BLOOD PRODUCTS ADVISORY COMMITTEE
110th Meeting, July 31, 2014
Great Room, White Oak Building 32, FDA
10903 New Hampshire Avenue
Silver Spring, MD

ISSUE SUMMARY

Topic I. Recombinant Human Hyaluronidase (rHuPH20) combined with Immune Globulin (Human) (IG10%), for treatment of subjects with Primary Immune Deficiency (PI) administered subcutaneously (SC): Benefit/Risk Considerations with particular focus on immunogenicity of recombinant human hyaluronidase.

Issue: FDA seeks recommendations from the Committee regarding the benefit/risk profile of HyQvia, including an assessment of the long term clinical impact of rHuPH20 directed antibodies and risk mitigation strategies, if needed.

Questions to the Committee

1. Do the available data indicate a favorable benefit/risk ratio for HyQvia taking into consideration the antibodies detected against PH20 which bind human tissues namely, reproductive tract, neurological and gastrointestinal tract tissues?

2. Would the Benefit:Risk assessment be influenced by mitigation strategies, including:

    a. restricted labeling for certain PI population subsets, e.g., pregnant women, male children, patients with inflammatory conditions involving the nervous system or gastrointestinal tract;
    b. implementation of risk communications to help subjects and physicians make informed decisions about whether to use HyQvia?

Background:

HyQvia is a combination product consisting of one vial of IG, (10%) and one vial of recombinant human hyaluronidase (rHuPH20; 160 U/ml), intended for treatment of PI. The IG component is manufactured by Baxter and is identical to Gammagard Liquid IGIV, licensed by the FDA on April 27, 2005 (STN 125105). The rHuPH20 component is a fully human PH20 lacking the carboxy-terminal lipid anchor. The rHuPH20 is manufactured by Halozyme, the manufacturer of HYLENEX, which was approved by FDA on

December 2, 2005 (NDA 21-859). The IG and rHuPH20 components are administered sequentially and subcutaneously. The rHuPH20 product is administered first in order to transiently degrade the glycosaminoglycan hyaluronan in the extracellular matrix of the SC space. IG is then administered through the same infusion set. The rHuPH20 is intended to permit larger volumes of IG to be infused subcutaneously than would be possible with IG alone, resulting in less frequent administrations, namely, dosing every 3 to 4 weeks instead of weekly and reduced total time required to complete an infusion. The potential advantages of HyQvia over current SC products are to allow for a larger total volume of infusion and a reduced total infusion time with the overall goal to extend the dosing interval from once weekly to once every 3-4 weeks. Use of HyQvia SC, therefore, would mimic the IGIV dosing schedule using the IGSC route of administration. In this document, HyQvia and, IGSC 10% and rHuPH20, are used interchangeably.

Baxter's IG product (Gammagard) is licensed as replacement therapy for primary immunodeficiency diseases (PI) in adult and pediatric patients two years of age or older; the proposed indication for HyQvia is PI for adult patients (≥16 years of age)..

Polyclonal immune globulin products carry different indications for use in a wide variety of diseases including: PI (including but not limited to common variable immunodeficiency such as hypogammaglobulinemia, X linked agammaglobulinemia, Wiskott Aldrich Syndrome, severe combined immunodeficiency), idiopathic immune thrombocytopenia, chronic inflammatory demyelinating polyneuropathy, chronic lymphocytic leukemia, Kawasaki disease, multifocal motor neuropathy and prevention of infection in pediatric HIV infected patients.

IG products can be administered intravenously (IV), subcutaneously (SC) or intramuscularly (IM). There are currently ten US-licensed IGIV products (Gammagard Liquid, Gammagard S/D, Gammaplex, Carimune NF, Privigen, Flebogamma 5% and 10%, Octagam 5%, Gamunex-C, and Bivigam), three licensed IGSC products (Gammagard Liquid, Gamunex-C and Hizentra) and one licensed IGIM product (GamaSTAN S/D). All products are indicated for PI and typically are given once weekly or biweekly (once every two weeks), or every 3-4 weeks, depending on the disease being treated. Patients with PI receive life-long treatment starting from a very young age.

IGIV doses in patients with PI are typically 300-800 mg/kg at 3-4 week intervals. When switching a patient from IGIV to SC dosing, the total monthly dose is divided into weekly or biweekly doses using a correction factor listed in the product labeling, thus increasing the frequency of infusions. IGSC can be more easily administered at home and is preferred by some patients for its convenience. However, IGSC products are somewhat limited in terms of the total dose that can be administered to a patient at any given time. There are limits to the amount (volume) of the product that can be infused into each injection site and greater volumes may require a greater number of sites for infusion.

Section 1: HyQvia Clinical Studies: Efficacy and Safety

Clinical Trial Roster

The HyQvia development plan consisted of prospective clinical trials conducted in the pediatric, adolescent and/or adult populations from 2006-2010. It was comprised of two phase 1 trials in healthy adult volunteers (161001, 170901) and three trials in PI subjects: a phase 1/2 trial (160602), a phase 2/3 trial (160601) and a phase 3 trial (**160603**) in PI subjects. Two other clinical trials, a 160603 extension study (160902) and a phase 2/3 safety study (161101), were initiated subsequently to assess longer-term safety. Following a discussion with FDA regarding a request for additional nonclinical data related to anti-rHuPH20 antibodies, treatment with rHuPH20 was discontinued at FDA's request in both ongoing studies. **APPENDIX 1** provides an overview of these trials in terms of study design, sample size, population, study objectives, and investigational products used.

Pharmacokinetics

A PK study consisting of three arms, namely IGIV, IGSC and IGSC with rHuPH20 (HyQvia), was performed. The results of the study indicated that the bioavailability of HyQvia (92% compared to IGIV) was increased by 20% compared to IGSC without hyaluronidase (72% compared to IGIV). As a consequence, the correction factor for dosing HyQvia in patients previously receiving IGIV would be minimal i.e 108% of the IGIV dose. In contrast, the correction factor for dosing IGSC without hyaluronidase in patients previously receiving IGIV, is 137% of the IGIV dose.

Subject Disposition

Subjects were withdrawn prematurely for various reasons. See **APPENDIX 2**.

Summary of Efficacy

Three data sets from the phase 3 trial (Study 160603) were analyzed for efficacy:

(a) Full Analysis Set (N=83): all subjects exposed to either or both study drugs (IGIV or HyQvia) and who provided data for the primary endpoint for any period of time

(b) Per-Protocol Data Set (N=74): subjects who completed at least 6 months of HyQvia treatment after the "ramp up" (the term used to describe a gradual increase in treatment interval and/or dose during initial exposure of the subject to the product)

(c) IGSC Naïve Subjects Data Set (N=44): subjects who had not previously been exposed to IGSC without rHuPh20.

The primary endpoint was met: the rate of annualized Acute Serious Bacterial Infections (ASBI) was significantly <1.0 (threshold for demonstrating substantial evidence of efficacy, FDA Guidance)[1]. The outcomes of the secondary efficacy endpoints were comparable between IGIV and HyQvia: days off from school/work, days on antibiotics, number of acute physician visits, days in hospital, days in hospital due to infection, and reduction in all infections. See **APPENDIX 3, Tables 3A, 3B, 3C and 3D.**

Summary of Safety

[1] FDA Guidance to Industry: Safety, Efficacy, and Pharmacokinetic Studies to Support Marketing of Immune Globulin Intravenous (Human) as Replacement Therapy for Primary Humoral Immunodeficiency, June 2008

Serious adverse events (SAE) and non-serious adverse events (AE) captured in clinical trials of PI subjects were subdivided into systemic and local adverse reactions (AR). The commonest AE in HyQvia subjects was infusion site pain.

In the phase 3 trial (Study 160603), 87 subjects received 365 infusions of IGIV and 1359 infusions of HyQvia (ramp-up phase: 230 infusions; efficacy phase: 1129 infusions);this was expanded to 1600 infusions of HyQvia during the Extension Study 160902. Although the AE rate per infusion was slightly lower overall in the HyQvia cohort than in the IGIV cohort, the rate of local AEs of mild and moderate intensity was disproportionately higher in the HyQvia arm (local AEs of severe intentity were uncommon and no different between groups); infusion site pain was the commonest local AE reported in HyQvia subjects. See **APPENDIX 4, Tables 4A, 4B and 4C.**

The applicant reported 9 of 87 cases with hemolysis. These subjects were asymptomatic. Upon further analysis of these cases including a review of the laboratory data, it was concluded that the degree of hemolysis was not clinically significant.

IMMUNOGENICITY OF rHuPH20

Section 1: Immunogenicity of rHuPH20 in Clinical Trials

The Applicant has provided data to show that rHuPH20 in HyQvia is a truncated, soluble version of the native human protein, lacking the carboxy-terminal GPI anchor present in native PH20. The Applicant's recombinant PH20 enzyme is expressed in Chinese hamster ovary cells transfected with a PH20 expression vector. A multi-step column chromatographic system is used to purify rHuPH20, in a process which limits host cell contaminating proteins in the final product. The Applicant has demonstrated that the purification process results in concentrated hyaluronidase activity that is 100-fold greater than that found in tissue extracts. The purified enzyme has a molecular mass of 65 kDa, with O-linked and N-linked glycosylation sites. ----------
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Of the subjects receiving HyQvia in Study 160603, 13/81 (16%) tested positive for PH20-directed antibodies, using a sensitive in vitro assay. An additional 2 patients developed PH20-directed antibodies during the extension study. Longitudinal analyses of PH20-binding antibodies in these patients are presented in **Appendix 5A**. This was an unexpected finding as PI subjects characteristically have reduced ability to develop an antibody response when challenged with antigens. In addition, the human rHuPH20 was expected to have similar features to native PH20, and elicit low or no immune responses similar to a self-antigen. No specific adverse events were found related to the PH20-directed antibody response during study 160603 or in the extension study 160602, although these studies were not designed to perform an evaluation of possible immunogenicity-related long term adverse events.

In order to better understand the possible risks associated with anti-PH20 antibody responses, the Applicant performed immunohistochemical studies first to evaluate endogenous PH20 expression (**Appendix 5B**). A high titer experimental (rabbit) antiserum generated against rHuPH20 demonstrated clear specific binding in the seminiferous tubules of testis, as expected. Additional weak specific immunoreactivity was unexpectedly observed in human enteric plexus tissue. In a follow-up study, the Applicant observed that treatment-emergent anti-PH20 antibodies were able to bind endogenous PH20 in male reproductive tissue. However treatment-emergent antibodies did not bind to enteric plexus tissue, nor did other antibody reagents directed against PH20 (summarized in **Appendix 5B**).

In response to an information request, the Applicant performed several new analyses of PH20 directed antibodies in the general population, and a retrospective analysis of subject samples collected in previous clinical trials using rHuPH20 (Table 1). In the prospective study, it was found that low titers (typically between 20 and 40) of non-neutralizing anti-PH20 antibodies do occur in a small subset of the general population, with an incidence of approximately 5-7%. Similarly, in previous clinical study experience, PH20 directed antibodies were observed in approximately 5-7% of study participants at baseline. The retrospective analysis of stored sera from previous trials showed no notable increase in incidence or titer of anti-PH20 antibodies in subjects receiving repeated SC administrations of rHuPH20. No risk of a PH20-directed antibody response had been detected, and no data associating Hylenex with anti-PH20 antibody responses has emerged from passive reporting or other clinical studies. In contrast, PI subjects receiving rHuPH20 demonstrated a distinct increase in PH20-directed antibodies, with some sustained titers substantially higher than observed in the general population (>10,000; Table 1).

Shortly after these analyses were submitted, FDA received reports of high-titer PH20-directed antibodies in a separate ongoing clinical evaluation. In this second study, subjects with hereditary angioedema (HAE) received repeated, rHuPH20-facilitated subcutaneous doses of plasma-derived C1 esterase inhibitor. The sponsor of the C1 esterase inhibitor clinical trial chose to terminate the study ahead of planned completion due to rHuPH20 immunogenicity. Extended follow up on these subjects is not currently available. The following table shows antibody titers against PH20 when used with other products.

