anti-PH20 antibodies.
 - a. Tissue cross-reactivity studies indicate that anti-PH20 antibodies bind to the enteric plexus and male reproductive tissue. The BLA does not contain information on the effect of life-time exposure to these antibodies, including in these tissues.
2. Possible toxicity of anti-PH20 antibodies in the developing fetus and the young.
 - a. Transplacental transfer of anti-PH20 antibodies is expected. The BLA does not contain information on possible developmental immunotoxicity of the proposed product.
3. Possible toxicity of the anti-PH20 antibodies in pediatric patients.
 - a. Pediatric patients may be especially susceptible to the effect of anti-PH20 antibodies. The BLA does not contain information from juvenile animal

studies to assess the effect of anti-PH20 antibodies, especially in the enteric plexus and male reproductive tissue.

4. Possible effect of anti-PH20 antibodies on male and female fertility.
 - a. A signed study report which includes a description of test article, immunization details, antibody response analysis and reactivity, etc should be submitted, for the report referenced in Halozyne Study Report 10059, reference number 108 (b)(4).

Table of Content

<u>1. Background</u>	4
1.1 Indication	4
1.2 Dose	4
<u>2. Mode of Action as it is Presently Known</u>	4
2.1 Receptor/Binding	4
2.2 Species Cross Reactivity	5
2.3 Tissue Cross Reactivity	5
<u>3. The Principal Model(s) Used for Efficacy, <i>in vivo</i></u>	5
3.1 Primary Pharmacodynamics	5
3.2 Secondary Pharmacodynamics	5
<u>4. Toxicology</u>	6
4.1 Combination Product	6
4.2 rHuPH20	6
Margin of Safety	6
Conclusions, Toxicology Studies	7
<u>5. PK Studies</u>	9
5.1. Combination Product	9
5.2. RHuPH20 Alone	9
<u>6. Distribution and Metabolism</u>	9
<u>7. Single-Dose Toxicity Studies</u>	10
7.1. rHuPH20	10
<u>8. Repeat-Dose Toxicity Studies</u>	10
8.1. Study Number: --- (b)(4) ----- “A 39-Week Toxicity Study of rHuPH20 Administered Subcutaneously in --- (b)(4) ----- Monkeys with a Recovery Phase”	10
8.2. Study Number: 08056 “A 7-Day Repeat Dose Intravenous and Subcutaneous Toxicity Study of rHuPH20 in --- (b)(4) ----- Monkeys”	11
8.3. Report R05049 “Safety and potential toxicity of ascending doses of rHuPH20 (b)(4)-- administered IP in rats”	12
<u>9. Reproductive and Developmental Toxicity</u>	13
9.1. Study Number R09058 “Subcutaneous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of rHuPH20 in Mice, Including a Postnatal Behavioral/Functional Evaluation”	13
9.2. Study 08176 “Subcutaneous developmental toxicity study of rHuPH20 in mice”	15
9.3. Study R07046 “Nonpivotal, dose range-finding embryo-fetal development study in mice”	16
<u>10. Local Tolerance, IG, 10% with rHuPH20</u>	16
10.1. Study R09131 “Local Tolerance Feasibility Study After Repeated Subcutaneous (Bolos) Administration of Gammagard 10% with rHuPH20 in (b)(4) Mice”	16
10.2. Study --- (b)(4) --- “Preclinical studies of the subcutaneous application of Gammagard Liquid after Hylenex pretreatment: Local Tolerance in the rabbit”	17
10.3. Report --- (b)(4) ----- “Local tolerance in the rabbit, (b)(4) effect”	17
<u>References</u>	17

1. Background

This BLA is for SC IG 10% co-packaged and administered in combination with recombinant human hyaluronidase PH20 (rHuPH20).

The immune globulin component of this product is identical to Gammagard Liquid 10% approved in 2005 under BLA 125105 for IV administration.

The recombinant human hyaluronidase PH20 (rHuPH20) component was also approved in 2005 under the trade name HYLENEX (hyaluronidase human injection). It is indicated as an adjuvant to increase the absorption and dispersion of other injected drugs; for subcutaneous fluid administration; and as an adjunct in subcutaneous urography for improving resorption of radiopaque agents. RHuPH20 is synthesized in Chinese hamster ovary (CHO) cells that have been transfected with a plasmid containing the DNA sequence encoding human hyaluronidase PH20. The approved preparation is made with what the submission refers to as “process (b)(4)”, which results in rHuPH20 concentrated at 160 U/mL. -----

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

1.1 Indication

HyQvia is indicated for the treatment of patients with primary immunodeficiency (PI) associated with defects in humoral immunity.

1.2 Dose

The recommended dose of Immune Globulin Infusion (Human), 10% of HyQvia for patients with PI is 300 to 600 mg/kg body weight infused at 3 to 4 week intervals. However, in the clinical trial, higher doses were used, with a maximum dose of 1.8 g/kg per 4 weeks (Table 14.3-28, Clinical Study 160603).

The amount of rHuPH20 co-packaged with IgG is 80 U/g IgG. Thus, for individuals taking the recommended dose of 0.6 g/kg IgG per month, the corresponding dose for rHuPH20 is 48 U/kg. Given the specific potency of the rHuPH20 preparation of --- (b)(4) ----- the dose of rHuPH20 becomes --- (b)(4) -----.

For off-label uses, IgG up to 2 g/kg can be used, corresponding to a dose of rHuPH20 of 160 U/kg or 0.0013 mg/kg.

2. Mode of Action as it is Presently Known

Human PH20 hyaluronidase depolymerizes hyaluronan (HA) at neutral pH. Hyaluronan is a glycosaminoglycan polymer with MW 10^7 Da whose main function is to create a barrier to bulk fluid flow in the extracellular matrix (ECM).

HA rapidly turns over in the body. For example, 50% of the HA in the body is in the skin where it has a half life of less than 1.5 days (1). Most of the HA is degraded in the ECM to slightly smaller molecules of HA (MW 10^6 Da) resulting in increased mobility and migration to the lymph nodes where they are further degraded and cleared. Up to 85% of HA is cleared in the lymphatics via the HA receptor for endocytosis, HARE (2). The rest 15% of HA enters the circulation through the thoracic duct, and it is cleared by the endothelial liver sinusoidal cells (LSC), and to a lesser extent, spleen (3, 4).

