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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 (Hylanex, (hyaluronidase human injection))

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

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

CC: Jennifer Reed, PhD

Applicant: Baxter Healthcare Corporation

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

Subject: Preclinical Pharm-Tox Review

Introduction

This review is an addendum of the BLA review (attached) completed on July 27, 2012 that recommended complete response (CR).

Baxter's response to the CR letter was submitted as a series of amendments during the period of March-December 2013 (#26, 27, 29 and 30, 32, 33). Pharmacology and toxicology studies submitted in these amendments, as well as amendment 35 (April 2014), are reviewed here.

Conclusions

This BLA can be approved with Post-Marketing Commitment to assess safety of the product in a chronic study in animals.

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Executive Summary

See Tables 1 and 2 for listings of toxicology studies performed in support of this BLA; main findings from these studies are outlined below.

1. There was an increase in splenic weight in ---(b)(4)----- monkeys following repeated weekly SC administration of rHuPh20 for 39 weeks (report R09050). No dose relationship was seen and no microscopic observations were noted. These reactions could be due to immune response following repeated administration of the human recombinant protein (see item number 2). No other systemic toxicities were attributed to rHuPH20 administration in the 39 week monkey study.
 - a. A dose-related spleen enlargement was also seen in female mice at the end of study R07046, following repeated administration.
2. Weekly administration of rHuPH20 for 39 weeks in a subchronic study in ----(b)(4)--- monkeys (study number 09050) resulted in very high anti-rHuPH20 titers.
 - a. These antibodies were neutralizing to endogenous monkey PH20 (Study 12032).
 - b. These antibodies did not bind to the enteric nerve plexus (ENP) of these monkeys (Study 12192 (b)(4), nor were deposited to the ENP during the duration of study (study number 12184 (b)(4).
 - c. These antibodies bound to the interstitial connective tissue of the seminiferous tubules but did not penetrate the seminiferous tubules lumen or epididymal lumen (Study 12184). There were no adverse male and female fertility outcomes in this study.
3. Reproduction and development, including male and female fertility studies were performed in mice and rabbits. Reproduction endpoints were included in the 39 week study performed in monkeys.
 - a. In reproduction and development studies in mice (RT08176 and R09058) anti-rhPH20 antibodies were generated which cross-reacted with murine PH20 and HYAL5 (study 12096). The fetus was exposed to these antibodies during late gestation (Gestation Day 18) and lactation (study number 12096).
 - i. The developmental NOAEL for the fetuses was 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.
 - ii. Maternal NOAEL of rHuPH20 was 18 mg/kg/day (Table 2).
 - iii. The NOAEL for reproduction in the dams 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 was 9 mg/kg/day (study R09058 in mice).
 - b. In a reproduction and development study, including fertility in female rabbits (study number 12195) high titers of anti-rHuPH20 antibodies were generated in F0 rabbits pre-implantation and maintained during gestation. These antibodies transferred to the fetus (F1) in late gestation (gestation day 20). Antibody titers peaked on LD4 and gradually declined at the end of weaning.

- i. 60% of F0 and 30% of F1 rabbits developed antibodies that were cross reactive to rabbit PH20. Antibodies produced from a similar protocol in rabbits (studies 12124 and 12124-(b)(4)) weakly stained ENP and strongly stained testes.
 - ii. Exposure to anti-rHuPH20 antibodies prior to mating and throughout gestation had no effect on female mating and fertility. Also no effects were seen on embryo-fetal and postnatal development of the offspring including developmental milestones, growth, behavior, maturation or offspring mating and fertility.
- c. In a fertility study in male rabbits (study number 12208) high titer and cross-reactive antibodies were generated prior to mating. No adverse outcomes in male reproductive functions, mating behavior or fertility were observed.
- d. Endpoints of reproductive organ morphology and function in male and female ---(b)(4)----- monkeys were included in the 39 week subchronic study (study number 09050). The NOAEL was determined to be 2 mg/kg, the highest dose tested.
4. Local reactions were seen following combination product and to a much smaller extent, following rHuPH20.
 - a. 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 [~150X human dose] and 2 mg/kg) in study report R09050. Recovery was seen after the 4-week recovery period.
 - b. Gammagard 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. rHuPH20 SC administration with or without Gammagard were not judged to be different from the saline control in (b)(4) mice (Study R09131).
5. To address the remaining concerns outlined in the CR letter the sponsor will include restrictions of use in the label and commits to perform the following studies as a PMC.
 - a. To assess the possible toxicity of life-time exposure to anti-PH20 antibodies, a chronic study in mice will be performed.
 - b. To assess the possible toxicity of the anti-PH20 antibodies in pediatric patients, a study in juvenile mice will be performed.

