UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

NOVARTIS GENE THERAPIES, INC. &
NOVARTIS PHARMACEUTICALS CORPORATION,
Petitioner

v.

GENZYME CORPORATION,
Patent Owner.

CASE: IPR2023-00608
PATENT NO. 9,051,542

DECLARATION OF MARTYN C. DAVIES

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I. INTRODUCTION

1. I submit this declaration in connection with Patent Owner's Preliminary Response in the *Inter Partes* Review IPR2023-00608 (the "IPR") of claims 5 and 6 ("challenged claims")[1] of U.S. Patent No. 9,051,542 (the "'542 patent"; Ex. 1001). I have been retained by Dechert LLP, counsel for Patent Owner Genzyme Corporation in this matter. I am being compensated for my time at my standard rate of £ 600 per hour (£ 650 per hour for deposition testimony), plus actual expenses. The opinions in this declaration are my own, and my compensation is not dependent in any way upon the outcome of the IPR of the '542 patent.

II. QUALIFICATIONS

2. I have over 35 years' experience in the formulation of pharmaceutical and biologic therapeutics and drug delivery fields. Outside of my academic responsibilities, in 1997 I co-founded Molecular Profiles Ltd., a pharmaceutical development, advanced formulation characterization, and clinical trial manufacturing company. I worked on client projects within this company from 1997 through its acquisition by Juniper Pharmaceuticals and until the latter was

[1] I understand that Patent Owner has disclaimed claims 1 and 2, which are no longer at issue here.

acquired by Catalent in 2018 (collectively referred to hereafter as "Molecular Profiles"). I have since acted as a pharmaceutical consultant providing services for the pharmaceutical industry worldwide.

3. In 1980, I graduated first in my class with a First Class Honours degree in Pharmacy from Brighton Polytechnic in the UK. In 1985, I was awarded a Ph.D. from the Chelsea School of Pharmacy, University of London (now Kings College, University of London) in the UK. I obtained my D.Sc. from the University of Nottingham in 2018.

4. After completing my doctorate degree, I held a lectureship at the Department of Pharmacy at the University of Manchester for one year. In 1985, I was appointed to a lectureship position at the University of Nottingham. I was then appointed a Reader in 1991 and Professor in 1996.

5. From 1985 until I recently retired, I have taught a range of pharmaceutical technology, analysis, and drug delivery classes to undergraduate students at the University of Nottingham. Between 1995 and 1997, I acted as the University's Head of Life, Health & Agricultural Sciences Division, Graduate School. In 1999, I became Deputy Head of the School of Pharmaceutical Sciences at the University. In 2000, I was promoted to Head of the School of Pharmaceutical Sciences and the School of Pharmacy at the University of Nottingham. I held this position until 2003.

6. My academic career has primarily focused on the area of the development and characterization of pharmaceutical formulations, advanced drug delivery, and biomedical systems. In the course of my academic career and in my role at Molecular Profiles and Juniper Pharmaceuticals, I developed extensive experience in the production and characterization of many different types of pharmaceutical dosage forms.

7. Over the course of my studies, I have authored over 380 peer-reviewed papers, reviews and book chapters.

8. I have extensive practical experience in the development and characterization of pharmaceutical formulations. I have gained this experience through laboratory work with doctoral students I supervised, my teaching work at the University of Nottingham, industry and business partnerships and consulting roles whilst working at the Laboratory of Biophysics and Surface Analysis ("LBSA") (a Research Division of the School of Pharmacy at the University of Nottingham that I co-founded), and in the course of performing various consulting projects through my work at Molecular Profiles with pharmaceutical companies. I have extensive experience with the formulation of pharmaceutical products and advanced drug delivery systems. I have participated in the making and characterization of many pharmaceutical preparations from 1985 through the present.

9. I have experience in the formulation and study of the structure, function and stability of biologic formulations both from my academic work (teaching and research) and also within my commercial pharmaceutical company. Formulation and delivery of biologics was also a significant feature of the scientific contributions to the Controlled Release Society for which I was Scientific Secretary and later President.

10. I have supervised many undergraduate, graduate, and post-doctoral students in my area of study, including over 100 post-doctoral and postgraduate students whose work has ranged from pre-formulation testing through tablet design and drug delivery systems. Most of the students that I supervised have gone on to work in positions within the pharmaceutical industry or academia.

11. I am active in various national and international societies, including the Royal Pharmaceutical Society of Great Britain (where I was elected a Fellow), the Royal Society of Chemistry (where I was elected a Fellow), and the Controlled Release Society (where I was elected a Fellow). I have served as President (2011 and 2012), Vice-President (2009), and Scientific Secretary (2001) of the Controlled Release Society, the premier international society in drug delivery. I am also a member of the Engineering and Physical Sciences Research Council Peer Review College, the Research Assessment Exercise 2008 Pharmacy Panel, and was a participant in the European Science Foundation Forward Look on

Nanomedicine. I also was elected as a Fellow of the Academy of Pharmaceutical Sciences in the United Kingdom.

12. I have been on the editorial boards of a number of the leading international scientific journals in formulation science, including the Journal of Pharmaceutical Sciences, Advanced Drug Delivery Reviews, the European Journal of Pharmaceutical Sciences, the Journal of Controlled Release and the Journal of Biopharmaceutics and Biotechnology.

13. I am a Fellow of the Royal Society of Chemistry, the Controlled Release Society and Royal Pharmaceutical Society of Great Britain and was awarded a Fellowship by the Academy of Pharmaceutical Sciences in the UK for my contribution in the field of pharmaceutical science.

14. In 1997, I co-founded the private sector company Molecular Profiles and served as the founding Chairman until 2013, when the company was acquired. In 2013, I was appointed as an Advisor to the Board of Directors and also chaired and served on the Scientific Advisory Board.

15. During my time with the company, Molecular Profiles was a pharmaceutical development and advanced formulation characterization company that provided services to top pharmaceutical companies worldwide. We delivered customized formulation, characterization, and development across a broad range of therapies. We also provided pharmaceutical development, clinical trial

manufacturing, advanced analysis, and consulting services and developed and packaged formulations for use by pharmaceutical companies in Phase I and Phase II clinical trials.

16. In 2007 and 2011, Molecular Profiles was awarded a Queen's Award for Enterprise in recognition of its commitment to bringing high quality innovative science to the pharmaceutical industry.