PH20 may play a role in water re-absorption in kidney (5), or may have other functions, but the mechanisms are not yet known.

2.1 Receptor/Binding

Hyaluronan (HA)

2.2 Species Cross Reactivity

PH20 is conserved across mammalian species. Based on a BLAST sequence alignment performed by this reviewer 90% homology or higher with mice, rat, dog, and monkey at the protein sequence level was noted.

2.3 Tissue Cross Reactivity

RHuPH20 binds to HA present in ECM such as in skin, cartilage, bone etc. Following subcutaneous injection rHuPH20 in animals there is no (or very little) exposure of the biologic in the blood.

Anti-PH20 antibodies from rabbits bind to human enteric plexus and male reproductive tissue.

3. The Principal Model(s) Used for Efficacy, *in vivo*

3.1 Primary Pharmacodynamics

Study No	Brief Description	Species
R03002	----(b)(4)----- assay	Mouse, ---(b)(4)-----

Permeation enhancement was tested by sc injection of *either* rHuPH20---(b)(4)----- Hyaluronidase reference standard (RS) *and* -----(b)(4)----- mice. rHuPH20 temporarily increased ---(b)(4)----- in a dose-dependent fashion and showed equivalent activity as the RS.

3.2 Secondary Pharmacodynamics

Study No	Brief Description	Species
R03003	Dermal reconstitution	Mouse, ---(b)(4)-----
R04002	---(b)(4)----- assay after IV administration	Mouse, ---(b)(4)-----
R04013	---(b)(4)----- assay	Mouse, ---(b)(4)-----
R08104	Dermal reconstitution	Mouse, ---(b)(4)--- <i>homozygous</i>
R08107	---(b)(4)----- assay	Mouse, ---(b)(4)--- <i>homozygous</i>

Study R03003 showed that the action of rHuPH20 was transient after intradermal injection; its effects were not seen at 24 h after injection in (b)(4) mice skin as measured by area of -----(b)(4)-----.

Study R04002 showed that IV administration of rHuPH20 in (b)(4) mice, increased ---(b)(4)----- -- (injected ID) area of ----(b)(4)----- in a dose-dependent manner.

Study R04013 showed that, in mice, rHuPH20 induced ---(b)(4)----- enhancement depends on the size of particles co-administered with it (ID). Significantly increased --(b)(4)--- was only seen for particles up to (b)(4) nm in diameter.

Study R08107 (“*in vivo* potency”) showed mostly similar effects of the (b)(4) rHuPH20 products (made by -----(b)(4)----- as measured by ---(b)(4)----- area.

Study R08104 showed that area of ---(b)(4)----- was significantly larger for rHuPH20 (made by -----(b)(4)----- than the vehicle control by (b)(4) hours or more suggesting that dermal reconstitution was complete at (b)(4) hrs (for rHuPH20 made with -- (b)(4)-- process) or -----(b)(4)-----.

4. Toxicology

4.1 Combination Product

Table 1. Tabulation of toxicology studies performed with a combination IgG10% and rHuPH20

Type of Study	Species and Strain	Method of Administration	GLP	Study No.
Local tolerance, combination product	(b)(4) Rabbits	SC	Yes	----(b)(4)----- and ----(b)(4)-----
Local tolerance, combination product	(b)(4) mice	SC	No	Study R09131

4.2 rHuPH20

Table 2. Tabulation of toxicology studies with rHuPH20 (modified from submission)

Type of Study	Species and Strain	Method of Administration	GLP	Study No.
Single-Dose (b)(4)	Rat, (b)(4)	IV	No	03-007/ R03005
Repeat-Dose Toxicity (b)(4) Days 1, 4, 8	Monkey(b)(4)	Peribulbar, SC, once daily	No	---(b)(4)---/ R05015
Repeat-Dose Toxicity (b)(4) Days 1, 8	Monkey, (b)(4)	Peribulbar, SC, once daily	Yes	---(b)(4)---/ R05014
Repeat-Dose Toxicity (b)(4) 7 days	Monkey, ---(b)(4)-----	IV and SC, once daily	Yes	---(b)(4)---/ R08056
Repeat-Dose Toxicity (b)(4) 6 weeks	Monkey, ---(b)(4)-----	Intravesical, once weekly	Yes	1005-1253/ R05108
Repeat-Dose Toxicity (b)(4) 39 weeks	Monkey, ---(b)(4)-----	SC, once weekly	Yes	258.01/ R09050
Embryo-fetal Development (b)(4) DG 6-15	Mouse, ---(b)(4)-----	SC, once daily	No	---(b)(4)---/ R07046
Embryo-fetal Development (b)(4) DG 6-15	Mouse, ---(b)(4)-----	SC, once daily	Yes	---(b)(4)---/ R08176
Pre/postnatal Development (b)(4) DG 6-DL 20 or DG 22	Mouse, ---(b)(4)-----	SC, once daily	Yes	---(b)(4)---/ R09058
Local Tolerance (b)(4) Days 1, 3, 7	Rat, (b)(4)	IP, once daily	No	04-007/ R05049

Margin of Safety

Table 3. NOAEL Determined in Toxicity Studies with rHuPH20 and the calculated Margin of Safety

Species	NOAEL (mg/kg)	HED ¹ (mg/kg)	Study Numbers	Margin of Safety ²	
				Based on HED	Based on mg/kg dose
---(b)(4)----- monkey (7 days)	5	1.613	---(b)(4)-----/ R08056	1,240	3,846
---(b)(4)----- monkey (39 weeks)	2	0.645	---(b)(4)-----/ R09050	496	1,538
Mice (developmental)	maternal: 18 fetal: 3	1.463 0.244	---(b)(4)-----/ R08176	1,125 188	13,846 2,308

¹ Human Equivalent Dose (calculated based on relative body surface area)

² Compared to the maximum intended human dose of 0.0013 mg/kg body weight (based on off-label dose of 2 g/kg IgG, the rHuPH20 amount in the package of 80 U/g IgG, and specific activity of HuPH20 --- (b)(4) -----).