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

Table 2: Tabulation of GLP toxicology studies with rHuPH20

Repeat-Dose Toxicity (b)(4)-- 7 days	Monkey, ----(b)(4)----	IV and SC, once daily	Yes	----(b)(4)----/ R08056
Repeat-Dose Toxicity (b)(4)-- 39 weeks	Monkey, ----(b)(4)----	SC, once weekly	Yes	258.01/ R09050
Embryo-fetal Development (b)(4)-- GD 6-15	Mouse, ----(b)(4)-----	SC, once daily	Yes	----(b)(4)----/ R08176
Pre/postnatal Development (b)(4)--- GD 6-DL 20 or GD 22	Mouse, ----(b)(4)-----	SC, once daily	Yes	----(b)(4)----/ R09058
Effects of Anti-rHuPH20 Antibodies on Male Fertility and General Reproduction	----(b)(4)----- ----- Rabbit	SC weekly for 5 weeks with 2 biweekly boosting	Yes	12208
Effects of Anti-rHuPH20 Antibodies on Female Fertility and Embryo-Fetal Development with Postnatal Assessments	----(b)(4)----- ----- Rabbit	SC weekly for 5 weeks with 2 biweekly boosting	Yes	12195
A GLP Juvenile Toxicity Study with an Extension Arm to Assess Chronic Exposure of Anti-rHuPH20 Antibodies	Mouse	Weekly or daily, PND7-PND108 and PND180	Yes	Preliminary stage under way; Study planned as PMC

Table 3: NOEL Determined in Toxicity Studies with rHuPH20 and the calculated Margin of Safety

Species	NOEL (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)---- U/mg).

Studies in the mouse

Study Number 12091; this study is an addendum to reproductive and development studies RT08176 and R09058.

Title: ADA response to rHuPH20 after SC administration to mice

Aim: This study was conducted to retrospectively evaluate the incidence and time course of antibodies to PH20 in nonpregnant female (b)(4) mice.

Design: Three dose and one control groups received daily administration of rHuPH20 for wither 5, 10 or 16 days, as shown in the table below (from the submission). Blood was collected via cardiocentesis on day 7, 12, 18, 36, 45 and 75.

Table 4

Group	No. of Mice¹	rHuPH20 Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Cessation Time points²	Blood Collection Time points³
1	12	0	5	SD16	Predose, SD75
2	36	3	5	SD5, SD10, or SD16	SD7, SD12, SD18, SD36, SD45, SD75
3	36	6	5		
4	36	9	5		

¹Six mice/group were sacrificed at each collection time point. In the event an insufficient number of animals were available for scheduled sacrifice, a decision was made regarding time point precedence. All sacrifice dates were

recorded in laboratory notebook(s).

²Corresponds to dosing through GD10, GD15, and GD21 (PND1) for Groups 2-4. Six mice/ treated group received

their final dose at SD5 and SD10. All remaining animals received their final dose on SD16.

³Blood was collected approximately 48h after respective animals' last dose on SD7, SD12, and SD18 to allow for a drug washout period. Samples collected on SD36, SD45 and SD75 were used to evaluate antibody persistence.

Outcome measures: Presence of anti-rHuPH20 binding antibodies and their cross-reactivity to murine PH20 by two methods: ----(b)(4)----- analyses against purified recombinant murine PH20 (rMuPH20) and a -----(b)(4)----- immunoassay to rMuPH20.

Results: All animals at the three levels groups of rHuPH20 generated anti-rHuPH20 antibodies by Study Day 12 with titers ranging from 1:50,000 to 1:31,250,000. High titers (~1:6,000,000) persist up to Day 75. The vast majority of the antibodies produced were cross-reactive to the mouse PH20.

Study Number: 12096; This study is an addendum to mouse reproductive and developmental toxicity studies R09058 and R08176.

Title: Evaluation of anti-drug antibodies (ADA) following administration of rHuPH20 by subcutaneous injection (development perinatal/postnatal study design)

Aim: To quantify the time course and extent of anti-rHuPH20 antibody exposure in pregnant female ----(b)(4)----- mice and their offspring (fetuses and pups) at time points that mimic the relevant developmental stages evaluated in prior GLP mouse embryo-fetal development (EFD) and peri/postnatal development (PPND) studies (R08176 and R09058, respectively).

Design: Four groups of pregnant (b)(4) mice received daily subcutaneous injections of rHuPH20 at 3, 6 or 9 mg/kg or vehicle control beginning on GD 6. Each group was further divided into subsets and subgroups as shown in table below (modified from the submission to include bleeding day). There were 6-12 animals per each subgroup (6-3 for the negative control group).

