

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

HALOZYME, INC.,
Petitioner,

v.

ALTEOGEN, INC.,
Patent Owner.

Case No. IPR2026-00176
Patent No. 12,221,638

DECLARATION OF DR. MICHAEL BUTLER

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I, Michael Butler, declare as follows:

I. QUALIFICATIONS

1. I have been retained by Counsel on behalf of Halozyme, Inc. to provide opinions in this matter related to my experience and expertise. My opinions are based on my skills, knowledge, training, education, and experience in matters of this nature, and my examination of the materials utilized in preparing this report.

2. I have a Ph.D. in Biochemistry from King's College at the University of London, U.K. and over 30 years of experience in the production of recombinant proteins in animal cells.

3. I am currently a Principal Investigator in Cell Technology at NIBRT in Ireland, an Adjunct Professor at the University College Dublin, and a Distinguished Professor Emeritus in the Department of Microbiology at the University of Manitoba, Canada.

4. I previously served as the Chief Scientific Officer for NIBRT from 2016 to 2019, and was the Director of Mabnet, a national network for monoclonal antibody production, from 2010 to 2017.

5. My research currently focuses on Mammalian Cell Technology for the production of recombinant proteins, including antibodies. My research concerns many areas of recombinant protein production, including protein glycosylation,

development of cell culture media, and bioprocess development among other things.

6. I founded a company called Biogro Technologies Inc., which develops serum-free media formulations for the growth of commercially important mammalian cell lines, such as CHO cells.

7. I am also a past or current member of a number of scientific societies that focus on the production of recombinant proteins such as the European Society for Animal Cell Technology and the International Society for Protein Expression.

8. I have published over 200 articles, book chapters, and textbooks related to mammalian cell culture, production of proteins, and manufacturing processes. Many of my publications directly relate to the issues relevant here. For example, I have published on fed-batch protein production processes in CHO cells, mammalian cell metabolism, strategies for enhancement of recombinant protein production in mammalian cells, and supplements to cell culture media. To date, my work has been cited over 6000 times.

9. A copy of my *Curriculum Vitae*, attached as EX1003, contains further details on my education, experience, publications, and other qualifications to render an expert opinion in this matter.

II. SCOPE OF WORK

10. I understand that a petition is being filed with the United States Patent and Trademark Office for *Inter Partes* Review of U.S. Patent No. 12,221,638 to Park, et al. (“the ’638 patent,” attached as EX1001), entitled “Method for Producing Recombinant Hyaluronidase.” I have been retained by Halozyme, Inc. (“Halozyme”) to offer an expert opinion on the validity of claims 1-15 of the ’638 patent assigned to Alteogen, Inc. (“Alteogen”). I receive \$600 per hour for my services. No part of my compensation is dependent on my opinions or on the outcome of this proceeding.

11. I have been specifically asked to provide my opinion on the unpatentability of claims 1-15 of the ’638 patent. In connection with this analysis, I have reviewed the ’638 patent. I have also reviewed and considered various other documents in arriving at my opinions and may cite to them in this declaration.

III. LEGAL STANDARDS

12. I understand that a claim is not patentable under 35 U.S.C. § 102 as anticipated if the claim as a whole is described, either expressly or inherently, in a single prior art reference. I understand that this also requires that the prior art reference disclose that each claim element is arranged as recited in the claims.

13. I understand that differences between the prior art reference and a claimed invention, however slight, invoke the question of obviousness, not

anticipation. I have been advised that a claimed invention is not patentable under 35 U.S.C. § 103 if it is obvious. A patent claim is unpatentable if the claimed invention would have been obvious to a person of ordinary skill in the field at the time the claimed invention was made. This means that even if all of the requirements of the claim cannot be found in a single prior art reference that would anticipate the claim, a person of ordinary skill in the relevant field who knew about all this prior art would have come up with the claimed invention.

14. I have further been advised that the ultimate conclusion of whether a claim is obvious should be based upon several factual determinations. That is, a determination of obviousness requires inquiries into: (1) the level of ordinary skill in the field; (2) the scope and content of the prior art; (3) what difference, if any, existed between the claimed invention and the prior art; and (4) any secondary evidence bearing on obviousness.

15. I have been advised that, in determining the level of ordinary skill in the field that someone would have had at the time the claimed invention was made, I should consider: (1) the levels of education and experience of persons working in the field; (2) the types of problems encountered in the field; and (3) the sophistication of the technology.

16. I have also been advised that, in determining the scope and content of the prior art, in order to be considered as prior art, a reference must be reasonably

related to the claimed invention of the patent. A reference is reasonably related if it is in the same field as the claimed invention or is from another field to which a person of ordinary skill in the field would look to solve a known problem.

17. I have been advised that a patent claim composed of several elements is not proved obvious merely by demonstrating that each of its elements was independently known in the prior art. In evaluating whether such a claim would have been obvious, I may consider whether there is a reason that would have prompted a person of ordinary skill in the field to combine the elements or concepts from the prior art in the same way as in the claimed invention.

18. I have been advised that any obviousness rationale for modifying or combining prior art must include a showing that a person of ordinary skill would have had a reasonable expectation of success.

19. I have been further advised that there is no single way to define the line between true inventiveness on the one hand (which is patentable) and the application of common sense and ordinary skill to solve a problem on the other hand (which is not patentable). For example, market forces or other design incentives may be what produced a change, rather than true inventiveness. I may consider whether the change was merely the predictable result of using prior art elements according to their known functions, or whether it was the result of true inventiveness. I may also consider whether there is some teaching or suggestion in

the prior art to make the modification or combination of elements claimed in the patent. I may consider whether the innovation applies a known technique that had been used to improve a similar device or method in a similar way. I may also consider whether the claimed invention would have been obvious to try, meaning that the claimed innovation was one of a relatively small number of possible approaches to the problem with a reasonable expectation of success by those skilled in the art.

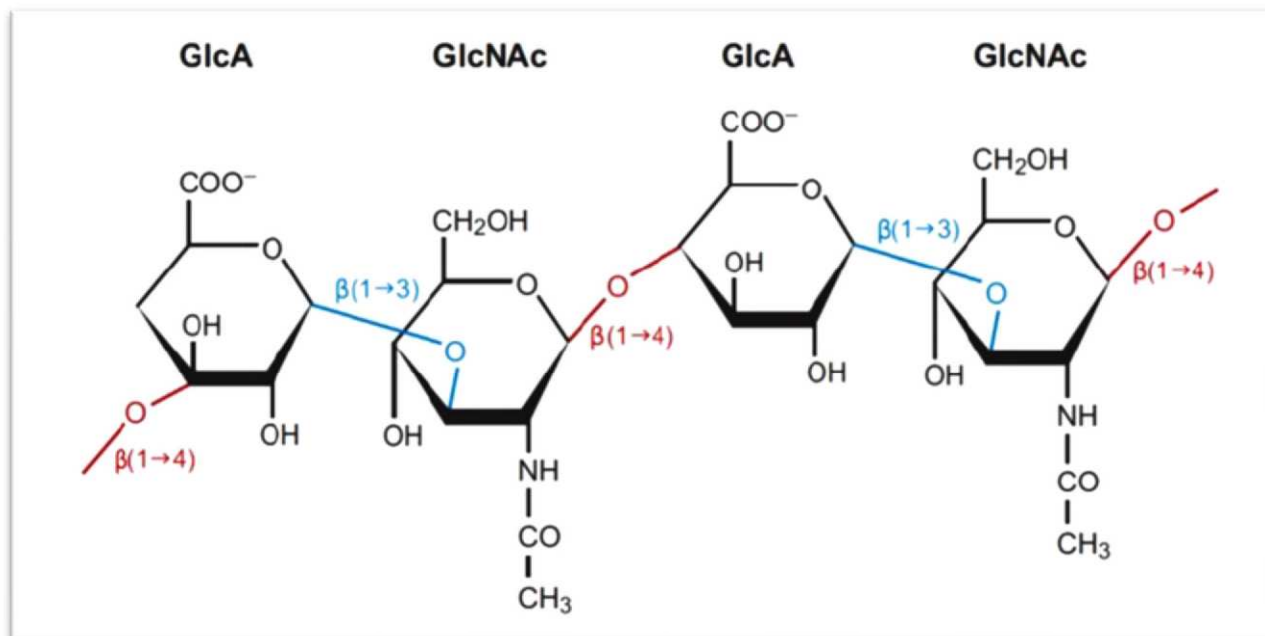
20. I understand that hindsight must not be used when opining on the patentability of claims of a patent. Therefore, my opinion will reflect the understanding of a person of skill in the art as of the relevant patent application filing dates, which I have been instructed is August 7, 2020, for this matter, the earliest date to which applicants have claimed priority for the claims of the '638 patent.

IV. OVERVIEW

21. The '638 patent is directed to methods for producing and purifying recombinant PH20 hyaluronidase.

22. Hyaluronidase is an enzyme that catalyzes the breakdown of hyaluronic acid which is a component of the extracellular matrix. EX1001, 1:39-40; EX1006, [0002]. Hyaluronic acid is a sugar polymer comprising repeating glucuronic acid (GlcA) and N-acetylglucosamine (GlcNAc) units. EX1029, Figure

1 (reproduced in part below). Mammalian hyaluronidases catalyze hydrolysis of the β -1,4 glycosidic bond that links GlcNAc to GlcA. EX1006, [0111].



23. Hyaluronidase lowers the viscosity of hyaluronic acid and increases tissue permeability. EX1001, 1:40-43; EX1006, [0108]. This property of hyaluronidase has been used in the medical context for many decades to, among other things, enhance absorption of injected fluids and drugs. EX1001, 1:44-54; EX1006, [0109], [0255]; EX1030, 230-231.

24. While there are several different forms of hyaluronidase expressed in mammals, the testes-specific hyaluronidase, PH20, is typically used in medical applications because it is active at a neutral pH typical of the physiologic environment. EX1030, 230-231; EX1006, [0108]. Prior to 2005, PH20 hyaluronidase used clinically was provided as an extract from animal sources such

as bovine. EX1030, 231. These extracts were not ideal for clinical use because, among other reasons, they contained only small amounts of hyaluronidase (e.g., <1%) and were associated with allergic reactions in some patients. *Id.*, 239 (“The clinical preparations used to date are non-human, unpurified, and can elicit immune reactions with repeat administration. Some of the bovine preparations were reported to elicit allergic responses in as much as 10% of a naïve population.”).

25. Halozyme developed methods for producing recombinant human PH20 hyaluronidase (“rHuPH20”) from cultured animal cells (e.g., Chinese Hamster Ovary cells, abbreviated as “CHO cells”). EX1031, 432-433 (“Development of a manufacturing process for rHuPH20”); EX1006, [0255]. Halozyme also developed methods for producing highly purified rHuPH20. *Id.* In 2005, Halozyme’s rHuPH20 product was approved by the FDA for several applications. EX1031, 435-436 (“Hylanex is indicated as an adjuvant to increase the absorption and dispersion of other injected drugs, for hypodermoclysis, and as an adjunct in subcutaneous urography for improving resorption of radiopaque agents.”). The approved indications for Hylanex have since expanded and now include co-administration with therapeutic antibodies. EX1032, 98, 100.

26. The ’638 patent claims methods for producing and purifying recombinant PH20 hyaluronidase in cultured animal cells (e.g., CHO cells). But

these claimed methods do not meaningfully differ from the prior art, including Halozyme's published methods for producing its FDA approved recombinant PH20 hyaluronidase.

V. BACKGROUND IN THE ART

A. Production of recombinant glycoproteins in cell culture

27. Cultured animal cells were used long before the '638 patent for the production of therapeutic recombinant proteins, such as antibodies, enzymes, and hormones. EX1007, Table 1; EX1008, 1. In particular, the Chinese Hamster Ovary ("CHO") cell line was routinely used for producing recombinant glycoproteins. EX1007, Table 1, 1111-1113; EX1011, 8.

28. CHO cells are frequently used because, among other reasons, they are capable of generating high yields of recombinant protein (e.g., grams per liter) and are well-adapted to large-scale production methods. For instance, CHO cells may be grown in suspension culture in large bioreactors using serum-free chemically defined media. EX1007, 1113-1114.

29. Because CHO cells were the main workhorse for production of therapeutic recombinant proteins, including PH20, much was known about their optimal culturing conditions. In addition, considerable resources were available that facilitated their use including commercially available growth media and even CHO lines selected for high level protein production.

1. Temperature Shift or “Biphasic” Culturing Methods

30. It was known that CHO cells are “highly tolerant” to changes in temperature. EX1007, 1113-1114. Capitalizing on this tolerance, culturing methods often included a temperature shift designed to maximize yields of recombinant protein.

Temperature shift (TS) to a hypothermic condition has been intensively studied to balance cell growth, protein productivity, and quality in both microbial fermentation and mammalian cell culture. Such a TS strategy holds the potential to improve fermentation and cell culture performance for a prolonged period with better overall productivity and quality. Since temperature downshifts have been reported to improve protein productivity by many different CHO cell lines, it is widely used as an effective platform strategy for cell culture process development and manufacturing in the biopharmaceutical industry.

EX1012, 191.

31. Temperature shift culturing methods conventionally included growth at or near physiologic temperature (37°C) to encourage proliferation and accumulation and the desired cell density followed by a shift to sub-physiologic temperatures (<37°C) to maintain the viability of the culture and prolong production of the recombinant protein (rP). Use of temperature shifts when producing recombinant proteins in CHO cells is well documented in the literature. For example, Xu describes:

The effect of TS on CHO cell culture and quality attributes depend on cell line, recombinant protein product, culture media, and other bioreactor conditions. In response to sub-physiological temperatures, the cell doubling time increases and the death rate is reduced with cells arrested in the G1 phase of the cell cycle. In order to maintain high viable cell density (VCD) during cell culture, a bi-phasic culture strategy is often used with cell growth at around 37 ° C during the exponential growth phase, followed by a shift to a lower temperature for protein production during the late log and/or the stationary phases. A mild temperature downshift decreases cell apoptosis, metabolism, nutrient utilization, ATP consumption and toxic metabolite accumulation. Lower oxygen consumption and shear stress resistance at lower temperatures are beneficial to scale-up for manufacturing with respect to less foaming, lower power input, and more process robustness. The effects of temperature downshift in CHO cell cultures on key quality attributes, including protein aggregation, charge variants, N-glycans and host cell proteins, has been reported. Therefore, it is necessary to consider both titer and quality attributes when developing a temperature shift strategy for CHO cell culture. In addition to bi-phasic cultures, tri-phasic cultures have also been reported to improve upstream titer and product quality attributes.

EX1012, 191.

32. Becerra similarly describes the use of temperature shift to improve productivity of the cultured cells.

With regard to the quality of the r-protein, it seems that in most cases, there are no significant changes in the glycosylation profiles, including

the degree of terminal sialylation. Accordingly, these r-proteins produced under mildly hypothermic conditions show the same in vitro biological activity as the r-proteins produced at 37 °C. In addition, the specific growth rate decreases considerably with decreasing temperature, and cell viability increases. Thus, decreasing the culture temperature has been suggested as a tool for the improvement of the production process, essentially defining biphasic culture strategies. During the first stage, normal cell growth is established at 37 °C, and once the expected cell density is reached, the temperature is decreased to increase r-protein productivity and extend culture viability.

EX1014, 2; *see also id.*, (“In the past ten years, there have been frequent reports indicating that a decrease of a few degrees in temperature from 37 °C, the usual culture temperature, until 28 °C, which is called mild hypothermia, allows an incremental increase in the specific productivity of r-proteins in CHO cell culture (Table 2).”).

33. The first temperature range of a temperature shift method was typically in a range close to normal physiologic temperature (37°C). EX1016, 11378 (“Usually the recommended temperature is 36–37 °C); EX1017, 21 (“Most human and mammalian cell lines are maintained at 36°C to 37°C for optimal growth”); EX1018, 2 (“36°C to 38°C”).

34. The second temperature range was lower than normal physiologic temperature and was considered mildly hypothermic. EX1005, [0021] (“further cultured at a temperature of less than 37°C and greater than 30°C, less than 35°C

and greater than 31°C, or at a temperature of about 32°C”); EX1006, Examples 13 & 15 (exemplifying 35.5°C); EX1012, Table 2; EX1014, 2 (“a decrease of a few degrees in temperature from 37°C, the usual culture temperature, until 28°C, which is called mild hypothermia, allows an incremental increase in the specific productivity of r-proteins”), Table 2 (collecting examples).