17. In January 2018, I was appointed as a Commander of the Order of the British Empire ("CBE") in Her Majesty's 2018 New Year's Honours List in recognition of my contribution to pharmacy and pharmaceutical research.

18. Attached as Exhibit 2005 is my curriculum vitae, which summarizes my background, credentials, and includes a list of my publications.

III. MATERIALS CONSIDERED

19. My analysis is based on my years of education, research, experience, and background, as well as my investigation and study of relevant materials for this declaration. When developing the opinions set forth in this declaration, I was asked by counsel to assume the perspective of a person having ordinary skill in the art ("POSA"), provided in the Petition and Dr. Amiji's declaration (Ex. 1025). Petition, 16-17; Amiji, ¶ 82. In addition to the Petition, I reviewed and considered the materials identified in the list below.

Exhibit	Description
1001	U.S. Patent No. 9,051,542 B2 to Wright, et al.
1002	File History of U.S. Patent No. 9,051,542 B2 to Wright, et al.
1003	International Patent Pub. No. WO 01/66137 A1 to Evans and Volkin.
1004	International Patent Pub. No. WO 99/41416 to Frei, et al.
1005	Huang J, Gao, et al., "Aggregation of AAV vectors, its impact on liver directed gene transfer and development of vector formulations to prevent and dissolve aggregation and enhance gene transfer efficiency," *Mol Ther.* 1:S286 (2000).
1006	Mingozzi, et al., "Improved Hepatic Gene Transfer by Using an Adeno-Associated Virus Serotype 5 Vector," *J Virol.* 76:10497-502 (2002).
1007	Wright et al., "Recombinant Adeno-Associated Virus: Formulation Challenges and Strategies for a Gene Therapy Vector," *Curr. Opin. Drug Disc. Dev.* 6(2): 174-178 (2003).
1008	Gatlin, L., Chapter 17, "Formulation and Administration Techniques to Minimize Injection Pain and Tissue Damage Associated with Parental Products," in *Injectable Drug Development: Techniques to Reduce Pain and Irritation,* (eds. P. Gupta and G. Brazeau), pp. 401-421 (1999).
1009	International Patent Pub. No. WO 03/039459 A2 to Liu, et al.
1010	International Patent Pub. No. WO 03/046142 A2 to Lochrie and Colosi.
1011	Potter M., et, al. Chapter 24, Streamlined Large-Scale Production of Recombinant Adeno-Associated Virus (rAAV) Vectors" in *Methods in Enzymol*., 346:413 (2002)
1012	Clark, K., Recent advances in recombinant adeno-associated virus vector production, *Kidney Int'l*, Vol. 61, Symposium 1, pp. S9–S15 (2002).
1013	Croyle, et al., "Development of Formulations That Enhance Physical Stability of Viral Vectors for Gene Therapy," *Gene Ther*., 8:1281-1290 (2001).
1014	*Cambridge English dictionary*. Retrieved from

Exhibit	Description
	https://dictionary.cambridge.org/us/doctionary/english/about.
1015	U.S. Patent No. 6,261,823 B1 to Tang et al.
1016	Freireich, E., et al., "Quantitative Comparison of Toxicity of Anticancer Agents in Mouse, Rat, Hamster, Dog, Monkey, and Man," *Cancer Chemoth Rep*. 50(4): 219-244 (1966).
1017	Center for Drug Evaluation and Research (CDER). *Guidance for Industry Stability Testing of Drug Substances and Drug Products*, US Department of Health and Human Services, Food and Drug Administration: Rockville, MD (July 2005).
1018	Carpenter, J. F. & Manning, M.C. (Eds.), *Rational Design of Stable Protein Formulations: Theory and Practice*, Vol. 13 (2002).
1019	F. B. Johnson and A. S. Bodily, *Effects of Environmental pH on Adenovirus-Associated Virus* (39085), Procs. of the Soc. for Experimental Biology and Med., pp. 585-90 (1975).
1020	*Remington: The Science and Practice of Pharmacy*, Nineteenth Ed., Vol. I, Chapters 17, 18, 23, and 36 (1995).
1021	*Remington: The Science and Practice of Pharmacy*, Nineteenth Ed., Vol. II, Chapter 87(1995).
1022	Ainley Wade and Paul J Weller (Eds.), *Handbook of Pharmaceutical Excipients*. The Pharmaceutical Press: London, 1994 (pp. 454-458).
1023	Joint Claim Construction Brief, Case Number, C.A. No. 21-1736-RGA (U.S. Dist. Ct. Del.), served by Aventis Inc., and Genzyme Corporation on January 20, 2023.
1024	Appendix 2A to Laboratory Stock Solutions and Equipment, Common Buffers and Stock Solutions, *Current Protocols in Nucleic Acid Chemistry*, pp. A.2A.1-A.2A.12 (2000).
1025	Declaration of Dr. Mansoor M. Amiji, Ph.D.
1026	U.S. Patent No. 6,146,874 to Zolotukhin et al.
1027	U.S. Patent Application Publication No. 2004/0166122 A1 to Evans, et al.

Exhibit	Description
1028	U.S. Patent Application Publication No. 2002/0041884 A1 to Evans, et al.
1029	File History of U.S. Patent No. 7,704,721 B2 to Wright, et al.
2006	Xie et al., "*Large-Scale Production, Purification and Crystallization of Wild-Type Adeno-Associated Virus-2*," 122 J. Virol. Methods 17 (2004) ("Xie")
2007	J.F. Wright et al., "*Identification of Factors that Contribute to Recombinant AAV2 Particle Aggregation and Methods to Prevent Its Occurrence during Vector Purification and Formulation*," 12 Molecular Therapy 171 (July 2005)
2008	A.E. Smith, "*Gene Therapy: Where Are We*," 354 The Lancet SI1 (July 1999)
2009	J.O. Konz et al. "*Development of a Purification Process for Adenovirus: Controlling Virus Aggregation to Improve the Clearance of Host Cell DNA*," 21 Biotechnol. Prog. 466 (2005) ("Konz")

IV. SUMMARY OF MY OPINIONS

20. First, it is my opinion that Ground 1, alleging that the challenged claims are obvious over Evans (Ex. 1003), Huang (Ex. 1005), and Mingozzi (Ex. 1006), does not establish the obviousness of claims 5 and 6 of the '542 patent.