Table 5

Subset	Subgroup	Dosing Days ¹	Blood Collection Days
1	NA	GD 6 to GD 16	GD 18
2	A	GD 6 to LD 4	LD 8
	B	GD 6 to LD 13	LD 15
	C	GD 6 to LD 21	LD 23
3	NA	GD 6 to LD 21	LD 23
rHuPH20 plasma concentration	NA	GD 6 to GD 18	LD 23

¹ GD - Gestation day; LD - Lactation day; NA – Not applicable

Outcome measures: Blood samples were collected via cardiocentesis in F0 maternal mice and their F1 fetuses/pups, the plasma isolated and samples assayed for the presence of anti-rHuPH20 binding antibodies. Anti-rHuPH20 antibody positive plasma samples from F0 maternal and F1 offspring were characterized for ability to cross-react to murine PH20 and murine Hyal5 by ---(b)(4)----- immunoassays.

Results: All F0 animals injected with rHuPH20 generated circulating anti-rHuPH20 binding antibodies by GD 18 (first time point sampled), median titer 51,200. The antibodies persisted up to LD 23. No clear dose-effect relationship was observed.

There was transplacental transfer to fetuses on GD18 with a median titer of 1:19,200, i.e an average fetal:maternal ratio ~1:4. Both maternal and pup titers increased from GD 18 through LD 21 (Figures 1 and 2, respectively). Whereas maternal titers increased slightly on LD23, pups titers declined then and at all points thereafter, still remaining at appreciable levels.

Figure 1: Maternal Anti-rHuPH20 Antibody Titers (Groups 2-4)

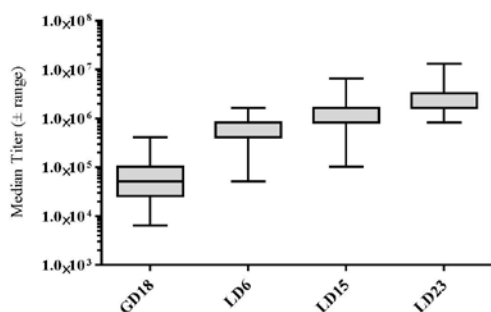
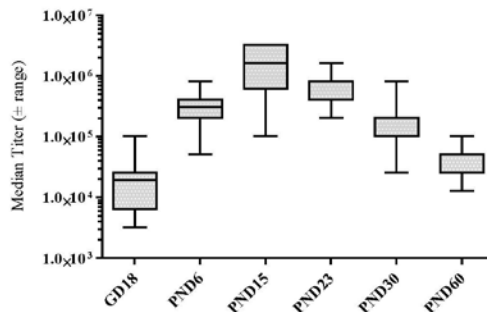


Figure 2: Fetal/Pup Anti-rHuPH20 Antibody Titers (Groups 2-4)



(b)(4) immunoassay analyses showed that 10-50% of the anti-rHPh20 antibodies detected in the fetal samples on GD 18 cross-react to rMuPH20 and rMuHyal5, respectively. This increases to 100% on LD 23. A significant correlation was observed between the titer and the likelihood of antibodies cross-reacting with murine endogenous hyaluronidases.

Conclusion: GLP (b)(4) mouse studies R09058 and R08176 did not reveal any effects on pre- and postnatal development in mice. Although the exposure to anti-PH20 antibodies in fetuses was not measured in that study, the present study shows that antibodies were likely generated starting on GD 18 and they persisted to PND 60. Drawback of the study remains that it did not measure antibody formation or transfer to fetuses at earlier time points, such as during implantation and organogenesis.

Study Number: 12115 (b)(4); This study is an addendum to study R08176

Title: Anti-rHuPH20 Antibodies from Mouse Embryo Fetal Developmental Toxicity Study

Aim: Ascertain that anti-rHuPH20 antibodies titers were present in samples collected on GD 15 in the dams in the embryo fetal developmental toxicity study (number R08176).

Design: this was a retrospective study assessing IgG levels in the dams on GD 15 from the previously collected TK blood samples. These analyses showed that in all three rHuPH20-treatment groups (3, 9, and 18 mg/kg/day), all F0 mothers had developed anti-rHuPH20 antibodies on GD 15 of the IgG isotype. There was a dose response effect in the highest titers of antibodies produced, however the variation of the individual titers within groups was significant.

Studies in the monkey

Study Number: 12116

Title: Retrospective analysis of immunization study in monkeys, signed report

A breeding study in ---(b)(4)--- monkey was conducted at the -----(b)(4)----- to assess if immunization with PH20 could prevent fertilization in females. No reduction in birth rate was seen in females immunized with recombinant ---(b)(4)--- PH20 protein prior to and during pregnancy. No adverse effects of anti-PH20 antibodies on fertility or adverse effects on offspring development were reported in this study.