Conclusions, Toxicology Studies

1. Tissue cross-reactivity studies indicated that anti-PH20 antibodies cross-react with enteric plexus and male reproductive tissue. Given that anti-PH20 antibodies are generated in the clinical studies, and the lack of information on possible toxicity of these antibodies following chronic use and use by susceptible populations, the sponsor should provide to the BLA information on:
 - a. Possible toxicity of life-time exposure to anti-PH20 antibodies.
 - i. A chronic study in relevant animal species with a study design that mimics clinical situation should be performed.
 - b. Possible toxicity of the anti-PH20 antibodies in pediatric patients.
 - i. Pediatric patients may be especially susceptible to the effect of anti-PH20 antibodies. The BLA does not contain information from juvenile animal studies to assess the effect of anti-PH20 antibodies, especially in the enteric plexus and male reproductive tissue.
2. Repeated administration of rHuPH20 resulted in neutralizing antibody formation in adult --- (b)(4) ----- monkeys.
 - a. In a 39 week subchronic study --- (b)(4) ----- very high antibody titers were generated. The titers correlated to dose and increased over time. Titers dropped following the 4-week recovery period.
 - b. These antibodies bound and neutralized rhPH20. They bound but did not neutralize monkey PH20. No data was submitted whether these antibodies bound to the enteric plexus of these monkeys. Upon necropsy, no damage to enteric plexus was seen in this study.
3. Transplacental transfer of anti-PH20 antibodies is expected. The BLA does not contain information on possible developmental immunotoxicity of the proposed product in a relevant species.
4. Reproductive and development studies were performed in mice.
 - a. Anti-rhPH20 antibodies were generated in mice. The study did not determine if the fetus had any exposure to these antibodies during the intra-uterine development.
 - i. Some antibody transfer in the mouse fetus via the egg sac can occur. However, the extent and the timing of the fetus exposure to the anti-PH20 antibodies in this study have not been determined.
 - b. The developmental NOAEL for the fetuses is 3 mg/kg/day in mice (study 08176). Reductions in fetal weight and increases in the number of late resorptions occurred in the 9 and 18 mg/kg/day dosage group. Total litter losses were seen at SC doses of 30 mg/kg/day in mice (study --- (b)(4) -----, report R07046). As such, at very high doses, rHuPH20 was embryofetotoxic but did not have a teratogenic effect.
 - c. Maternal NOAEL of rHuPH20 is 18 mg/kg/day.
5. Effects of rHuPH20 on fertilization were not performed. It is not known if chronic anti-PH20 neutralizing antibodies would have any effect in fertilization in men or women.
 - a. Endpoints of reproductive organ morphology and function in male and female --- (b)(4) ----- monkeys were included in the 39 week subchronic study. The NOAEL was determined to be 2 mg/kg, the highest dose tested.

- b. The NOAEL for reproduction in the dams (i.e. animals who are already pregnant) and for viability and growth in offspring (i.e. F1 animals that were exposed to rHuPH20 *in utero*), including sexual maturation, learning and memory and the ability to produce an F2 generation is 9 mg/kg/day (study R09058 in mice).
 - c. Studies described in the BLA (in footnote 108 of Halozyme study report 10059) indicate that monkeys (male and female) immunized with PH20 did not display impaired fertility, despite anti-PH20 antibody formation.
6. Local reactions were seen following combination product and to a much smaller extent, following rHuPH20.
 - a. rhPH20 SC administration with or without Gammagard were not judged to be different from the saline control in (b)(4) mice (Study R09131).
 - b. IgG administration was associated with local site inflammation that increased in severity after repeated administration in rabbits (Report ---(b)(4)---), likely due to immune reaction of rabbit's immune cells with anti-galactose α 1, 3 galactose antibodies in human IgG. Mild reactions were found in the group where rHuPH20 and saline was administered. The -(b)(4)- of the preparation did not affect the local changes.
 - c. Minimal, dose related subcutaneous perivascular lymphoplasmacytic infiltration of plasma cells and lymphocytes were seen in the rHuPH20 injection site at the two highest dose groups in ---(b)(4)---- monkeys (0.2 mg/kg, or ~150X human dose and 2 mg/kg) in study ---(b)(4)----. Recovery was seen after the 4-week recovery period.
7. There was an increase in splenic weight in -----(b)(4)----- monkeys following 39 week repeated SC administration of rHuPh20 (report R09050). No dose relationship was seen and no microscopic observations were noted. A dose-related spleen enlargement is seen in female mice at the end of study R07046, following repeated administration. These reactions could be due to immune response following repeated administration of the human recombinant protein.
8. There are signs of hepatotoxicity in male ---(b)(4)----- monkeys (Report 08056) following repeated (7 days) SC administration of rHuPH20, namely hepatocytic vacuolation in 2/2 males in the SC group with or without ALT, AST and CK elevation. This sign is not seen after IV administration in the same study or following 39 weekly SC dosing in ---(b)(4)---- monkeys and is considered incidental.
9. Slight renal tubule dilation with the lumina filled with "hyaline casts" is seen in male rats following single IV administration of rHuPH20 at a dose ~65X human dose (R03005). Uterine (hydrometra) and kidney toxicity (endothelial changes) were seen in female rats after single doses (~9X clinical dose) administered IP (Report R05049). Due to the different route of administration, it is not clear what the relevance of these findings would be in the clinic.
10. Local tolerance studies in rabbits and (b)(4) mice were performed for IGSC, 10% with and without rHuPH20 using SC route of administration.
 - a. In rabbits, the IgG administration was associated with local site inflammation that increased in severity after repeated administration, likely due to immune reaction with human IgG. The -(b)(4)- of the preparation did not affect these changes (Report ---(b)(4)-----).
 - b. In (b)(4) mice reactions associated with the administration of rhPH20 with or without Gammagard were not judged to be different from the saline control.