21. Second, it is my opinion that Ground 3, alleging that the challenged claims are obvious over Frei (Ex. 1003), Huang (Ex. 1005), and Mingozzi (Ex. 1006), does not establish the obviousness of claims 5 and 6 of the '542 patent.

V. LEGAL STANDARD FOR OBVIOUSNESS

22. I understand that the Petitioner has the burden of proving the challenged claims obvious by a preponderance of evidence, which means proving that the challenged claims are more likely than not obvious.

23. I have been informed by counsel that a patent claim may be unpatentable if it would have been obvious in view of a combination of prior-art references. I have further been informed that a patent claim is obvious if the differences between the subject matter of the claim and the prior art are such that the subject matter as a whole would have been obvious to the POSA in the relevant field at the time the invention was made. Specifically, I understand that the obviousness question involves a consideration of the following factors:

- the scope and content of the prior art;
- the differences between the prior art and the claims at issue;
- the knowledge of a person of ordinary skill in the pertinent art; and
- if present, objective factors indicative of non-obviousness, sometimes referred to as "secondary considerations."

24. I have been informed that for a claimed invention to be considered obvious over a combination of references, the POSA must have had a motivation and/or reason for combining teachings from multiple prior-art references in the manner proposed to arrive at the claim. I further understand that in determining whether a prior-art reference would have been combined with other prior art or with other information within the knowledge of the POSA, the following are examples of approaches and rationales that may be considered:

- combining prior-art elements according to known methods to yield

predictable results;

- simple substitution of one known element for another to obtain predictable results;
- use of a known technique to improve similar devices in the same way;
- applying a known technique to a known device ready for improvement to yield predictable results;
- applying a technique or approach that would have been "obvious to try," i.e., choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success;
- known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations would have been predictable to one of ordinary skill in the art;
- some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior-art reference or to combine prior-art reference teachings to arrive at the claimed invention.

25. I understand that this motivation or reason to combine may come from a prior-art reference or based on the POSA's knowledge or common sense.

26. I have been informed that for a combination of references to render the claimed invention obvious, the POSA must have been able to arrive at the

claimed invention by altering or combining the applied references, and must have had a reasonable expectation of success in doing so. I understand that the claim is considered as a whole, so the obviousness analysis requires consideration of whether the claimed combination of elements was rendered obvious by the cited prior art.

27. I also have been informed that a claim can be obvious if it is the product of routine experimentation or optimization. For ranges recited in a claim, I have been informed that a particular parameter must first be recognized as a result-effective variable (i.e., a variable which achieves a recognized result), before the determination of the optimum or workable ranges of said variable might be characterized as routine experimentation.

28. Finally, I understand that it is important to guard against slipping into the use of hindsight and to resist the temptation to read into the prior art the teachings of the invention in issue. I have been informed it is improper to rely on the inventor's own path to support a conclusion of obviousness; what matters is the path that the POSA would have followed, as evidenced by the pertinent prior art.

VI. BACKGROUND SCIENTIFIC CONCEPTS RELAVENT TO THE '542 PATENT

A. Osmolarity and Tonicity

29. The osmotic pressure of a solution is determined by the concentration of particles of solute in that solution. Ex. 1020, 613. The osmotic pressure of an

aqueous solution can be increased by adding a salt as a solute to that solution, such as sodium chloride (NaCl). When added to solution, NaCl dissociates into its Na^+ and Cl^- ions, so for every molecule of NaCl added to the solution there are two particles of solute present in the solution.

30. Consideration of osmotic pressure is important for pharmaceutical and biologic formulations because the osmotic pressure of a composition impacts the biological response of cells and tissue in proximity to the site where the composition enters the body. When a solution is placed in contact with a semipermeable membrane, like a cell membrane, if the osmotic pressure on one side of the membrane is different than the osmotic pressure on the other side of the membrane, molecules of solvent, but not the solute, will move across the membrane to equilibrate osmotic pressure on both sides of the membrane. *Id.*, 613. When the osmotic pressures on both sides of the cell membrane are the same, then the solutions on either side of the membrane are considered isosmotic. *Id.*

31. Osmotic pressure can be measured in osmolarity, which is a measure of the number of particles of solute per liter of solution. *Id.*, 614. Osmolarity is determined by multiplying the molarity of the solute in solution by the number of ions into which it dissociates. *Id.*, 615-16.

32. When NaCl dissolves in water there are two solute particles, Na^+ ions and Cl^- ions. Each ion contributes to the osmotic pressure of the solution. So, one

mole (M) of NaCl becomes two osmoles (Osm) in solution. So the osmolarity of salts of monovalent ions such as NaCl will be double the molarity in solution *Id.*, 616. For example, a 100 mM NaCl solution will have an osmolarity of 200 mOsm as shown below:

100 mmol NaCl x 2 = 200 mOsm

33. As another example, magnesium chloride ($MgCl_2$) dissociates in water into three particles of solute, one Mg^{2+} and two Cl^- ions, so its osmolarity is triple the molarity of $MgCl_2$. Thus, a 100 mM $MgCl_2$ solution will have an osmolarity of 300 mOsm as shown below.

100 mmol $MgCl_2$ x 3 = 300 mOsm

34. Tonicity and osmolarity are related topics. As Remington explains, the "term isotonic, meaning equal tone, is, in medical usage commonly used interchangeably with isosmotic. However, terms such as isotonic and tonicity should be used only with reference to a physiologic fluid." *Id.*, 613. A composition that is isosmotic with the tissue and cells it is in contact with is considered isotonic. For example, serum osmolarity is about 300 mOsm, so a 300 mOsm salt solution is isotonic with serum. *Id.*, 615. "Physiological solutions with an osmotic pressure lower than that of body fluids, or of 0.9% sodium chloride solution, are referred to commonly, as being hypotonic. Physiological solutions

having a greater osmotic pressure are termed hypertonic." *Id.*, 613. In molarity, 0.9% NaCl is equal to ~150 mM NaCl. Ex. 1001, 5:15-16.

35. In the timeframe of June 2004, the POSA would have sought to make rAAV compositions that minimize deviation from isotonicity to avoid causing tissue damage and pain when administered. Consistent with my opinion, Dr. Amiji's declaration states that "[i]t was well known ***as of June 2004*** that it is preferrable to ***maintain the osmolarity*** of a pharmaceutical composition to be ***as close to isotonic as possible, especially for parenteral administration***, to reduce injection pain." Amiji, ¶ 65 (citing Ex. 1008, 410-411; Ex. 1021, 1525 ("Solutions to be administered subcutaneously ***require strict attention to tonicity*** adjustment.")).