Table 1. Baseline and Post treatment Prevalence and Titers of anti-PH20 Observed in rHuPH20 Clinical Studies and in Normal Volunteers*

Biotherapeutic	Population	rHuPH20 Dose	Baseline Prevalence	Baseline Titer Range	Post-Treatment Prevalence	Post-Treatment Titer Range
Insulins	--(b)(4)-- ------	----(b)(4)---------- --------- ----------	--(b)(4)-------- --------	--(b)(4)-------- --------	--(b)(4)------- ---------	--(b)(4)------ ----------
Herceptin (trastuzumb)	---------(b)(4)-------- ---------- ----------	---(b)(4)----	--(b)(4)-------- ---------	--(b)(4)------- ---------	--(b)(4)------- ---------	--(b)(4)------ ----------
MabThera (rituximab)	----------b)(4)-------- ---------- ----------	---(b)(4)---------- --	--(b)(4)-------- ----------	--(b)(4)-------- - -------	--(b)(4)--------- - ------	--(b)(4)------- -------
Immune Globulin for Injection, 10%	Primary Immunodeficiency	---(b)(4)---------- ---	1/81 (1.1%)	Negative-160	15/87 (17.2%)	Negative-81,920
C1 Esterase Inhibitor	Hereditary angioedema	24,000-48,000 units	3/47 (6.4%)	Negative-1,280	18/46 (39.1%)	Negative-5,242,880
----------	Plasma donors	None	56/961 (5.8%)	Negative-640	n/a	n/a
----------	General population	None	35/692 (5.1%)	Negative-1,280	n/a	n/a

**Summary data provided by the Applicant in June 2013, updated to include most recent information from a study of plasma-derived C1 esterase inhibitor administered with rHuPH20 (discontinued, final study information not publicly available). An increased incidence and titer of anti-PH20 is apparent in the two most recent studies, in which subjects received subcutaneous therapeutic preparations of plasma-derived Ig or plasma-derived C1 esterase inhibitor facilitated by rHuPH20.*

Overall, these findings could suggest a special risk of immunogenicity when rHuPH20 is chronically administered with complex biological therapeutics such as plasma-derived products. Gammagard Liquid, derived from the plasma of human donors, does contain PH20-directed antibodies at low titer. The Applicant's data suggested that PI patients receiving systemic Ig replacement would demonstrate a maximum anti-PH20 titer of 160. It is theoretically possible that rHuPH20 could form complexes with anti-PH20 in Gammagard Liquid during or after infusion, and that these complexes augment presentation of rHuPH20 antigens to the immune system. Alternatively or in addition, changes in manufacturing, formulation, or stability of rHuPH20 could underlie the apparently increased immunogenicity observed in recent clinical studies. The increased anti-PH20 responses in recent studies might also reflect the already recognized tendency toward autoantibody formation in both HAE and PI subjects (1,2). Whether these, or other possible mechanisms, contribute to anti-PH20 responses remains an open question requiring further study.

In all cases, treatment-emergent anti-PH20 antibodies analyzed to date have been characterized as non-neutralizing and lacking cross-reactivity to other human hyaluronidases. Since HyQvia subjects will

experience lifetime exposure to the rHuPH20 protein, and will likely experience complex health issues over that time, changes in immune recognition of rHuPH20 could be envisioned. Such changes could include the development of PH20-neutralizing antibodies, isotype switching to include IgE, cytolytic T lymphocyte responses, or epitope spreading resulting in antibody binding to, or neutralization of, other hyaluronidases (reviewed in 3). There are 6 members of the human hyaluronidase gene family, with divergent tissue distribution (Table 2).

Table 2. The Human Hyaluronidase Gene Family**

Name	Distribution	Deficiency results in
Hyal1	Lysosomal hyaluronidase with broad tissue distribution	Mucopolysaccharidosis IX and erosive arthritis (4)
Hyal2	Lysosomal hyaluronidase with broad tissue distribution	Increased plasma HA, some skeletal and hematological abnormalities (5)
Hyal3	Expression in GI, brain	No information on deficiency
PH20	Male reproductive tissue; lower levels in other tissues, including CNS	Anti-PH20 antibodies - possible fertilization impact in some animal models (6,7)
Hyal4	Placenta, skeletal muscle	No information on deficiency
HyalP1	Pseudogene	No information on deficiency

***Distribution and deficiency phenotypes associated with human hyaluronidases are shown. Summary of FDA analysis, June 2013.*

Disruption in Hyal1 or Hyal2 has been linked with bone and joint abnormalities (4,5), while clinical syndromes associated with deficiencies in Hyal3, Hyal4, or HyalP1 have not been described. Primary sequence similarity among the human hyaluronidases is approximately 30%, but increased homology is present in regions associated with enzymatic activity. An example of sequence similarity across hyaluronidases is provided in Table 3, using one immunodominant epitope associated with PH20 neutralization in guinea pigs and cross-neutralization of PH20 of unrelated species (7). These data could suggest that, with repeated exposure to rHuPH20, an antibody response recognizing an alternative hyaluronidase might be possible. The likelihood of generating cross-binding or cross-neutralizing antibodies in subjects receiving chronic administration of HyQvia is unknown. The potential clinical impact to HyQvia subjects of generating antibody responses to other members of the hyaluronidase family is unknown and uncertain.

Table 3. Comparison of Sequence Conservation for One Immunodominant PH20 Epitope***

Species	Protein	% Identity	% Homology
Guinea pig	PH20	100	100

Mouse	PH20	88	88
Chimpanzee	PH20	84	88
Rhesus macaque	PH20	84	88
Cow	PH20	80	84
Rabbit	PH20	80	88
Human	PH20	80	84
Human	Hyal2	68	77
Human	Hyal4	63	70

****An immunodominant peptide associated with cross-species neutralizing activity was identified within residues 94-119 of guinea pig PH20 (7). This peptide sequence was used to perform a BLAST search. Resultant hits are listed from highest to lowest similarity. FDA analysis, June 2013.*

Section 2: "Spreading Factor" Extracts Versus rHuPH20: Product Features Possibly Related to Immunogenicity

The many differences between recombinant and extract-based PH20 products argue against any assumptions regarding similarities in safety profile. Dispersion facilitating agents ("spreading factors") derived from bovine testes extracts have been commercially available since 1948 (reviewed in 8). Use of spreading factors was limited to acute (one time) applications, largely due to hypersensitivity issues. Reactivity to animal hyaluronidase extracts have been demonstrated via skin-prick testing, using whole extract as the challenge, such that immune responses specific to bovine PH20 protein itself were not evaluated. Whether humans exposed to animal PH20 protein-containing extracts break tolerance to endogenous PH20 has not been explored. Decades after commercial introduction of spreading factors, purification of hyaluronidase enzymes, sequencing, and eventual cloning of the human hyaluronidase gene family made it possible to prepare PH20 via recombinant DNA technology (reviewed in ref. 8). The Applicant has suggested that recombinant hyaluronidase, compared with tissue extract hyaluronidase, presents reduced risk of immune reactions and immunogenicity, due to human origin of the primary sequence and increased purity of rHuPH20 . While this could be true, much is unknown. Available data about immune responses to rHuPH20, prior to HyQvia studies, were again largely focused on skin-prick testing. IgE-associated wheal reactions to intradermally applied Hylenex are reduced, as compared to animal hyaluronidase extracts, possibly related to the absence of immunoglobulin, albumin, and other contaminants of animal origin (9). Further evaluation of PH20 specific immune reactions was not conducted, consistent with the single-use applications originally envisioned for rHuPH20.

The Applicant has shown that preparations of treatment-emergent anti-PH20 antibodies from subjects receiving HyQvia are able to bind endogenous PH20 in human tissue (Appendix 5B), suggesting that tolerance to native PH20 was broken as a result of receiving repeated doses of rHuPH20. Binding of anti-PH20 to native PH20 in tissues might elicit or enhance local inflammation, through, for example, deposition of complement or recruitment of effector cells through Fc-mediated interactions. None of the subjects receiving HyQvia generated PH20 neutralizing antibodies, or antibodies that were able to bind to other hyaluronidases. Nevertheless it is conceivable over a lifetime of rHuPH20 exposure, epitope spreading might result in neutralizing or cross-reactive antibodies. If such antibodies interacted with hyaluronidase protein in, for example, joint, neuronal or testicular tissues, increased inflammation or reduction in function could be a potential outcome.

Section 3: Risk Assessment for PH20-Directed Antibodies

General

Essential to understanding immunogenicity risk is a complete description of the expression pattern of the protein target. The expression of PH20 in human tissues has not been fully evaluated, particularly under stress conditions such as infection and injury. There are also considerable uncertainties surrounding PH20 expression profiles in different potential HyQvia recipients, e.g., young versus older subjects, pregnant women and the developing fetus, and subjects with acute injury, inflammation or infection.

PH20 (also known as SPAM1) expression has been described in mouse, guinea pig, rabbit, monkey, and human (10-14). High levels of PH20 expression have been documented in sperm cells and epididymis in the testis (15). Other published articles describe PH20 expression in tissues of the male and female reproductive tract (16-19); in synoviocytes, chondrocytes, and skin fibroblasts (20); in brain oligodendrocytes (21-22); in various neoplasias, melanoma and glioma (23-27); and at low levels in small intestine, muscle, liver, kidney, and heart (17). PH20 mRNA has been detected by Northern analysis in testis, while various authors report that signals from low frequency PH20 transcripts can be amplified and detected by RT-PCR in murine embryonic stem cells and other tissues (29). With rare exception, PH20 expression data come from studies of normal tissues. While it has been suggested that PH20 expression may increase in inflamed or injured tissue (22), there is little confirmatory data available, particularly in human tissue. A summary of in vitro and in vivo data related to PH20 expression in humans and animal models is provided in ***Appendix 5C***, and studies performed by the Applicant to evaluate the safety of PH20 are summarized in ***Appendix 6***. Three organ systems of special interest are highlighted in the following discussion, based on the Applicant's PH20 expression data generated for the

BLA and/or published reports. For each organ system, potential risks associated with PH20-directed antibody exposure, and gaps in our knowledge, are described.

Reproductive System

The primary site of PH20 expression is in male reproductive tissue, in which PH20 has been associated with sperm maturation and the process of fertilization. In several systems, hyaluronidase activity of PH20 facilitates penetration of sperm through the oocyte cumulus layer. PH20 additionally binds to the zona pellucida and contributes to sperm activation.

These functions of PH20 led some researchers to evaluate PH20 as a potential contraceptive vaccine target. Results from such studies are summarized in Table 4. In studies in guinea pig, neutralizing anti-PH20 antibodies were associated with low sperm counts, experimental autoimmune orchitis, and reversible infertility (6). Infertility in female guinea pigs was also observed, by a mechanism that was insufficiently characterized but thought to be interference of sperm:egg interactions (6). In other in vivo model systems, experimental immunization with PH20 protein and/or peptides did not reduce fertility. In rabbits, high titers of PH20 neutralizing antibodies did not result in impaired fertility, although the serum antibodies did block sperm:egg interactions in vitro. In mice, genetically defined SPAM-1 deficient animals demonstrated normal fertility, with only modest changes in the time course of in vitro fertilization. A second hyaluronidase, HYAL5, is proposed to compensate for PH20 inactivity in the PH20 deficient mouse, and in mice with anti-PH20 antibody production. Hyal5 is not expressed in humans. Similarly, linear epitopes derived from macaque PH20 presented in emulsion adjuvants yielded PH20 directed antibodies which bound to sperm but did not impair fertility.

Table 4: Summary of Results from In Vivo Models Assessing Impact of anti-PH20 on Fertility

Species	Males	Females
Rabbit	No effect on fertility	No effect on fertility
Guinea Pig	Reversible infertility	Reversible infertility
Cynomolgus Macaque	Not tested	No effect on fertility
Mouse	No effect on fertility	No effect on fertility

Summary on anti-PH20 impact on fertility in animal models provided by the Applicant. Note that mice are unique in the expression of Hyal5, with expression and function proposed to be redundant with PH20. In guinea pigs the effect on fertility was proposed to be humoral, i.e. neutralization of sperm:egg interaction. This observation was the foundation for the contraceptive vaccine.