35. The table below summarizes the first and second temperatures used in prior art temperature shift methods that I discuss above.

Reference	First Temperature	Second Temperature
Wei (EX1006)	37°C	less than 37°C (e.g., 35.5, 36.0, 36.5°C)
Zmuda (EX1005)	37°C	30 to less than 37°C (e.g., 32°C)
Xu (EX1012)	36.5°C	33 to 35°C
Becerra (EX1014)	37°C	28 to less than 37°C

36. It was appreciated by persons of ordinary skill in the art that development of a culturing protocol with a temperature shift for producing recombinant protein involved the routine optimization of the lower temperature. Specifically, it was known that the optimal lower temperature might differ based on the cell line and the recombinant protein among other variables. EX1012, 191 (“The effect of TS on CHO cell culture and quality attributes depend on cell line, recombinant protein product, culture media, and other bioreactor conditions.”); EX1014, 2 (“A general analysis of the background shows that the magnitude of the

effect of reducing the culture temperature over the specific production rate depends markedly on the cell line and the r-protein itself.”).

37. Accordingly, persons of ordinary skill in the art appreciated that the lower temperature was a result-effect variable that was routinely optimized when producing recombinant proteins in CHO cells.

2. Culturing modes and media

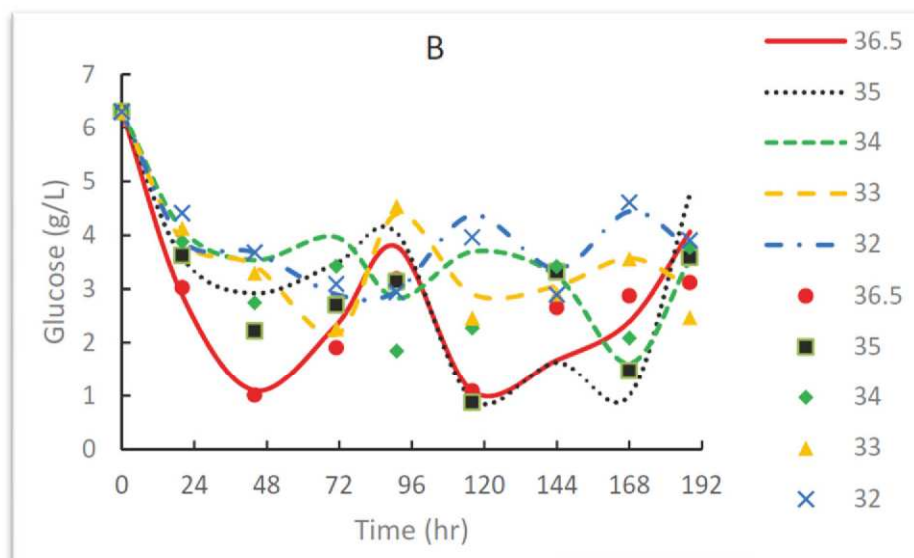
38. A number of cell culture production modes were commonly used in connection with CHO cells including “batch, fed-batch, and perfusion” modes. EX1024, 942; *see also* EX1019, 57 (“batch or fed-batch”); EX1011, 8 (“Fed-batch cultures are now in routine use, prolonging the cell viability by delivering optimised feeds on specifically chosen days”); EX1014, Table 2 (listing production modes including batch, fed-batch, continuous, and perfusion). *See generally* EX1011 (“3.2. Cultivation methods”). Wei, for example, describes a fed-batch method in which the cells are fed on specifically chosen days during the culturing. EX1006, [0360], [0373].

39. Prior to the ’638 patent it was routine to manipulate culturing conditions to optimize yields of produced recombinant proteins. In addition to temperature, parameters such as glucose concentration and pH were maintained in favorable ranges.

40. Glucose was typically used as a carbon source for cultured CHO cells. Defined media for CHO cells often contained around 25-35 mM (4.5-6.5 g/L) glucose (sometimes referred to as dextrose). EX1009, 772. Skilled artisans appreciated that glucose concentrations would affect the quality of produced recombinant glycoproteins. EX1009, 772 (explaining that high glucose levels lead to build up of lactate which has “adverse effects on mammalian cell growth, therapeutic protein productivity and quality”); EX1024, Fig. 3, Table II (low concentrations of glucose block glycosylation).

41. It was further known that glucose consumption by the cells would reduce the concentration of glucose in the culturing media. EX1012, Fig. 1; EX1009, Fig. 1. Accordingly, it was conventional to “feed” the cells with media comprising glucose during the culturing to maintain levels of this nutrient. EX1009, 772; EX1006, [0354], [0360], [0373], EX1012, 200 (“Additional glucose was supplied to maintain the concentration range.”); EX1005, [0041], [0233], [0236], Table 1, Fig. 5, 6. For example, it was known that “4.4 mM [glucose] represents a common target concentration in the biopharmaceutical industry for controlled fed-batch cultures.” EX1009, Abstract. A person of ordinary skill in the art would have known that 4.4 mM glucose is about 0.8 g/L ($0.0044 \text{ mol/L} \times 180.16 \text{ g/mol} = 0.79 \text{ g/L}$). It was also known to maintain the glucose concentration

between 1 g/L and 4 g/L when culturing CHO cells. EX1012, Figure 1B (reproduced in part below).



42. It was known that the optimal pH for growth of CHO cells was around neutral pH (e.g., 7.0). EX1019, 57 (“The optimum pH was in the region of 7.0 to 7.2”); EX1006, [0360] (“Parameters were ...; pH: 7.2”); EX1012, 200 (“the pH was controlled at pH 7.1”); EX1020, 4 (“Typical pH set points for CHO cells are neutral to slightly alkaline (7.0–7.2).”); EX1021, 1036 (“At reduced pH levels (6.90 and 6.80) the cultivation time could be prolonged to 9 and 12 days.”).

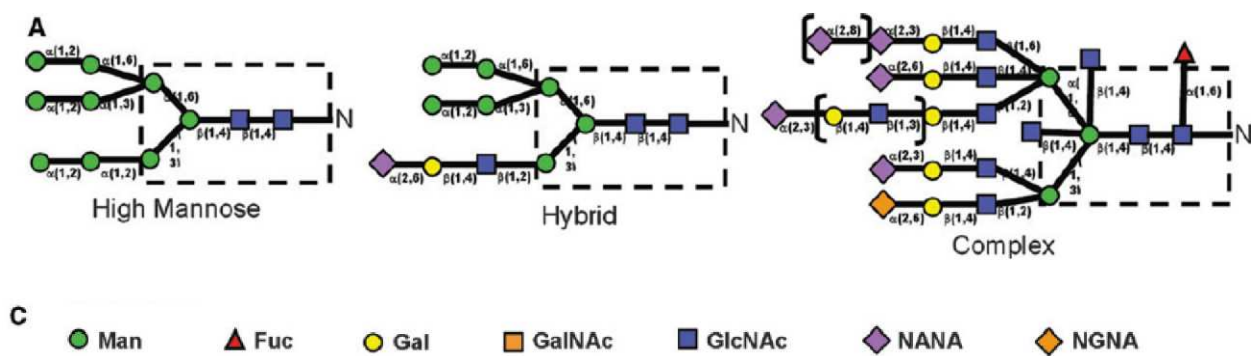
43. It was also known that the pH of cultured cells would decrease over time due to the accumulation of lactate in the culture medium. As a result, it was conventional to maintain the pH of the culture medium by adding base. EX1022, 1052 (“Sodium carbonate and sodium hydroxide are commonly used for pH control in bioreactors.”). It was further known to use “buffering agents suitable for

use in cell culture applications,” such as MOPS, HEPES, and phosphate which are optimal for buffering at physiological pH levels. EX1005, [0147].

B. N-Linked Glycosylation

44. Another reason CHO cells were frequently used as an expression host for recombinant proteins is that they produce glycoproteins with human-like glycan structures. EX1011, 12; EX1006, [0130]. Glycosylation was known to “significantly modulate yield, bioactivity, solubility, stability against proteolysis, immunogenicity, and clearance rate from circulation.” EX1023, 148; *see also* EX1006, [0114]-[0115].

45. Recombinant glycoproteins may be glycosylated on asparagine residues; this is typically referred to as N-linked glycosylation. N-linked glycans take a variety of forms which are divided into three categories: high mannose, hybrid, and complex. EX1024, Fig. 1A, C (reproduced below); *see also* EX1006, [0018], Fig. 2. The structures shown below are representative, and many forms are known within each category. *See, e.g.*, EX1025, Table II.



46. High mannose glycans, as their name implies, are made up mostly of mannose (green circle). High mannose glycans contain neither galactose nor sialic acid. Hybrid glycans have one arm made up of mannose; the other arm includes additional sugars such as galactose (yellow circle) and NANA (purple diamond). NANA and NGNA (orange circle) are abbreviations for N-acetylneuraminic acid and N-glycolylneuraminic acid, respectively, and are colloquially referred to as “sialic acid.” Complex glycans are heavily processed and include both galactose and sialic acid.

47. Galactose is only present on hybrid and complex glycans. Because sialic acid (e.g., NANA, NGNA) is only added to galactose, it too is found only in hybrid and complex glycans.

48. It was further known that N-linked glycosylation may be removed from proteins enzymatically. For example, a person of ordinary skill in the art would have understood that sialidase—an enzyme that cleaves sialic acid from N-glycans— accumulates in bioreactors due to its release from dead cells. EX1024, 943 (“Extracellular sialidase and glycosidases decrease sialic acid, cell viability can be a critical factor in final sialic acid levels...Extracellular sialidase will reduce sialylation”), 944 (“High-cell viability (less sialidase activity) in conjunction with high cell productivity (shorter residence time) may diminish the overall effect on sialylation.”). Accordingly, the level of sialylation on recombinant

proteins relates not just to the enzymatic addition of sialic acid within the producing cells but also to the build-up of sialidase outside the cells.

C. N-linked Glycosylation of Recombinant PH20

49. Prior to the '638 patent, recombinant PH20 hyaluronidase ("rHuPH20") was known to be glycosylated at several asparagine residues (i.e., N-linked glycosylation). For example, rHuPH20 is glycosylated at six different asparagine residues, including N82, N166, N235, N254, N368, and N393. *E.g.*, EX1006, [0031], [0114], [0136], [0137].

50. It was further known that of the six glycosylated asparagine residues in rHuPH20 only three asparagine residues were linked to complex glycans. EX1006, [0366].

VI. PRIOR ART

A. Wei – European Patent 3037529

51. European Patent 3037529 to Ge Wei, Krishnasamy Paneerselvam, Gregory I. Frost, and Louis Bookbinder is titled "Extended Soluble PH20 Polypeptides and Uses Thereof" ("Wei," EX1006). Wei was filed on December 9, 2009, was first published on June 29, 2016, and issued on March 27, 2019. I understand that Wei may be considered in evaluating the patentability of the claims of the '638 patent.

52. Wei describes temperature shift culturing methods specifically for production of recombinant PH20 hyaluronidase. Among other recombinant PH20

variants, Wei describes production and purification of “rHuPH20”—also known as SEQ ID NO:122—which is a recombinant human PH20 protein. The rHuPH20 protein differs from the 509 amino acid human PH20 protein in that it has 447 amino acids and lacks the 35 amino acid signal peptide at the N-terminus and 27 amino acids at the C-terminus of the molecule. EX1006, [0029], [0205], [0321] (“rHuPH20 (SEQ ID NO:122)”), [0386], Tables 29-30, pp. 292-293 (SEQ ID NO:122); *see also* EX1031, 430 (“509 amino acid human PH20 sequence”), 432 (describing rHuPH0 as “a 447 amino acid 61 kDa glycoprotein with a properly processed amino terminus and 6 N-linked glycosylation sites.”).

53. Wei discloses, for example, methods referred to as Gen1 and Gen2 for producing rHuPH20 in bioreactors. EX1006, Examples 13 & 15. Each of these methods include growing the cells initially at 37°C followed by decreasing the temperature in a stepwise fashion to 35.5°C and maintaining the culture at that lower temperature for 3 days. EX1006, [0360], [0373]. Gen1 and Gen2 methods for producing rHuPH20 control both pH and glucose concentration. EX1006, [0360], [0373].

54. Wei further discloses that rHuPH20 made according to Example 15C (Gen2) results in rHuPH20 with N-linked glycosylation.

rHuPH20 is glycosylated at six different asparagine residues, including N82, N166, N235, N254, N368, and N393. The results show that N254 is approximately 75% occupied, N393 is

approximately 85% occupied, and the four remaining sites, N82, N166, N235 and N368, are greater than 99% occupied. All of the three types of N-glycans, high mannose, hybrid and complex types, are present in rHuPH20. In general, rHuPH20 contains about 45% high mannose glycans, 45% complex glycans and 10% hybrid glycans. About 35% of the total glycans are anionic, of which 25% contain a sialic acid and the remaining 10% contain an unknown anionic group, possibly a phosphate group.

EX1006, [0324]; *see also id.*, [0322] (“Briefly, rHuPH20 (as produced in Example 15C)...”).

55. Wei further discloses producing crude soluble rHuPH20 with an enzymatic activity of 17,000 U/mL. EX1006, [0373]. Wei also discloses purification of crude soluble rHuPH20 and achieving enzymatic activity levels that exceed the level required by the claims. EX1006, [0362], Table 20 (reporting enzymatic activity of four independent preparations of 24,493, 26,683, 18,321, 21,052 U/mL), Tables 21 & 23, [0361]-[0367] (describing concentration and purification).

B. Zmuda – International Patent Publication WO2017/011598

56. International Patent Publication WO 2017/011598 to Jonathan Zmuda and Chao Yan Liu is titled “System and Method for Improved Transient Protein Expression in CHO Cells” (“Zmuda,” EX1005). Zmuda was filed on July 13,

2016, and was published on January 19, 2017. I understand that Zmuda may be considered in evaluating the patentability of the claims of the '638 patent.

57. Zmuda describes a temperature shift culturing method for production of recombinant proteins in an improved CHO cell line.

58. Zmuda discloses culturing CHO cells first at the physiologic temperature of 37°C then decreasing the temperature to sub-physiologic temperatures to improve yields of recombinant protein. Zmuda describes the sub-physiologic temperature ranges as “less than 37°C and greater than 30°C, less than 35°C and greater than 31°C, or at a temperature of about 32°C.” EX1005, [0021]; *see also id.*, Fig. 5, p. 82. Zmuda exemplifies a second lower temperature of 32°C in its “high titer” and “max titer” protocols. EX1005, 82.

59. Zmuda further describes an improved CHO cell line—referred to as “ExpiCHO”—and explains that the line is capable of generating “gram per liter or greater levels of protein expression.” EX1005, [0012], [0058], [0241].

C. Wei 2013 – WO 2013/102144

60. International Patent Publication WO 2013/102144 to Ge Wei, Michael H. Shepard, Qiping Zhao, and Robert James Conner is titled “PH20 Polypeptide Variants, Formulations and Uses Thereof” (“Wei 2013,” EX1037). Wei was filed on December 28, 2012, and was published on July 4, 2013. I understand that Wei

2013 may be considered in evaluating the patentability of the claims of the '638 patent.

61. Wei 2013 discloses recombinant PH20 hyaluronidase variants that have “increased stability and/or increased activity.” EX1037, Abstract.

VII. THE '638 PATENT

62. The '638 patent is titled “Method for Producing Recombinant Hyaluronidase.” EX1001, cover (54). The '638 patent issued on February 11, 2025 with 15 claims directed to methods of producing recombinant PH20 hyaluronidase. I have reviewed the specification of the '638 patent in its entirety and it describes culturing methods for producing recombinant PH20 hyaluronidase in CHO cells that are not inventive. Instead, the disclosed culturing methods include ranges for parameters long known to be associated with recombinant protein production such as temperature shift and maintaining pH and residual glucose concentration.

63. The '638 patent acknowledges that Halozyme developed and sold recombinant PH20 hyaluronidase under the tradename Hylenex™. EX1001, 1:54-60 (discussing Hylenex™ and recombinant PH20 expression) (citing Bookbinder 2006); *see also* EX1030, 231 (discussing production of a soluble recombinant form of human PH20 (“rHuPh20”) in CHO cells and its purification).