Study Number: 12032 (b)(4); this study is an addendum to study R09050

Title: ---(b)(4)----- Monkey Anti-rHuPH20 Antibody Neutralization of (b)(4)-PH20

IgG---(b)(4)--- from the blood samples collected from animals in the TK arm of the ---(b)(4)--- monkey study R09050 (see BLA memo for study design details, # R09050) were isolated and were assayed for 1) binding and 2) neutralization activity. ---(b)(4)--- PH20 was isolated from monkey sperm. 5/8 monkeys contained neutralizing activity against endogenous monkey PH20 at different titers. These antibodies were able to stain monkey testis (Halozyne Report No. 12192). The study R09050 had determined that these monkeys developed antibodies that also bound and neutralized rHuPH20.

Study Number: 12192 (b)(4)

Title: Cross-Reactivity of Anti-rHuPH20 Antibodies to Monkey Tissues

Aim: To investigate if monkey anti-rHuPH20 antibodies generated in the 39-week toxicity study R09050 bound to monkey tissues. Polyclonal IgG from plasma of one monkey with high titer of anti-rHuPH20 antibody, rabbit polyclonal anti-rHuPH20 (pAb), and a mouse anti-rHuPH20 monoclonal antibody (mAb) cocktail specific for human testis were used in this study. Staining of the seminiferous tubules in monkey testis was shown for all antibody preparations. Only the rabbit pAb had what is described as weak diffuse staining in colon enteric nerve plexus (ENP). No staining was observed in colon tissue with mouse mAb cocktail or the monkey pAb preparation.

Study Number: 12184 (b)(4)

Title: IgG Deposition in the Enteric Nerve Plexus and Testis of Monkeys

Aim: to determine whether IgG molecules could penetrate into the enteric nerve plexus (ENP) of ---(b)(4)--- monkeys that had been treated with rHuPH20 for 39 weeks and had developed anti-rHuPH20 antibodies.

Slides of formalin fixed paraffin embedded colon sections and ---(b)(4)--- fixed paraffin embedded testis and epididymis sections (5 µm) from a GLP 39 week subcutaneous toxicity study of rHuPH20 (number R09050) were treated with biotinylated goat anti-monkey IgG primary antibody. Negative control was antibody blocked with monkey IgG.

566 plexuses from colon sections from the majority of male and female animals in all dose groups were analyzed. 7 ENPs (1.2%) showed low intensity staining for IgG within the plexus which was determined an artifact of fixing/processing or organ collection at necropsy (located at the edge of the tissue sections, close to folded tissue, or adjacent to tissue tearing); there was no statistically significant dose-effect relationship.

Testis sections from 16 males (4/dose group) in each dose group showed monkey IgG labeling was restricted to the interstitial connective tissue between the seminiferous tubules, but no staining was seen in the lumen.

~250 epididymal lumen profiles for each of 16 males in the study were examined (~4000 individual lumens). Weak staining was observed in one lumen of three animals, one each in negative control, low dose, and high dose groups. This was also considered artifact of processing.

Studies in the rabbit

Study Number: 12195

Title: Effects of Anti-rHuPH20 Antibodies on Female Fertility and Embryo-Fetal Development with Postnatal Assessments in the -----(b)(4)----- Rabbit

Objective: to investigate potential adverse effects of anti-rHuPH20 antibodies during mating and fertility and on offspring development following exposure of the female rabbits to anti-rHuPH20 antibodies before mating and through mating, implantation and closure of the hard palate.

Animal Species: Rabbit

Justification: The sponsor considers rabbit the appropriate species for the study based on observations of transplacental transfer of the antibodies at the end of gestation.

Study Design (GLP): Eighty-eight female rabbits were randomized into a buffer control group (N=44) and a rHuPH20 dosed group (N=44). The 44 rabbits per group were equally allocated to the Cesarean section or natural delivery cohorts. The test facility's historical control database was also used for interpretation of any findings. Female rabbits were dosed with single subcutaneous injections of 0.76 mg/kg/dose rHuPH20 on study day (SD) 1, 8, 15, 22, 29 and 42 in order to generate and maintain anti-rHuPH20 antibody titers prior to mating (based on results of the pilot immunization study in rabbits. Anti-rHuPH20 antibody titers were measured in maternal plasma samples at weekly intervals from baseline through SD29, on SD42, and on GD15 and GD29. The rHuPH20 dosed females were mated to non-dosed male breeders on SD44.

All females were super-ovulated with human chorionic gonadotropin after mating and the dose group received a boost of rHuPH20 on GD15 to maintain antibody titers throughout gestation.

The Cesarean section phase was evaluated on GD29 (fetal external, visceral and skeletal examinations). The dams in the natural delivery phase were euthanized at the end of lactation.

Prewearing, pups were evaluated for survival, clinical signs, body weight and developmental landmarks (hair growth, eye opening, air righting, acoustic startle and pupil constriction). An equal number of male and female pups from each litter (1/sex/litter, where possible) were randomly selected at weaning for additional postnatal observations through adulthood, including body weight, food consumption, sexual maturation, neurobehavioral testing and assessment of fertility and mating outcome.