36. Dr. Amiji also cites Gatlin (Ex. 1008), which "***emphasizes*** *'the two formulation parameters, pH and* ***tonicity***, that are usually associated with tissue damage and injection pain,' and 'provides a parenteral product development outline' that includes guidance on ways in which pH and tonicity 'may be modified to minimize tissue damage and pain caused by a parenteral product.'" Amiji, ¶ 131 (citing Ex. 1008, 401-402). Gatlin explains:

> The primary purpose for adjusting product osmolality is to ***minimize red blood cell lysis, tissue damage, and pain*** when the product is administered …. If cells are placed into a hypertonic solution, the cells may lose

> water and shrink (crenation) …. In fact, the British Pharmacopoeia states that aqueous solutions for SC, intradermal, or ***IM [intramuscular] injections should be made isotonic if possible***.

Ex. 1008, 411.

37. Carpenter made a similar observation, stating that "[i]f a protein drug is to be administered by intravenous bolus injection or subcutaneously, rather than by continuous infusion, there are ***strict isotonicity and pH considerations that have to be met*** for a pain-free injection." Ex. 1018, 182. Thus, it is my opinion that in developing a composition for human parenteral administration, the POSA would have avoided formulation conditions that resulted in significant deviations from isotonicity.

B. Ionic strength

38. Ionic strength is another parameter of pharmaceutical compositions that "is a measure of the intensity of the electrical field in a solution and may be expressed as the following equation:

$$\mu = \tfrac{1}{2} \sum c_i z_i^2$$

where z_i is the valence of ion i" (e.g., z_i is one for monovalent ions, Na^+ and Cl^- and two for divalent Mg^{2+}) and c_i is the molar concentration of the salt. Ex. 1020, 616.

39. For a 100 mM NaCl solution, the ionic strength would be:

$$\mu = \tfrac{1}{2}(0.1 \times 1^2 + 0.1 \times 1^2) = 100 \text{ mM } (0.1\text{M})$$

Ex. 1020, 616. Whereas for a 100 mM $MgCl_2$ solution, the ionic strength would be:

$$\mu = \frac{1}{2}(0.1 \times 2^2 + 0.1 \times 1^2 + 0.1 \times 1^2) = 300 \text{ mM } (0.3\text{M})$$

Ex. 1020, 616.

40. To summarize, a 100 mM NaCl solution has an osmolarity of 200 mOsm and an ionic strength of 100 mM, whereas a 100 mM $MgCl_2$ solution has an osmolarity of 300 mOsm and an ionic strength of 300 mM. This illustrates for solutions of the same molarity, the ionic strength of solutions of multivalent ion-containing salts (e.g., $MgCl_2$) is higher than for solutions of salts of monovalent ions (e.g., NaCl).

41. Buffer ionic strength requires consideration of the pH-dependent dissociation and ionization of buffer species. Remington uses the example of the ionization of phosphoric acid (H_3PO_4), which can dissociate into three species having different charges, including dihydrogen phosphate ($H_2PO_4^-$), hydrogen phosphate (HPO_4^{2-}), and phosphate (PO_4^{3-}). Ex. 1020, 219, 226. Depending on pH, different concentrations of each species will be present in solution, and those concentrations will impact ionic strength. Around neutral pH values, $H_2PO_4^-$ and HPO_4^{2-} predominate.

42. For buffer ionic strength, Carpenter explains that "50mM sodium phosphate containing 0.1M NaCl, [is] a buffer that has a relatively high ionic

strength" where the POSA would understand the various phosphate species provide the buffering effect. Ex. 1018, 32. A "buffer of moderate ionic strength" is "10mM Tris, pH 8.5, 100 mM NaCl, l mM EDTA" (*id.*, 33), where the mixture of Tris and EDTA are the compounds that provide the buffering effect at pH 8.5. Both the "moderate" and "high" ionic strength buffer solutions have the same NaCl concentrations (0.1 M = 100 mM), so the difference in their ionic strengths is from the buffering species not NaCl, because the NaCl contributes the same level of ionic strength (μ = 100 mM) to both systems.

C. Detection of Aggregates

43. Determining whether there is significant aggregation of rAAV vector particles in a composition requires analyses that can detect particles that are not visible to the naked eye. For example, AAV-2 "particles have an icosahedral structure of ~26 nm in diameter." Ex. 1007, 174. For visualization of particles, "approximately 50 μm [50,000 nm] is the lower limit unless the Tyndall effect is used whereby particles as small as 10 μm [10,000 nm] can be seen by the light scattered from them." Ex. 1021, 1547. Given that 10 μm = 10,000 nm, the smallest particles that can possibly be seen with the naked eye (employing light scattering techniques) are at least ~384 times the size of a monomeric viral vector particle, ~26 nm in diameter. For further context, the largest average particle radius detected in the aggregation studies of the '542 patent was less than 140 nm

(diameter = 280 nm) (Ex. 1001, Figs. 1A, 1B), which is still magnitudes smaller than the10,000 nm threshold for detection by the naked eye. Thus, visual inspection of a composition for a lack of precipitation is not an adequate measure of the presence of aggregates.

44. Average particle radius is one measure of whether there is significant aggregation presence in a composition. An average radius of Rh < 20 nm indicates that there is effectively no aggregation whereas "Rh values >20 nm are deemed to indicate the occurrence of some level of aggregation." Ex. 1001, 9:26-27.

45. Methods including dynamic light scattering ("DLS") can be used to determine average particle radius. An AAV-2 particle, for example, has a radius of ~13 nm (Ex. 1007, 174), so an aggregate of two AAV-2 particles would have a radius exceeding 20 nm. The POSA would understand that measuring average particle radius is a sensitive method to measure aggregation in biologics. As aggregates are of a larger particle size (and radius) they will have a greater scattering intensity than particles of a smaller size. Thus, techniques for measuring average particle size, such as DLS, are sensitive to the presence of aggregates as the average particle radius of the sample will increase from that of the unaggregated particles.