64. The '638 patent discloses two recombinant human PH20 proteins that it refers to as “wild-type human PH20” and “HM46.” *E.g.*, EX1001, Table 1. The

'638 patent does not describe the wild-type human PH20 or HM46 proteins but instead points to an International Patent Publication for their descriptions. 16:65-67 (“The sequences of wild-type human PH20 and HM46 are disclosed in International Patent Publication No. 2020/022791.”); *see also* EX1013 (WO2020/022791 which is a publication of PCT/KR2019/009215); EX1015 (US2021/0155913 claiming priority to PCT/KR2019/009215).

65. International Patent Publication No. 2020/022791 (EX1013) was published in the Korean language and is also published in the English language as US 2021/0155913A1 (“the ’913 application,” EX1015). According to the ’913 application, wild-type human PH20 is identified as SEQ ID NO: 1. EX1015, Abstract (“wild-type PH20 having the amino acid sequence of SEQ ID NO: 1”), pp. 36-37 (SEQ ID NO: 1). According to the ’913 application, wild-type human PH20 has 509 amino acids. The ’913 application does not disclose a “HM46” variant.

66. The ’638 patent acknowledges that the ExpiCHO cell line used in all disclosed experiments was obtained from a commercial source. EX1001, 17:8-10 (“transfecting ExpiCHO cells with recombinant genes using ExpiFectamine CHO Reagent (Gibco, USA)”; *see also id.*, 24:33 (“expression in ExpiCHO cells”), 25:2-3 (“Expression was performed using the ExpiCHO expression system.”). The ExpiCHO cell line used in the ’638 patent was initially described by Zmuda and

others for high titer production of recombinant glycoproteins. *See, e.g.*, EX1005, [0009]-[0026]; EX1036, 1 (“To address the significant unmet need for higher transient CHO protein titers, we employed systems-based approaches whereby the latest advances in cell culture media, feeds, transfection reagents, and expression enhancers were optimized in conjunction with a new high-expressing CHO cell clone.”); *see also* Section VI.B.

67. The ’638 patent exemplifies culturing methods that include a temperature shift and exclusively uses a first culturing temperature of 37°C and a lower second temperature of 32°C. EX1001, 17:36-42, 17:43-49, 17:51-59, 17:59-65, 17:67-18:9, 18:53-61, 19:28-30, 19:60-62, 20:66-67, 25:10-12. This is the same temperature shift temperatures exemplified in Zmuda. EX1005, p. 88; *see also* EX1036, 3-4 (describing feeding and shifting cells to 32°C); Section VI.B.

68. The ’638 patent claims a method for producing PH20 and variants thereof. *See* EX1001, claim 1 (“PH20 or a variant thereof”), 4 (“wherein the recombinant protein has a specific enzymatic activity as hyaluronidase at least 10% higher than a specific enzymatic activity of a wild human PH20”), 7 (“substitution of one or more amino acid residues when compared to an amino acid sequence of a wild human PH20”), 13 (“truncation of at least one amino acid residue of N-terminus when compared to an amino acid sequence of a wild human PH20”), 14 (“comprises truncation of at least one amino acid residue of C-terminus when

compared to an amino acid sequence of a wild human PH20”), 15 (“comprises truncation of at least one amino acid residue of N-terminus and at least one amino acid residue of C-terminus when compared to an amino acid sequence of a wild human PH20”).

69. The '638 patent takes a broad interpretation of the term “PH20 variant,” explaining that the term encompasses amino acid substitutions, deletions, insertions, and truncations so long as the variant has hyaluronidase enzymatic activity.

Human-derived “hyaluronidase PH20” or “PH20 variant” according to the present invention is exemplified in International Patent Publication No. 2020/022791 and U.S. Pat. No. 9,447,401, but is not limited thereto, and is interpreted to include any hyaluronidase or a variant which includes substitutions, deletions and insertions of one or more amino acid residues, optionally includes truncations of one or more amino acid residues of the N-terminal and/or C-terminal amino acid residues in the amino acid sequence of “hyaluronidase PH20” or “PH20”, and has hyaluronidase enzymatic activity.

EX1001, 10:11-21.

70. The '638 patent provides exemplary data only for “wild-type human PH20” and one variant. With respect to the wild-type human PH20, I have reviewed the '638 patent in its entirety and was unable to find any description of the method used to produce the protein. Accordingly, it is unknown whether the

claimed method or some other method was used. With respect to PH20 variants, only one is exemplified and that is HM46. *See* EX1001, Table 1, 17:4-18:14.

71. The '638 patent does not disclose the integral viable cell density (IVCD) reached during the first culturing in any of its examples. *See* EX1001, 17:42-43 (“when the integral viable cell density reached a predetermined level”), 17:49-50 (same), 17:58-59 (same), 17:65-66 (same), 18:9-10, 18:61-62 (“when the integral viable cell density (IVCD) reached a shift range”), 19:30-31 (“when the integral viable cell density reached the change range,”), 19:62-63 (same), 20:67-21:1 (same).

72. The '638 patent provides experimental data only for a second culturing lasting 5, 12, or 13 days. EX1001, 17:51, 17:67, 18:11, 25:12.

73. The '638 patent describes the invention as relating to culturing conditions that allegedly improve enzymatic activity.

In the present invention, when the host cells for producing recombinant hyaluronidase PH20 or a variant thereof are cultured under specific culture conditions, the N-glycosylation characteristics of the produced recombinant hyaluronidase PH20 or a variant thereof, and furthermore, the enzymatic activity of the produced hyaluronidase PH20 or a variant thereof, are remarkably improved.
EX1001, 4:49-55.

74. The '638 patent links the alleged improved enzymatic activity to sialylation, galactosylation and/or mannosylation levels.

Specifically, it was found that it is effective to increase the activity of the produced hyaluronidase PH20 or a variant thereof by controlling the sialylation, galactosylation and/or mannosylation levels, in particular, the sialylation level in the N-glycans, and that the enzymatic activity and productivity of the hyaluronidase PH20 or a variant thereof according to the present invention can be remarkably improved by performing culture for a certain time period after changing the culture temperature.

EX1001, 4:56-64.

75. Despite alleging a linkage between N-glycan levels and enzymatic activity, the broad claims of the '638 patent encompass much of the practical range for each of these physical properties. Specifically, the claims require a minimum of 10,000 units/mL hyaluronidase enzymatic activity and 1 to 38% sialylation. As for hyaluronidase enzymatic activity, the specification states only:

All of Cell line test cultures #2, #3, #4, and Cell line cultures #1, #2, #3, #4 shown in Table 1, which are obtained by culture methods using the results of Examples 2, 3, 4 and 5, exhibited an enzyme expression level of 10,000 unit/mL or more....

EX1001, 18:44-49.

76. With respect to the sialylation levels, the specification provides values in a range of 2.5 to 28%. *E.g.*, EX1001, Table 1. As for galactosylation and mannosylation the specification exemplifies a range of 14.8 to 49.6% and 47.9 to 58.3%, respectively. EX1001, Table 1. Despite that all exemplified values for

sialylation, galactosylation, and mannosylation are within the claimed ranges, not all culture methods used to produce recombinant PH20 are within the scope of the claims. EX1001, Table 1, 17:24-26 (“Cell line clone test culture #1 is a 2 L batch culture performed at 37° C. without shift the culture temperature”).

77. The alleged improvements in glycosylation and activity levels are not different than what was known in the art. For example, Wei describes producing recombinant PH20 hyaluronidase that meets the claimed activity and sialylation level requirements. *Compare* EX1001, claim 1 (“wherein sialylation of N-glycan of the produced recombinant protein is 1 to 38%”) *with*, EX1006, [0324] (“25% [of N-glycans] contain a sialic acid”). Wei further describes the activity level for the produced recombinant PH20 hyaluronidase as higher than 10,000 units/mL. EX1001, claim 1 (“hyaluronidase enzymatic activity at 10,000 units/mL or higher”), 7:33-51 (explaining the glycosylation “numerical values may also include an error of 10%”); EX1006, [0373] (“a final productivity of 17,000 Units per ml”), Tables 20, 23; *see also* Section VI.A.

78. Furthermore, the claimed method of producing recombinant PH20 hyaluronidase is nearly identical to Wei’s method. Wei describes a temperature shift method that maintains pH and glucose concentrations within the claimed ranges. To the extent Wei did not expressly disclose the claimed lower temperature range, it was disclosed by Zmuda among several other references.

79. As I explain in further detail below, a person of ordinary skill in the art would have considered the '638 patent's claimed method of producing recombinant PH20 hyaluronidase obvious in view of prior art such as Wei and Zmuda.

VIII. OVERVIEW OF THE LEVEL OF SKILL

80. I have been advised that "a person of ordinary skill in the relevant field" (or "person of ordinary skill in the art," or "POSA") is a hypothetical person to whom one could assign a routine task with reasonable confidence that the task would be successfully carried out. I have been advised further that, in determining the level of ordinary skill in the art that someone would have had at the time the claimed invention was made, I should consider: (1) the levels of education and experience of persons working in the field; (2) the types of problems encountered in the field; and (3) the sophistication of the technology.

81. In my opinion, a person of ordinary skill in the relevant field as of August 7, 2020, would typically have an M.S. or Ph.D. in cell biology, biochemistry or related life sciences discipline, or could have less education but significant professional experience in one or more of these fields.

IX. CLAIM CONSTRUCTION

82. I have been advised that the claim terms of the '638 patent claim are to be given the plain and ordinary meaning that the terms would have to a person

of ordinary skill in the art in question at the relevant priority date. I understand that the analysis focuses on how the patentee used the claim term in the claims, specification, and prosecution history. I also understand that dictionaries or other sources may assist in determining the plain and ordinary meaning but cannot override a meaning that is unambiguous from the patent itself. I have followed these principles in my analysis throughout this declaration.

X. GROUND 1 – CLAIMS 1-15 ARE OBVIOUS AS ILLUSTRATED BY WEI AND ZMUDA

83. As discussed in detail herein, a person of ordinary skill in the art would have considered claims 1-15 obvious. The combination of Wei and Zmuda, for example, describes a method of producing recombinant PH20 hyaluronidase that is indistinguishable from what is claimed. Below, I note specific prior art disclosure that satisfies each element of each challenged claim. After each claim element, I provide a chart identifying certain exemplary prior art disclosure that is relevant to that element.

84. Zmuda describes an optimized CHO cell line (ExpiCHO) along with culturing methods for producing high titers (> 2 g/L) of recombinant glycoproteins. EX1005, [0013]; *see* Section VI.B. According to Zmuda, titers are substantially increased (e.g., 2- to 3-fold) when CHO cells are grown first at 37°C and are then shifted to a lower second temperature below 37°C. EX1005, [0021], [00245], Figs. 5, 6, 8.

85. Wei discloses methods for producing recombinant hyaluronidase PH20 in cultured animal cells. *E.g.*, EX1006, Examples 13 & 15. The disclosed methods include a temperature shift in which cells are first cultured at 37°C and then are shifted to a series of lower second temperatures below 37°C including 35.5°C. EX1006, [0360], [0373]. Wei further discloses culturing the cells for 3 days at 35.5°C prior to harvesting. EX1006, [0360], [0373].

86. Wei teaches culturing cells in a bioreactor at a pH set to 7.2 and further teaches sampling daily for “pH verification.” EX1006, [0360], [0373]. Wei also teaches equipping the bioreactor “with instrumentation that monitors and controls pH.” EX1006, Table 27.

87. Wei teaches feeding the cultured cells with media comprising glucose during the second culturing. EX1006, [0360], [0373], Table 27.

88. Wei discloses producing recombinant hyaluronidase that meets the sialylation requirement. Wei discloses:

In general, rHuPH20 contains about 45% high mannose glycans, 45% complex glycans and 10% hybrid glycans. About 35% of the total glycans are anionic, of which 25% contain a sialic acid and the remaining 10% contain an unknown anionic group, possibly a phosphate group.

EX1006, [0324].

89. Wei discloses that its methods produce recombinant hyaluronidase with enzymatic activity at 10,000 units/mL or higher. EX1006, [0373] (“17,000 Units per ml”). Wei further discloses that its method produces concentrated and partially purified recombinant hyaluronidase with enzymatic activity in excess of that claimed. EX1006, Tables 19, 20, 23.

90. A person of ordinary skill in the art would have had good reason to combine the teachings of Wei and Zmuda to achieve what is recited in the claims and would have reasonably expected success in doing so.

91. Zmuda discloses a temperature shift protocol for producing recombinant glycoproteins and further teaches that the second culturing temperature is in the range of 30-37°C. *See* Section VI.B. Wei discloses a temperature shift protocol for producing recombinant PH20 hyaluronidase that uses temperatures below 37°C and exemplifies 35.5°C as a lower second culturing temperature. *See* Section VI.A. A person of ordinary skill in the art would have been motivated to combine the teachings of Wei and Zmuda and would have expected success because it was well established and documented in the literature that optimizing the lower second culturing temperature would improve yields. *See* Section V.A.

92. Reasons to combine Wei and Zmuda may be derived from, among other places, the references themselves. For example, Zmuda teaches lowering the

culture temperature within the range of 30-37°C. EX1005, [0021] (“then further cultured at a temperature of less than 37°C and greater than 30°C, less than 35°C and greater than 31°C, or at a temperature of about 32°C.”). Zmuda further teaches that decreasing the temperature from 37°C to a temperature within the disclosed range (e.g., 32°C) may yield 2-3 times greater recombinant protein titers than cultures grown at a constant 37°C. EX1005, 82.

93. Prior art teachings regarding the benefits of optimizing the lower culturing temperature are discussed not only in Zmuda but are well supported in other relevant literature at the time. It was considered routine to optimize the second culturing temperature when producing recombinant proteins in animal cells. *E.g.*, EX1014, 2 (“In the past ten years, there have been frequent reports indicating that a decrease of a few degrees in temperature from 37°C, the usual culture temperature, until 28°C, which is called mild hypothermia, allows an incremental increase in the specific productivity of r-proteins in CHO cell culture (Table 2).”), Table 2 (collecting examples of mild hypothermia); EX1012, 191 (“Since temperature downshifts have been reported to improve protein productivity by many different CHO cell lines, it is widely used as an effective platform strategy for cell culture process development and manufacturing in the biopharmaceutical industry.”); EX1022, 1055 (“shifts to lower incubation

temperatures are commonly used in industry to increase culture duration and titer”).

94. A person of ordinary skill in the art would have expected reductions in the culture temperature to result in improvements to yield and quality of the produced recombinant protein. EX1012, 191 (“Such a TS strategy holds the potential to improve fermentation and cell culture performance for a prolonged period with better overall productivity and quality.”), *id.* (“The effects of temperature downshift in CHO cell cultures on key quality attributes, including protein aggregation, charge variants, N-glycans and host cell proteins, has been reported.”), EX1014, 2 (“Thus, decreasing the culture temperature has been suggested as a tool for the improvement of the production process,...”). Such improvements to yield and quality of the produced recombinant protein would have been expected to result in increases in hyaluronidase activity.

95. A person of ordinary skill in the art would have reasonably expected improvements in overall recombinant protein yield to result from optimization of the lower second culturing temperature. However, as confirmed by the relevant prior art, a skilled artisan would have expected only modest changes in N-linked glycosylation levels, including sialylation levels. EX1020, 8 (“no decrease in Epo-Fc sialylation is anticipated at low cultivation temperature (30°C) and acidic pH (6.75),”); EX1027, 196 (“These results suggest that the commonly used

temperature shift strategy to enhance productivity is generally a safe practice from a protein glycosylation perspective..."); EX1028, Figure 6A (showing only minor differences in sialylation in cultures grown at 30°C as opposed to 37°C); EX1014, 2 ("With regard to the quality of the r-protein, it seems that in most cases, there are no significant changes in the glycosylation profiles, including the degree of terminal sialylation."). Accordingly, a person of ordinary skill in the art would have had good reason to combine Wei and Zmuda and would have reasonably expected success in achieving the claimed method. In my opinion, claims 1-15 are obvious.