5. **PK Studies**

5.1. ***Combination Product***

Pharmacokinetics for IG, 10% with rHuPH20 were investigated in rabbits and dogs.

In rabbits (Report ---(b)(4)-----), IgG 10% administered SC (500 mg/kg) with or without rHuPH20 or saline had no influence on any of the PK parameters of IgG, 10% in rabbits.

In dogs (Report R06019 & R06025), IgG 10% (1,000 mg/kg) administered SC with or without rHuPH20 at different ratios (as admix or sequentially) showed that:

1. Co-formulation of IgG with 100 U/mL rHuPH20 significantly increased C_{max} (146%) and decreased T_{max} to (30%) as compared to SC administration of IgG alone.
2. Sequential administration or mixture with lower amounts of rHuPH20 did not affect PK parameters.

5.2. ***RHuPH20 Alone***

PK studies were performed in mice, rats, ---(b)(4)----- monkeys.

In rats (Report R05107), single IV doses of rHuPH20 (86, 860 and 8,600 U/kg) resulted in detectable plasma hyaluronidase activity at 1 min post-injection only in the highest dose, falling below the level of detection ((b)(4) U/mL) at 5 min post-injection.

In mice (Report R09023), single IV doses of 48,000 U/kg rHuPH20 of ----(b)(4)----- preparations resulted in half-lives less than 5 minutes and plasma activity of rHuPH20 below lower limit of quantitation by 30 minutes.

In ---(b)(4)----- monkeys (Report R07060) it was shown that after single-doses of rHuPH20 (b)(4)-, the half-life of hyaluronidase activity in plasma was ~5 minutes and 10.3 to 15.7 hours after IV and SC administration respectively. Estimated bioavailability after SC administration is 1.5-4%. At SC doses of 1 mg/kg, most of the plasma PH20 activity were below the assay's quantification limit.

Toxicokinetic profiles were evaluated in repeated dose studies in ---(b)(4)----- monkeys (reports R08056 and R09050) confirming low systemic exposure after SC administration. As shown in report R09050, plasma activity of rHuPH20 at SC doses of 0.02 and 0.2 mg/kg was below baseline levels. Only for the animals that received the highest dose (2.0 mg/kg/week) of rHuPH20 (b)(4)--, plasma hyaluronidase activity exceeded pre-dose levels. Due to antibody formation at all dose levels, the C_{max} and AUC of PH20 after peaking on day 85 for M and 183 for F, decrease at later time points. The exposures are consistently higher for F than M. T_{max} at all time-points is 6 hours or less with most group average plasma levels below quantitation limit at 12 hours.

6. **Distribution and Metabolism**

Limited studies were performed with rHuPH20 regarding absorption and distribution following subcutaneous administration in animals.

37,000 U/kg rHuPH20 administered in female ---(b)(4)----- mice (report R08127) resulted in injection site activity half-life between 13 to 20 minutes - much shorter than plasma half life in --(b)(4)----- monkeys following SC administration (~10-16 hrs). Low rHuPH20 activity was observed in lymph tissue homogenates up to 24 hours post dose.

Metabolism studies were not performed with rHuPH20. Like other glycoproteins and depending on the glycosylation pattern, PH20 is likely to be internalized intracellularly via glycan-binding lectins such as mannose, and asyalo-glycoprotein receptors and undergo proteolysis and catabolism. Likely sites of clearance are the injection sites, the draining lymph nodes, endothelial cells of blood vessels and the liver.

7. Single-Dose Toxicity Studies

7.1. *rHuPH20*

Report R03005:

Single-dose toxicity in rats (non GLP toxicology study)

10 ----(b)(4)----- rats (5 male and 5 female) were administered a single dose of 10,500 U/kg *rHuPH20* -(b)(4)-, i.e. 0.09 mg/kg IV. N=1M and 1F received only a vehicle formulation.

No clinical signs, symptoms, or behavioral abnormalities were noted over the 2-week observation period of the study. Animals were sacrificed on Day 14. No gross abnormalities were noted at necropsy. Histological analysis revealed *slight renal tubule dilation* with the lumina containing an amorphous material consistent with hyaline casts in all 5 male rats. All other tissues evaluated were within normal limits.

8. Repeat-Dose Toxicity Studies

8.1. Study Number: ---(b)(4)----- “A 39-Week Toxicity Study of *rHuPH20* Administered Subcutaneously in ---(b)(4)----- Monkeys with a Recovery Phase”

Testing Facility: (b)(4) USA, Ltd.

Aim: To evaluate responses in ---(b)(4)----- monkeys of *rHuPH20* when administered subcutaneously once weekly for 39 consecutive weeks, followed by a 4-week recovery period. Study design: N=24 M and 24 F 4.21 to 7.12 years old and 2.7 kg to 10.3 kg, were assigned to the four groups receiving either -----(b)(4)----- buffer control (----- (b)(4)-- or *rHuPH20* (lot numbers: HUB0701EA, HUB0702CA) at three doses weekly for 39 weeks as subcutaneous injections. 2 animals/sex/group were allowed to recover for 4 weeks.

Group	Dose Level (mg/kg/day)	Number of Animals (Male/Female)	Necropsy (Male/Female)	
			Terminal	Recovery
1	0	6/6	4/4	2/2
2	0.02	6/6	4/4	2/2
3	0.2	6/6	4/4	2/2
4	2	6/6	4/4	2/2

Outcome measurements:

Clinical observations, menstrual monitoring (females), food consumption, and body weights were routinely assessed. Electrocardiography, blood pressure, respiratory rate, ophthalmology, testicular volume (males), urinalysis, semen analysis (males), hematology, coagulation, serum chemistry, testosterone and luteinizing hormones (male), toxicokinetic profiles, and anti-drug activity responses were assessed at specific time points. Organ weight analysis and anatomic pathology was performed at the end of dosing and recovery.

Results and Conclusions

1. Both total and neutralizing anti-*rHuPH20* antibodies were seen in all the dose groups. **The titer was correlated to dose** and generally increased over time. Antibody levels were detected in all groups **six weeks** after dosing began.
 - c. A low level of neutralizing activity against *rHuPH20* was detected in 4/6 F and 2/6 M or half of the animals receiving 0.02 mg/kg (Group 2) after 13 weeks. The titers range 95 to 1210.