Overall, differences in the impact of anti-PH20 on fertility in these studies likely arise from multiple causes, which complicate interpretation. The form of PH20 (purified native protein, recombinant, linear peptide) and adjuvant used for experimental immunization varied, possibly resulting in varied immune responses in the animals. Orchitis in immunized guinea pigs was suggested to result from cell-mediated

cytotoxic responses, which might not have been elicited in the other models (6). Whether cell-mediated cytotoxic responses could be elicited in humans after repeated exposure to rHuPH20 is unknown. If neutralizing antibodies to PH20 were formed in patients receiving rHuPH20, the mitigating effect of other compensatory hyaluronidases might or might not be expected Taken together, it is not clear from these data which of the models best reflects the role of PH20 in human fertility, or which immune responses elicited in animals might be possible or predicted in patients chronically exposed to rHuPH20.

It has been suggested that generation of PH20-directed antibodies would not be clinically relevant, since the blood:reproductive tissue barrier would be expected to block interaction of anti-PH20 with its target. However, a breakdown in the barrier between blood and reproductive tissue can result in autoantibody formation such as anti-sperm antibody formation (30-31). Compromise of the blood:testes barrier may occur with testicular trauma and torsion, or secondary to infections or autoimmune conditions. Compromise of the blood:testes barrier might permit PH20 directed antibodies to target tissue. It is feasible that reduced fertility could result from anti-PH20 antibody exposure, due to interference with the role of PH20 in sperm maturation and function. Reduction in fertility due to anti-PH20 antibody formation could be of particular concern in PI subjects with autoimmune-spectrum phenotypes, who may already experience limitations in fertility due to autoantibody production (32-33). Other adverse events could be envisioned due to Fc-mediated recruitment / deposition of effectors such as complement or cytotoxic cells, leading to tissue injury and possible loss of function. Finally, immunoprivilege is incompletely understood in developing reproductive tissue in the human fetus and neonate. If HyQvia was to be administered during pregnancy, interference with fetal reproductive tissue development and possible tissue loss could occur in the presence of PH20-directed antibodies.

To address the roles of PH20 in fertility, the Applicant has provided a series of preclinical in vivo studies.

a) In a 39 week study in monkeys, PH20 directed antibodies had no effects on sperm, hormone production, or reproductive organ histology. IgG was not found to deposit in testis and epididymis. The work was done in normal healthy animals. The studies provided do not help to understand whether anti-PH20 accesses tissue during injury or infection, and what impact antibody binding might have under those conditions. The data set provided by the Applicant does not exclude the possibility of PH20 directed antibodies accessing reproductive tissue and impacting reproductive function.

b) A GLP fertility study in rabbits demonstrated no effect of PH20 directed antibodies on fertility, fetal development, or fertility of the offspring. The level of conservation of PH20 function in fertility across species is poorly understood. Whether the rabbit model is predictive of PH20 functions in human fertility is unclear. Whether anti-PH20 antibodies might access PH20 in tissue during injury or infection was not addressed by this work. The data set provided by the Applicant does not exclude the possibility of PH20 directed antibodies accessing reproductive tissue and impacting reproductive function.

Neurological Tissue

Data suggesting PH20 expression in the central nervous system is very recent. Published reports from two laboratories describe PH20 expression in oligodendrocytes and their precursors (21-22). Other

hyaluronidases, such as Hyal1 and Hyal2, are also reported to be expressed in oligodendrocytes, and hyaluronan remodeling is reported in CNS lesions, suggesting that regulation of hyaluronan by local expression and secretion of hyaluronidases may be important for some oligodendrocyte functions (21, 34). One published paper describes increased PH20 expression in demyelinated lesions in a mouse model of autoimmune encephalomyelitis, and in cortical lesions of multiple sclerosis (MS) subjects (22). There are several published studies, in addition to the Applicant's own unpublished studies, countering these findings, in which PH20 protein and mRNA were not detected in normal CNS or oligodendrocytes in vitro or in vivo, or in CNS lesions. A tabulation of these studies is provided in Appendix 5. Differences in the kinds of normal and diseased tissue evaluated, and in the methods and reagents used to detect PH20, contribute to variability in these studies' results and complicate interpretation. The possibility that PH20 expression is increased in CNS under stress conditions such as injury and inflammatory disease, as suggested by increased detection in cortical lesions and in a variety of cancers, is a relevant consideration that will require additional study.

Even if PH20 is expressed in the CNS, access of anti-PH20 antibodies to the target would be blocked under normal conditions by the intact blood:brain barrier. However, under conditions such as acute disseminated encephalomyelitis (ADEM; 35), where the blood:brain barrier is compromised, there is a possibility that PH20-directed antibodies might have access to PH20 expressed in CNS. Other occurrences such as concussion, multiple sclerosis, or stroke might also result in blood:brain barrier disruption (36-37), with the potential for increased access of anti-PH20 to PH20 expressed in neurological tissue. If binding of antibodies to PH20-expressing cells, such as oligodendrocytes, were to occur, it is conceivable that effects on cellular functions might result, leading to adverse events. For example, in the context of an existing neurological lesion, alterations in oligodendrocyte function could alter or slow lesion repair. If recruitment / deposition of effectors such as complement or cytotoxic cells were to occur, increased tissue injury or accelerated loss of function could be envisioned. At least one study has implicated PH20 in fetal CNS development in mice. If PH20 is expressed in the CNS of the human fetus, a possible risk to developing CNS tissue could be envisioned if PH20-directed antibody binding were to occur, with or without recruitment / deposition of effectors such as complement or cytotoxic cells.

To address the neurological roles of PH20, the Applicant has provided a package of preclinical in vivo studies. The studies provided do not help to understand changes in PH20 expression in human neurological tissue, especially during injury or infection. Furthermore these studies do not address whether PH20-binding antibodies access neurological tissue or impact functions particularly during injury or illness. The data set provided by the Applicant does not exclude the possibility of PH20 directed antibodies impacting neurological tissue development and function.

a) The Applicant has noted that genetically defined mice entirely lacking PH20 have no detectable changes in brain histology, myelination, or neuronal function. This constitutive knockout mouse provides a very interesting tool for evaluating whether other hyaluronidases might compensate for deficiency in PH20, in reproductive tissue and in other tissues. How the constitutive PH20 knockout mouse predicts the roles of PH20 in human neurological tissue, at in healthy tissue or during injury and

repair, is unclear.b) The Applicant has performed nonclinical safety studies in mouse and rabbit, in which PH20 directed antibodies were not associated with adverse changes in neurological development or function at any stage of development. Postnatal development of neurological tissues were normal. Because this work was performed in animals without injury or illness, any increased expression and/or function for PH20 in injured neurological tissue would have been missed. The level of conservation of PH20 expression and functions across species, during fetal development and during injury or infection, are areas that have not been sufficiently studied. Whether the models are predictive of PH20 functions in human neurological tissue is unclear.

Gastrointestinal Tract

A single unpublished tissue-cross reactivity study, sponsored by the Applicant at a contract facility, demonstrated binding of an experimental rabbit anti-human PH20 reagent to colonic enteric nerve plexus. Expression of PH20 in enteric plexus in humans or in animal models has not been previously published. Follow-up unpublished data sponsored by the Applicant did not confirm expression of PH20 protein in enteric plexus, in humans without known GI disease or in GI tissue of animal models at baseline, using different antibody reagents from those which originally generated a specific binding signal . Researchers in the Applicant's lab also failed to detect PH20 mRNA in enteric plexus preparations. Confirmation of these data by a third party would strengthen the negative finding. Whether PH20 might be upregulated in a subset of plexus cells, for example during infection or other proinflammatory event, is a relevant consideration that has not been studied previously. If PH20 is expressed in enteric plexus, access of anti-PH20 antibodies to the target would only be expected if the blood:enteric plexus barrier were disrupted, as might be envisioned during gastrointestinal infection or inflammation. Gastrointestinal complications such as chronic bacterial or enteroviral infection and colitis are common in primary immunodeficiency, with incidence of 20-60% in various clinical reports (38). If PH20-directed antibodies were able to target enteric plexus cells, with possible deposition of complement and/or recruitment of cytotoxic effector cells, a potential risk of interference with enteric nerve activity and negative impact on GI function could be envisioned.

To address the roles of PH20 in the enteric plexus, the Applicant has provided a package of preclinical in vivo studies. Specifically, the Applicant has shown that in a GLP 39-week study in monkeys, anti-PH20 antibodies were not found deposited in colonic enteric nerve plexus, and no changes in enteric plexus were found in microscopic evaluation of colon tissue. In addition, no adverse findings in GI development or function were found in studies performed in mice, rabbits, or monkeys. This work does not help to understand changes in PH20 expression in human enteric plexus, especially during injury or infection, and does not address whether PH20-binding antibodies access enteric plexus during infection or injury. The data set provided by the Applicant does not exclude the possibility of PH20 directed antibodies impacting enteric plexus development and function. Importantly, the level of conservation of PH20 expression and functions across species, especially during fetal development and during injury or infection, are areas that have not been sufficiently studied. If PH20 expression is increased in injured or repairing enteric nerves in people, such a signal would have been missed in the in

the Applicant's in vivo studies, which were performed in healthy animals. Whether these models are sufficiently predictive of PH20 functions in human enteric plexus is unclear.

In conclusion, treatment with this recombinant therapeutic biologic product could elicit unwanted immune responses that impact subject safety. FDA's concern over potential risks associated with long-term exposure of PI subjects to rHuPH20 led to a discontinuation of treatment with rHuPH20 with rHuPH20 in on-going clinical trials (160902 and 1611). Subjects enrolled in these clinical studies were transitioned to IG therapy (IV or SC) only and the respective protcols were amended accordingly.

BENEFIT-RISK

Benefit

Clinical benefit was investigated in PI subjects (N=87), including pediatric subjects 2-12 years of age (N=14) and 12-16 years of age N=(9) in an open-label, single-arm, phase 3 study (Study 160603).

HyQvia was effective in reducing acute serious bacterial infections as defined in FDA's Guidance. The rate of acute serious bacterial infections per subject-year was 0.025 (upper limit of the 99% CI: 0.046), which is significantly lower ($p<0.0001$) than the 1.0 rate threshold considered to provide substantial evidence of efficacy.

HyQvia administered at the same dosing interval as IV administration (every 3-4 weeks) resulted in similar IgG trough levels as IGIV. In contrast to IGSC without PH20, higher bioavailability was observed with HyQvia as determined by AUC per dose/kg (20% higher with HyQvia). Every 3-4 week administration is an advantage when compared with weekly administration of IGSC 10% without PH20. Median duration of infusion of HyQvia versus IGIV in adults and children ≥12 years was comparable (2.13 vs. 2.33 h) but the duration was shorter in children 2-<12 years of age (1.73 vs. 2.49 h). This observed shortening of median infusion duration may not be clinically significant

Risk

Subjects with PI who are treated with HyQvia would be exposed to risks of antibody formation associated with chronic rHuPH20 exposure. The available data show that PI patients exposed to HyQvia develop antibodies against PH20. Whether these antibodies will penetrate tissues in testes, bowel and brain and bind locally expressed PH20 is not known. Furthermore, it is not known whether binding of PH20 in these tissues will cause adverse events such as infertility, and inflammation in the bowel and brain (discussed in more detail above). With lifelong exposure to HyQvia, it is possible that some patients' antibodies will progress from binding non-neutralizing to neutralizing antibodies due to epitope spreading. A pharmacovigilance plan that would be able to predict or monitor these types of risks may not be feasible as many years of subject and offspring follow up may be required for observation of antibody related adverse events. Reproductive toxicity studies would not help in predicting the concern for the effect of these antibodies.

Benefit-risk assessment

Risk-benefit considerations are very different for HyQvia compared to other IG products because the main immunogenic component of concern (rHuPH20) is not a life-saving therapeutic; rather,

subcutaneous administration of rHuPH20 is designed primarily to increase the convenience of treatment in PI subjects. The main advantage of HyQvia compared with IGSC without rHuPH20 is the lower frequency of dosing. Patients receiving HyQvia can be treated at intervals of 3-4 weeks, compared with weekly injections for IGSC without rHuPH20. Prolongation of the treatment interval is due to infusion of larger volumes of IG which is facilitated by increased permeability of the subcutaneous tissue following rHuPH20 pre-administration. Short-term risks associated with HyQvia, (e.g., infusion site pain/erythema/pruritus, headache, nausea) in clinical trials were acceptable, but the long-term risks associated with antibodies to rHuPR20 remain unknown. Absent HyQvia, these subjects can still receive a wide variety of IG products to treat their PI without exposure to rHuPH20.