96. The prior art knowledge that the glycosylation levels do not appreciably change with temperature is further corroborated by the '638 patent itself. In Table 1 (reproduced below), the culture in the red box was grown at 37°C whereas the culture in the blue box was grown at 37°C then shifted to 32°C. EX1001, 17:24-25 ("Cell line clone test culture #1 is a 2 L batch culture performed at 37°C."), 17:36-42 ("Cell line test culture #1 was obtained by culturing at a culture temperature of 37°C ... then shifted the culture temperature to 32°C."), Table 1. There is no significant difference in the N-glycan content of these two cultures. A person of ordinary skill in the art would understand these data to support the lack of strong correlation between growth temperature and sialylation values that was already taught in the prior art.

TABLE 1

Hyaluronidase type	Preparation	Relative activity	N-Glycan content		
			Galactosylation (%)	Sialylation (%)	Mannosylation (%)
Wild-type human PH20	Temporary expression culture	100%	32.9	15.2	57.2
Wild-type human PH20	Cell line culture	100%	42.5	21.7	54.8
HM46	Temporary expression culture	167%	37.1	7.4	48.1
HM46	Cell line clone test culture #1	115%	46.9	18.6	48.9
HM46	Cell line clone test culture #2	161%	40.6	16.4	54.4
	purified fraction #1				
HM46	Cell line clone test culture #2	86%	42.1	28	56.8
	purified fraction #2				
HM46	Cell line test culture #1	158%	49.6	14.4	48.1

A. Claim 1

0. Preamble

A method for producing a recombinant protein that comprises at least one of hyaluronidase PH20 or a variant thereof, the method comprising

97. Wei teaches the preamble of claim 1.
98. Wei discloses methods for producing recombinant hyaluronidases.

PH20 polypeptides, including esPH20 polypeptides and C-terminally truncated PH20 polypeptides provided herein, can be produced by any method known to those of skill in the art including in vivo and in vitro methods. Desired proteins can be expressed in any organism suitable to produce the required amounts and forms of the proteins, such as for example, needed for administration and treatment. Expression hosts include prokaryotic and eukaryotic organisms such as *E. coli*, yeast, plants, insect cells, mammalian cells, including human cell lines and transgenic animals. Expression hosts can differ in their protein production levels as well as the types of post-translational modification that are present on the expressed proteins. The choice of expression host can be made based on these and other factors, such as regulatory and safety considerations, production costs and the need and methods for purification.

EX1006, [0186].

99. Wei also discloses that mammalian cell lines, including CHO cells, may be used as expression hosts for producing recombinant PH20 hyaluronidase.

Many cell lines are available for mammalian expression including mouse, rat human, monkey, chicken and hamster cells. Exemplary cell lines include but are not limited to CHO, Balb/3T3, HeLa, MT2, mouse NS0 (nonsecreting) and other myeloma cell lines, hybridoma and heterohybridoma cell lines, lymphocytes, fibroblasts, Sp2/0, COS, NIH3T3, HEK293, 293S, 2B8, and HKB cells. Cell lines also are available adapted to serum-free media which facilitates purification of secreted proteins from the cell culture media. Examples include CHO-S cells (Invitrogen, Carlsbad, CA, cat #11619-012) and the serum free

EBNA-1 cell line (Pham et al., (2003) Biotechnol. Bioeng. 84:332-42.). Cell lines also are available that are adapted to grow in special mediums optimized for maximal expression. For example, DG44 CHO cells are adapted to grow in suspension culture in a chemically defined, animal product-free medium.

EX1006, [0198].

100. Wei discloses that its methods include producing recombinant human hyaluronidase PH20 and variants thereof.

Human soluble esPH20 polypeptides include, in addition to residues 36-490, one or more contiguous amino acids from amino acid residue position 491 of SEQ ID NO:107, inclusive, such that the resulting polypeptide is soluble. Exemplary human esPH20 soluble polypeptides are those that have amino acids residues corresponding to amino acids 36-491, 36-492, 36-493, 36-494, 36-495, 36-496 and 36-497 of SEQ ID NO.107. Exemplary of these are those with an amino acid sequence set forth in any of SEQ ID NOS:60-63 and 102-104. Also included are allelic variants and other variants, such as any with 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence identity with the corresponding polypeptides of SEQ ID NOS: 60-63 and 102-104 that retain neutral activity and are soluble. Reference to sequence identity refers to variants with amino acid substitutions.

EX1006, [0026].

101. Wei further exemplifies using CHO cells to produce soluble recombinant human PH20 hyaluronidase (“rHuPH20”). EX1006, Examples 13 & 15.

102. Zmuda also discloses a method for producing recombinant protein. For example, Zmuda describes an improved CHO cell line—referred to as “ExpiCHO”—and explains that the line is capable of generating “gram per liter or greater levels of protein expression.” EX1005, [0012], [0058], [0241]. Zmuda discloses improving the production of recombinant proteins in CHO cells by performing a temperature shift for the protein production phase after the cell proliferation stage. EX1005, [0021], Fig. 5, p. 82. Although Zmuda does not explicitly mention hyaluronidase PH20, a person of ordinary skill in the art would have understood that ExpiCHO cells were useful for recombinant hyaluronidase PH20 production.

'638 patent	Prior Art
[Preamble] A method for producing a recombinant protein that comprises at least one of hyaluronidase PH20 or a variant thereof, the method comprising:	Wei: Example 11 (“Generation of a soluble rHuPH20-expressing cell line”) Example 14 (“Production Gen2 Cells Containing Soluble human PH20 (rHuPH20)”) <i>See also</i> EX1006, [0012], [0013], [0025]-[0034], [0072], [0123]-[0130], [0135]-[0157]; EX1031, 430-433; Section V.

103. Accordingly, Wei and Zmuda teach the preamble of claim 1.

1. Element 1.1

first culturing host animal cells expressing the recombinant protein until an integral viable cell density of the host animal cells reaches 20×10^6 to 120×10^6 cells $\times$ day/mL, wherein first culturing is performed within a first temperature range from 35°C to 38°C; and

104. Wei and Zmuda teach Element 1.1.

105. Zmuda describes culturing methods for producing high titers (> 2 g/L) of recombinant glycoproteins in host animal cells (i.e., CHO cells). EX1005, [0013]; *see* Section VI.B. According to Zmuda, titers are substantially increased (e.g., 2- to 3-fold) when CHO cells are grown first at 37°C and are then shifted to a lower second temperature below 37°C. EX1005, [0021], [00245], Figs. 5, 6, 8.

106. Wei discloses generating host animal cells (e.g., CHO) that express recombinant PH20. Specifically, Wei describes generation of two expression hosts—referred to as 3D35M and 2B2—that produce soluble recombinant PH20 hyaluronidase (“rHuPH20”). EX1006, Examples 11 & 14. The “Gen1” method disclosed in Wei, is a method for producing and purifying rHuPH20 from 3D35M cells. EX1006, Example 13. The “Gen2” method disclosed in Wei, is a method for producing and purifying rHuPH20 from 2B2 cells. EX1006, Example 15.

107. While the production and purification methods of Gen1 and Gen2 are similar, the Gen2 method generally produces more rHuPH20. *Compare* EX1006, [0360] (“The process resulted in production of soluble rHuPH20 with an enzymatic

activity of 1600 Units/ml...) *with, id.*, [0373] (“The process resulted in a final productivity of 17,000 Units per ml ...”).

108. Wei teaches a first culturing of host animal cells expressing the recombinant protein at a first temperature of 37°C. Specifically, both Gen1 and Gen2 methods use a temperature shift and culture cells at a first temperature of 37°C. EX1006, [0360], [0373].

109. A person of ordinary skill in the art would have understood the claimed integrated viable cell density (IVCD) range to be conventional. Wei, for example, teaches first culturing host animal cells expressing the recombinant protein until an integral viable cell density of the host animal cells reaches 20×10^6 to 120×10^6 cells $\times$ day/mL.

110. A person of ordinary skill in the art would have understood “integral of viable cell density” or “IVCD” to be a measure of the prolongation of viable cell density (VCD) over time. In a batch culture of mammalian cells, exponential growth continues from the cell inoculum to a maximum viable cell density before entering a decline phase. In a fed-batch culture the decline phase may be delayed or the rate of decline reduced through feeding of the cells. Delaying or reducing the decline rate has the effect of viable cells being maintained over a longer period of time and results in a higher overall product yield. EX1034, 887. IVCD represents the integral of viable cell density values from time zero to the time point

of interest (e.g. temperature shift, harvest). That is, IVCD is typically measured by the area under the growth curve.

111. Wei does not disclose values for viable cell density over time but does disclose data regarding inoculation and maximum cell densities and growth rates that might be used to estimate IVCD over time. For example, Wei discloses that the bioreactor is seeded with between 0.33 and 0.46×10^6 cells/mL at time zero. EX1006, Table 19. Wei further discloses that the doubling time for the cells during logarithmic growth is between 23.5 and 29.8 h. *Id.* Wei also discloses that the maximum cell density is between 5.65 and 9.70×10^6 cells/mL. *Id.* Based on these data, I would estimate the IVCD for the culture to be within the claimed range at day 6 and later.

112. Below I show a calculation made to explain my estimation based on the data for HUA0420C in Table 19 of Wei. This data reveals that the cells were inoculated at a concentration of 0.46×10^6 cells/mL and grew to a maximum cell density of 9.70×10^6 cells/mL by logarithmic growth with a doubling time of 23.5 h.

113. Assuming exponential growth, I can calculate the time (t) it would take for the cells to reach maximum cell density (X_f) from the initial cell density (X_0) with a doubling time (DT) using the formula:

$$t = DT \cdot \log_2 \left(\frac{X_f}{X_0} \right)$$

$$t = 23.5 \cdot \log_2 \left(\frac{9.7}{0.46} \right) = 103.4 \text{ h} = 4.31 \text{ days}$$

114. The IVCD over this time period (0 to 4.31 days) was calculated using the exponential formula:

$$\text{IVCD} = \frac{(X_f - X_0)}{\ln(2)} \cdot DT$$

$$\text{IVCD} = \frac{9.7 - 0.46}{0.693} \cdot 23.5 = 313.3 \times 10^6 \text{ cells} \cdot \text{hour/mL}$$

$$\text{IVCD}_{\text{days}} = \frac{313.3}{24} = 13.05 \times 10^6 \text{ cells} \cdot \text{day/mL}$$

115. From the data, the culture continued to day 13 when the cell viability was 41%. To calculate the VCD at this point, it can be assumed that at maximum cell density the % viability would likely be around 95%. Therefore, at day 13 the VCD was estimated at:

$$\text{VCD}_{\text{day13}} = \frac{41}{95} \cdot 9.7 \times 10^6 \text{ cells/mL} = 4.2 \times 10^6 \text{ cells/mL}$$

116. In the Table presented below I have assumed a gradual linear decrease in viable cell density during the decline period after maximum cell density (day 4.3) to day 13 to interpolate the data and calculate the cumulative IVCD using the trapezoid method from the equation:

$$\text{IVCD}_i = \frac{\text{IVCD}_{i-1} + \text{IVCD}_i}{2} \cdot (t_i - t_{i-1})$$

The cumulative IVCD values were calculated from time zero

Time (day)	VCD (x 10⁶ cells/mL)	Cumulative IVCD (x 10⁶ cells.day/mL)
0	0.46	0
4.3	9.7	13.05
5	9.25	19.68
6	8.62	28.62
8	7.36	44.60
10	6.10	58.06
11	5.47	63.85
12	4.83	68.99
13	4.2	73.51

117. The cumulative values in the Table show that the critical IVCD of 20×10^6 cells*day/mL claimed in the '638 patent was attained in the culture (HUA0420C) cited in Table 19 of Wei at day 6 and was maintained within the claimed range of 20×10^6 to 120×10^6 cells*day/mL throughout the culturing.

118. My opinion regarding the IVCD values for the CHO cells expressing recombinant PH20 hyaluronidase described in Wei is confirmed by an additional Halozyme patent that describes the Gen2 rHuPH20 production method (Baker, EX1035). For example, Baker discloses values for viable cell density over time for the Gen2 rHuPH20 production method. EX1035, Example 7, Table 24

119. From the data included in Table 24 of Baker, I calculated the cumulative integral of viable cell density (IVCD) values using the standard trapezoidal integration formulation:

$$IVCD = \sum_{t_1}^{t_2} \left(\frac{VCD_1 + VCD_2}{2} \right) \cdot (t_2 - t_1)$$

Hours	VCD (x10 ⁵ /ml)	IVCD 10 ⁵ cells.hours/ml	IVCD 10 ⁶ cells.day/ml
0	4.7	0	0
24	8.9	163.2	0.68
50	19.3	529.8	2.21
76	36.7	1257.8	5.24
120	73.6	3684.4	15.35
145	84.3	5658.15	23.58
165	98.8	7489.15	31.20
190	95.3	9915.4	41.31
201	105	11017.05	45.90
214	95.9	12322.9	51.35
242	81.2	14802.3	61.68
268	51.9	16532.6	68.89
287	38.4	17390.45	72.46
308	31.6	18125.450	75.52

120. As with Wei, the growth curves in Baker demonstrate that the critical IVCD of 20 x 10⁶ cells*day/mL claimed in the '638 patent was attained in at day 6

and was maintained within the claimed range of 20×10^6 to 120×10^6 cells*day/mL throughout the culturing.

121. Accordingly, Wei teaches culturing cells at a first temperature (37°C) until an IVCD in a range of 20×10^6 to 120×10^6 cells*day/mL is achieved.

'638 patent	Prior Art
[1.1] first culturing host animal cells expressing the recombinant protein until an integral viable cell density of the host animal cells reaches 20×10^6 to 120×10^6 cells*day/mL, wherein first culturing is performed within a first temperature range from 35°C to 38°C ; and	<i>See also</i> EX1006, Table 19; EX1014, 2, Table 2; EX1016, 11378; EX1017, 21; EX1018; Section V.A

122. Accordingly, Wei and Zmuda teach Element 1.1.

2. Element 1.2

subsequently second culturing at least part of the host animal cells from first culturing for 2 to 18 days, which produces the recombinant protein, wherein second culturing is performed within a second temperature range from 28°C to 34°C

123. Wei and Zmuda teach Element 1.2.

124. Wei discloses a temperature shift method for producing rHuPH20.

Both Gen1 and Gen2 methods use a temperature shift in which the temperature of the culture is reduced from 37°C to a second culturing temperature. Both Gen1

and Gen2 methods culture of the host animal cells at the reduced temperature for between 2 and 18 days to produce the recombinant PH20 protein. Specifically, Wei discloses decreasing the temperature to 35.5°C and culturing the cells for an additional 3 days. EX1006, [0360], [0373].

125. Temperature shift culturing is also described by Zmuda in relation to production of recombinant proteins in ExpiCHO cells, an improved CHO cell line. Zmuda explains that the ExpiCHO cell line is capable of generating “gram per liter or greater levels of protein expression.” EX1005, [0012], [0058], [0241]. The ExpiCHO cell line used by Zmuda satisfies element 1.2, which status within the claim scope is confirmed by its use throughout the ’638 patent. EX1001, 17:8 (“produced by transfecting ExpiCHO cells”), 24:33 (“expression in ExpiCHO cells”), 25:2-3 (“Expression was performed using the ExpiCHO expression system.”).

126. Zmuda describes culturing methods that include a temperature shift in which cells are “further cultured at a temperature of less than 37°C and greater than 30°C, less than 35°C and greater than 31°C, or at a temperature of about 32°C.” EX1005, [0021]. Zmuda exemplifies shifting from 37°C to a second culturing temperature of 32°C in its “high titer” and “max titer” protocols. EX1005, 82, 88.

127. Zmuda discloses performing the second culturing for 2 to 18 days.

Zmuda discloses that the shift to the second culturing temperature of 32°C occurs day 1 post-transfection for each protocol, and that the cells are harvested on Day 10-12 post-transfection in the “high titer” protocol and Day 12-14 post-transfection in the “max titer” protocol. EX1005, 81-82, 88.

128. A person of ordinary skill in the art would have reasonably expected improvements in overall recombinant protein yield to result from routine optimization of the lower second culturing temperature. The routine nature of culture temperature optimization and its positive effects on recombinant protein production are well-established in the relevant literature, including Zmuda. EX1005, [0021], [0245], Figs. 5, 6, 8; EX1014, 2, Table 2; EX1012, 191; EX1022, 1055; *see also* Section V.A.