46. The sensitivity of average particle radius measurement techniques is illustrated by a study of recombinant Ad5 ("rAd5") viral particles, which have a

diameter of 80 nm. Ex. 2009, 466 ("Konz"). Konz compared three methods of detecting the presence of significant aggregation in viral particle compositions: DLS particle diameter detection (which is equivalent to radius detection) in the composition, UV/SDS yield assay following filtration of the composition through a 0.22 μm filter, and the A_{320}/A_{260} absorbance ratio of the composition. *Id.*, 469-70.

47. According to DLS analysis, unfiltered rAd5 Lot B contained 58% monomer and 42% aggregate, which resulted in a "bimodal distribution ha[ving] peaks centered around 120 and 500 nm [diameter], corresponding to monomeric and aggregated virus, respectively." *Id.*, 470. Even if the majority of viral particles remain monomeric in Lot B, the POSA would have understood because the aggregates in Konz had over four times the detected diameter of the monomer the aggregates skewed the average diameter upward. Indeed, Konz's "mass-weighted-mean aggregate contain[ed] roughly 15 particles," showing a relatively small amount of formation can radically increase average particle diameter/radius. *Id.*, 470. Thus, the POSA would have understood that for a composition to have an average diameter (or radius) value close to that of the monomer, indicates a lack of significant aggregation.

48. Konz also determined the yield after "0.22 μm absolute filtration" as a measure of the degree of viral particle aggregation within the composition. *Id.* For Lot B the "yield across filtration" was 75% as determined by UV/SDS assay, "a

measure of total rAd5." *Id.*, 469-70. As noted prior to filtration, significant aggregation (~58% monomer) was present in Lot B, and following filtration the composition still contained only 77% monomer. Konz's results confirm that a high percentage viral particle recovery following filtration of the composition of said AAV vector particles through a 0.22 μm filter is a measure reflective of whether significant aggregation occurs, as discussed further in the context of the '542 patent. Section VII, below.

49. The third method analyzed by Konz was the A_{320}/A_{260} UV absorbance ratio. Dr. Amiji's declaration stated that "[a]s of June 2004, the UV absorbance ratio for the wavelengths A_{320}/A_{260}, which is a measure of the turbidity of a dispersion, was known to 'indicate the aggregation of the virus particles' in prepared adenovirus formulations." Amiji, ¶ 58 (citing Frei, 12:12-17). Dr. Amiji's declaration further asserted that "[i]t was also known that '[p]urified, free [adeno]virus particles display a [A_{320}/A_{260}] ratio of about 0.22-0.30:1.'" *Id.* (citing Ex. 1015, 6:59-63). This is not an accurate description of how the POSA would have understood this reference. The patent of Ex. 1015 states "[p]urified, free virus particles display a light scattering ratio of about 0.22-0.30:1." Ex 1015, 6:62-63. The POSA would have understood a value in this range merely indicates that free virus particles are present but does not provide the POSA with a determination that aggregates are not present.

50. As of the June 2004 filing date, the A_{320}/A_{260} was an unproven method for identifying the absence of aggregates. Although Konz published in 2005, shortly after the earliest June 1, 204 filing of the '542 patent, Konz reflects the what the POSA would have known; the A_{320}/A_{260} absorbance ratio in the range of 0.22-0.30 indicates the presence of free virus particles but does not suggest that no aggregates are present. In fact, Konz shows that using established assays, such as average particle radius/diameter and product recovery after filtration can detect the presence of aggregates in samples which fall within the A_{320}/A_{260} absorbance ratio range of 0.22-0.30.

51. Konz challenges the notion that the A_{320}/A_{260} absorbance ratio, and particularly ratios in the range of 0.22-0.30, can be used as a method to indicate the absence of aggregates. For example, Konz's "Lot B, having a A_{320}/A_{260} ratio of 0.23, resulted in a bimodal distribution with peaks at about 130 and 260 nm (Figure 8)" according to DLS analysis. Ex. 2009, 470. "In this case, ***77% of the sample was monomeric,***" so there were a significant number of aggregates. Recovery after filtration through a 0.22 μm filter resulted in a yield of 75% for Lot B, also indicating significant aggregation. *Id*. (Table 3). Thus, it is my opinion that the POSA would not have considered the A_{320}/A_{260} absorbance ratio as an indicator of whether an rAAV composition exhibited "significant aggregation" or the lack of it, in a composition, particularly compared to other measures of the

presence of aggregates in the ’542 patent, including average particle ratio and product recovery after filtration.

52. As explained below in Section IX.C.3, for Ground 3 the Petition and Dr. Amiji’s declaration both rely on Frei’s A_{320}/A_{260} absorbance ratio to “indicat[e] no aggregation.” Petition, 49; Amiji, ¶ 249 (“Frei’s compositions are stable with no virus aggregation during storage.”). The POSA, however, would not have interpreted Frei’s data to indicate no aggregation was present in Frei’s compositions. Moreover, Konz’s testing is contrary to Amiji’s assertion that A_{320}/A_{260} absorbance values within Frei’s data “indicat[e] no aggregation.” A_{320}/A_{260} absorbance values are far less sensitive to aggregation than average particle radius and product recovery following filtration recited in the ’542 patent’s claims 5 and 6, respectively.