A graphical representation of the study is shown in figure 3 (From submission).

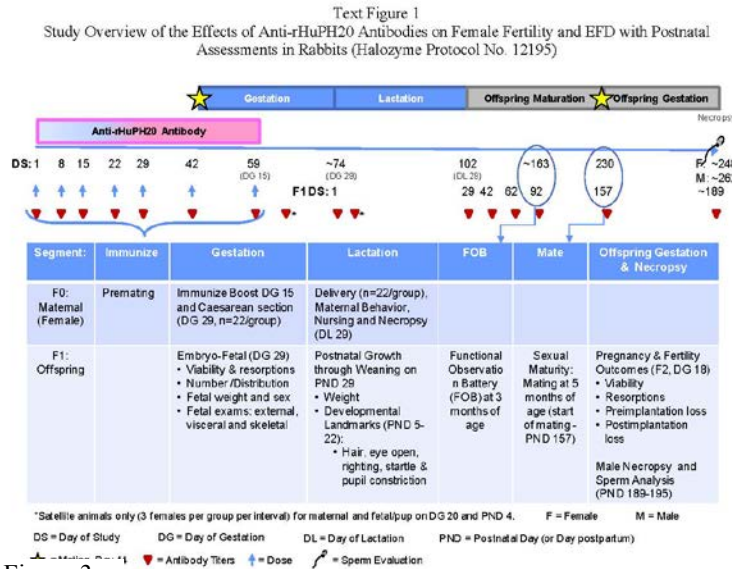


Figure 3

Text Figure 1: The day of the study (DS) is indicated along the timeline. The day of mating was DS 44 and was designated Day 0 of Gestation (DG 0). The blue arrows denote dosing days. Female rabbits (F0 generation) were administered rHuPH20 or buffer control subcutaneously (blue arrows) prior to mating and also received an additional dose on DG 15 to ensure titers remained elevated throughout gestation. Plasma samples were collected for analysis of antibodies as indicated (red triangles). The rabbits in the Caesarean-sectioning phase (n=22/group) were evaluated for fertility and embryo-fetal development near the end of gestation (DG 29). The rabbits in the natural delivery phase (n=22/group) were evaluated for maternal behavior and pup survival, growth and development. The day of delivery (birth) was designated as Postnatal Day (PND) 1 and is also referred to as Lactation Day 1 (LD) 1, Day of Lactation (DL) 1 or Day 1 of postpartum. The end of weaning for the offspring (F1 generation) was PND 29 (LD 29 or Day 29 postpartum). Pups (1/sex/litter, where possible) were randomly selected for postweaning assessments including neurobehavioral testing, fertility and mating outcomes (Caesarean-sectioning evaluated on DG 18 of F2 generation).

Results: All the does have high titers of anti-PH20 antibody starting on day of study (DS) 15. In late gestation (GD20), there were high titers in fetuses.

Peak median titers were maintained from SD42 through GD29 (1:3,276,800). Three rHuPH20-treated satellite females were sacrificed at GD20, and the anti-rHuPH20 antibody titers determined in maternal and fetal plasma (pooled per litter). Titers in GD20 does ranged from 1:204,800 to 1:1,638,400. The titers in all three pooled fetal samples were 1:204,800. These data indicate transfer of anti-rHuPH20 Ig to fetuses in late gestation.

In the F1 generation, 100% incidence of exposure to anti-rHuPH20 binding antibodies was observed up to PND 62, declining thereafter. Peak median titer was observed in the F1 generation at LD 4 (median 3,276,800). Thereafter, the median titer in the F1 generation gradually declined to the end of weaning (LD 29). A median titer of 200 was observed in the F1 generation animals that maintained detectable anti-

rHuPH20 antibodies on the Day of Mating (~PND 152). 60% of F0 and 30% of F1 rabbits developed antibodies that were cross reactive to rabbit PH20.

There were 3 mortalities – 2 in the control and one in the dosing group. Mortalities and clinical observations were not directly attributed to rHuPH20 treatment or anti-rHuPH20 antibodies. Mating (96% and 100%) and fertility (94% and 88%) were comparable between control and treatment groups. In the cesarean section group, there were no gross lesions in does and fetuses and no differences in the numbers of corpora lutea, implantations, litter sizes, live fetuses, fetal weights and pre- and post-implantation loss between the buffer control-treated and rHuPH20-treated groups.