129. Accordingly, a person of ordinary skill in the art would have had good reason to optimize the second culturing temperature and would have reasonably expected success in achieving what is claimed.

'638 patent	Prior Art
[1.2] subsequently second culturing at least part of the host animal cells from first culturing for 2 to 18 days, which produces the recombinant protein,	EX1005, [0058]; EX1014, 2, Table 2; EX1016, 11378; EX1017, 21; EX1018; Section V.A

wherein second culturing is performed within a second temperature range from 28°C to 34°C,	
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130. Accordingly, Wei and Zmuda teach Element 1.2.

3. Element 1.3

wherein the second culturing maintains (a) a residual glucose concentration within a range from 0.001 g/L to 4.5 g/L and/or (b) pH within a range from 6.8 to 7.2 in a culture medium therefor,

131. Wei teaches Element 1.3.

132. As an initial matter, Element 1.3 is satisfied when the second culturing maintains the recited glucose concentration *or* pH range, even if both are not maintained. However, as explained below, Wei teaches both limitations.

133. A person of ordinary skill in the art would have known that maintaining glucose concentrations within an optimal range was beneficial for recombinant protein production. It was also known that cultured cells would deplete glucose in the media over time. To that end, it was conventional to feed cells during the culturing with media comprising glucose to maintain optimal glucose levels (e.g., 1 g/L to 4 g/L). EX1005, [004], [007], [0041], [0233], [0236], [0241], pp. 82, 86-88, Figs. 5, 6; EX1006, [0354], [0360], [0373], EX1009, 772; EX1012, 200; EX1034, 888; *see also* Section V.A.

134. Wei teaches maintaining the glucose concentration in an optimal range. For example, the Gen1 method (Example 13) includes feeding the cultured cells on day 11—the day the cells are shifted to 35.5°C—with a supplement that comprises glucose. EX1006, [0360]. More specifically, the cells are provided with Feed #3 which includes CD CHO media (4.5-6.5 g/L glucose) and 50 g/L glucose. The total concentration of glucose in Feed #3 is around 54 to 56 g/L. The volume of Feed #3 is 3.7 L and the volume of the culture after Feed #3 is 95.6 L (i.e., 85 L + the volume of each of the three feeds). Thus, the added concentration of glucose is 2.1 g/L (i.e., $\sim 54 \text{ g/L} \times (3.7 \text{ L} / 95.6 \text{ L})$).

135. Example 15 also describes feeding cells on day 11—the day the cells are shifted to 35.5°C—with a supplement that comprises glucose. EX1006, [0373]. More specifically, the cells are provided with Feed #4 which includes CD CHO media (4.5-6.5 g/L glucose) and 33 g/L glucose. The total concentration of glucose in Feed #4 is 40 to 42 g/L. The volume of Feed #4 is 10.8 L and the volume of the culture after Feed #4 is 303 L (i.e., 260 L + the volume of each of the four feeds). Thus, the added concentration of glucose is $\sim 1.4 \text{ g/L}$ (i.e., $\sim 40 \text{ g/L} \times (10.8 \text{ L} / 303 \text{ L})$).

136. While Wei does not disclose the amount of glucose in the media before or after feeding the cells, a person of ordinary skill would have expected the concentration to be within the claimed range. First, a person of ordinary skill

would not have expected the residual glucose concentration to be below 0.001 g/L because glucose is a required nutrient. Allowing the glucose concentration to dip below 0.001 g/L would result in the death of the cultured cells.

137. Second, a person of ordinary skill would not have expected the residual glucose concentration to be above 4.5 g/L during the second culturing (i.e., days 11 and beyond). Wei describes growing cells in defined CHO media (e.g., CD CHO) starting at time zero. A person of ordinary skill in the art would have known that defined CHO media typically has a glucose concentration between 4.5 and 6.5 g/L.

138. It was well known and documented in the literature, that CHO cells deplete glucose in the media over time. For example, during exponential growth phase of culturing (e.g., days 0 to 2), metabolism of glucose by CHO cells will cause the concentration of glucose to decrease from ~6.5 g/L to ~1 g/L. EX1012, Fig. 1B. During the stationary phase, CHO cells will cause the concentration of glucose to decrease from 4.0 g/L to 1 g/L in approximately 1.5 days (e.g., between 85 and 120 h). *Id.*

139. Accordingly, a person of ordinary skill in the art would not expect adding 1.4 g/L or 2.1 g/L glucose to the cultured cells to cause the total glucose in culture to exceed 4.5 g/L.

140. To the extent Wei does not teach maintaining the residual glucose concentration in a range from 0.001 to 4.5 g/L, it would have been obvious to do. As I explain above, fed-batch cultures were used to maintain the viability of cultured cells over a longer period of time as compared to batch cultures. The increased viability over time was known to be a consequence of feeding cells to maintain nutrients within an optimal range. It was conventional to maintain residual glucose concentration in a range from 1.0 to 4.0 g/L through sequential feeding of the culture. *See* EX1012, Fig. 1B (exemplifying maintaining the glucose concentration in a range of 1-4 g/L), 200 (“Additional glucose was supplied to maintain the concentration range”); EX1009, Abstract (“The initial extracellular glucose concentrations used represented high (30 mM) and low (10 mM) glucose conditions, where at the time the transcriptomes were compared, the glucose concentrations were approximately 24 and 4.4 mM for the mid-exponential cultures, where 4.4 mM represents a common target concentration in the biopharmaceutical industry for controlled fed-batch cultures.”), 772 (“Other researchers have focused directly on maintaining low glucose concentrations (~5 mM)¹ via controlled fed-batch conditions interfaced with nonlinear models.”); *see also* Section V.A. Accordingly, a person of ordinary skill in the art would have

¹ 4.4 mM and 5 mM glucose is about 0.8 g/L and 0.9 g/L glucose, respectively.

considered it obvious to maintain the concentration of an essential nutrient (glucose) within a range concentrations known to promote viability of the cultured cells (e.g., 1.0 to 4.0 g/L).

141. As for pH, Wei teaches culturing cells in a bioreactor at a pH set to 7.2 and further teaches sampling daily for “pH verification.” EX1006, [0360], [0373]. In addition, Wei teaches equipping bioreactors with instrumentation that allows for monitoring and control of pH. EX1006, Table 27 (“Equipped with instrumentation that monitors and controls pH,”). This disclosure falls within the pH range of the claim.

142. As explained above, the conventionality of the claimed range is well-documented in the relevant literature. EX1019, 57 (“The optimum pH was in the region of 7.0 to 7.2”); EX1012, 200 (“the pH was controlled at pH 7.1”); EX1020, 4 (“Typical pH set points for CHO cells are neutral to slightly alkaline (7.0–7.2).”); EX1021, 1036 (“At reduced pH levels (6.90 and 6.80) the cultivation time could be prolonged to 9 and 12 days.”). Indeed, even the inventors of the ’638 patent admit that a pH range of 7.2 ± 0.4 —which overlaps with the claimed range — is “conventional.” EX1001, 21:9-11, Table 2. I agree with the inventors that this range is conventional for culturing CHO cells. It is thus unsurprising that maintaining pH within this range is disclosed in Wei.

143. Furthermore, Wei’s disclosure of the claimed pH is thus consistent with the literature, including the disclosure of Zmuda to use “buffering agents suitable for use in cell culture applications,” such as MOPS, HEPES, and phosphate which are optimal for buffering at physiological pH levels. EX1005, [0147].

'638 patent	Prior Art
[1.3] wherein the second culturing maintains (a) a residual glucose concentration within a range from 0.001 g/L to 4.5 g/L and/or (b) pH within a range from 6.8 to 7.2 in a culture medium therefor,	<i>See also</i> EX1009, 772; Section V.A

144. Accordingly, Wei and Zmuda teach Element 1.3.

4. Element 1.4

wherein sialylation of N-glycan of the produced recombinant protein is 1 to 38%, and

145. Wei teaches Element 1.4.

146. Wei further discloses performing a “glycan analysis study” on the rHuPH20 made according to Example 15. EX1006, Example 6; *see id.*, [0322] (“Briefly, rHuPH20 (as produced in Example 15C)...”).

147. Wei discloses that purified rHuPH20 has 25% sialylated N-glycans, which falls squarely within the claimed range:

rHuPH20 is glycosylated at six different asparagine residues, including N82, N166, N235, N254, N368, and N393. The results show that N254 is approximately 75% occupied, N393 is approximately 85% occupied, and the four remaining sites, N82, N166, N235 and N368, are greater than 99% occupied. All of the three types of N-glycans, high mannose, hybrid and complex types, are present in rHuPH20. In general, rHuPH20 contains about 45% high mannose glycans, 45% complex glycans and 10% hybrid glycans. About 35% of the total glycans are anionic, of which 25% contain a sialic acid and the remaining 10% contain an unknown anionic group, possibly a phosphate group.

EX1006, [0324].

148. In my opinion, a person of ordinary skill in the art would not have expected modest changes to the second culturing temperature to result in sialylation levels outside the claimed range. It was well established in the relevant literature at the time that sialylation levels may modestly decrease but would not be expected to increase in response to small decreases in culture temperature. *E.g.*, EX1020, 8 (“Product sialylation did not change between cultivation temperatures of 39–35°C, but decreased by 20% at 33°C and 40% at 30°C. However, Trummer et al. (2006b) later showed that reduced sialylation at cell culture temperature of 33°C and 30°C was prevented when used in combination with slightly acidic pH levels (pH set point 6.90).”); EX1027, 196 (“Reducing the culture temperature from 37 C to 33 C in a CHO cell line expressing human granulocyte macrophage

colony stimulating factor saw little change in protein glycosylation and terminal sialylation, but did enhance specific productivity [43].”); EX1028, Figure 6A; EX1014, 2 (“With regard to the quality of the r-protein, it seems that in most cases, there are no significant changes in the glycosylation profiles, including the degree of terminal sialylation. Accordingly, these r-proteins produced under mildly hypothermic conditions show the same in vitro biological activity as the r-proteins produced at 37°C”).

149. Accordingly, it is my opinion that a person of ordinary skill in the art would expect the small difference in temperature for the second culturing (35.5°C vs. 34°C) would not result in sialylation outside the claimed range. My opinion in this regard is further corroborated by the '638 patent itself which demonstrates that culturing temperature (e.g., 37°C v. 32°C) does not appreciably change glycosylation levels (e.g., sialylation). *See* EX1001, 17:24-25 17:36-42, Table 1; *see also* Section VII.

'638 patent	Prior Art
[1.4] wherein sialylation of N-glycan of the produced recombinant protein is 1 to 38%, and	Wei discloses: EX1006, Example 15 (“Production of Gen2 soluble rHuPH20 in 300L Bioreactor Cell Culture...”). “In this example a glycan analysis study of rHuPH20 [] was performed...Briefly, rHuPH20 (as produced in Example 15C)....” EX1006, [0321]-[0324] (Example 6).

	<p>“rHuPH20 is glycosylated at six different asparagine residues, including N82, N166, N235, N254, N368, and N393. The results show that N254 is approximately 75% occupied, N393 is approximately 85% occupied, and the four remaining sites, N82, N166, N235 and N368, are greater than 99% occupied. All of the three types of N-glycans, high mannose, hybrid and complex types, are present in rHuPH20. In general, rHuPH20 contains about 45% high mannose glycans, 45% complex glycans and 10% hybrid glycans. About 35% of the total glycans are anionic, of which 25% contain a sialic acid and the remaining 10% contain an unknown anionic group, possibly a phosphate group.” EX1006, [0324].</p> <p><i>See also</i> EX1006, [0031]-[0034], [0041], [0115], [0135]-[0157]</p>
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150. Accordingly, Wei teaches element 1.4.

5. Element 1.5

wherein the produced recombinant protein has a hyaluronidase enzymatic activity at 10,000 units/mL or higher

151. Wei teaches Element 1.5.

152. Wei teaches Element 1.5 in multiple ways. For example, Wei discloses the Gen2 method produces crude rHuPH20 with “a final productivity of 17,000 Units per ml.” EX1006, [0373]. A person of ordinary skill in the art would not expect a modest reduction in culture temperature (e.g., 35.5°C to 34°C) to result in a final productivity below 10,000 units per mL. First of all, the Gen2 method produces hyaluronidase enzymatic activity in vast excess of what is

claimed and such a modest reduction in temperature would not be expected to dramatically alter productivity.

153. To the extent a modest reduction in temperature results in an alteration to productivity, a person of ordinary skill in the art would have expected improved productivity. For example, skilled artisans appreciated that lower culture temperatures tend to correlate with increased productivity of the cells and improvements to the quality of the produced recombinant protein. EX1012, 191 (“improve fermentation and cell culture performance for a prolonged period with better overall productivity and quality”); EX1014, 2 (“the temperature is decreased to increase r-protein productivity and extend culture viability”); *see also* Section V.A. For example, Zmuda explains that dropping the temperature from 37°C to 32°C improved recombinant protein yields 2-3 fold. EX1005, [0021], [00245] (“The Max Titer protocol generated approximately 3-fold higher yields than the Standard protocol, while the High Titer protocol generated approximately 2-fold higher yields.”), Figs. 5, 6, 8.

154. Increasing cell productivity routinely results in increased protein yield. Moreover, improvements to quality result in production of increased levels of active protein due to, for instance, reducing the amount of damaged protein (e.g., denatured or aggregated forms). *E.g.*, EX1012, 191 (discussing improved quality attributes including reductions to “protein aggregation” at lower culture

temperature). Such improvements to cellular productivity and quality would have been expected to increase the final productivity of the culture.

155. Furthermore, while a skilled artisan would have expected increases to the productivity of the culture (e.g., yield, quality), the claims are not limited to just crude hyaluronidase enzymatic activity production levels. The claims encompass hyaluronidase enzymatic activity levels obtained following concentration or purification of the recombinant hyaluronidase such as described by Wei. For example, Wei specifically teaches that hyaluronidase enzymatic activity may be increased using concentration and purification. Wei discloses that the Gen1 method produces “a final productivity of 1600 Units per ml.” EX1006, [0354]. However, Wei further discloses concentrating and purifying the rHuPH20 produced with the Gen1 method such that the hyaluronidase enzymatic activity levels exceed 10,000 units/mL. Wei describes concentrating the rHuPH20 produced using MWCO (molecular weight cut off) filters. EX1006, [0361], Table 19 (reproduced in part below with highlighting). The concentration step increases the harvest hyaluronidase enzymatic activity levels approximately 10-fold.

Table 19. Monitoring data for cell culture, harvest, concentration and buffer exchange steps.				
Parameter	HUA0406C	HUA04010C	HUA0415C	HUA0420C
Time from thaw to inoculate 100 L bioreactor (days)	21	19	17	18
100 L inoculation density ($\times 10^6$ cells/mL)	0.45	0.33	0.44	0.46
Doubling time in logarithmic growth (hr)	29.8	27.3	29.2	23.5
Max. cell density ($\times 10^6$ cells/mL)	5.65	8.70	6.07	9.70
Harvest viability (%)	41	48	41	41
Harvest titer (U/ml)	1964	1670	991	1319
Time in 100-L bioreactor (days)	13	13	12	13
Clarified harvest volume (mL)	81800	93300	91800	89100
Clarified harvest enzyme assay (U/mL)	2385	1768	1039	1425
Concentrate enzyme assay (U/mL)	22954	17091	8561	17785

156. Wei further describes partially purifying the concentrated rHuPH20 on a Q Sepharose column. EX1006, [0362], Table 20 (reproduced below with highlighting). This column chromatography step further increased the hyaluronidase enzymatic activity levels such that all lots exceed the claimed 10,000 units/mL.

Table 20. Q sepharose column data				
Parameter	HUA0406C	HUA0410C	HUA0415C	HUA0420C
Load volume (mL)	10647	13524	12852	20418
Load Volume/Resin Volume ratio	3.1	4.9	4.5	7.3
Column Volume (mL)	2770	3840	2850	2880
Eluate volume (mL)	6108	5923	5759	6284
Protein Conc. of Eluate (mg/mL)	2.8	3.05	2.80	2.86
Eluate Enzyme Assay (U/mL)	24493	26683	18321	21052
Enzyme Yield (%)	65	107	87	76

157. Accordingly, a skilled artisan would have expected a modest reduction in culture temperature (e.g., 35.5°C to 34°C) to produce rHuPH20 with a final productivity in excess of 10,000 units/mL. Such a skilled artisan would have also expected, based on Wei's teachings, to produce concentrated or purified rHuPH20 in excess of 10,000 units/mL.