The guidance of the Committee is sought in evaluating the benefit/risk profile of HyQvia, and seeking the Committee's recommendations regarding additional studies and possible risk mitigations.

Questions to the Committee

1. Do the available data indicate a favorable benefit/risk ratio for HyQvia taking into consideration the antibodies detected against PH20 which bind human tissues namely, reproductive tract, neurological and gastrointestinal tract tissues?

2. Would the Benefit:Risk assessment be influenced by mitigation strategies, including:

    a. restricted labeling for certain PI population subsets, e.g., pregnant women, male children, patients with inflammatory conditions involving the nervous system or gastrointestinal tract;
    b. implementation of risk communications to help subjects and physicians make informed decisions about whether to use HyQvia?

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APPENDIX 1: CLINICAL TRIAL ROSTER

Study ID	Study Phase and Design	No. of Subjects Age Range Duration/Subject	Study Objectives	Products Studied
161001	Phase 1, prospective, randomized, placebo controlled (HSA or LR), sequential, within subject + between subject, single-center study	53 adult healthy volunteers 19-65 years; 6 weeks	Evaluate effectiveness of rHuPH20 in enhancing SC administration of IG 10%	rHuPH20 with/without IGSC 10%
170901 Part 4	Phase 1, prospective, double-blind, randomized, controlled (formulation buffer) study	12 adult healthy volunteers; 26-64 years; 8 weeks	Assess safety, tolerability, infusion pressure, and maximal flow rate of human IGSC infusion administered with and without rHuPH20	rHuPH20 with IGSC 10%; IGSC 10%

160602	Phase 1/2, prospective, open-label, sequential, multicenter, two-arm (Arm 1: HyQvia; Arm 2: IGIV) study in PI subjects	11 subjects; 20-76 years; Arm 1: 8-65 days Arm 2: 133-165 days	Determine the dose of rHuPH20 enabling up to 600 mg/kg of IG, 10% to be administered SC in a single infusion site (HyQvia) in PI subjects.	IGIV 10%; IGSC 10% + rHuPH20
160601	Phase 2/3, prospective, open-label, non-controlled, multi-center study in PI subjects	49 subjects; 3-77 years; ≥10 months each	Evaluate tolerability and PK of IGIV 10% compared with IGSC 10% in PI subjects and to evaluate efficacy in Acute Serious Bacterial Infections (ASBI)	IGIV 10%; IGSC 10%
160603	Phase 3, prospective, open-label, non-controlled, multi-center study, 2 sequential cohorts (Epoch 1 [IGIV 10%] followed by Epoch 2 [HyQvia]) study in PI subjects	87 subjects; 4-78 years; Epoch 1: 3 months Epoch 2: 12 months	Evaluate efficacy, tolerability and PK; comparison of IGIV, 10% administered IV or HyQvia SC in PI subjects	IGIV 10%; HyQvia
	Phase 3, prospective, open-label, non-controlled, multi-center Extension study 160902*	66 subjects;	Long-term safety and efficacy	IGIV 10% HyQvia
161101*	Phase 2/3, prospective, non-controlled, multi-center	37 subjects;	Safety, tolerability, PK and efficacy	HyQvia

***Prematurely terminated at FDA request due to concerns over potential toxicity of chronic exposure to rHuPH20**

APPENDIX 2: Subject Disposition

Studies enrolling healthy volunteers

Phase 1 Study 161001

2/53 randomized subjects did not complete the trial (1 due to hypotension following rHuPH20 administration but prior to IGSC 10% administration; 1 lost to follow-up)

Phase 1 Study 170901 part 4

5/12 randomized subjects did not complete the trial (2 due to severe hemolytic anemia following IGSC administration and 3 others were withdrawn due to premature termination of the study). The study was terminated early at the applicant's request, because of the occurrence of a combination of two severe cases of hemolytic anemia in the HyQvia treatment group and non-serious "flu-like" symptoms in a third subject), also in the HyQvia cohort. The applicant attributed the cases of hemolysis to an outbreak of influenza. FDA review concluded that a the possibility that this was product-related could not be excluded, subsequent studies suggest that the rate of hemolysis with this product is not a concern.

Studies enrolling PI subjects

Phase 1/2 Study 160602

1/11 HyQvia subjects did not complete the trial due to local discomfort

Phase 2/3 Study 160601

5/49 subjects did not complete the trial for reasons stated below:

2 to <12 y/o (N=14)

IGIV: two pediatric subjects did not complete the trial: conflict with vacation plans in one case and refusal of the child to continue in another case.

≥12 y/o (N=35)

IGSC: three subjects ≥12 y/o did not complete the trial: a family emergency in one case; refusal to continue in a second; and decreased quality of life described as increased fatigue and general malaise in a third.

Phase 3 Study 160603

16/87 subjects did not complete the trial

Epoch 1 (IGIV):

3 subjects did not complete Epoch 1:

One subject 2-<12 y/o withdrew due to difficult IV access; two subjects ≥12 y/o missed two consecutive visits

Epoch 2 (HyQvia)

13 subjects did not complete Epoch 2:

2-<12 y/o (N=14): geographic factors (2 subjects); status epilepticus SAE (in a subject with past medical history of epilepsy), respiratory failure SAE (1 subject), upper abdominal pain (1 subject); fear of SC abdominal needle stick (1 subject).

≥12 y/o (N=75): lost to follow up (1 subject); moved out of state where no other research site was available (1 subject); infusion site edema (1 subject); inconvenience of home care (1 subject); infusion site pain (1 subject); infusion site pain for 3 days (1 subject); grand mal seizure SAE (1 subject with past medical history of epilepsy); acute orthopedic SAE (1 subject).

APPENDIX 3: EFFICACY

Table 3A. Primary Endpoint of Acute Serious Bacterial Infections (ASBI) Compared to a Control Rate of 1 ASBI per annum (Study 160603)

Analysis Set	Point Estimate	Upper Limit of 99% CI	p-Value
Full Analysis Set (N=81)	0.025	0.046	<0.0001
Per-Protocol Analysis Set (N=74)	0.023	0.048	<0.0001
IGSC Naïve Subject Analysis Set (N=44)	0.000	0.130	<0.0001

Table 3B. Analysis of All Infections (Study 160603)

Analysis Set	Treatment	Point Estimate (Upper Limit of 99% CI)
Full Analysis Set (N=81)	IGIV	4.51 (3.50 to 5.69)
	HyQvia	2.97 (2.51 to 3.47)
Per-Protocol Analysis Set (N=74)	IGIV	4.44 (3.38 to 5.72)
	HyQvia	2.95 (2.48 to 3.48)
IGSC Naïve Subject Analysis Set (N=44)	IGIV	4.42 (3.13 to 6.03)
	HyQvia	3.50 (2.79 to 4.32)

Table 3C. Acute Serious Bacterial Infections (ASBI) Compared to a Control Rate of 1 ASBI per annum (Study 160603 and Extension Study 160902)

	Point Estimate	Upper Limit of 99% Confidence Interval	P-value
Study 160603 (N=81)	0.025	0.046	<0.0001
Extension Study 160902 (N=66)	0.020	0.045	<0.0001

Table 3D. Secondary Efficacy Endpoints Expressed as Monthly Rates (Study 160603)

Parameter	Treatment	Point Estimate (Upper Limit of 99% CI)
Days off school work	IGIV	0.23 (0.15 to 0.34)
	HyQvia	0.28 (0.20 to 0.37)
Days on antibiotics	IGIV	3.15 (2.19 to 4.35)
	HyQvia	1.69 (1.29 to 2.16)
Acute physician visits	IGIV	0.33 (0.23 to 0.45)
	HyQvia	0.40 (0.32 to 0.49)
Days in hospital	IGIV	0.06 (0.03 to 0.10)
	HyQvia	0.02 (0.01 to 0.03)
Days in hospital due to infections	IGIV	0.03 (0.01 to 0.05)
	HyQvia	0.00 (0.00 to 0.01)

APPENDIX 4: SAFETY

Table 4A. Serious Adverse Events in PI Subjects

Study ID	Treatment Cohort	Safety Event
161101 (N=11)	HyQvia	Anaphylaxis (non lethal)
160601 (N=47)	IGIV 10%	Sinusitis
		Seizure
	IGSC 10%	Acute cholecycstitis
		Left-sided chest pain
160603 (N=87)	IGIV 10%	GI hemorrhage + asthmatic bronchitis
	HyQvia	Status epilepticus + respiratory failure + gastroenteritis + tonsillar hypertrophy
		Thrombosis RUE
		Cervical disk infection
		Headache
		Acute adrenocortical insufficiency
		Lobar pneumonia
		Oral leukoplakia
		Cervical dysplasia
		Grand mal + petit mal
		Acute orthopedic event requiring surgery
160902 Extension Study (N=66)	IGIV 10%	None reported
	HyQvia	Death secondary to mixed drug toxicity
		Death secondary to cardiac arrest
		Cystocele, vaginal prolapse, altered mental status, TIA
		Spinal meningeal cyst
		COPD, pneumonia
		Heart failure, worsening aortic insufficiency
		Appendicitis
		Abdominal adhesions
		Femur fracture
		Hypertension, atrial fibrillation
		Cryptosporidiosis infection
		Cellulitis LLE
161101 (N=37)	HyQvia	

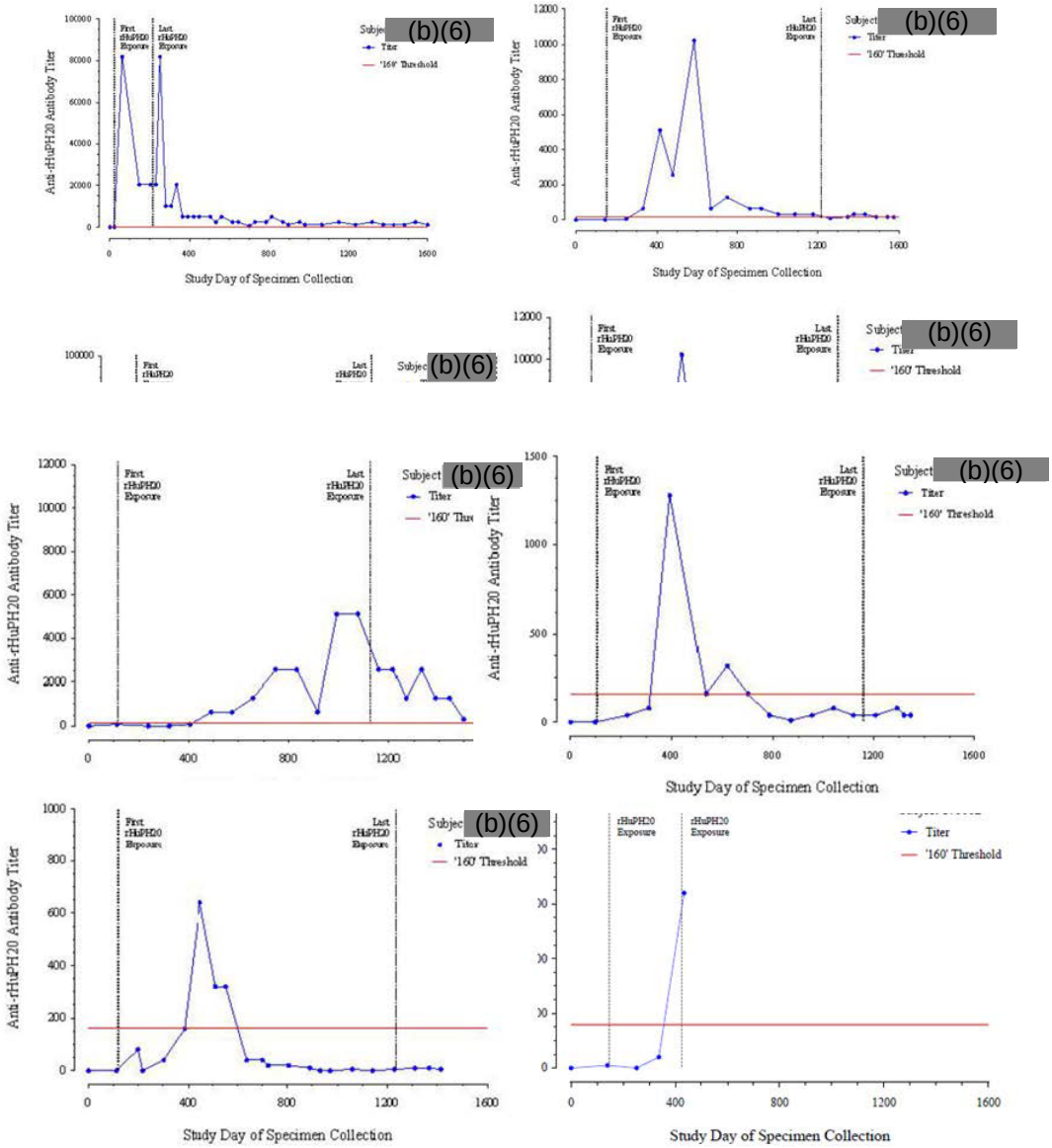
Table 4B. Local and Systemic Nonserious Adverse Events (Study 160603)

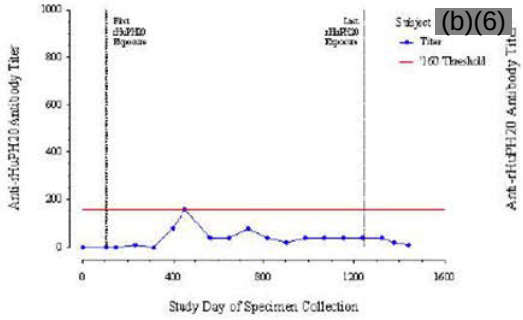
Route of Administration (No. of Infusions)	Mild AE	Moderate AE	Severe AE	TOTAL AE
IGIV (365)				
Local AE	5	0	0	5
Systemic AE	235	132	11	378
Total AE	240	132	11	383
IGSC (1129)				
Local AE	166	66	3	235
Systemic AE	522	305	12	839
Total AE	688	371	15	1074

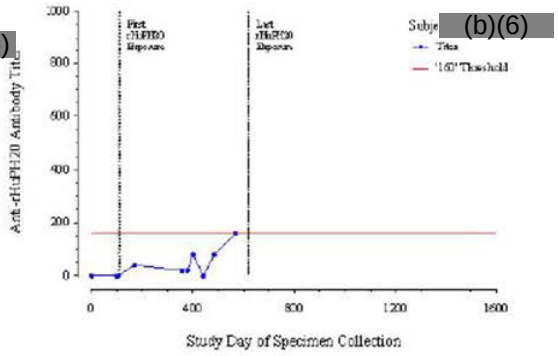
Table 4C. Nonserious Adverse Events (Study 160603 and Extension Study 160902)

Product	No. of Infusions	No. of AEs	AE Rate per Subject	AE Rate per Infusion	AEs by Intensity AE Rate per Subject AE Rate per Infusion		
					Mild	Moderate	Severe
IGIV	365	383	4.40	1.05	240 2.76 0.66	132 1.52 0.36	11 0.13 0.03
HyQvia							
Study #160603	1129	1074	13.26	0.95	688 8.49 0.61	371 4.58 0.33	15 0.19 0.01
Study #160603 + #1600902	1600	1229		0.77			

Appendix 5A: Longitudinal Analysis of PH20-Directed Antibody Titers in HyQvia Recipients (Data supplied by the Applicant)




Anti-rHuPH20 Antibody Titer
Study Day of Specimen Collection
Subject (b)(6)
Titer
'160' Threshold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Study Day of Specimen Collection
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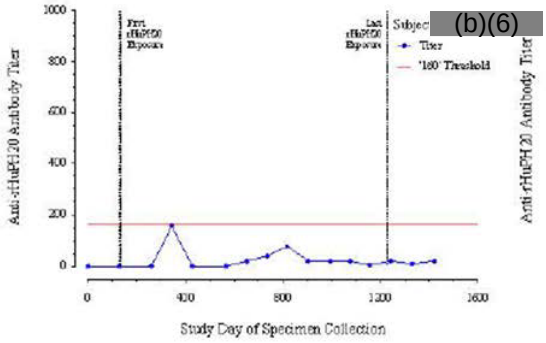
'160' Threshold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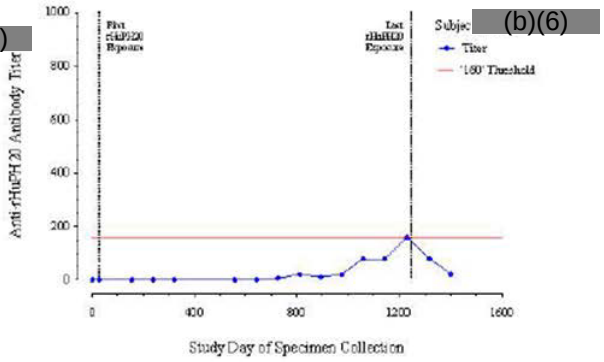
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Study Day of Specimen Collection
Subject (b)(6)
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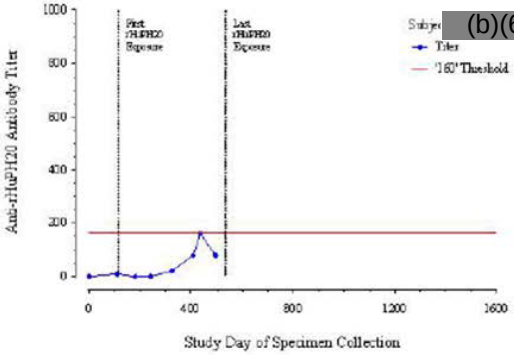
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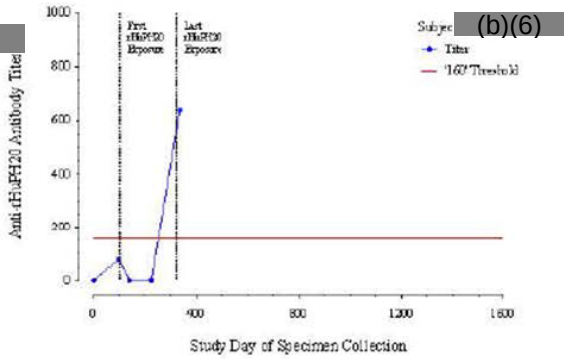
Subject (b)(6)
Titer
'160' Threshold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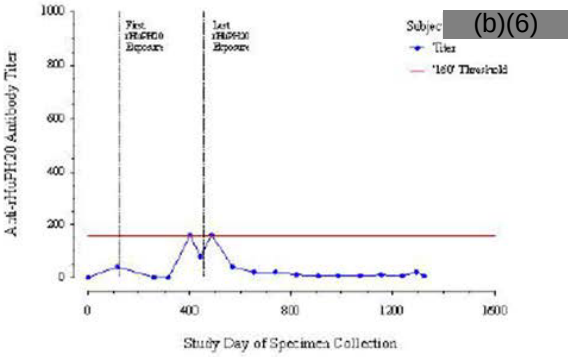
Anti-rHuPH20 Antibody Titer
Study Day of Specimen Collection
Subject (b)(6)
Titer
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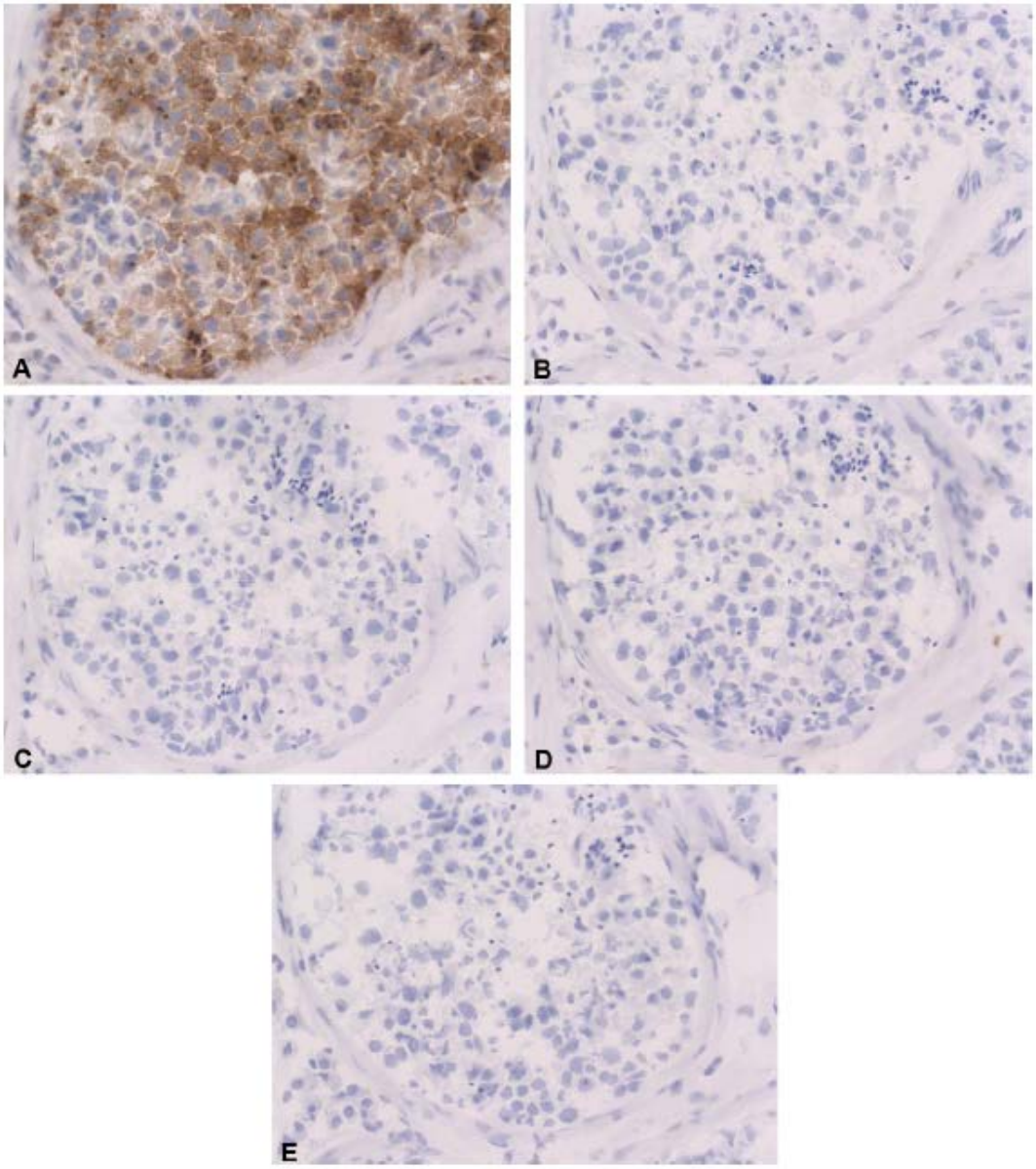
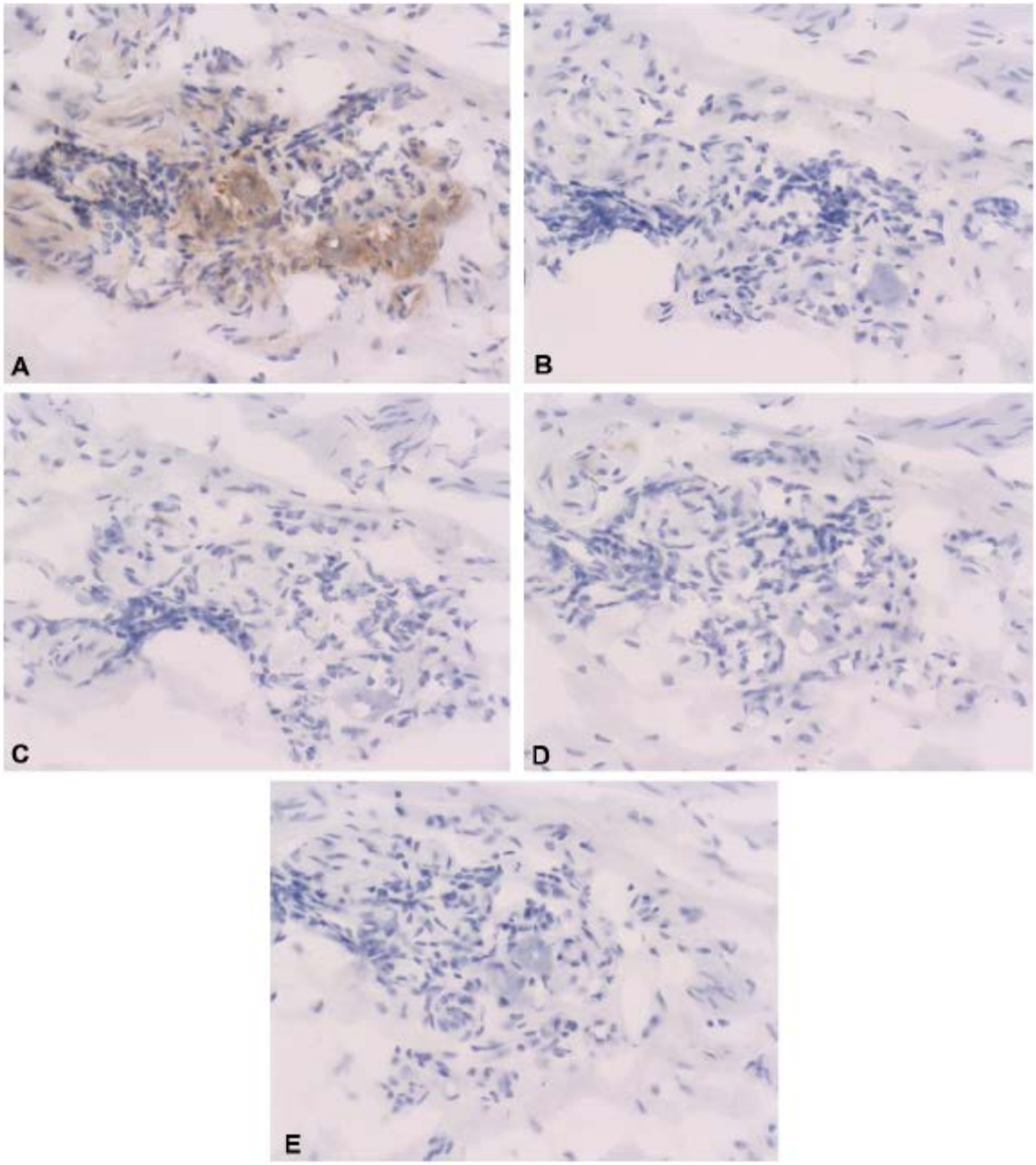
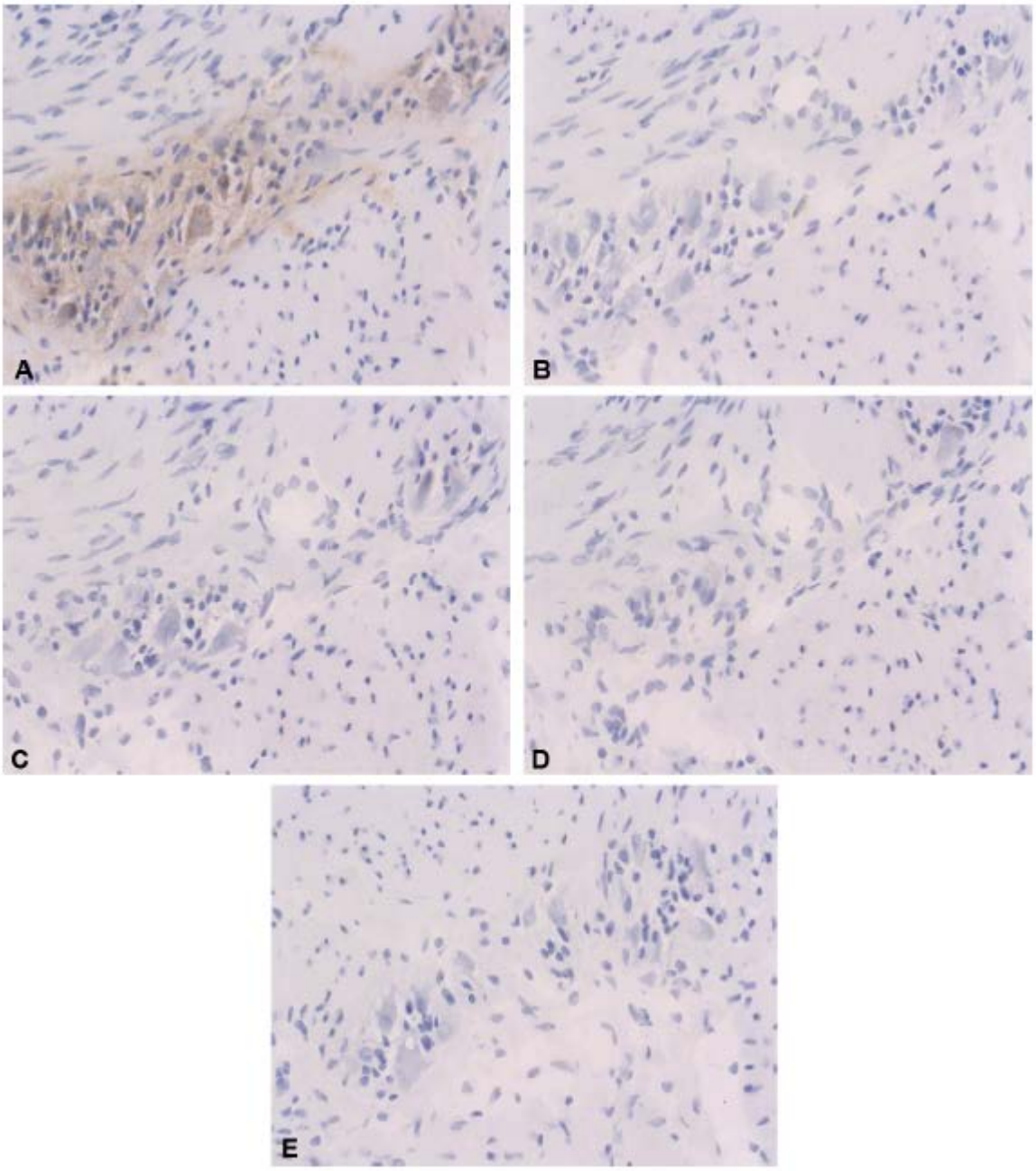
Appendix 5B: Representative Data From PH20 Expression Studies and Binding of HuPH20 Directed Antibody Preparations in Different Assay Formats