In the natural delivery group there were no anti-rHuPH20 antibody-related maternal or pup findings. All rHuPH20-treated females delivered a litter and there were no stillborn pups. The duration of gestation, number of pups per litter, pup survival and pup body weights were comparable between the buffer control-treated and rHuPH20-treated groups. At necropsy of does, no gross lesions occurred. Developmental landmarks of normal growth and neurological development at the end of weaning were comparable between the groups. There were no unusual necropsy observations in the pups examined prior to weaning or in the pups not selected for continued evaluations and euthanized at weaning. All F1 generation rabbits survived to adulthood except for one rHuPH20-group female found dead on postpartum day 69. No clear cause of death could be determined. Clinical signs noted for this rabbit included fecal stained fur and soft or liquid feces on PND 68. A general body weight gain was seen in this rabbit from PNDs 43 to 64 and a 12% (219 g) body weight loss was seen from PNDs 64 to 69. This was not considered related to the presence of anti-rHuPH20 antibodies because it was a single event. Male F1 offspring did not have changes in sperm parameters, male reproductive organ weights or mating and fertility outcomes. There were no gross necropsy observations in the F1 generation male and female rabbits.

Conclusion: Maternal exposure to anti-rHuPH20 antibodies prior to mating and throughout gestation had no effect on mating and fertility. Maternal anti-rHuPH20 antibodies transferred to their offspring at the end of gestation and had no effect on embryo-fetal or postnatal development of offspring including developmental milestones, growth, behavior, maturation or offspring mating and fertility.

Study Number: 12208

Title: Effects of Anti-rHuPH20 Antibodies on Male Fertility and General Reproduction in the -----
----- (b)(4) ----- Rabbit

Objective: To detect potential adverse effects of anti-rHuPH20 antibodies on male fertility and early embryo-fetal development. Antibodies were induced by treatment of male (b)(4) rabbits with rHuPH20 for a period of time prior to mating to untreated female rabbits.

Study Design (GLP): Forty-four male rabbits at least 4 months old at the time of arrival at facility, were randomized into a buffer control group (N=22) and a rHuPH20 treated group (N=22). Forty-four virgin untreated female rabbits were also included in the study for 1:1 mating to assess fertility endpoints. The vaccination/boosting schedule was similar to the study in females and is schematically shown in Figure 4 (from submission).

Figure 4

Study Overview of the Fertility and Early Embryo-Fetal Development Study of Anti-rHuPH20 Antibodies in the Male Rabbits



The day of the study is indicated along the timeline (thin blue arrow from left to right). Male rabbits were treated with rHuPH20 to generate anti-rHuPH20 antibodies or buffer control (short blue arrows) prior to mating with untreated females. Plasma samples were collected for analysis of anti-rHuPH20 antibodies as indicated (red triangle). Semen samples were collected on DS 30, 37, 49 and 71 (as indicated by sperm figures). DS 30 served as a semen sample collection test run, therefore semen samples were collected but not analyzed.

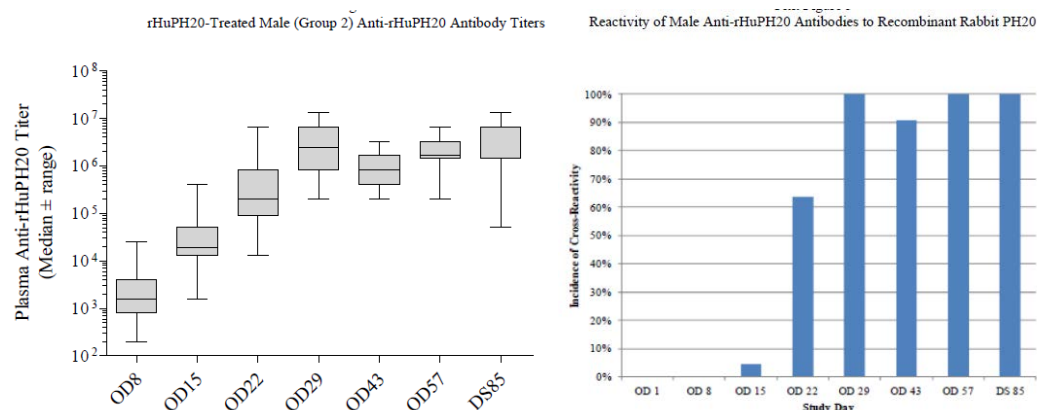
Sperm analysis was performed prior to mating (DS 37), after mating (DS 49) and prior to necropsy on DS 71. The untreated female rabbits underwent Caesarean sectioning on DS 63/day of gestation (DG) 18 to assess fertility outcome (parameters presented in text table 1 above: terminal evaluations-females). The male rabbits were retained on study until the outcome of mating was known and received an additional dose of rHuPH20 as a booster on DS 57 to maintain anti-rHuPH20 antibody titers until terminal necropsy. The terminal necropsy of the males was on SD85, after all reproductive assessments were complete.

Blood samples were collected prior to dosing, and on day of study (DS, also study day SD or day of observation, DO) 1, 8, 15, 22, 29, 43 and 57, and on the day of necropsy (DS 85) and were tested for anti-rHuPH20 binding antibody titers, binding to recombinant rabbit PH20 (rRbPH20) and anti-rHuPH20 neutralizing activity. Semen samples were collected for all buffer control-treated or rHuPH20-treated male rabbits prior to mating on DS 37, following mating on DS 49, and again on DS 71 and were analyzed for motility and sperm count utilizing the ----(b)(4)----- for computer-assisted sperm analysis (CASA) or manually for sperm morphology. On DS 44, naïve female rabbits were mated with rHuPH20-treated or buffer control-treated male rabbits at a 1:1 ratio. Females with evidence of mating were super-ovulated with human chorionic gonadotropin within two hours of mating. On DG 18, the females underwent ovarian and uterine examinations to assess the fertility by the number and distribution of corpora lutea, implantation sites, placentae (size, color or shape) and viable and nonviable embryos. A total of 17 and 21 confirmed pregnant female rabbits per buffer control-treated and rHuPH20-treated groups, respectively, were evaluated for fertility index and overall fetal viability.

Results: The time course and magnitude of the anti-rHuPH20 antibody response in rHuPH20-treated males is presented in Figure 5. By DS 8, all rHuPH20-treated males were positive for anti-rHuPH20 binding antibodies which were maintained through terminal necropsy (DS 85). Median titers increased from 1,600 on DS 8 to 2,457,600 on DS 29. Median titers of $\geq 819,200$ were maintained from DO 29 (2,357,600) through DS 85 (6,553,600). These antibodies were neutralizing to rHuPH20. Cross-reactivity

to recombinant rabbit PH20 was first observed at DS 15, and then increased to 100% incidence by DS 29. Greater than 90% incidence of cross-reactivity to recombinant rabbit PH20 was maintained from DS 29 through DS 85.

Figure 5



12 of 22 of rHuPH20-treated males had erythema grade 1, 4 males had grade 2 erythema and 2 males had grade 3 erythema at the injection site after the 5th dose. There were no statistically significant changes in body weight compared to buffer-treated controls. Semen analyzed on DS 37, 49 and 71. The only statistically significant difference was reduced number of static or non-motile sperm in the rHuPH20-treated males on DS 37 but not on DS 49 or DS 71. This was not regarded as related to anti-rHuPH20 antibodies, and was not considered adverse. There were no adverse findings on necropsy. After mating, there were no statistical differences in the fertility index, number of corpora lutea, implantation sites, or viable and nonviable embryos or pre-/postimplantation loss.

Conclusions: In this study there were no adverse outcomes following exposure to anti-PH20 antibodies with respect to male reproductive functions, mating behavior or fertility.

Study Number: 12124 and 12124-(b)(4)

Title: Antibody Response against rHuPH20 in -----(b)(4)----- Rabbits Following Three Separate Immunization Procedures

Aim: To evaluate the incidence, time course and magnitude of anti-rHuPH20 antibodies in non-pregnant female and male -----(b)(4)----- rabbits to aid in the design of traditional rabbit embryo-fetal development (EFD) and peri/postnatal development (PPND) studies.

Design: A total of sixteen female (b)(4) rabbits and two male (b)(4) rabbits were dosed either once daily with rHuPH20 (----- (b)(4) -----) for 28 days, with a booster injection thereafter or once weekly ----- (b)(4) ----- for five weeks, with a booster on day 50. A vehicle control group was also included.

Outcome measures: Anti-rHuPH20 antibody titers, cross-reactivity to recombinant rabbit PH20 (rRbPH20), tissue binding of purified generated antibodies to colon ENP and testes.

Results: Either a daily or weekly subcutaneous dosing of female rabbits can generate high-titer, sustained levels of anti-rHuPH20 antibodies that cross-react to rRbPH20 at a high incidence (Study 12124).

Anti-rHuPH20 antibodies specifically bind rabbit PH20 in spermatozoa and testis tissue, irrespective of the dosing regimen or IHC method. The staining of rabbit colon containing ENP by anti-rHuPH20 antibodies is determined to be nonspecific (Study 12124-(b)(4)).

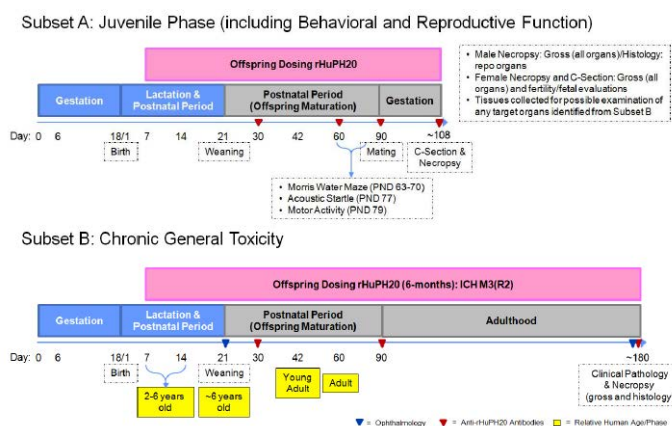
PMC Study

Number – Not assigned

Title: Protocol Synopsis: A GLP Juvenile Toxicity Study with an Extension Arm to Assess Chronic Exposure of Anti-rHuPH20 Antibodies in the Mouse