'638 patent	Prior Art
[1.5] wherein the produced recombinant protein has a hyaluronidase enzymatic activity at 10,000 units/mL or higher.	<i>See also</i> EX1006, [0041], [0122], [0129], [0138], [0144], [0156]; Section VI

158. Thus, Wei teaches element 1.5.

159. Accordingly, the prior art renders claim 1 obvious.

B. Claim 2

The method according to claim 1, wherein galactosylation of N-glycan is 1 to 68%, and mannosylation of N-glycan is 40 to 63%.

160. Wei teaches the additional limitation of claim 2.

161. Wei describes its purified rHuPH20 has N-linked glycosylation, including galactosylated and mannosylated N-glycans in amounts falling within the ranges recited in the claim. For example, Wei states:

rHuPH20 is glycosylated at six different asparagine residues, including N82, N166, N235, N254, N368, and N393. The results

show that N254 is approximately 75% occupied, N393 is approximately 85% occupied, and the four remaining sites, N82, N166, N235 and N368, are greater than 99% occupied. All of the three types of N-glycans, high mannose, hybrid and complex types, are present in rHuPH20. In general, rHuPH20 contains about 45% high mannose glycans, 45% complex glycans and 10% hybrid glycans. About 35% of the total glycans are anionic, of which 25% contain a sialic acid and the remaining 10% contain an unknown anionic group, possibly a phosphate group.

EX1006, [0324].

162. Wei confirms that rHuPH20 has mannosylated glycans within the broad claimed range of 40 to 63%. As I discuss in detail above, high mannose glycans do not contain galactose or sialylation and are largely made up of mannose. Thus, a person of ordinary skill in the art would understand disclosure of 45% high mannose glycans to be equivalent to disclosure of 45% mannosylated N-glycans. *See* Section V.B.

163. Wei further confirms that rHuPH20 has galactosylated N-glycans within the broad range of 1 to 68%. While Wei does not provide a precise amount of galactosylated N-glycans, a skilled artisan however would have known how to derive a range for the percentage of these N-glycans. First of all, high mannose N-glycans do not contain galactose; it is only the complex and hybrid N-glycans that may contain galactose. *See* Section V.B. Thus, the total amount of complex (45%)

and hybrid (10%) N-glycans—that is, 55%—is an upper limit on the amount of galactosylated N-glycans. Additionally, sialic acid is only added to N-glycans with galactose. Because the amount of sialylated N-glycans is 25%, this number is a lower limit on the amount of galactosylated N-glycans.

164. Accordingly, the amount of galactosylated N-glycans on the recombinant PH20 hyaluronidase produced according to Wei's method is between 25 and 55% which is well within the claimed range.

165. In my opinion, a person of ordinary skill in the art would not have expected modest changes to the second culturing temperature to result in mannosylation or galactosylation levels outside the ranges of claim 2. It was well established in the relevant literature at the time that glycosylation levels are not appreciably changed by modest reductions to culture temperature. *E.g.*, EX1027, 196 (“Reducing the culture temperature from 37 C to 33 C in a CHO cell line expressing human granulocyte macrophage colony stimulating factor saw little change in protein glycosylation and terminal sialylation, but did enhance specific productivity [43].”); EX1028, Figure 6A; EX1014, 2 (“With regard to the quality of the r-protein, it seems that in most cases, there are no significant changes in the glycosylation profiles, including the degree of terminal sialylation. Accordingly, these r-proteins produced under mildly hypothermic conditions show the same in vitro biological activity as the r-proteins produced at 37°C”).

166. The prior art knowledge that the glycosylation levels do not appreciably change with temperature is further corroborated by the '638 patent itself. *See* EX1001, 17:24-25 17:36-42, Table 1; *see also* Section X.A.4.

'638 patent	Prior Art
2. The method according to claim 1, wherein galactosylation of N-glycan is 1 to 68%, and mannosylation of N-glycan is 40 to 63%.	<i>See also</i> Sections V.B-C

167. Accordingly, the prior art renders claim 2 obvious.

C. Claim 3

The method according to claim 1, wherein sialylation of N-glycan is 1 to 30%.

168. Wei teaches the additional limitation of claim 3. As I discuss above with respect to Element 1.4, Wei discloses wherein sialylation of N-glycan is 1 to 30%. *See Supra*, Section X.A.4. Specifically, Wei discloses that purified rHuPH20 has 25% sialylated N-glycans, which falls squarely within the range of claim 3. EX1006, [0324]. In my opinion, a person of ordinary skill in the art would not have expected modest changes to the second culturing temperature to result in sialylation levels outside the claimed range. It was well established in the relevant literature at the time that glycosylation levels are not appreciably changed by

culturing temperature. *E.g.*, EX1020, 8; EX1027, 196; EX1028, Figure 6A; EX1014, 2.

169. The prior art knowledge that the glycosylation levels do not appreciably change with temperature is further corroborated by the '638 patent itself. *See* EX1001, 17:24-25 17:36-42, Table 1; *see also* Section X.A.4.

'638 patent	Prior Art
<p>3. The method according to claim 1, wherein sialylation of N-glycan is 1 to 30%.</p>	<p>Wei discloses:</p> <p>EX1006, Example 15 (“Production of Gen2 soluble rHuPH20 in 300L Bioreactor Cell Culture...”).</p> <p>“In this example a glycan analysis study of rHuPH20 [] was performed...Briefly, rHuPH20 (as produced in Example 15C)....” EX1006, [0322].</p> <p>“rHuPH20 is glycosylated at six different asparagine residues, including N82, N166, N235, N254, N368, and N393. The results show that N254 is approximately 75% occupied, N393 is approximately 85% occupied, and the four remaining sites, N82, N166, N235 and N368, are greater than 99% occupied. All of the three types of N-glycans, high mannose, hybrid and complex types, are present in rHuPH20. In general, rHuPH20 contains about 45% high mannose glycans, 45% complex glycans and 10% hybrid glycans. About 35% of the total glycans are anionic, of which 25% contain a sialic acid and the remaining 10% contain an unknown anionic group, possibly a phosphate group.” EX1006, [0324].</p>

170. Accordingly, the prior art renders claim 3 obvious.

D. Claim 4

The method according to claim 1, wherein the recombinant protein has a specific enzymatic activity as hyaluronidase at least 10% higher than a specific enzymatic activity of a wild human PH20.

171. Wei teaches the additional limitation of claim 4. For example, Wei discloses variant human PH20 hyaluronidases with enzymatic activity at least 10% higher than wild human PH20.

The human esPH20 polypeptides provided herein can exhibit hyaluronidase activity that is increased or decreased compared to the wild-type GPI-anchored form of PH20. For example, the human esPH20 polypeptides provided herein can exhibit hyaluronidase activity that is 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more of the hyaluronidase activity exhibited by the wildtype GPI-anchored form. In some examples, human esPH20 polypeptides exhibit increased hyaluronidase activity compared to the wildtype GPI-anchored form. The hyaluronidase activity of human esPH20 polypeptides can be increased by 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more compared to the hyaluronidase activity of the wildtype GPI-anchored form.

EX1006, [0128].

172. While Wei describes hyaluronidase enzymatic activity (e.g., units/mL), a person of ordinary skill in the art would have appreciated that these variants would also have increased specific enzymatic activity (e.g., units/mg). *See,*

e.g., EX1006, [0353] (“Specific activity (Units/mg) was calculated by dividing the hyaluronidase activity (U/ml) by the protein concentration (mg/mL).”). This is because a more active variant will have more units of activity on a milligram-by-milligram basis.

173. That a more active PH20 variant will have both higher activity and higher specific activity is supported by the '638 patent:

As described above, in the hyaluronidase PH20 or variant thereof produced by the production method according to the present invention, the specific activity of the enzyme can be increased by 10% or more compared to that produced by a general method, in particular, compared to the specific activity of wild-type human PH20. This increase in enzymatic activity is due to changes in N-glycosylation properties and/or charge modification of the hyaluronidase PH20 or variant thereof produced by the method according to the present invention.

EX1001, 10:66-11:8.

174. Furthermore, a person of ordinary skill in the art would have known that specific activity is a function of purity of the recombinant protein. *See, e.g.*, EX1006, [0071] (“A substantially chemically pure compound, however, can be a mixture of stereoisomers. In such instances, further purification might increase the specific activity of the compound.”). Purified wild-type human PH20 has higher specific activity than a corresponding unpurified wild-type human PH20. This is because impurities increase the weight (mg) but do not increase hyaluronidase

activity. EX1006, [0071] (“As used herein, isolated or purified polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue from which the protein is derived,”). Accordingly, a person of ordinary skill in the art would have known that a preparation of purified wild-type recombinant PH20 will have at least 10% higher specific activity than the corresponding unpurified wild-type recombinant PH20.

'638 patent	Prior Art
4. The method according to claim 1, wherein the recombinant protein has a specific enzymatic activity as hyaluronidase at least 10% higher than a specific enzymatic activity of a wild human PH20.	<p>“As used herein, neutral active refers to the ability of a PH20 polypeptide to enzymatically catalyze the cleavage of hyaluronic acid at neutral pH. A neutral active C-terminally truncated or N-partially glycosylated PH20 provided herein has or has about 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more activity compared to the hyaluronidase activity of a corresponding neutral active PH20 that is not C-terminally truncated or N-partially glycosylated.” EX1006, [0041]</p> <p><i>See also</i> EX1006, [0122], [0129], [0138], [0144], [0156].</p>

175. Accordingly, the prior art renders claim 4 obvious.

E. Claim 5

The method according to claim 1, wherein at least one of the first culturing or the second culturing are performed by one or more methods selected from the group consisting of batch culture, repeated batch culture, fed-batch culture,

repeated fed-batch culture, continuous culture, and perfusion culture.

176. Wei teaches the additional limitation of claim 5.

177. A person of ordinary skill in the art would understand Wei to disclose the Gen1 and Gen2 culturing methods of Wei as fed-batch cultures. Specifically, Wei describes feeding cultures in bioreactors during the first and second culturing. EX1006, [0360] (“The reactor was sampled daily for cell counts, pH verification, media analysis, protein production and retention. Nutrient feeds were added during the run.”), [0373] (same).

178. Moreover, the methods listed in claim 5 were those in common use at the time including for culturing CHO cells. *E.g.*, EX1011, 8-10 (discussing batch, fed-batch, continuous and perfusion culture modes); *see also* Section V.A.

'638 patent	Prior Art
5. The method according to claim 1, wherein at least one of the first culturing or the second culturing are performed by one or more methods selected from the group consisting of batch culture, repeated batch culture, fed-batch culture, repeated fed-batch culture, continuous	<i>See also</i> EX1005, [0006]; EX1024, 942; EX1019, 57; EX1014, Table 2; Section V.A

culture, and perfusion culture.	
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179. Accordingly, the prior art renders claim 5 obvious.

F. Claim 6

The method according to claim 1, wherein at least one of the first culturing or the second culturing are performed under one or more conditions selected from the group consisting of:

(i) a condition in which an ammonia concentration is maintained at 5 mM or higher in a culture medium thereof;

(ii) a condition in which one or more substances is added to the culture medium thereof, wherein the one or more substances are selected from the group consisting of glutamine, glucosamine, uridine, glucosamine, and sodium butyrate; and

(iii) a condition in which galactose and manNAc are not added to the culture medium thereof.

180. Wei teaches the additional limitation of claim 6.

181. Wei discloses the culturing conditions of claim 6(ii). For example, Wei discloses feed medium supplemented with sodium butyrate. EX1006, [0360], [0373].

182. Wei further discloses the culturing conditions of claim 6(iii). The Gen1 and Gen2 methods describe the media components used for culturing. However, none of listed components include galactose or manNAc, which discloses they were not added to the culture medium. *See, e.g.*, EX1006, Examples 13 & 15.

183. Conventional culturing methods typically did not include galactose or manNAc in the medium. A person of ordinary skill in the art had good reason not to add galactose or ManNAc as substitutes for glucose in cell culture media as these sugars would be costly for large-scale bioprocessing.

'638 patent	Prior Art
<p>6. The method according to claim 1, wherein at least one of the first culturing or the second culturing are performed under one or more conditions selected from the group consisting of:</p> <p>(i) a condition in which an ammonia concentration is maintained at 5 mM or higher in a culture medium thereof;</p> <p>(ii) a condition in which one or more substances is added to the culture medium thereof, wherein the one or more substances are selected from the group consisting of glutamine, glucosamine, uridine, glucosamine, and sodium butyrate; and</p> <p>(iii) a condition in which galactose and manNAc</p>	<p><i>See also</i> EX1006, Table 27: Feed #2, #3, and #4 contain sodium butyrate</p>

are not added to the culture medium thereof.	
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184. Accordingly, the prior art renders claim 6 obvious.

G. Claim 7

7. The method according to claim 1, wherein the recombinant protein comprises substitution of one or more amino acid residues when compared to an amino acid sequence of a wild human PH20.

185. Wei teaches the additional limitation of claim 7.

186. Wei teaches methods directed to producing soluble recombinant human PH20 hyaluronidase (“rHuPH20”). EX1006, [0023] (“PH20 includes those of any origin including, but not limited to, human, ...”), [0029]. Wei broadly defines soluble recombinant human PH20 hyaluronidase as “any form of PH20 that is soluble under physiologic conditions.” EX1006, [0024].

187. Wei discloses broadly soluble recombinant human PH20 hyaluronidases including variants thereof. For example, Wei discloses variants that comprise substitutions relative to the wild-type human PH20 as required by claim 7.

Also included are allelic variants and other variants, such as any with 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence identity with the corresponding polypeptides of SEQ ID NOS: 60-63 and 102-

104 that retain neutral activity and are soluble. Reference to sequence identity refers to variants with amino acid substitutions.

EX1006, [0026]; *see also id.*, [0023], [0127].

188. Wei also discloses producing variant PH20 hyaluronidases that include substitutions at positions 82, 166, 235, 254, 368, and 393. EX1006, [0355], [0336], Table 16.

'638 patent	Prior Art
7. The method according to claim 1, wherein the recombinant protein comprises substitution of one or more amino acid residues when compared to an amino acid sequence of a wild human PH20.	<i>See also</i> EX1006, [0008]-[0010], [0023], [0027], [0029], [0065]-[0067], [0121], [0124], [0126], [0135]-[0137], SEQ ID NO: 187, 202-205, 208, 189, 206-209, 190

189. Accordingly, the prior art renders claim 7 obvious.

H. Claim 8

8. The method according to claim 1, further comprising separating and purifying the produced recombinant protein.

190. Wei teaches the additional limitations of claim 8.

191. Wei broadly teaches separating and purifying the produced recombinant protein. For example, Wei teaches separating the produced recombinant protein from the culture media. EX1006, [0355] (“Conditioned media from the 3D35M clone was clarified by depth filtration and tangential flow

diafiltration.”), [0361], Table 27 (“Filtration of bioreactor cell culture” & “Concentration and buffer exchange prior to chromatography”).

192. Wei also broadly teaches purifying the produced recombinant protein using chromatography, including ionic exchange chromatography.

Proteins, such as soluble PH20 polypeptides, including esPH20 polypeptides, or other C-terminal truncated PH20 polypeptides, can be purified using standard protein purification techniques known in the art including but not limited to, SDS-PAGE, size fractionation and size exclusion chromatography, ammonium sulfate precipitation and ionic exchange chromatography, such as anion exchange.

EX1006, [0202].

'638 patent	Prior Art
8. The method according to claim 1, further comprising separating and purifying the produced recombinant protein.	<i>See also</i> EX1005, [0008], [0223], [0241]

193. Accordingly, the prior art renders claim 8 obvious.

I. Claims 9 and 10

9. The method according to claim 8, wherein separating and purifying the produced recombinant protein are performed using ionic bond and/or hydrophobic interaction characteristics thereof, rather than affinity binding.