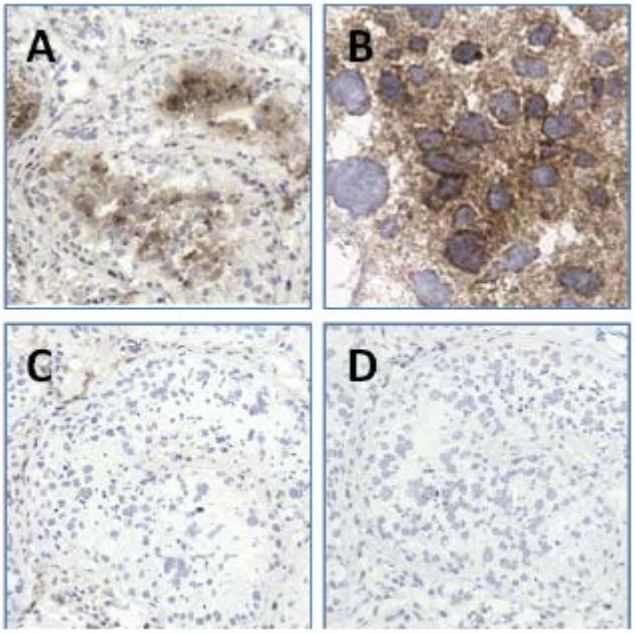
Original ---(b)(4)---- Tissue Cross Reactivity Study: Testis Tissue Expression of Human PH20. For this study, the Applicant chose a highly specific, affinity purified experimental antiserum generated in rabbits to detect human PH20 protein in a panel of tissues from human volunteers without known disease. Strong specific staining was observed in human testis (A). Staining was absent when primary antibody was preincubated with rHuPH20 (B). Negative control antibody yielded no staining, either in the presence (C) or absence (D) of rHuPH20 preincubation. There was no staining in the absence of primary antibody (E).



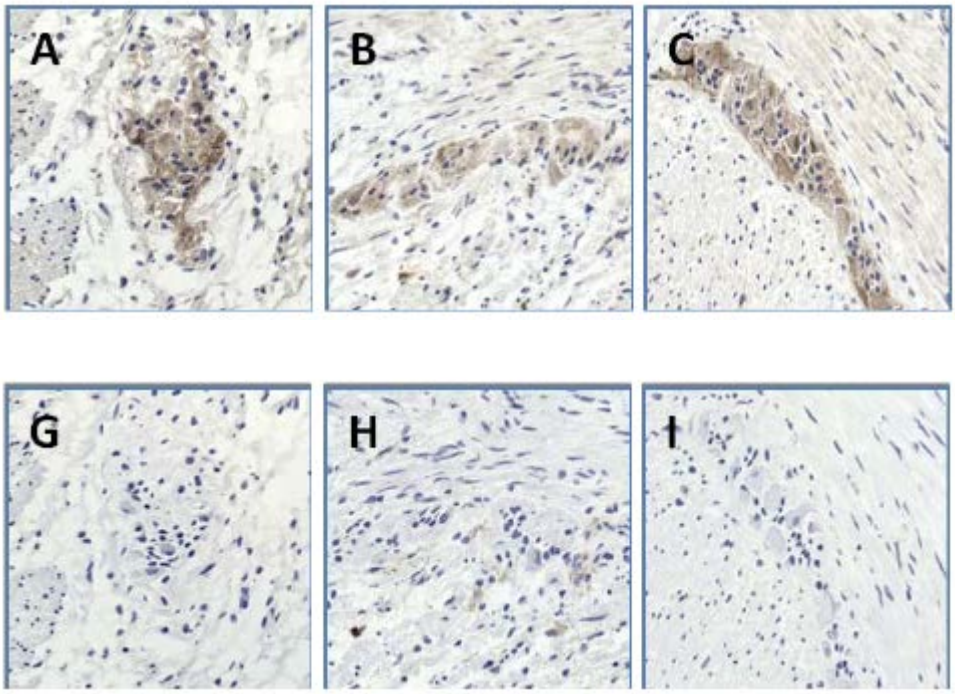
Original ---(b)(4)---- Tissue Cross Reactivity Study: Upper Gastrointestinal Tissue Expression of Human PH20. For this study, the Applicant chose a highly specific, affinity purified experimental antiserum generated in rabbits to detect human PH20 protein in a panel of tissues from human volunteers without known disease. Weak specific staining was observed in the ganglion of enteric plexus of human esophageal tissue (A). Staining was absent when primary antibody was preincubated with rHuPH20 (B). Negative control antibody yielded no staining, either in the presence (C) or absence (D) of rHuPH20 preincubation. There was no staining in the absence of primary antibody (E).



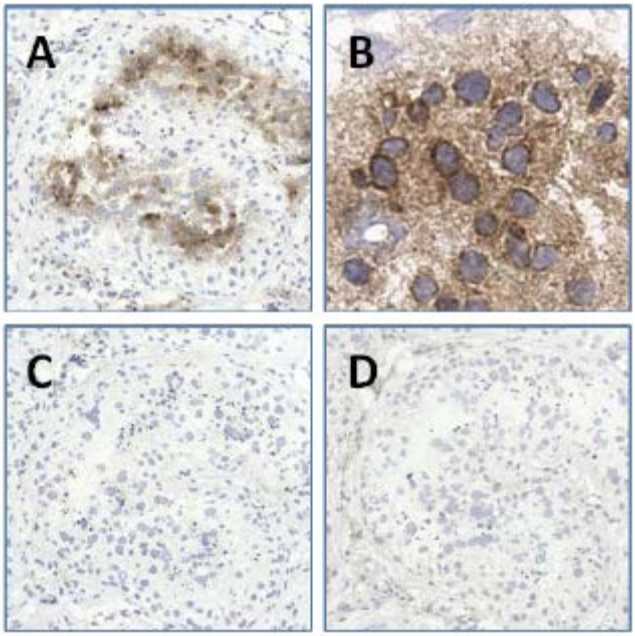
Original ---(b)(4)---- Tissue Cross Reactivity Study: Lower Gastrointestinal Tissue Expression of Human PH20. For this study, the Applicant chose a highly specific, affinity purified experimental antiserum generated in rabbits to detect human PH20 protein in a panel of tissues from human volunteers without known disease. Weak specific staining detected in ganglion of myenteric plexus of human colon tissue (A). Staining was absent when primary antibody was preincubated with rHuPH20 (B). Negative control antibody yielded no staining, either in the presence (C) or absence (D) of rHuPH20 preincubation. There was no staining in the absence of primary antibody (E).



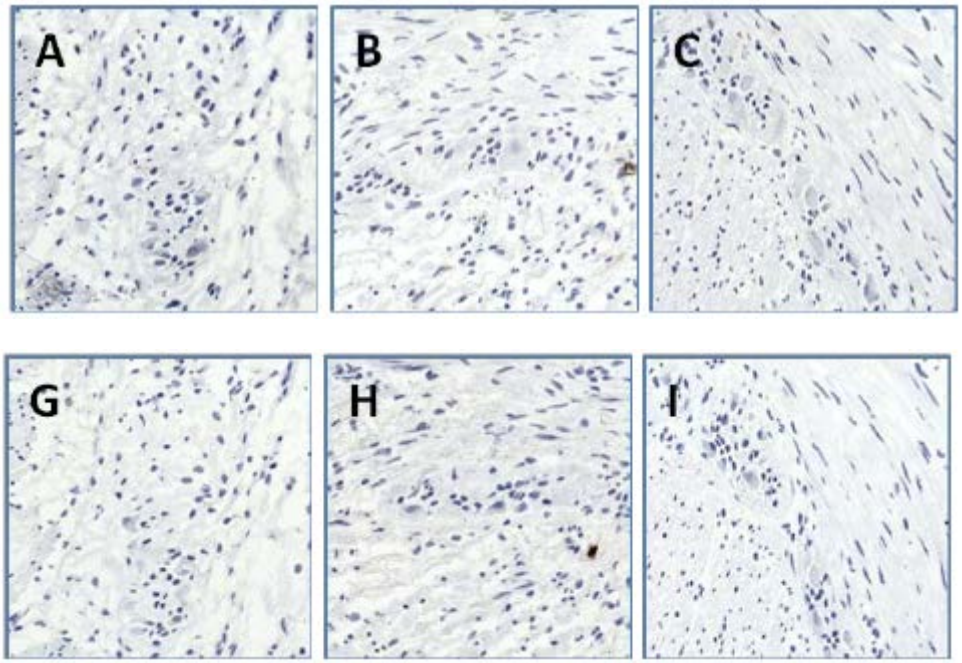
Applicant's Repeat Tissue Cross Reactivity Study: Rabbit anti-PH20 Staining in Testis Tissue. The Applicant chose a highly specific, affinity purified experimental antiserum generated in rabbits to detect human PH20 protein in two fresh-frozen testis tissue samples (A-B). Primary antibody was preincubated with rHuPH20 to demonstrate specificity (C-D).



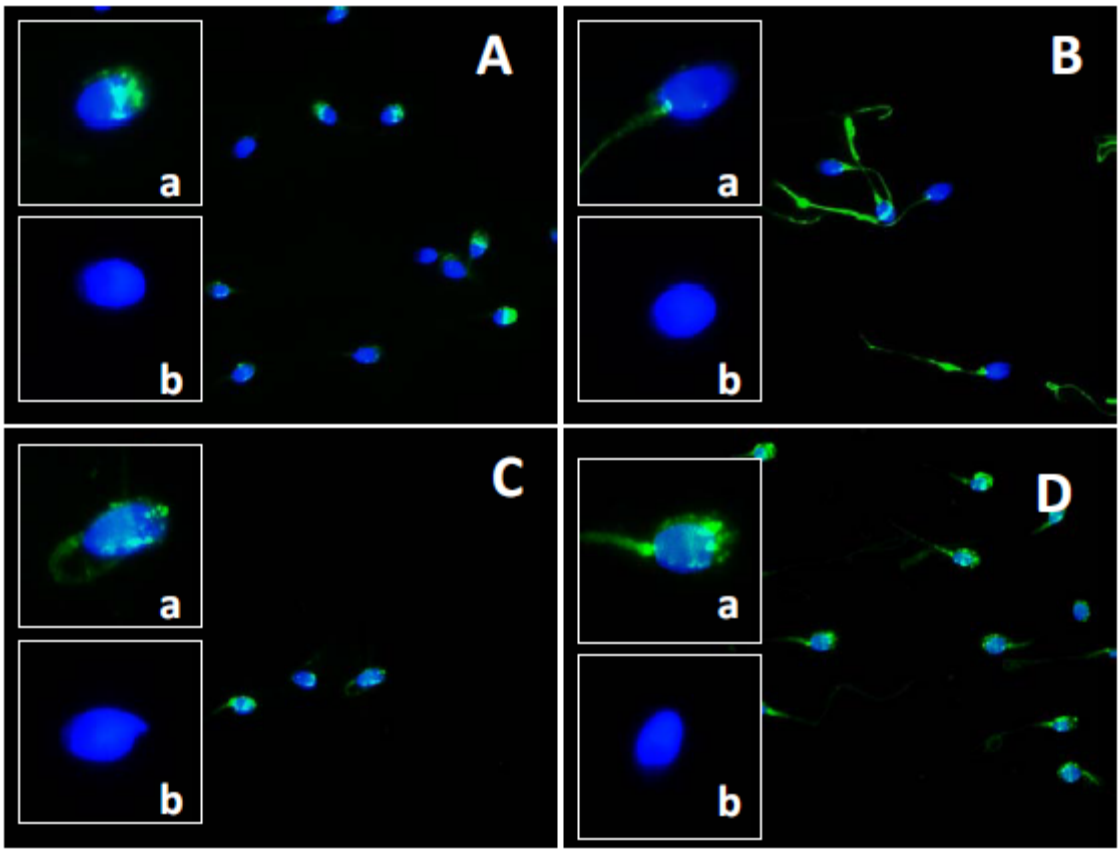
Applicant's Repeat Tissue Cross Reactivity Study: Rabbit anti-PH20 Staining in Gastrointestinal Tissue. The Applicant chose a highly specific, affinity purified experimental antiserum generated in rabbits to detect human PH20 protein in three samples of fresh-frozen colon tissue (A-C). Primary antibody was preincubated with rHuPH20 to demonstrate specificity (G-I).



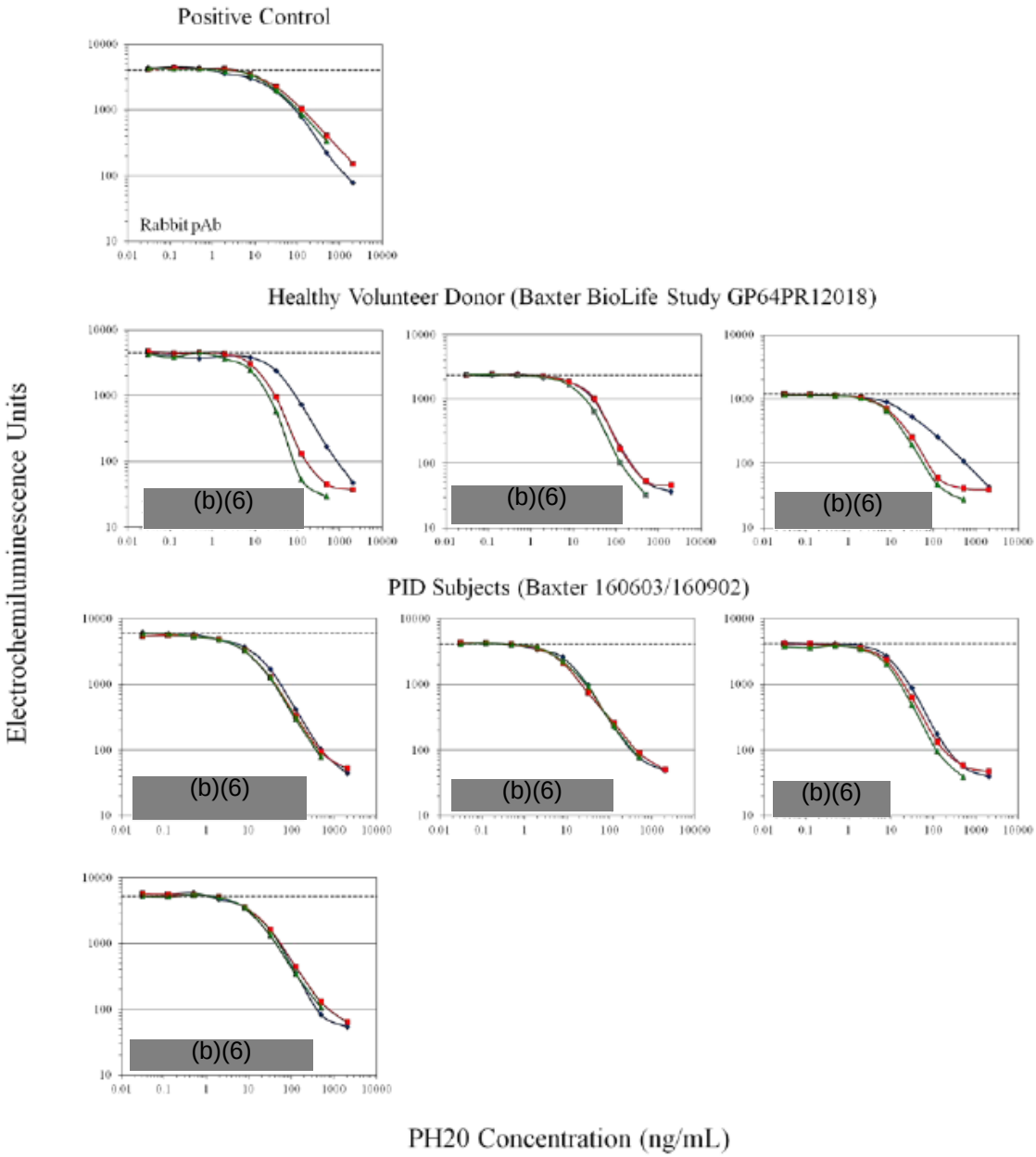
Applicant's Repeat Tissue Cross Reactivity Study: Mouse mAb Cocktail anti-PH20 Staining in Testis Tissue. The Applicant combined (b)(4) mouse mAbs directed against human PH20 protein as a primary antibody preparation. Staining was detected in two fresh-frozen testis tissue samples (A-B). The primary antibody cocktail was preincubated with rHuPH20 to demonstrate specificity (C-D).