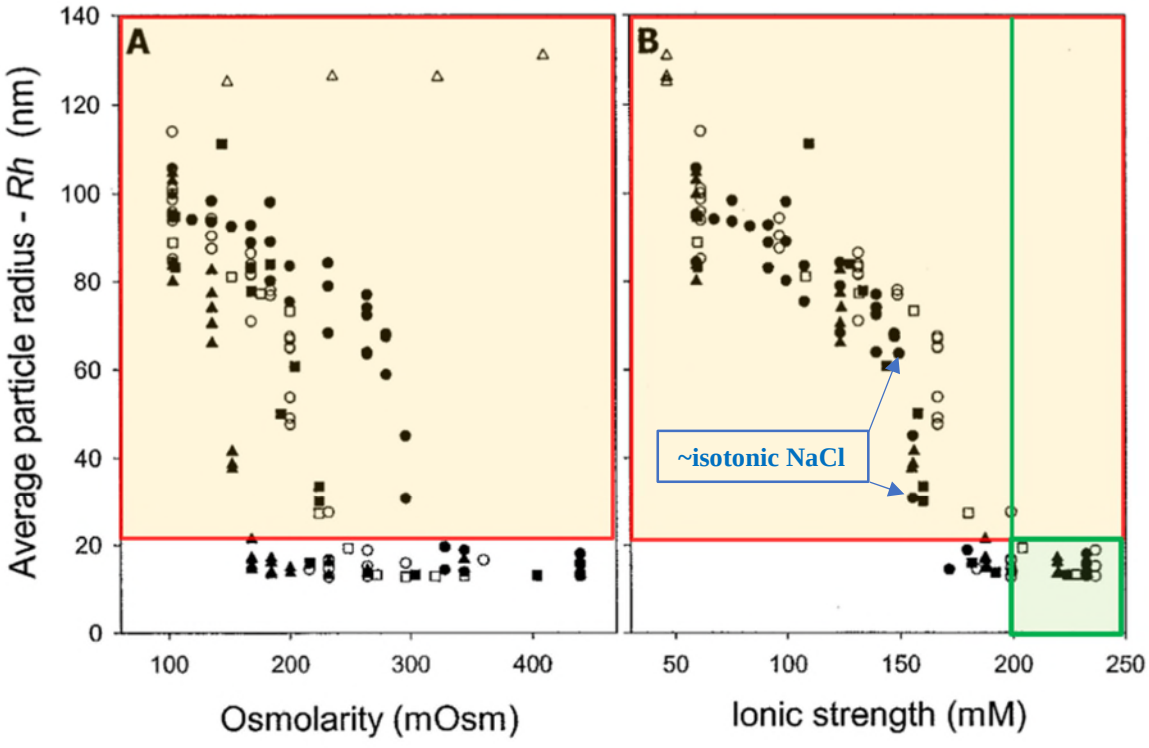
VII. THE ’542 PATENT

53. The inventors studied vector aggregation in solutions of sodium chloride, sodium citrate, sodium phosphate, sodium sulfate, magnesium sulfate, and glycerol, among other e.g., amino acids, and tracked aggregation as a function of two parameters, osmolarity (Figure 1A) and ionic strength (Figure 1B). Ex. 1001, 6:63-65, 12:33-67 (Example 3), FIGS. 1A, 1B.

54. In Figures 1A and 1B, “[a]verage particle radius is measured by dynamic light scattering (DLS) following vector dilution in varying concentrations

of excipients buffered with 10 mM sodium phosphate at pH 7.5." Ex. 1001, 4:18-21. For DLS, Given the average radius of these viral particles is approximately 13nm, "Rh values >20 nm are deemed to indicate the occurrence of some level of aggregation," whereas Rh < 20 nm indicate a lack of significant aggregation. *Id.*, 9:25-27; Section VI.C, above.

55. In annotated Figures 1A and 1B below, the salts and excipients are denoted as follows: sodium chloride (●), sodium citrate (◯), sodium phosphate (▪), sodium sulfate (□), magnesium sulfate (▲), and glycerol (Δ):



Id., FIGS. 1A, 1B (annotation added). In both Figures, compositions falling within the orange shaded portion of the plots exhibited significant aggregation (Rh > 20

nm), whereas compositions in the unshaded or green shaded portions of the plot did not (Rh < 20 nm).

56. The results of Figure 1A, which plots particle radius as a function of osmolarity, showed no consistent relationship between aggregation and osmolarity across the tested excipients. Multivalent "magnesium sulfate [(▲)] prevented aggregation at >200 mOsm," whereas monovalent "sodium chloride [(●)] required ≧350 mOsm to achieve a similar effect." *Id.*, FIG. 1A, 7:1-8 (Stating that "[s]odium citrate, sodium sulfate, and sodium phosphate are intermediate in their potency to prevent vector aggregation"). Glycerol (Δ), which is a polyol, never prevented aggregation even when osmolarity of the composition reached osmolarity values above 400 mOsm.

57. Figure 1B shows data from the same experiment "plotted as a function of the calculated ionic strength, rather than osmolarity, for each excipient." *Id.*, 7:18-20. In contrast to the plot of particle radius versus osmolarity, Figure 1B's plot of particle radius versus ionic strength shows a clear trend; "vector aggregation is prevented when ***ionic strength is ~200 mM or greater*** regardless of which salt is used" as shown by the green line at 200 mM ionic strength in annotated Figure 1B, above. *Id.*, 7:21-22. These data led to the breakthrough "that the ionic strength (μ) of a solution … is the ***primary factor affecting aggregation***." *Id.*, 7:22-25. The green box in annotated Figure 1B illustrates the scope of claim 5

requiring both an "ionic strength … greater than 200 mM" and a "an average particle radius (Rh) of less than about 20 nm as measured by dynamic light scattering." *Id.*, 14:34-37.

58. The identification of ionic strength as a key composition parameter that prevents aggregation unlocked the path to isotonic compositions for human parenteral administration by employing multivalent ions to adjust ionic strength. As the inventors observed, "[o]f practical concern, commonly used buffered saline solutions have insufficient ionic strength to prevent AAV2 vector aggregation at concentrations exceeding 10^{13} particles/mL." *Id.*, 4:65-67. "Isotonic (150 mM) [NaCl] has an ionic strength of 150 mM, a value insufficient to maintain AAV2 solubility at high vector concentrations," as shown in annotated Figure 1B. *Id.*, 5:15-17. Thus, making a concentrated rAAV composition in which the ionic strength is attributable to higher NaCl concentrations can render the composition hypertonic. *Id.*, 5:4-7.

59. Because of the exponential relationship between ionic strength and charge valency, "multivalent ions" (i.e., ions having a charge of two or more) "achieve a similar degree of inhibition of aggregation at lower concentrations than monovalent [NaCl]," for which Na^+ and Cl^- only contribute one charge each. *Id.*, 7:1-8. The inventors recognized and capitalized on the "exponential relationship of ionic strength with charge [v]alency … to develop isotonic formulations with

high ionic strength." *Id.*, 5:7-10. Compositions containing multivalent ions, including pharmaceutically acceptable excipients containing citrate, sulfate, magnesium, and/or phosphate can have an ionic strength of greater than 200 mM, while remaining isotonic, because multivalent ions at low concentrations have high ionic strength.