10. The method according to claim 9, wherein separating and purifying the recombinant protein are performed using

hydrophobic interaction chromatography and ion exchange chromatography, rather than affinity chromatography.

194. As discussed above, Wei and Zmuda render claim 8 obvious. Wei also teaches the additional limitations of claims 9 and 10 which depend from claim 8.

195. Wei broadly teaches that the produced recombinant protein may be purified with chromatography, including ionic bond chromatography.

Proteins, such as soluble PH20 polypeptides, including esPH20 polypeptides, or other C-terminal truncated PH20 polypeptides, can be purified using standard protein purification techniques known in the art including but not limited to, SDS-PAGE, size fractionation and size exclusion chromatography, ammonium sulfate precipitation and ionic exchange chromatography, such as anion exchange.

EX1006, [0202].

196. Wei further teaches the use of hydrophobic bond chromatography.

EX1006, [0363] (“Phenyl-Sepharose (Pharmacia) hydrophobic interaction chromatography was next performed.”), [0377] (same).

197. While Wei discloses purification of rHuPH20 using a combination of ion exchange, hydrophobic, and affinity chromatography, claims 9 and 10 do not require any particular purification level for the produced recombinant protein. Thus, to the extent either claim is construed to exclude use of “affinity chromatography,” it would have been obvious to perform only the ion exchange and reverse-phase chromatography steps of Wei’s method.

'638 patent	Prior Art
<p>9. The method according to claim 8, wherein separating and purifying the produced recombinant protein are performed using ionic bond and/or hydrophobic interaction characteristics thereof, rather than affinity binding.</p> <p>10. The method according to claim 9, wherein separating and purifying the recombinant protein are performed using hydrophobic interaction chromatography and ion exchange chromatography, rather than affinity chromatography.</p>	EX1006, [0071], [0200]-[0202].

198. Accordingly, the prior art renders each of claims 9 and 10 obvious.

J. Claims 11 and 12

11. The method according to claim 8, wherein purifying further comprises removing at least part of acidic recombinant protein.

12. The method according to claim 11, wherein removing the acidic recombinant protein uses ion exchange chromatography.

199. Wei teaches the additional limitations of claims 11 and 12.

200. For example, Wei teaches separating and purifying the produced recombinant protein from the culture media. EX1006, [0355] (“Conditioned media from the 3D35M clone was clarified by depth filtration and tangential flow diafiltration.”), [0361], Table 27 (“Filtration of bioreactor cell culture” & “Concentration and buffer exchange prior to chromatography”). Wei broadly teaches purifying the produced recombinant protein using chromatography, including ionic exchange chromatography.

Proteins, such as soluble PH20 polypeptides, including esPH20 polypeptides, or other C-terminal truncated PH20 polypeptides, can be purified using standard protein purification techniques known in the art including but not limited to, SDS-PAGE, size fractionation and size exclusion chromatography, ammonium sulfate precipitation and ionic exchange chromatography, such as anion exchange. EX1006, [0202].

201. Claims 11 and 12 do not require removal of any particular amount of “acidic recombinant protein.” Thus, any removal of acidic recombinant protein on an ion exchange column would satisfy both claims.

202. Wei further teaches using a Q Sepharose ion exchange column to purify rHuPH20. EX1006, [0355] (“Soluble rHuPH20 was then purified by sequential chromatography on Q Sepharose (Pharmacia) ion exchange, ...”).

203. Q Sepharose is a positively charged ion exchange column that binds negatively charged moieties such as acidic groups (e.g., sialic acid). A person of

ordinary skill in the art would understand that Q Sepharose ion exchange chromatograph operates by binding acidic recombinant proteins to the Q Sepharose. However, column chromatography, including with Q Sepharose, is not a quantitative method and some loss of protein is to be expected. For example, it would be expected that at least a portion of the acidic recombinant proteins will not bind to the Q Sepharose column in the first instance and will not purify with the bulk of the recombinant protein. It would also be expected that elution of bound recombinant protein from the column will also fail to dislodge a portion of the acidic recombinant protein. In either instance, at least a portion of the acidic recombinant protein will be removed by the Q Sepharose column. *See, e.g.,* EX1006, Table 20 (reporting yields from Q Sepharose column as low as 65%). A POSA thus would understand Wei discloses at least a portion of the acidic recombinant protein being removed by the Q Sepharose column.

'638 patent	Prior Art
11. The method according to claim 8, wherein purifying further comprises removing at least part of acidic recombinant protein. 12. The method according to claim 11, wherein removing the acidic recombinant	<i>See also</i> EX1005, [0008], [0223], [0241]; EX1006, [0071], [0200]-[0202].

protein uses ion exchange chromatography.	
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204. Accordingly, the prior art renders each of claims 11 and 12 obvious.

K. Claims 13-15

13. The method according to claim 7, wherein the recombinant protein further comprises truncation of at least one amino acid residue of N-terminus when compared to an amino acid sequence of a wild human PH20.

14. The method according to claim 7, wherein the recombinant protein further comprises truncation of at least one amino acid residue of C-terminus when compared to an amino acid sequence of a wild human PH20.

15. The method according to claim 7, wherein the recombinant protein further comprises truncation of at least one amino acid residue of N-terminus and at least one amino acid residue of C-terminus when compared to an amino acid sequence of a wild human PH20.

205. Wei teaches the additional limitations of claims 13-15.

206. The '638 patent explains that the sequence of wild human PH20 is disclosed in a Korean PCT application. EX1001, 16:65-67 ("The sequences of wild-type human PH20 and HM46 are disclosed in International Patent Publication No. 2020/022791."); *see also* EX1013 (WO2020/022791 which is a publication of PCT/KR2019/009215). The sequence of wild human PH20 is also disclosed in a U.S. patent application claiming priority to the PCT application EX1015 (US2021/0155913 claiming priority to PCT/KR2019/009215).

207. According to the '913 application, wild-type human PH20 is identified as SEQ ID NO: 1. EX1015, Abstract (“wild-type PH20 having the amino acid sequence of SEQ ID NO: 1”), pp. 36-37 (SEQ ID NO: 1). The wild-type human PH20 of SEQ ID NO: 1 has 509 amino acids. EX1015, pp. 36-37.

208. Wei describes production and purification of “rHuPH20”—also known as SEQ ID NO:122—which is a recombinant human PH20 protein. The rHuPH20 protein differs from the 509 amino acid wild human PH20 protein in that it has 447 amino acids and lacks the 35 amino acid signal peptide at the N-terminus and 27 amino acids at the C-terminus of the molecule. EX1006, [0029], [0205], [0321] (“rHuPH20 (SEQ ID NO:122)”), [0386], Tables 29-30, pp. 292-293 (SEQ ID NO:122); *see also* EX1031, 430 (“509 amino acid human PH20 sequence”), 432 (describing rHuPH0 as “a 447 amino acid 61 kDa glycoprotein with a properly processed amino terminus and 6 N-linked glycosylation sites.”). Accordingly, because rHuPH20 has both an N- and C-terminal truncation of at least one amino acid, it satisfies the requirements of claims 13-15.

209. Wei further teaches methods broadly directed to producing soluble recombinant human PH20 hyaluronidase (“rHuPH20”). EX1006, [0023] (“PH20 includes those of any origin including, but not limited to, human, ...”), [0029]. Wei broadly defines soluble recombinant human PH20 hyaluronidase as “any form of PH20 that is soluble under physiologic conditions.” EX1006, [0024].

210. Wei teaches that soluble recombinant human PH20 hyaluronidases include truncated forms of the protein.

Reference to PH20 includes precursor PH20 polypeptides and mature PH20 polypeptides (such as those in which a signal sequence has been removed), truncated forms thereof that have activity, and includes allelic variants and species variants, variants encoded by splice variants, and other variants, including polypeptides that have at least 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the precursor polypeptides set forth in SEQ ID NO:107 and 109, or the mature forms thereof.

EX1006, [0023]; *see also id.*, [0018], Fig. 1 (demonstrating SEQ ID NO: 107 is the sequence of the full-length wild-type human PH20).

211. Wei discloses that soluble human PH20 hyaluronidases include variants that comprise truncations at the C-terminus relative to the wild-type human PH20 as required by claim 14.

The PH20 polypeptides provided herein are soluble proteins that are truncated at the C-terminus and include those that lack all of the GPI-anchor attachment signal sequence (e.g. are truncated at amino acid positions 450 to 490).

EX1006, [0006]; *see also id.*, [0023], [0025], [0026], [0041], [0108], [0109], [0119]-[0128].

212. Wei discloses that soluble human PH20 hyaluronidases variants that comprise truncations at the N- and C-terminus relative to the wild-type human PH20 as required by claims 13 and 15. For example, Wei describes soluble human PH20 hyaluronidases (i.e., those that lack the C-terminus) that also lack the N-terminal signal peptide and thus begin at amino acid 36. EX1006, [0009], [0012], [0013], [0026], [0030], [0124]-[0127]; *see also id.*, [0171] (explaining that the PH20 signal peptide sequence may be replaced with a heterologous sequence).

'638 patent	Prior Art
<p>13. The method according to claim 7, wherein the recombinant protein further comprises truncation of at least one amino acid residue of N-terminus when compared to an amino acid sequence of a wild human PH20.</p> <p>14. The method according to claim 7, wherein the recombinant protein further comprises truncation of at least one amino acid residue of C-terminus when compared to an amino acid sequence of a wild human PH20.</p> <p>15. The method according to claim 7,</p>	<p>EX1006, [0140], [0146]-[0158], [0180], [0303]-[0314], Table 2</p>

<p>wherein the recombinant protein further comprises truncation of at least one amino acid residue of N-terminus and at least one amino acid residue of C-terminus when compared to an amino acid sequence of a wild human PH20.</p>	
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213. Accordingly, the prior art renders each of claims 13-15 obvious.

XI. GROUND 2 – CLAIMS 4 AND 7 ARE OBVIOUS AS ILLUSTRATED BY WEI, ZMUDA, AND WEI 2013

214. As discussed in detail above, a person of ordinary skill in the art would have considered claims 1-15 obvious in view of Wei and Zmuda. To the extent the limitations of claims 4 and 7 are not taught by Wei and Zmuda, they would have been obvious in further view of Wei 2013.

215. The combination of Wei and Zmuda, for example, describes a method of producing recombinant PH20 hyaluronidase that is indistinguishable from what is claimed. *See* Section X. Wei 2013 further teaches recombinant PH20 hyaluronidase variants with “increased stability and/or increased activity.” EX1037, Abstract.

216. Wei 2013 discloses “modified PH20 polypeptides that contain at least one amino acid replacement in a PH20 polypeptide, where the modified PH20 polypeptide exhibits increased hyaluronidase activity compared to the PH20

polypeptide not containing the amino acid replacement.” EX1037, 13:22-25. More specifically, Wei 2013 discloses over 600 PH20 hyaluronidase variants with base substitutions that have “activity increased relative to wildtype.” According to Wei 2013:

[A]bout 536 mutants exhibit 120% or greater than 120% of the hyaluronidase activity of wildtype PH20 and about 75 of the mutants exhibit 300% or greater than 300%[] of the hyaluronidase activity of wildtype PH20. In particular, the results in Table 9 show that that hyaluronidase activity compared to wildtype of mutant S69A is about 22-fold; mutant S69R is about 14-fold; mutant I70A is about 27-fold; mutant I70K is about 14-fold; mutant I70R is about 14-fold; and mutant I271L is about 10-fold.

EX1037, 265:4-22; *see also id.*, Table 9.

217. Wei 2013 discloses that the active mutants have base substitutions compared with “the sequence of amino acids of PH20 set forth in SEQ ID NO:3.” EX1037, 265:5-7. SEQ ID NO: 3 is a “soluble C-terminal truncated variant[]” of human PH20. EX1037, 50:1-11; *see also id.*, 54:27-31 (“As used herein, soluble human PH20 (sHuPH20) includes human PH20 polypeptides that lack a contiguous sequence of amino acids from the C-terminus of human PH20 that includes all or a portion of the glycosylphosphatidylinositol (GPI) anchor sequence ...”); Figure 1 (showing SEQ ID NO:3 ends at the underlined “Y” residue).

218. Wei 2013 also discloses methods for producing and purifying soluble recombinant PH20 hyaluronidase. For example, Wei 2013 discloses the same method as Wei for production of “Gen2 soluble rHuPH20.” *Compare* EX1037, 228:17-229:19 *with*, EX1006, [0372]-[0373].

219. A person of ordinary skill in the art would have had good reason to combine the teachings of Wei and Zmuda for the reasons I discuss above. In particular, Zmuda discloses a temperature shift protocol for producing recombinant glycoproteins and further teaches that the second culturing temperature is in the range of 30-37°C. *See* Section VI.B. Wei discloses a temperature shift protocol for producing recombinant PH20 hyaluronidase that uses 35.5°C as a lower second culturing temperature. *See* Section VI.A. A person of ordinary skill in the art would have been motivated to combine the teachings of Wei and Zmuda and would have expected success because it was well established and documented in the literature that optimizing the lower second culturing temperature would improve yields. *See* Section V.A.1 & X.

220. A person of ordinary skill in the art would also have had good reason to produce and purify Wei 2013’s soluble variant PH20 hyaluronidases using the combined method of Wei and Zmuda. Moreover, such a skilled artisan would have reasonably expected success. My opinion is supported by Wei itself, which explains that the disclosed methods apply to PH20 and variants thereof. EX1006,

[0008]-[0010], [0023], [0027], [0029], [0065]-[0067], [0121], [0124], [0126], [0135]-[0137], SEQ ID NO: 187, 202-205, 208, 189, 206-209, 190.

221. Accordingly, a person of ordinary skill in the art would have had good reason to combine Wei, Zmuda, and Wei 2013 and would have reasonably expected success in achieving the claimed method. In my opinion, claims 4 and 7 are obvious

A. Claim 4

The method according to claim 1, wherein the recombinant protein has a specific enzymatic activity as hyaluronidase at least 10% higher than a specific enzymatic activity of a wild human PH20.

222. Wei teaches the additional limitation of claim 4. For example, Wei discloses variant human PH20 hyaluronidases with enzymatic activity at least 10% higher than wild type human PH20.

The human esPH20 polypeptides provided herein can exhibit hyaluronidase activity that is increased or decreased compared to the wild-type GPI-anchored form of PH20. For example, the human esPH20 polypeptides provided herein can exhibit hyaluronidase activity that is 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more of the hyaluronidase activity exhibited by the wildtype GPI-anchored form. In some examples, human esPH20 polypeptides exhibit increased hyaluronidase activity compared to the wildtype GPI-anchored form. The hyaluronidase activity of human esPH20

polypeptides can be increased by 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more compared to the hyaluronidase activity of the wildtype GPI-anchored form.

EX1006, [0128].

223. Wei 2013 discloses over 600 variant human PH20 hyaluronidases with activity at least 10% higher than wild type human PH20:

[A]bout 536 mutants exhibit 120% or greater than 120% of the hyaluronidase activity of wildtype PH20 and about 75 of the mutants exhibit 300% or greater than 300%[] of the hyaluronidase activity of wildtype PH20. In particular, the results in Table 9 show that that hyaluronidase activity compared to wildtype of mutant S69A is about 22-fold; mutant S69R is about 14-fold; mutant I70A is about 27-fold; mutant I70K is about 14-fold; mutant I70R is about 14-fold; and mutant I271L is about 10-fold.

EX1037, 265:4-22; *see also id.*, Table 9, 13:22-25.

224. While Wei and Wei 2013 describe hyaluronidase enzymatic activity (e.g., units/mL), a person of ordinary skill in the art would have appreciated that these variants would also have increased specific enzymatic activity (e.g., units/mg). *See, e.g.*, EX1006, [0353] (“Specific activity (Units/mg) was calculated by dividing the hyaluronidase activity (U/ml) by the protein concentration (mg/mL).”). This is because a more active variant will have more units of activity on a milligram-by-milligram basis.

225. That a more active PH20 variant will have both higher activity and specific activity is supported by the '638 patent:

As described above, in the hyaluronidase PH20 or variant thereof produced by the production method according to the present invention, the specific activity of the enzyme can be increased by 10% or more compared to that produced by a general method, in particular, compared to the specific activity of wild-type human PH20. This increase in enzymatic activity is due to changes in N-glycosylation properties and/or charge modification of the hyaluronidase PH20 or variant thereof produced by the method according to the present invention.