- d. Neutralizing activity was detected in 11 of 12 animals in Group 3 generally starting on week 13 (one animal on week 10) with titers ranging 145-14,700.
 - e. Neutralizing activity was detected in 11 of 12 animals in Group 4 starting on week 13 with titers ranging 240-50,950.
 - f. After the 4-week recovery period, hyaluronidase neutralizing activity in most of the samples, but not all, dropped approximately 50% from peak levels.
2. Minimal subcutaneous perivascular lymphoplasmacytic infiltration of plasma cells and lymphocytes were present in the injection site of all animals administered 2.0 mg/kg rHuPH20 and in 2/8 animals in 0.2 mg/kg rHuPH20 dose group. This indicates that there may be some local bleeding/edema. At the end of the recovery period 3/4 recovery animals at the high dose group and all the animals at the other dose groups did not show this symptom.
 3. Plasma hyaluronidase activity was only detected following a dose of 2.0 mg/kg and it remained near physiologic levels (i.e. between 10 and 25 U/mL). Thus, rHuPH20 administered once-weekly via subcutaneous administration at a very high dose of 2.0 mg/kg has only limited systemic exposure.
 4. There was an increase in splenic weight in males at the end of treatment and females in the end of recovery period. No dose relationship was observed. This symptom could be related to immune response to rHuPH20 or perhaps clearance of HA degradation products. A dose-related spleen enlargement is seen in female mice at the end Study R07046.
 5. Other findings, not considered related to the test article administration due to lack of dose response and/or presence in the negative controls, are:
 - g. minimal erythrocytosis in mandibular lymph nodes (M and F, not dose related);
 - h. cysts in thyroid/parathyroid at 2/4 high dose M and 1/4 F ("cystic follicles" in 1/8 controls), stomach in 1/4 high dose M, and in the thymus (both 4/12 M and 3/11 F in most doses, not dose related; also seen in 1/8 controls)
 - i. 1/4 M had minimal and 1/4 M had marked decreased cellularity in thymus at high dose; correlates with decrease in thymus weight in M and it may be due to aging-related thymic involution
 - j. sporadic, non-dose related minimal mononuclear infiltration in myocardium (2/4 low dose M, 1/4 and 2/4 low and mid-dose F); also seen in 1/4 negative control M
 6. (D.E.Scott addendum) No changes were observed at week 39 with respect to semen analysis (sperm concentration, motility, morphology), testicular volume, testosterone, or luteinizing hormone.

Reviewer's conclusions

The antibody response seen in monkeys was neutralizing to rHuPH20, but there appears to be no clear clinical effects of such neutralizing response after 39 weeks of use.

The neutralizing activity was dose related. Low neutralizing titers were seen at weekly doses of 0.02 mg/kg/day or 10X maximal clinical dose for the cohort 1B (18,000 U or 0.15 mg or 0.002 mg/kg). This study could not identify any clinical effect of neutralizing antibodies. However, it remains unclear what the effect of endogenous HuPH20 neutralization would be in the human population.

The No-Observed-Adverse-Effect-Level (NOAEL) for rHuPH20 under the conditions of this study was 2.0 mg/kg or 240,000 U/kg, the highest dose level administered.

8.2. Study Number: 08056 "A 7-Day Repeat Dose Intravenous and Subcutaneous Toxicity Study of rHuPH20 in ---(b)(4)----- Monkeys"

Performing Laboratory: (b)(4) (GLP)

Aim: To evaluate responses in ---(b)(4)----- monkeys to intravenous bolus and subcutaneous administrations of rHuPH20 once daily for 7 consecutive days.

Study Design: 6M/6F ---(b)(4)----- monkeys of -----(b)(4)----- origins, 3.17 to 3.49 years old and weighing 2.044 kg to 3.643 kg were treated either with control buffer (----- (b)(4)-----) or with rHuPH20 IV or SC at 5 mg/kg daily for 7 days.

Group	Nominal Dose Level (mg/kg)	Route of Administration	Number of Animals (Male/Female)
1	0	SC and IV Bolus	2/2
2	5	IV Bolus	2/2
3	5	SC	2/2

Outcome measurements: Clinical observations (twice daily), body weights (once weekly), food consumption (daily), ophthalmology, hematology, serum chemistry, coagulation, urinalysis and toxicokinetic parameters.

Results:

Low plasma hyaluronidase activities were observed following subcutaneous administration. The median time to peak activity was 45.0 min. The AUC from the subcutaneous dose was 38,500 U•min/mL which was relatively low compared to the AUC of 713,000 U•min/mL from the intravenous dose. After 7 daily doses, there was a slight drop in AUC value in both intravenous and subcutaneous administration showing slight neutralizing antibody activity.

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine kinase (CK) levels were elevated from pre-dosing levels, for one male of the subcutaneous starting at day 2. The same subject also displayed minimal, focal hepatocytic vacuolation upon post-mortem evaluation, also seen in the other male in the group.

Conclusions:

There are signs of hepatotoxicity namely hepatocytic vacuolation in 2/2 males in the SC group with or without ALT, AST and CK elevation. It is unclear if this is test item-related, although not likely as the systemic exposure is much lower after SC administration than after IV administration and the sign is not seen after IV administration. In addition, this effect is not seen in 39 week study in monkeys. The vacuolation could be due to postmortem procedure, also seen in mice(6).

8.3. Report R05049 “Safety and potential toxicity of ascending doses of rHuPH20 (b)(4)--administered IP in rats”

Non-GLP toxicity study

Aim: evaluate the safety and potential toxicity of ascending doses of rHuPH20 (b)(4)--administered directly into the peritoneum of rats on Days 1, 3, and 6.

Design: A total of 21 -----(b)(4)----- female rats were dosed with either control saline (3 rats), or 150 U/kg, 1,500 U/kg, or 15,000 U/kg of rHuPH20 or 0.00125, 0.0125 and 0.125 mg/kg (6 rats for each dose), and evaluated at necropsy (3 rats from each dose group after dosing on Day 6 and on Day 28 of the study).