60. Particle size detection by DLS *(id.,* 9:5-50 (Table 3), claim 5) and percent product recovery following filtration through a 0.22 μm filter (*id.,* 7:65-8:40 (Table 2), claim 6), further confirmed that high ionic strength compositions did not exhibit significant rAAV aggregation. The inventors prepared three solutions for AAV2-AADC vectors: "Control Formulation (**CF**: 140 mM sodium chloride, 10 mM sodium phosphate, 5% sorbitol, pH 7.3); Test Formulation 1 (**TF1**: 150 mM sodium phosphate, pH 7.5); and Test Formulation 2 (**TF2**: 100 mM sodium citrate, 10 mM Tris, pH8.0)." *Id.*, 11:66-12:3. In Experiment 1 the samples contained 2.5×10^{13} vg/ml vector, and in Experiment 2 the samples contained 6.7×10^{13} vg/ml vector.

61. In Example 2, titled "Ultrafiltration and Diafiltration to Detect AAV Aggregation," the exemplary formulations were filtered through a 0.22 μm filter. *Id.*, 8:1-10, 11:53-12:29. Table 2 summarizes the results of Experiments 1 and 2, including the ionic strength of each formulation. *Id.*, 8:19-44.

TABLE 2

AAV VECTOR RECOVERY AT PROCESS SCALE

Experiment	Formulation	μ (mM)	Target (vg/mL)	Actual (vg/mL)	Yield % (RSD)
1	CF	160	2.5E13	1.93E13	77 (6.6)
1	TF1	310	2.5E13	2.38E13	95 (7.4)
1	TF2	510	2.5E13	2.33E13	93 (7.4)
2	CF	160	6.7E13	3.98E13	59 (6.0)
2	TF2	510	6.7E13	6.42E13	96 (4.4)

62. As Table 2 shows, recoveries exceeded 90% following filtration in formulations TF1 and TF2 having ionic strengths greater than 200 mM, whereas recovery from CF formulations, ionic strength 160 mM, was only 77% and 59% for experiments 1 and 2, respectively.

63. The inventors also conducted storage and freeze-thaw ("F/T") cycle studies on the CF, TF1, and TF2 formulations, with the results presented in Table 3, in which particle radius was measured by DLS to determine the presence of aggregates. *Id.*, 9:5-65.

TABLE 3

STABILITY OF AAV2 VECTORS

	Particle radius - Rh (nm)							
Formu-		4° C.	−20° C.			−80° C.		
lation	Pre	5 d	1 F/T	5 F/T	10 F/T	1 F/T	5 F/T	10 F/T
CF	14.5	27.0	22.4	56.1	94.5	20.6	57.5	141
TF1	13.8	16.3	TH	TH	TH	TH	TH	TH
TF2	13.8	14.4	14.2	14.0	14.1	13.8	21.3	50.9

Pre: DLS radius measured immediately following 0.2 μm filtration.
Vector concentrations (vg/mL): CF: 1.93E13, TF1: 2.38E13, TF2: 2.33E13.
TH: signal intensity is too high to measure because of extensive aggregation.

64. "As shown in Table 3 … CF shows some aggregation after 5 days of storage at 4° C, as well as following one or more F/T cycles at −20 or −80° C. For vector prepared in TF1, no aggregation occurs after 5 days [of storage] at 4° C …. For vector prepared in TF2, no aggregation is observed at 4° C., or following up to 10 F/T cycles at −20° C …." *Id.*, 9:44-55. These studies further confirmed the importance of increased ionic strength in preventing aggregation in concentrated rAAV compositions.

65. In summary, the inventors' identification of high ionic strength as a key factor in preventing rAAV aggregation enabled them to be the first to make storable concentrated rAAV compositions suitable for human parenteral use. *Id.*, 10:29-43.

VIII. PETITION GROUND 1: CLAIMS 5 AND 6 WOULD NOT HAVE BEEN OBVIOUS TO THE POSA IN VIEW OF EVANS, HUANG, AND MINGOZZI

66. In my opinion, the Petition and Dr. Amiji's declaration fail to consider the full scope of teachings in the prior art. Evans, Huang, and Mingozzi, when considered in the context of the prior art as a whole, would not have provided the POSA with a reason to develop the high-concentration rAAV compositions claimed in the '542 patent, or a reasonable expectation of success in doing so.

67. An issue with Evans—that neither the Petition nor Dr. Amiji's declaration point out—is that Evans is not about viral particle aggregation. In fact,

Evans does not consider the issue of aggregation at all. Instead, Evans is about suppressing vector instability caused by radical oxidation. Evans, 9:23-25 (A "centerpiece of [Evans'] formulations … relate[s] to inclusion of components that act as inhibitors of free radical oxidation."); *id.*, 13:8-11 ("An essential quality of the present invention is the finding that non-reducing free radical scavengers and/or chelators are important for maximizing both short and long term stability of viral formulations."). For this reason, Evans' focus is on identifying agents that prevent radical oxidate, such as radical scavengers and chelators. Evans, 10:6-11. For example, Evans identifies chelators such as ethylenediaminetetraacetic acid (EDTA) as being particularly important for improving composition stability. *Id.*, 33:12-15, 33:30-34:1 (Example 13).

68. The other references cited in Ground 1 of the Petition, Huang and Mingozzi, similarly would not have provided the POSA with any guidance to address rAAV aggregation. Huang is a one paragraph abstract that indicates aggregation is a problem for high-concentration rAAV compositions, stating that "at high concentrations, AAV virions form aggregates of different sizes in a range of different buffer systems and storage conditions. The size of aggregates appears to be concentration dependent." Huang, S286. Huang states "that some of our formulations could lead to a 30-50% ***reduction in the size*** of aggregates." *Id.* From this statement, the POSA would not have understood quantitatively what

Huang means, because there is no description of the size of the aggregates to which Huang refers. Moreover, Huang never suggests that its stored rAAV compositions could remain free of significant aggregation, and in fact suggests the opposite, that the presence of aggregates is an intractable problem. Huang also does not disclose any information about its formulations that led to reduced aggregate size.

69. Mingozzi, like Evans, never addresses aggregation. Mingozzi does not provide any information about its formulations.

70. Thus, Evans, Huang, and Mingozzi do not provide any indication of how to address the problem of rAAV aggregation in high-concentration compositions or any reasonable expectation of succeeding in doing so.