EX1001, 10:66-11:8.

226. Furthermore, a person of ordinary skill in the art would have known that specific activity is a function of purity of the recombinant protein. *See, e.g.*, EX1006, [0071] (“A substantially chemically pure compound, however, can be a mixture of stereoisomers. In such instances, further purification might increase the specific activity of the compound.”); EX1037, 74:2-4 (same). Purified wild-type human PH20 has higher specific activity than a corresponding unpurified wild-type human PH20. This is because impurities increase the weight (mg) but do not increase hyaluronidase activity. EX1006, [0071] (“As used herein, isolated or purified polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue from which the protein is derived,”); EX1037, 74:5-8 (same).

Accordingly, a person of ordinary skill in the art would have known that a preparation of purified wild-type recombinant PH20 will have at least 10% higher specific activity than the corresponding unpurified wild-type recombinant PH20.

'638 patent	Prior Art
<p>4. The method according to claim 1, wherein the recombinant protein has a specific enzymatic activity as hyaluronidase at least 10% higher than a specific enzymatic activity of a wild human PH20.</p>	<p>“As used herein, neutral active refers to the ability of a PH20 polypeptide to enzymatically catalyze the cleavage of hyaluronic acid at neutral pH. A neutral active C-terminally truncated or N-partially glycosylated PH20 provided herein has or has about 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 300%, 400%, 500%, 1000% or more activity compared to the hyaluronidase activity of a corresponding neutral active PH20 that is not C-terminally truncated or N-partially glycosylated.” EX1006, [0041]</p> <p>“In some instances, among the PH20 polypeptides provided herein, the modified PH20 polypeptide exhibits at least 120%, 130%, 135%, 140%, 145%, 150%, 160%, 170%, 180%, 200%, 250%, 300%, 350%, 400%, 500%, 1500%, 2000%, 3000%, 4000%, 5000% or more of the hyaluronidase activity of the PH20 polypeptide not containing the amino acid replacement(s).” EX1037, 4:9-13.</p> <p>“Provided herein are modified PH20 polypeptides that contain one or more amino acid replacements in a PH20 polypeptide and that exhibit hyaluronidase activity. The modified PH20 polypeptides can exhibit 40% to 5000% of the hyaluronidase activity of a wildtype or reference PH20 polypeptide, such as the polypeptide set forth in SEQ ID NOS: 3 or 7.” EX1037, 96:2-5.</p> <p><i>See also</i> EX1006, [0122], [0129], [0138], [0144], [0156]; EX1037, Tables 3, 8, 9, 11, 15, Example 6</p>

227. Accordingly, the prior art renders claim 4 obvious.

B. Claim 7

7. The method according to claim 1, wherein the recombinant protein comprises substitution of one or more amino acid residues when compared to an amino acid sequence of a wild human PH20.

228. Wei teaches the additional limitation of claim 7.

229. Wei teaches methods directed to producing soluble recombinant human PH20 hyaluronidase (“rHuPH20”). EX1006, [0023] (“PH20 includes those of any origin including, but not limited to, human, ...”), [0029]. Wei broadly defines soluble recombinant human PH20 hyaluronidase as “any form of PH20 that is soluble under physiologic conditions.” EX1006, [0024].

230. Wei discloses broadly soluble recombinant human PH20 hyaluronidases including variants thereof. For example, Wei discloses variants that comprise substitutions relative to the wild-type human PH20 as required by claim 7.

Also included are allelic variants and other variants, such as any with 40%, 45%, 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence identity with the corresponding polypeptides of SEQ ID NOS: 60-63 and 102-104 that retain neutral activity and are soluble. Reference to sequence identity refers to variants with amino acid substitutions. EX1006, [0026]; *see also id.*, [0127].

231. Wei also discloses producing variant PH20 hyaluronidases that include substitutions at positions 82, 166, 235, 254, 368, and 393. EX1006, [0336], Table 16.

232. Wei 2013 discloses variant PH20 hyaluronidases that comprise a substitution of one or more amino acids. For example, Wei 2013 discloses a long list of variant PH20 hyaluronidases that are active and comprise a substitution of one or more amino acids relative to wild type PH20. EX1037, Table 3. Wei 2013 also discloses a long list of variant PH20 hyaluronidases that are inactive and comprise a substitution of one or more amino acids relative to wild type PH20. EX1037, Table 5.

'638 patent	Prior Art
7. The method according to claim 1, wherein the recombinant protein comprises substitution of one or more amino acid residues when compared to an amino acid sequence of a wild human PH20.	<p><i>See also</i> EX1006, [0008]-[0010], [0023], [0027], [0029], [0065]-[0067], [0121], [0124], [0126], [0135]-[0137], SEQ ID NO: 187, 202-205, 208, 189, 206-209, 190</p> <p>EX1037, Tables 3, 8, 9, 11, 15, Example 6</p>

233. Accordingly, the prior art renders claim 7 obvious.

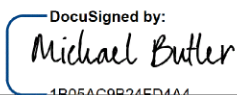
XII. CONCLUDING STATEMENTS

234. In signing this declaration, I understand that the declaration will be filed as evidence in a contested case before the Patent Trial and Appeal Board of

the United States Patent and Trademark Office. I acknowledge that I may be subject to cross-examination in this case and that cross-examination will take place within the United States. If cross-examination is required of me, I will appear for cross-examination within the United States during the time allotted for cross-examination.

235. I declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: December 10, 2025

By: 
1805AC0B24FD4A4...
Michael Butler

XIII. APPENDIX – LISTING OF CHALLENGED CLAIMS

1. [PREAMBLE] A method for producing a recombinant protein that comprises at least one of hyaluronidase PH20 or a variant thereof, the method comprising:

[ELEMENT 1.1] first culturing host animal cells expressing the recombinant protein until an integral viable cell density of the host animal cells reaches 20×10^6 to 120×10^6 cells $\times$ day/mL, wherein first culturing is performed within a first temperature range from 35° C. to 38° C.; and

[ELEMENT 1.2] subsequently second culturing at least part of the host animal cells from first culturing for 2 to 18 days, which produces the recombinant protein, wherein second culturing is performed within a second temperature range from 28° C. to 34° C.,

[ELEMENT 1.3] wherein the second culturing maintains (a) a residual glucose concentration within a range from 0.001 g/L to 4.5 g/L and/or (b) pH within a range from 6.8 to 7.2 in a culture medium therefor,

[ELEMENT 1.4] wherein sialylation of N-glycan of the produced recombinant protein is 1 to 38%, and

[ELEMENT 1.5] wherein the produced recombinant protein has a hyaluronidase enzymatic activity at 10,000 units/mL or higher.

2. The method according to claim 1, wherein galactosylation of N-glycan is 1 to 68%, and mannosylation of N-glycan is 40 to 63%.

3. The method according to claim 1, wherein sialylation of N-glycan is 1 to 30%.

4. The method according to claim 1, wherein the recombinant protein has a specific enzymatic activity as hyaluronidase at least 10% higher than a specific enzymatic activity of a wild human PH20.

5. The method according to claim 1, wherein at least one of the first culturing or the second culturing are performed by one or more methods selected from the group consisting of batch culture, repeated batch culture, fed-batch culture, repeated fed-batch culture, continuous culture, and perfusion culture.

6. The method according to claim 1, wherein at least one of the first culturing or the second culturing are performed under one or more conditions selected from the group consisting of:

(i) a condition in which an ammonia concentration is maintained at 5 mM or higher in a culture medium thereof;

(ii) a condition in which one or more substances is added to the culture medium thereof, wherein the one or more substances are selected from the group consisting of glutamine, glucosamine, uridine, glucosamine, and sodium butyrate; and

(iii) a condition in which galactose and manNAc are not added to the culture medium thereof.

7. The method according to claim 1, wherein the recombinant protein comprises substitution of one or more amino acid residues when compared to an amino acid sequence of a wild human PH20.

8. The method according to claim 1, further comprising separating and purifying the produced recombinant protein.

9. The method according to claim 8, wherein separating and purifying the produced recombinant protein are performed using ionic bond and/or hydrophobic interaction characteristics thereof, rather than affinity binding.

10. The method according to claim 9, wherein separating and purifying the recombinant protein are performed using hydrophobic interaction chromatography and ion exchange chromatography, rather than affinity chromatography.

11. The method according to claim 8, wherein purifying further comprises removing at least part of acidic recombinant protein.

12. The method according to claim 11, wherein removing the acidic recombinant protein uses ion exchange chromatography.

13. The method according to claim 7, wherein the recombinant protein further comprises truncation of at least one amino acid residue of N-terminus when compared to an amino acid sequence of a wild human PH20.

14. The method according to claim 7, wherein the recombinant protein further comprises truncation of at least one amino acid residue of C-terminus when compared to an amino acid sequence of a wild human PH20.

15. The method according to claim 7, wherein the recombinant protein further comprises truncation of at least one amino acid residue of N-terminus and at least one amino acid residue of C-terminus when compared to an amino acid sequence of a wild human PH20.

XIV. EXHIBIT LIST

Exhibit No.	Description
1001	U.S. Patent No. 12,221,638 to Park (“the ’638 patent”)
1003	Curriculum Vitae of Michael Butler, Ph.D.
1005	International Patent Publication WO2017/011598 to Jonathan Zmuda and Chao Yan Liu is entitled “System and Method for Improved Transient Protein Expression in CHO Cells”
1006	EP3037529 to Ge Wei, Krishnasamy Paneerselvam, Gregory I. Frost, and Louis Bookbinder is entitled “Extended Soluble PH20 Polypeptides and Uses Thereof” (“Wei”)
1007	“Human cell lines for biopharmaceutical manufacturing: history, status, and future perspectives” to Jennifer Dumont, Don Euwart, Baisong Mei, Scott Estes, and Rashmi Kshirsagar (“Dumont”)
1008	“Recombinant Proteins for Industrial versus Pharmaceutical Purposes: A Review of Process and Pricing” to John Puetz and Florian Wurm (“Puetz”)
1009	Gowtham YK, Saski CA, Harcum SW. “Low glucose concentrations within typical industrial operating conditions have minimal effect on the transcriptome of recombinant CHO cells.” Biotechnol Prog. 2017 May;33(3):771-785. (“Gowtham”)
1010	Intentionally left blank
1011	“Mammalian cell culture for production of recombinant proteins: A review of the critical steps in their biomanufacturing” to Róisín O’Flaherty, Adam Bergin, Evangelia Flampouri, Leticia Martins Mota, Ismael Obaidi, Andrew Quigley, Yongjing Xie, Michael Butler (“O’Flaherty”)
1012	“Systematic development of temperature shift strategies for Chinese hamster ovary cells based on short duration cultures and kinetic modeling” to Jianlin Xu, Peifeng Tang, Andrew Yongky, Barry Drew, Michael C. Borys, Shijie Liu & Zheng Jian Li (“Xu”)
1013	WO2020/022791 to Park
1014	“Exploring the effect of mild hypothermia on CHO cell productivity” to Silvana Becerra, Julio Berrios, Nelson Osses, Claudia Altamirano (“Becerra”)
1015	US20210155913A1 to Park
1016	“Miniaturized Integrated Platform for Electrical and Optical Monitoring of Cell Cultures” to Carmen Moldovan, Rodica Iosub, Cecilia Codreanu, Bogdan Firtat, Daniel Necula, Costin Brasoveanu and Ion Stan (“Moldovan”)
1017	Invitrogen CELL CULTURE BASICS Handbook
1018	Gibco CD CHO Medium User Guide
1019	“Optimizing CHO Cell Culture Conditions” to Christopher A. Sellick, Arfa R. Maqsood, Alexandra S. Croxford, Alan J. Dickson, Royston Goodacre, and Gill M. Stephens (“Sellick”)
1020	“Biphasic cultivation strategy to avoid Epo-Fc aggregation and optimize protein expression” to Christian Kaisermayer, David Reinhart, Andreas Gili, Martina Chang, Per-Mikael Aberg, Andreas Castan, Renate Kunert (“Kaisermayer”)

1021	“Process Parameter Shifting: Part I. Effect of DOT, pH, and Temperature on the Performance of Epo-Fc Expressing CHO Cells Cultivated in Controlled Batch Bioreactors” to Evelyn Trummer, Katharina Fauland, Silke Seidinger, Kornelia Schriebl, Christine Lattenmayer, Renate Kunert, Karola Vorauer-Uhl, Robert Weik, Nicole Borth, Hermann Katinger, Dethardt Muller (“Trummer”)
1022	“Effects of Culture Conditions on <i>N</i> -Glycolylneuraminic Acid (Neu5Gc) Content of a Recombinant Fusion Protein Produced in CHO Cells” to Michael C. Borys, Nimish G. Dalal, Nicholas R. Abu-Absi, Sarwat F. Khattak, Ying Jing, Zizhuo Xing, Zheng Jian Li (“Borys”)
1023	“Production platforms for biotherapeutic glycoproteins. Occurrence, impact, and challenges of non-human sialylation” to Darius Ghaderi, Mai Zhang, Nancy Hurtado-Ziola & Ajit Varki (“Ghaderi”)
1024	“Optimal and consistent protein glycosylation in mammalian cell culture” to Patrick Hossler, Sarwat F Khattak, and Zheng Jian Li (“Hossler 2009”)
1025	“NIST Interlaboratory Study on Glycosylation Analysis of Monoclonal Antibodies: Comparison of Results from Diverse Analytical Methods” to Maria Lorna A. De Leoz, David L. Duewer, Adam Fung, Lily Liu, Hoi Kei Yau, Oscar Potter, Gregory O. Staples, Kenichiro Furuki, Ruth Frenkel, Yunli Hu, Zoran Sosic, Peiqing Zhang, Friedrich Altmann, Clemens Grunwald-Grube, Chun Shao, Joseph Zaia, et al. (“De Leoz”)
1026	Intentionally left blank
1027	“Protein Glycosylation Control in Mammalian Cell Culture: Past Precedents and Contemporary Prospects” to Patrick Hossler (“Hossler 2012”)
1028	“The Combined Effect of Sodium Butyrate and Low Culture Temperature on the Production, Sialylation, and Biological Activity of an Antibody Produced in CHO Cells” to Fei Chen, Tianci Kou, Li Fan, Yan Zhou, Zhaoyang Ye, Liang Zhao, and Wen-Song Tan (“Chen”)
1029	“Hyaluronidase: An overview of its properties, applications, and side effects” to Hyunwook Jung (“Jung”)
1030	“A recombinant human enzyme for enhanced interstitial transport of therapeutics” to L.H. Bookbinder, A. Hofer, M.F. Haller, M.L. Zepeda, G.-A. Keller, J.E. Lim, T.S. Edgington, H.M. Shepard, J.S. Patton, G.I. Frost (“Bookbinder”)
1031	“Recombinant human hyaluronidase (rHuPH20): an enabling platform for subcutaneous drug and fluid administration” to Gregory I Frost (“Frost”)
1032	“ENHANZE® drug delivery technology: a novel approach to subcutaneous administration using recombinant human hyaluronidase PH20” to Kenneth W. Locke, Daniel C. Maneval and Michael J. LaBarre (“Locke”)
1033	“N-Glycosylation and enzymatic activity of the rHuPH20 expressed in Chinese hamster ovary cells” to Liu T, Li Y, Xu J, Guo Q, Zhang D, Song L, Li J, Qian W, Guo H, Zhou X, Hou S. (“Liu”)
1034	Butler M, Meneses-Acosta A. Recent advances in technology supporting biopharmaceutical production from mammalian cells. Appl Microbiol Biotechnol. 2012 Nov;96(4):885-94.
1035	U.S. Patent No. 8,187,855 to David Baker and Louis H. Bookbinder (“Baker”)

1036	Liu, Chao Yan, Virginia Spencer, Shyam Kumar, Jian Liu, Henry Chiou, and Jonathan Zmuda, “Attaining High Transient Titters in CHO Cells”
1037	International Patent Publication WO2017/011598 to Ge Wei, Michael H. Shepard, Qiping Zhao and Robert James Conner is titled “PH20 Polypeptide Variants, Formulations and Uses Thereof” (“Wei 2013”)