Results: Hydrometra was observed at the Day 6 necropsy in two animals from the 15,000 U/kg group and, at the Day 28 necropsy, in three animals from the 15,000 U/kg group and one from the 1,500 U/kg group. Microscopic examination of parenchymal organs was within normal limits for nearly all animals.

At the Day 28 necropsy interval, the kidneys of all 3 animals in the 15,000 U/kg group and 1 animal in the 1,500 U/kg group showed cytologic changes in the epithelium of the distal

convoluted tubules. These findings are not seen in any of other preclinical studies conducted with rHuPH20 dosed SC, IV, peribulbar or intravesical. However, changes in the renal tubule (dilation with fluid accumulation in the lumen) are seen in rats receiving a single high IV dose of rHuPH20 (Report R03005).

Based on the hydrometra and distal renal tubule changes observed mainly in the 15,000 U/kg group, the dose of 1,500 U/kg was considered to be the maximum tolerated dose in this study.

Reviewer conclusions: *IP administration of rHuPH20 results in uterine and kidney toxicity in female rats at doses similar to clinical doses. Only the lowest dose, ~1X human clinical dose, did not result in any toxicity. Due to the different route of administration, it is not clear what the relevance of these findings would be in the clinic.*

9. Reproductive and Developmental Toxicity

9.1. Study Number R09058 “Subcutaneous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of rHuPH20 in Mice, Including a Postnatal Behavioral/Functional Evaluation”

Performing Laboratory: -----(b)(4)-----

Aim: to detect adverse effects of rHuPH20 treatment of ---(b)(4)----- female mice from implantation through lactation and weaning on gestation, parturition, lactation and maternal behavior in female mice and on the development of the offspring of the treated female mice. This study was designed to evaluate ICH Harmonised Tripartite Guideline stages C through F of the reproductive process, but did not include an evaluation of Caesarean-delivered fetuses (stages C and D), because this evaluation was performed in a supplementary study. Because manifestations of effects induced during this period may be delayed, observations were continued through sexual maturity of the F1 generation mice.

Design:

25 pregnant female mice ----(b)(4)----- per dosage group were treated once daily SC with rHuPH20 to at dosages of 0 (Vehicle: -----(b)(4)-----), 3, 6, and 9 mg/kg/day from day 6 of presumed gestation and continuing through day 22 of presumed gestation (mice assigned to natural delivery that do not deliver a litter) or day 20 postpartum (mice assigned to natural delivery that deliver a litter).

Outcome measurements: cage-site observations twice each day of the study, for clinical observations, abortions, premature deliveries and deaths before and one to two hours after dosage administration and on the day sacrifice. Maternal behavior was recorded on DLs 1, 4, 7, 14 and 21. Each litter was evaluated for viability at least twice daily. The pups in each litter were counted once daily. Clinical observations were recorded once daily during the preweaning period. Pup body weights were recorded on DLs 1, 4, 7, 14, and 21. Viabilities, clinical observations, body weights, age of vaginal patency (female mice) or age of preputial separation (male mice), passive avoidance test (for learning, short-term retention and long-term retention), motor activity test and mating performance were recorded.

After completion of the 21-day postpartum period, surviving female mice were sacrificed and a gross necropsy was performed. The number and distribution of implantation sites were recorded. Mice that did not deliver a litter were sacrificed on DG 23 and examined for gross lesions and pregnancy status.

At necropsy, gross pathologic findings were recorded.

Female F1 mice were sacrificed on DG 18 (or an estimated DG 16 for female mice without a confirmed mating date. Uteri of apparently nonpregnant mice were examined while being pressed between glass plates to confirm the absence of implantation sites. Male mice were sacrificed after

completion of the cohabitation period, and testes and epididymides were excised and paired organ weights were recorded.

Results

F0

Mortality: 3 unscheduled deaths. These deaths were not considered to be direct effects of the test article but were probably related to these mice being unable to tolerate repeated administration of a proteinaceous material. The sponsor reports that “*sporadic deaths have been observed in other studies with repeated administration of proteinaceous material to mice*”.

1/25 mice in the 6 mg/kg/day groups was found dead on day 4 of lactation (DL 4), 74 minutes after the 16th daily dosage. The only adverse clinical observation before death was purple discoloration on the back on DG 16; all tissues appeared normal at necropsy. This dam had delivered a litter of 10 normal pups, one of which was found dead on DL 2. Mouse 6189 of 9 mg/kg dosage group was found dead on DG 17, before the 12th daily dosage. Adverse clinical observations for this mouse included cold to touch and purple tail on DG 15; all tissues appeared normal at necropsy. The litter consisted of 11 fetuses and 3 late resorptions.

Mouse 6193 in the 9 mg/kg dosage group was sacrificed due to adverse clinical observations on DG 16, approximately two hours after the 11th daily dosage. Adverse clinical observations for this mouse consisted of decreased motor activity, prostrate, pale ears, pale extremities, cold to touch, blue discolored extremities (nose, forepaws, hindlimbs), mild dehydration and bradypnea on DG 16; all tissues appeared normal at necropsy. The litter consisted of 13 fetuses.

One dead litter in the 6 mg/kg group was not viable having several deformities. No clinical or necropsy observations in the F1 generation pups were attributed to dosages of rHuPH20 as high as 9 mg/kg/day.

All clinical and necropsy observations during the gestation and lactation periods were considered unrelated to rHuPH20.

Fertility index was 88.0%, 80.0%, 84.0% and 80.0% of the 25 mated female mice in the 0 (Vehicle), 3, 6 and 9 mg/kg/day dosage groups, respectively.

F1 Generation Pups/Mice

No deaths related to maternal administration of rHuPH20 occurred. All F1 generation male mice survived to scheduled sacrifice. One F1 generation female mouse in the 0 (Vehicle) mg/kg/day maternal dosage group was found dead on postnatal day 31 (PND 31).