A. The Petition Overlooks that the POSA Would Have Understood that Evans' Reported Vector Particle Concentrations Were Well Below Corresponding Vector Genome Concentrations

71. The Petition does not provide any evidence or an adequate explanation for how the POSA would have possibly considered that Evans discloses a composition comprising viral particles at "a concentration exceeding 1 x 10^{13} vg/ml up to 6.4 x 10^{13} vg/ml" as claim 1 recites. Petition, 25, 30. The Petition cites Evans' claim directed to "a virus concentration in the range from about 1x10^{7}vp/mL to about 1x10^{13}vp/mL" to argue that the uppermost endpoint of this range (10^{13}vp/mL ± 5%) overlaps with the scope of the claims. Petition, 30 (citing Evans, claim 3). But in order for Evans' uppermost concentration, recited

as viral ***particles*** per milliliter (vp/ml), to meet the claimed concentration, recited in viral ***genomes*** per milliliter (vg/ml), the POSA would have had to "***[a]ssum[e] that 100% of the particles contain vector genomes***." *Id*. It is my opinion that the POSA would never make such an assumption.

72. Dr. Amiji's declaration, in fact, indicates that the POSA would not have made such an assumption. Dr. Amiji states that "Wright [Ex. 1007] teaches that $\geq 10^{14}$ capsid particles (cp)/ml corresponds to $\geq 10^{13}$ vg/ml." Amiji, ¶ 119 (citing Ex. 1007, 176). Based on Wright, the POSA would have understood that a concentration in vp/ml could have as much as a ***10-fold excess in empty capsids***, which would mean that Evan's most concentrated compositions of 10^{13} vp/ml could have a viral genome concentration of only 10^{12} vg/ml.

73. Lochrie (Ex. 1010) states that "***more than 80% of AAV material*** created during rAAV production ***may be empty capsids***, and current column chromatography purification techniques do not separate packaged capsids from empty capsids." Lochrie, 4:20-23. Evans, notably, states that its "recombinant Ad5gag virus was purified by ***column chromatography***," (Evans, 21:12-13), of which Lochrie says "column chromatography purification techniques d***o not separate packaged capsids from empty capsids***." Lochrie, 4:20-23.

74. Thus, it is my opinion that Dr. Amiji's declaration and the references it cites demonstrate that the POSA would have understood that Evans' composition

would not come close to containing vector genome concentration "exceeding 1 x 10^{13} vg/ml."

B. The Petition Does Not Establish that the POSA Would Have Been Motivated to Formulate a High-Concentration rAAV Vector Composition at an Ionic Strength Greater than 200 mM

75. The Petition does not provide a reason or motivation for why the POSA would develop a composition comprising an rAAV "concentration exceeding 1 x 10^{13} vg/ml," "one or more multivalent ions selected from … citrate, sulfate, magnesium, and phosphate," with an ionic strength "greater than 200mM."

76. Because of the unsolved issues with aggregation, in June 2004, the POSA would have avoided making an rAAV composition with a "concentration exceeding 1 x 10^{13} vg/ml." The Petition acknowledges the POSA understood that higher viral particle concentrations caused increased aggregation. Petition, 14 (citing Huang, S286). Croyle (Ex. 1013) explains that for rAAV composition, "[h]igh concentrations of protein may induce aggregate formation and precipitation upon freezing resulting in poor recovery of the product." Ex. 1013, 1286.

77. Huang explains that at high concentrations aggregation led to losses in infectivity so large that lower concentration viral vector compositions provided better infectivity than higher concentration compositions. Petition, 19, 32 (citing Huang, S286). Specifically, Huang stated that "when the concentration reached 5-10x10^{13}vg/ml, gene transfer efficiency was ***10-100-fold lower*** compared to the

same vector administered at the same dose but having a concentration of 1-5x10^{12}vg/ml." Huang, S286. As explained below in Sections VIII.B.1 and VIII.C.2 and other publications indicated that there were no known approaches to solving the high-concentration AAV aggregate issue.

78. Mingozzi, which the Petition cites to argue that the POSA would been motivated to administer "doses of 3.2x10^{13}vg for a 60kg human" at a "concentration exceeding 1 x 10^{13}" (Petition, 31), does not provide any formulation information, let alone any specifics on excipients and ionic strength.

79. Evans, Huang, and Mingozzi, individually and in combination do not teach how to make high-concentration rAAV compositions without significant aggregation. Moreover, these three references do not provide any support for the Petition's arguments that the POSA would have been motivated to develop a high-concentration rAAV composition with an ionic strength greater than 200 mM and the recited multivalent ion(s).

1. The Petition Does Not Identify Any Prior Art Teaching or Suggestion that Ionic Strength Likely Impacted rAAV Aggregation

80. I disagree with the Petition's argument that it would have been obvious to optimize the ionic strength of an rAAV composition "because ionic strength was a known condition that likely affects vector aggregation." Petition, 36 (citing Ex. 1007, 175; Amiji, ¶¶ 175-177). Wright (Ex. 1007), in fact, indicates

that the POSA would have been uncertain of what parameter(s) were relevant to rAAV aggregation. Wright states that the "mechanism of vector aggregation is ***not well understood***, and purification conditions that ***may affect aggregation include*** buffer ionic strength and pH, shear and vector concentration". Ex. 1007, 175. On a molecular level, Wright states that the "nature of the interparticle interactions that result in aggregation has ***not been well characterized***." *Id.*, 176.

81. The Petition never explains how Wright's statement that factors causing vector aggregation were "not well understood"—followed by a non-exclusive list of conditions that *may* impact aggregation, including "buffer ionic strength," would have led the POSA to increase ionic strength by increasing NaCl concentration. Petition, 35-36. Moreover, the POSA would have understood that adjustments to *buffer* ionic strength would not have been made by adding salts such as NaCl or $MgCl_2$, which are not buffers. *See* Section VI.B, above.

82. Buffers are added to modify and maintain a target pH of a composition. At physiological pH values, near neutral pH, "buffer action [is] attributed to systems of (1) weak acids and their conjugate bases, (2) weak bases and their conjugate acids and (3) certain acid-base pairs which can function in the manner either of System 1 or 2." Ex. 1020, 227. The chloride ion (Cl^-) in salts such as NaCl and $MgCl_2$ would be considered a conjugate base to hydrochloric acid (HCl), a strong acid, which would not have been considered a buffer in a near-

neutral pH solution. *Id.* (explaining "hydrochloric acid buffers" may be used at lower pH values, for example, "the pH range 1.2 to 2.2").

83. The POSA would have understood Wright's "buffer ionic strength" to refer to the intrinsic ionic strength of buffer systems that would have been used in the relevant pH range rAAV compositions. *See* Section VI.B, above. For buffer ionic strength selection, