FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

MEMORANDUM

Date: 8.25.2014

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

To: File for BLA 125402/0/41

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

Through: Dorothy Scott, M.D.; CBER/OBRR/DH/LPD; WO52-72 #4124; 301-827-3016

Cc: Cherie Ward-Peralta; CBER/OBRR/DBA; WO71 #4204; 240-402-8447

             Subject: 125402/0/41; Final product memo
             Product: Immune Globulin Infusion (Human), 10% with Recombinant Human
             Hyaluronidase: HYQVIA
             Submission Date: August 8, 2014
             Manufacturer: Baxter Healthcare Corporation

Summary and Recommendation:

The Applicant should commit to quantifying ----(b)(4)--- in rHuPH20 supplied with HyQvia. Based on
data collected, the Applicant should propose a specification for ----(b)(4)---- content across shelf life.

Review of Current Submission:

In this brief electronic submission, the Applicant submits revised versions of the proposed package insert
and patient information material, a PMC study regarding rHuPH20 immunogenicity and adverse events,
and a GLP lifetime study evaluating impact of anti-PH20 antibodies in otherwise healthy mice.

----(b)(4)---- are strongly linked with immunogenicity of recombinant products. The Applicant has
 -------------------- ----(b)(4)--
-------------- to its rHuPH20 product quality assessments. The Applicant should establish an assay to
specifically measure ----(b)(4)---- in rHuPH20 supplied with HyQvia. Based on data collected, the
Applicant should propose a specification for ----(b)(4)---- content across shelf life.

Background:

Of the subjects receiving HyQvia in pivotal studies, 15 tested positive for PH20-directed antibodies, using a sensitive in vitro assay. 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. A high titer experimental (rabbit) antiserum generated against rHuPH20 demonstrated strong staining in human male reproductive tissue, as expected. Additional specific staining 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.

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, below). In the prospective study, it was found that low titers (typically between 1:20 and 1:40) 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). At that time, the Applicant suggested immune responses to PH20 in PI subjects was exceptional, possibly related to the complexity of the IG therapeutic product. The Applicant stated, "The differential incidence, from baseline to treatment emergent, of PH20-Abs with other tested products to date is in sharp contrast to the HyQvia profiles," and concluded that "the unique titer levels and incidence of treatment emergent PH20-Abs seen in the HyQvia clinical studies is not extrapolatable to other settings and uses of rHuPH20."

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	Post-Treatment Prevalence	Post-Treatment Titer
Insulins	(b)(4) (b)(4)	(b)(4) ------------ ------------ ------------	(b)(4) (b)(4)	--(b)(4)-- ------	(b)(4) (b)(4)	----(b)(4)----- ----
Herceptin **(trastuzumab)**	-----(b)(4)------ ---------- ----------------------	--(b)(4)--	(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) (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-640	18/47 (38.3%)	Negative-5,242,880
------------	Plasma donors	None	56/961 (5.8%)	Negative-640	n/a	n/a
------------	General population	None	23/569 (4.9%)	Negative-1280	n/a	n/a

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 1:160. 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. Of note, the rHuPH20 formulation used in the PI and hereditary angioedema clinical trials compared to that used in mAb and insulin studies , ---
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------. 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
PH-20PH20	Male reproductive tissue; lower levels in other tissues, including CNS	Possible fertilization impact in some animal models (6,7)
Hyal4	Placenta, skeletal muscle	No information on deficiency
HyalP1	Pseudogene	No information on deficiency

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	PH-20PH20	100	100
Mouse	PH-20PH20	88	88
Chimpanzee	PH-20PH20	84	88
Rhesus macaque	PH-20PH20	84	88
Cow	PH-20PH20	80	84
Rabbit	PH-20PH20	80	88
Human	PH-20PH20	80	84
Human	Hyal2	68	77
Human	Hyal4	63	70

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 has 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 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. Thus the relative immunogenicity of bovine PH20, presented in low concentration within a tissue extract, versus a highly-concentrated recombinant version of human PH20, is unknown.

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

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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, indicating 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.

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. 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 not fully 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 kinetics 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

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 is unknown. 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. While anti-PH20 antibodies raised in monkeys were able to bind endogenous PH20 in immunohistochemistry studies, IgG was not found deposited in testis and epididymis in vivo. 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 that PH20 directed antibodies could access reproductive tissue and diminish fertility.

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. 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 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, in healthy tissue or during injury and repair, is unclear. The PH20 deficient mouse does not address the potential impact of PH20-directed antibodies on neurological tissue, if binding of the target were to occur. 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. The majority of preclinical work was performed in animals without injury or illness, thus any increased expression and/or function for PH20 in injured neurological tissue would have been missed. The Applicant performed one EAE study in mice constitutively deficient in Spam1/PH20. No impact of PH20 deficiency was noted by the Applicant in this model; however the potential compensatory role of Hyal5 in this mouse makes interpretation difficult. 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 performed in the Applicant's lab 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 commercially available antibody reagents different from those which originally generated the specific

binding signal. Researchers in the Applicant's lab also did not 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 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 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.

Risk Mitigation

Chronic treatment with this recombinant therapeutic biologic product could elicit unwanted immune responses that have the potential to impact patient safety. Some considerations for risk mitigation are noted below.

1) ----(b)(4)---- is a key feature of rHuPH20 that is likely to promote immunogenic responses. As a means to enhance product safety, it would be highly desirable to include a direct measurement of rHuPH20 ----(b)(4)---- content, and set a shelf life specification for ----(b)(4)----.

2) Since the pivotal clinical studies were not designed to systematically evaluate anti-PH20 responses, a PMC study of rHuPH20 immunogenicity would be very valuable. Based on previous information, a study size of 200 patients would be predicted to yield approximately 30 patients with elevated PH20-directed antibody responses, 6 of whom may demonstrate strong responses defined as titers greater than 1:10,000 and with evidence of antibody class switching. Study duration of 10 years could provide a more thorough evaluation of adverse events potentially associated with PH20-directed responses in this patient group. The study should feature a characterization of antibody levels every six months, with additional characterization (neutralizing and cross-reactive antibodies; antibody isotype and IgG subclass) for titers NLT 10,000, using the same assay method that was reported for clinical trial samples.

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