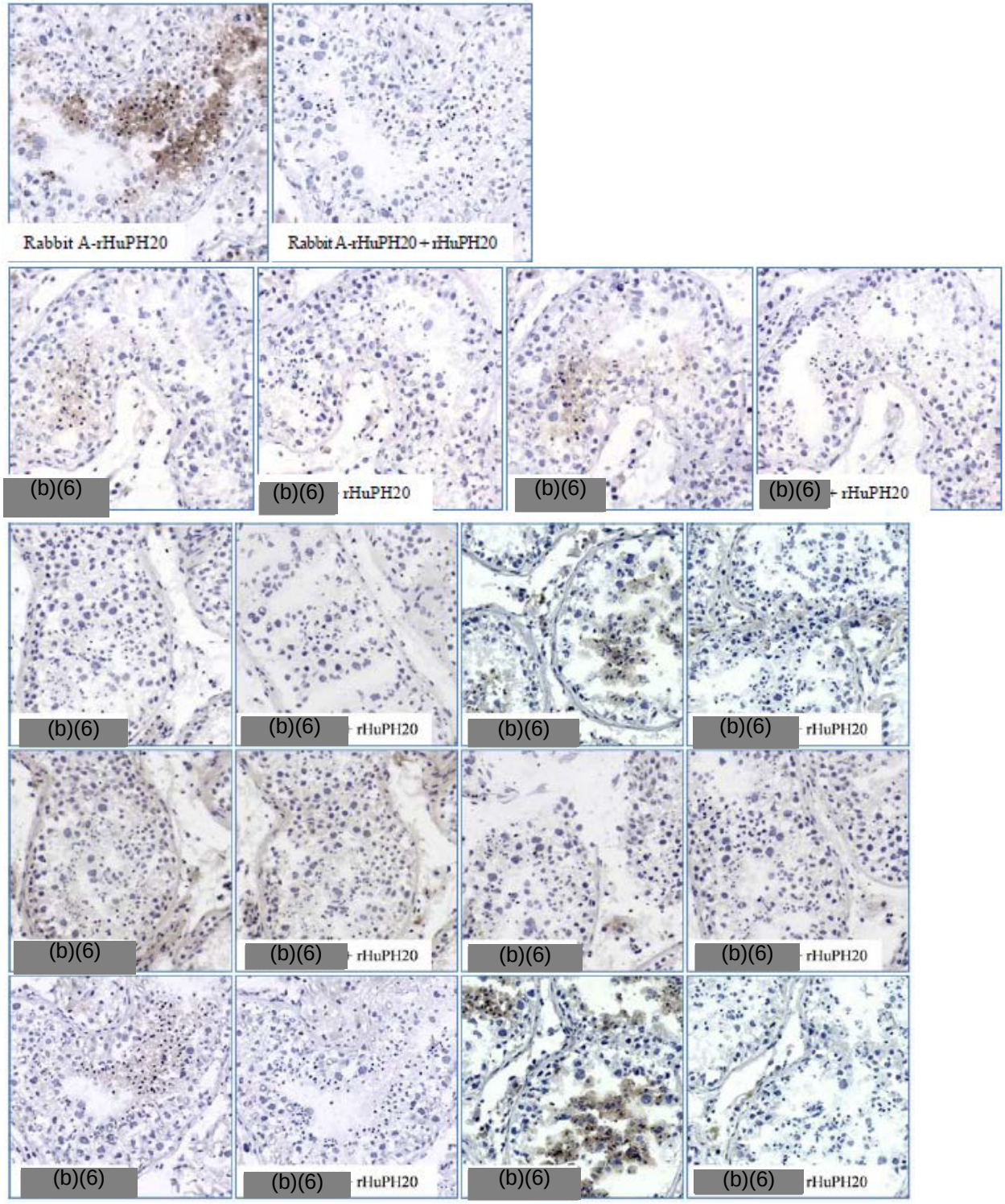
Applicant's Repeat Tissue Cross Reactivity Study: Mouse mAb Cocktail anti-PH20 Staining in Colon Tissue. The Applicant combined (b)(4) mouse mAbs directed against human PH20 protein as a primary antibody preparation. Staining was not detected in three fresh-frozen colon tissue samples (A-C). Preincubation with rHuPH20 did not impact signal (G-I).



Applicant's Immunoreactivity Study: ---(b)(4)---- Treatment Emergent Antibodies Bind Human PH20 on Sperm Smears. The Applicant performed immune---(b)(4)------ detection of PH20 in human sperm samples. (A): Affinity-purified, ---(b)(4)------ rabbit anti-rHuPH20 polyclonal antibody preparation, a positive control. (B-D) Human antibodies from an identified plasma donor with anti-PH20 reactivity (B), or from two PID patients that developed PH20 reactivity after receiving HyQvia (C-D), were affinity-purified using an rHu-PH20 ---(b)(4)------, then ---(b)(4)------ for use as immuno---(b)(4)------ reagents.



<u>Applicant's Competition Assay: Treatment Emergent Antibodies Bind Human PH20.</u> The Applicant evaluated PH20 binding of rabbit anti-PH20 (top), human anti-PH20 from identified plasma donors with anti-PH20 antibodies (middle), or treatment emergent anti-PH20 antibodies from PID patients receiving HyQvia (bottom). In all cases, binding to labeled rHuPH20 was effectively competed with rHuPH20 (blue). Binding was also competed with human PH20 released from the surface of sperm with (b)(4) treatment (green line), and recombinant PH20 released from the surface of CHO cells with (b)(4) treatment (red line). X-axis demonstrates increasing amounts of unlabeled competing PH20.



<u>Applicant's Repeat Tissue Cross Reactivity Study: Human Anti-PH20 Staining of Human Testis Tissue.</u> In samples of fresh-frozen human testis tissue, the Applicant evaluated binding of rabbit anti-PH20 (top), treatment emergent anti-PH20 antibodies from PID patients receiving HyQvia (middle), or human anti-PH20 from identified plasma donors with anti-PH20 antibodies (bottom). Weak positive staining is demonstrated with some human anti-PH20 antibody samples. Using the same samples, staining in human colon tissue was not detected (not shown).

1 page determined to be not releaseable

Appendix 5C: Studies of PH20 Protein and Transcript Expression in Human and Animal Tissues

Studies of Human PH20 Protein and Transcript Expression

PRESENT in epididymis, testes, prostate

Gmachl M et al. *1993. The human sperm protein PH20 has hyaluronidase activity. FEBS Lett 336: 545-548.*

Lathrop WF et al*. 1990. cDNA cloning reveals the molecular structure of a sperm surface protein, PH20, involved in sperm-egg adhesion and the wide distribution of its gene among mammals. J. Cell. Biol 111: 2939-2949.*

Deng X et al. *2000. Mouse Spam1 (PH20): evidence for its expression in the epididymis and for a new category of speramatogenic-expressed genes. J. Androl. 21: 822-832.*

Csoka AB et al. *1999. Expression analysis of six paralogous human hyaluronidase genes clustered on chromosomes 3p21 and 7q31. Genomics 60: 356-361.*

Evans EA et al. *2003. SPAM1 (PH20) protein and mRNA expression in the epididymides of humans and macaques. Reprod. Biol. Endocrin. 1: 54.*

Halozyme: *Treatment emergent anti-PH20 binds PH20 in human male reproductive tissue Report (b)(4)*

PRESENT in female reproductive tissue, synoviocytes, chondrocytes, fibroblasts

Csoka AB et al. *1999. Expression analysis of six paralogous human hyaluronidase genes clustered on chromosomes 3p21 and 7q31. Genomics 60: 356-361.*

Martin-DeLeon PA. *2003. Germ cell hyaluronidases: their role in sperm function. Int. J. Androl. 34: e306-e318.*

Beech DJ et al. *2003. Expression of PH20 in normal and neoplastic breast tissue. J. Surg. Res. 103: 203-207.*

El Hajjaji H et al. *2005. Chondrocytes, synoviocytes, and dermal fibroblasts all express PH20, a hyaluronidase active at neutral pH. Arthritis Res. Ther. 7: R756-R768.*

ABSENT in female reproductive tissue, spleen, liver

Jones MH et al. *1995. Expression analysis, genomic structure, and mapping to7q31 of the human sperm adhesion molecule gene SPAM1. Genomics 29: 796-800.*

PRESENT in oligodendrocytes, brain neurons, grey matter lesions MS

Sloane JA et al. *2010. Hyaluronan blocks oligodendrocyte progenitor maturation and remyelination through TLR2. Proc. Natl. Acad. Sci. 107: 11555-11560.*

Preston M et al. *2013. Digestion products of the PH20 hyaluronidase inhibit remyelination. Ann. Neurol. 73: 266-280.*

ABSENT in oligodendrocytes, brain neurons, white matter lesions MS

Patel S et al. *2002. Hyaluronidase gene profiling and role of Hyal-1 overexpression in an orthotopic model of prostate cancer. Int. J. Cancer 97: 416-424.*

Han M et al. *2008. Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets. Nature 451: 1076-1081.*

Halozyme: *Allen Brain database query*

Halozyme: *Multiple Sclerosis Proteome database query*

PRESENT in enteric plexus, normal adults
Halozyme/Asterand*: Tissue cross-reactivity study Report (b)(4)*

ABSENT in enteric plexus, normal adults or developing fetus
Halozyme: *ECL based immunoassay in ENP extracts, Report (b)(4)*
Halozyme: *Repeat tissue cross-reactivity study with additional antibodies, Report (b)(4)*
Halozyme: *Repeat tissue cross-reactivity study with differently-purified antibody prep Report --(b)(4)-----*
Halozyme: *Treatment emergent anti-PH20 fails to bind human enteric plexus, Report (b)(4)*
Halozyme: *RT-qPCR study, Report (b)(4)*
Halozyme: *In situ hybridizationstudy, Report (b)(4)*
Halozyme: *qPCR analysis, fetal enteric plexus Report (b)(4)*

Studies of Mouse PH20 Protein and Transcript Expression

PRESENT in male reproductive tissue
Zhang H and Martin-DeLeon PA. *2003. Mouse epididymal Spam1 (PH20) is released in the luminal fluid with its lipid anchor. J. Androl 24: 51-58.*
Thaler CD and Cardullo RA. *1995. Biochemical characterization of a glycosylphosphatidylinositol-linked hyaluronidase on mouse sperm. Biochemistry 34: 7788-7795.*
Deng X et al. *2000. Mouse Spam1 (PH20): evidence for its expression in the epididymis and for a new category of speramatogenic-expressed genes. J. Androl. 21: 822-832.*

PRESENT in kidney
Grigorieva A et al. *2007. Expression of SPAM1 (PH20) in the murine kidney is not accompanied by hyaluronidase activity: Evidence for potential roles in fluid and water reabsorption. Kidney Blood Press Res 30: 145-155.*

PRESENT in oligodendrocytes, brain (normal, EAE)
Preston M et al. *2013. Digestion products of the PH20 hyaluronidase inhibit remyelination. Ann. Neurol. 73: 266-280.*

ABSENT in oligodendrocytes, brain (normal, EAE)
Halozyme: *Deep RNA sequencing study (b)(4)*
Halozyme: *EAE Immunohistology Subreport (b)(4)*
Halozyme: *Immunihistochemistry Report (b)(4)*
Halozyme: *qRT-PCR Study (b)(4)*

ABSENT in enteric plexus, normal adult or developing fetus
Halozyme: *qPCR study Report (b)(4)*

Studies of Rabbit PH20 Protein and Transcript Expression
PRESENT in testis
Halozyme: *RT-qPCR Study (b)(4)*

ABSENT in enteric plexus
Halozyme: *RT-qPCR Study (b)(4)*

Studies of Rhesus Macaque PH20 Protein and Transcript Expression

PRESENT in male reproductive tissue
Evans EA et al. *2003. SPAM1 (PH20) protein and mRNA expression in the epididymides of humans and macaques. Reprod. Biol. Endocrin. 1: 54.*

Appendix 6: Safety Studies of rHuPH20

Table 6A. 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)------
Local tolerance, combination product	(b)(4) mice	SC	No	----(b)(4)-------

Table 6B. 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)----------
Repeat-Dose Toxicity *(b)(4)* **39 weeks**	Monkey, ---(b)(4)--------	SC, once weekly	Yes	(b)(4)/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	--(b)(4)---
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 to be performed as PMC

Table 6C. Tabulation of All Toxicology Animal Studies with rHuPH20

Type of Study	Species and Strain	Method of Administration	GLP	Study No.
Single-Dose *(b)(4)*	Rat, (b)(4)	IV	No	-----(b)(4)------- ----------
Repeat-Dose Toxicity *(b)(4)* **Days 1, 4, 8**	Monkey, (b)(4)	Peribulbar, SC, once daily	No	-----(b)(4)------- ----------
Repeat-Dose Toxicity *(b)(4)* **Days 1, 8**	Monkey, (b)(4)	Peribulbar, SC, once daily	Yes	-----(b)(4)------- ----------
Repeat-Dose Toxicity *(b)(4)* **7 days**	Monkey, ----(b)(4)-----	IV and SC, once daily	Yes	-----(b)(4)------- ----------
Repeat-Dose Toxicity *(b)(4)* **6 weeks**	Monkey, ---(b)(4)-----	Intravesical, once weekly	Yes	-----(b)(4)------- ----------
Repeat-Dose Toxicity *(b)(4)* **39 weeks**	Monkey, ---(b)(4)-------	SC, once weekly	Yes	(b)(4)/ R09050
Embryo-fetal Development *(b)(4)* **DG 6-15**	Mouse, ---(b)(4)------	SC, once daily	No	-----(b)(4)------- ----------
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	-----(b)(4)------- ----------
Effects of Anti-rHuPH20 Antibodies on Male Fertility and General Reproduction	---------(b)(4)------- Rabbit	SC weekly for 5 weeks with 2 biweekly boosting	Yes	(b)(4)
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 to be performed as PMC

Table 6D. 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)----------	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)-------.