F1 showed lower weights at weaning that reach statistical significance in some of the days but remain comparable for the entire postweaning period.

An increased number (5/24 litters) of male mice in the 9 mg/kg/day maternal dosage group had a bent tail. “*This alteration was not considered related to the test article because no increase in tail anomalies occurred in the developmental toxicity study (Protocol ---(b)(4)-----) or in the female mice in this study.*” Further, responding to an IR sent on 9-17-09, the sponsor states that 1) 4.8% of the litters in historical controls show gross observations 2) this change is not significant because it is not accompanied by changes in caudal vertebrae and 3) the change is not seen in females or the studies R08176 and R07046, where higher doses were used.

As such this reviewer agrees with the sponsor’s conclusions:

Sponsor Conclusion

On the basis of these data, the maternal no-observable-adverse-effect level (NOAEL) for rHuPH20 is 9 mg/kg/day.

The NOAEL for reproduction in the dams and for viability and growth in the offspring including sexual maturation, learning and memory and the ability to produce an F2 generation is also 9 mg/kg/day. No adverse effects occurred on these parameters at the highest dosage tested.

9.2. Study 08176 “Subcutaneous developmental toxicity study of rHuPH20 in mice”

Performing Laboratory: ----(b)(4)-----

Aim: to detect adverse effects of rHuPH20 on ---(b)(4)----- pregnant female mice and development of the embryo and fetus consequent to exposure of the dam from implantation to closure of the hard palate. This study evaluates ICH Harmonised Tripartite Guideline stages C and D of the reproductive process.

Design: One hundred pre-mated female mice ---(b)(4)----- were assigned to four groups 25 mice per dosage group (main study), one hundred and eight mated female ---(b)(4)----- were assigned to three groups with 36 in each of Groups II through IV (toxicokinetic study).

Mice were dosed with the test article or the vehicle control (buffer) once daily on days 6 through 15 of presumed gestation (DG 6-15), the period of organogenesis. Dosages were adjusted daily for body weight changes and given at approximately the same time each day. All surviving main study mice were Caesarean-sectioned on day 18 of presumed gestation. Toxicokinetic study mice were sacrificed and blood samples (1.0 mL \pm 20%) were collected on days 6, 7 (24 hour), 15 or 16 (24 hour) of presumed gestation.

Outcome Measurements: viability, clinical observations, body weight and body weight changes, feed consumption, mating performance, toxicokinetic and necropsy observations, Caesarean-sectioning observations, fetal sex, fetal body weights, fetal gross external and soft tissue and skeletal alterations.

The mice were examined for number and distribution of corpora lutea, implantation sites, live and dead fetuses, early and late resorptions.

Samples were collected prior to toxicokinetic dosage administration, and at approximately 30 minutes, 2 hours, 4 hours, 8 hours and 24 hours post dosage.

Results

One main study mouse and one toxicokinetic satellite mouse in the 18 mg/kg/day dosage group and two main study mice in the 9 mg/kg/day dosage group were found dead. *“The deaths were not believed to be related to the test article because the mice did not exhibit any signs of toxicity (adverse clinical signs, reduced body weight gains or body weight losses) and the deaths (two per group) were not dosage-dependent; as more deaths in the highest dosage groups on earlier gestation days would have been expected.”*

Fetal body weights (total, male and/or female) were significantly reduced ($p \leq 0.05$) in the 9 and 18 mg/kg/day dosage groups compared to the vehicle control group.

The number of late resorptions was increased in the 9 and 18 mg/kg/day dosage groups and was considered test article related.

The Cmax of rHuPH20 activity on Day 6 of gestation was 3.44, 30.5 and 49.6 U/mL for 3, 9 and 18 mg/kg/day doses, respectively. The corresponding AUC (0-24) was 5.45, 45.3 and 120 U·h/mL.

On Day 15, Cmax was 20.9, 17.1 and 99.2 U/mL and AUC (0-24) was 55.1, 79.0 and 363 U·h/mL for 3, 9 and 18 mg/kg/day doses, respectively.

Both Cmax and AUC (0-24) generally increased with dose on Days 6 and 15.

Cmax ratio (Day 15/Day 6) was 6.08, 0.561 and 2.00 and AUC (0-24) (Day 15/Day 6) ratio was 10.1, 1.74 and 3.03 for 3, 9 and 18 mg/kg/day doses, respectively.

There is an increase of exposure with dose.

Sponsor conclusions:

The maternal no-observable-adverse-effect-level (NOAEL) of rHuPH20 is 18 mg/kg/day.

The developmental NOAEL is 3 mg/kg/day. Reductions in fetal weight and increases in the number of late resorptions occurred in the 9 and 18 mg/kg/day dosage group.

rHuPH20 proved to be embryofetotoxic but did not show an overt dysmorphicogenic (i.e. teratogenic) potential.

In response to an IR on 09-17-09, sponsor states that maternal mortality less than 15% is seen in other studies with proteinaceous materials without clear cause. *“In addition, the dose range-finding embryo-fetal study in mice (R07046), found no maternal deaths, albeit with only eight mice per dose group, even when dosed at a higher dose of 30 mg/kg/day.”*

In light of this information, this reviewer agrees with the sponsor’s conclusions regarding maternal and fetal NOAEL.

9.3. Study R07046 “Nonpivotal, dose range-finding embryo-fetal development study in mice”

-(b)(4)- rHuPH20 was administered SC at 0 (vehicle control), 1, 3, 10 or 30 mg/kg on DG 6 through DG 15, and animals were sacrificed on DG 18. There were no test article-related fetal gross alterations. There were no test article-related effects on the dams or their litters in the 1 or 3 mg/kg/day dose groups.

Results:

1/8 and 3/8 mice in the 10 and 30 mg/kg/day dose groups, respectively, had enlarged spleens. 1/8 mice in the 10 mg/kg/day dose group had a clear fluid-filled bursal cyst on the right ovary. 4/8 dams in the 30 mg/kg/day dose group had resorbed conceptuses (100% resorptions). Also, 1/8 dams in the 10 mg/kg/day dose group had a litter that consisted of 69% resorptions. The live litter size of the 30 mg/kg/day dose group was reduced compared to controls.

10. Local Tolerance, IG, 10% with rHuPH20

10.1. Study R09131 “Local Tolerance Feasibility Study After Repeated Subcutaneous (Bolus) Administration of Gammagard 10% with rHuPH20 in (b)(4) Mice”

Aim: To evaluate the local tolerance to repeated subcutaneous administration of Gammagard liquid 10% with and without rHuPH20 in (b)(4) mice.

Performing Laboratory: Halozyme Therapeutics

Model: Male -----(b)(4)----- mice

Design: Thirty-six male ----(b)(4)----- mice approximately eight weeks old and weighing 22-32 grams were randomized in four groups of 9 animals each receiving the test article or buffer as indicated below at a volume of 0.2 ml. The procedure was repeated in a subset of 6 animals per group on Study Day 8 and in a subset of 3 animals per group on Study Day 15. Three animals/group were sacrificed on days 2, 9 and 16.

Group	No. of Mice/Group	Test Article	
		Left Thigh	Right Thigh
1	9	0.1 mL Low pH buffer + 0.1 mL 0.9% NaCl	
2		100 mg/mL Gammagard + 0.1 mL 0.9% NaCl	0.9% NaCl
3		1000 U/mL rHuPH20 + 0.1 mL 0.9% NaCl	
4		1000 U/mL rHuPH20 + 100 mg/mL Gammagard	

Outcome Measures: Animals were examined twice daily for mortality/morbidity. Body weights were recorded prior to dosing on Day 1 and prior to necropsy. Injection sites were observed daily for signs of reactions. The local sites of injection and lymph nodes were removed surgically and evaluated histopathologically.

Results:

Mixed leukocyte inflammation, edema, hemorrhage in the subcutis, exudative inflammation, necrosis and ulceration in the epidermis, and degeneration and regeneration of subcutaneous

skeletal muscle were the findings associated with the administration of control and test materials. Histologic findings in the inguinal lymph nodes were consistent with the immunodeficiency in this strain of mouse with no significant differences between the groups.

Conclusions:

In this study, it did not appear the local reactions associated with the administration of rhPH20 with or without Gammagard were different from the saline control. However, due to the small size of the model the application procedure was associated with local damage that could confound the results.

10.2. Study ---(b)(4)----- “Preclinical studies of the subcutaneous application of Gammagard Liquid after Hylenex pretreatment: Local Tolerance in the rabbit”

Aim: To evaluate the local tolerance of IG, 10% with and without rHuPH20 -(b)(4)-- after repeated SC application in rabbits.

Species: (b)(4) rabbits

Design: 5 groups, N=2M and 2F/group each injected with Gammagard liquid with rHuPH20 at a dose of 100 U/mL or 1,000 U/mL IG, 10% or with equivalent volumes of saline in the right flank and negative control (saline) in the left. As an additional control group, rabbits were treated with an equivalent volume of saline in combination with the high dose (1,000 U/mL) of rHuPH20 on the right flank and with the equivalent volume of saline alone on the left flank.

Outcome measures:

Cage-side behavior observation, macroscopic examination of the injection sites daily. Twenty four hours after administration, the group was euthanized and tissue samples were collected and fixed for histopathologic evaluation (H+E staining). The tissue samples included skin, subcutaneous tissue and muscle from the injection site along with regional lymph nodes and spleen.

Results: No local irritation at the injection site. A microscopic evaluation of the injection sites revealed inflammatory reactions in all groups treated with rHuPH20 and IG, 10% and saline and IG, 10%. These reactions increased in severity after repeated administration. “A remarkable enlargement of the local lymph nodes” was seen in IG groups. Very mild reactions were found in the group where rHuPH20 and saline was administered.

The inflammatory reactions after repeated administration appear to be due to anti-human IgG immune response. Also human anti-galactose α 1, 3 galactose antibodies binding to galactosyl epitopes on rabbit fibroblasts might also lead to immediate inflammatory reactions.

The animals treated with rHuPH20 alone had slight to moderate inflammation and enlargement of the lymph nodes.

10.3. Report ---(b)(4)---- “Local tolerance in the rabbit, (b)(4) effect”

In this local tolerance study in rabbits laboratory preparations of IG, 10% with different -----(b)(4)----- were administered SC. A total of three injections, one week apart were tested. In a similar result to study ---(b)(4)-----, IG 10% administration resulted in local inflammation. The (b)(4) of the preparation did not influence the inflammatory reaction.

References

1. Tammi R, Saamanen AM, Maibach HI, & Tammi M (1991); Degradation of Newly Synthesized High Molecular Mass Hyaluronan in the Epidermal and Dermal Compartments of Human Skin in Organ Culture. The Journal of Investigative Dermatology 97, 126-130.

2. Harris EN, Weigel JA, & Weigel PH (2004); Endocytic Function, Glycosaminoglycan Specificity, and Antibody Sensitivity of the Recombinant Human 190-KDa Hyaluronan Receptor for Endocytosis (HARE). *The Journal of Biological Chemistry* 279, 36201-36209.
3. Weigel PH & Yik JH (2002); Glycans As Endocytosis Signals: the Cases of the Asialoglycoprotein and Hyaluronan/Chondroitin Sulfate Receptors. *Biochimica Et Biophysica Acta* 1572, 341-363.
4. Laurent TC & Fraser JR (1992); Hyaluronan. *The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology* 6, 2397-2404.
5. Grigorieva A, Griffiths GS, Zhang H, Lavery G, Shao M, Taylor L, & Martin-DeLeon PA (2007); Expression of SPAM1 (PH-20) in the Murine Kidney Is Not Accompanied by Hyaluronidase Activity: Evidence for Potential Roles in Fluid and Water Reabsorption. *Kidney & Blood Pressure Research* 30, 145-155.
6. Li X, Elwell MR, Ryan AM, & Ochoa R (2003); Morphogenesis of Postmortem Hepatocyte Vacuolation and Liver Weight Increases in Sprague-Dawley Rats. *Toxicologic Pathology* 31, 682-688.