Aim: to identify potential toxicity of persistent anti-rHuPH20 antibodies exposure in juvenile mice dosed with recombinant human hyaluronidase (rHuPH20) from postnatal day (PND) 7 to adulthood (PND 90) and the potential impact on adult neurobehavioral and reproductive function (Subset A). An extension arm of the study will further assess the potential toxicity of chronic exposure of anti-rHuPH20 antibodies from juvenile exposure through maturity into adulthood.

Study Design: The study animals will be divided into two subsets: subset A, the juvenile phase study (n=30/sex/group) will assess behavior, mating and fertility. Subset B, the chronic phase, (n=20/sex/group) includes general toxicity assessments through 6 months of age (e.g., gross necropsy and histology on all tissues). Animals in both Subset A and B will be dosed subcutaneously either daily or weekly based on results of an ongoing dose-range finding juvenile study. Only one dose level will be used based on the observation that antibody titers do not generally increase linearly with the dose. A schematic of the study design is shown in Figure 6.



Outcome measures: Clinical signs, body weights will be evaluated in all animals; plasma anti-rHuPH20 antibody titers from all animals in Subset A on postnatal days (PND): 30, 60, 90 (prior to mating) and 108 (maternal and male necropsy) and from all animals in subset B on PND 30, 90 and 180 days (6-months necropsy). All the analyses are shown in Table 6 (from submission).

Table 6

Parameter	Subset A Juvenile Phase	Subset B Chronic
Animal Number (N)	30/sex/group	20/sex/group

Dose Period	PND 7 to PND 108 ^a	PND 7 to PND 180
Clinical Observations/ Body Weight	Yes	Yes
Sexual Maturation	PND 21 (female) and PND 27 (male)	NA
Morris Water Maze	PND 63-70	NA
Acoustic Startle	PND 77	NA
Motor Activity	PND 79	NA
Anti-rHuPH20 antibody titers	PND 30, 60, 90 and PND 108 (end of in-life) ^a	PND 30, 90 and 180
Clinical Pathology	NA	PND 160
Ophthalmology	NA	PND 21 and ~180
Start of Mating	PND 90 ^a	NA
Fertility Endpoints and Caesarean Section	GD 18	NA
Fetal Examinations: sex, weight, external, visceral and skeletal	Yes	NA
Necropsy	Male and Female (C-section): PND 108 ^a	PND 180
Tissue Collection/Organ weights	Standard tissue panel	Standard tissue panel
Histology	Male reproductive organs & Target organs from Subset B (if any)	Standard tissue panel

Justification for study design:

Study No. 12115 showed that anti-rHuPH20 antibodies that develop in juvenile mice are cross-reactive to endogenous mouse PH20.

The rationale for the duration of rHuPH20 dosing of juvenile from PND 7 through PND 90 for Subset A was selected to capture the developmental periods when neurogenesis, sexual maturation and fertility assessments are typically performed in mice and when there are large historical control data sets for each of these developmental milestones. 6-Month Chronic Assessments (Subset B): The rationale for selection of the 6-month termination for assessment of chronic effects of anti-rHuPH20 antibodies was based on ICH guidance S6(R1) and M3 (R3) and (Clarke *et al.*, 2008).

To increase the power of the study an observation in a juvenile animal will be compared to the buffer treated controls and to the historical control data at the -----(b)(4)----- The type, severity, frequency, and progression of an effect, its biological significance in the absence of dose-response information will also be considered. The juvenile mice will be placed on study with litter mates to serve as controls. Any potential findings will also be evaluated for possible familial associations and other possible non-study related causes.

Reviewer's conclusions: The study as design is acceptable based on ICH guidance and literature surveys. One possible drawback of the species selection is that PH20 insufficiency in the mouse may be compensated by HYAL5 making effects in fertility harder to detect. To mitigate this, the label will include restrictions of use and a clinical study to be performed as a PMC.

References

Clarke, J., Hurst, C., Martin, P., Vahle, J., Ponce, R., Mounho, B., Heidel, S., Andrews, L., Reynolds, T., & Cavagnaro, J. (2008). Duration of chronic toxicity studies for biotechnology-derived pharmaceuticals: is 6 months still appropriate? *Regul. Toxicol. Pharmacol.* 50, 2-22, doi:S0273-2300(07)00107-9 [pii];10.1016/j.yrtph.2007.08.001 [doi].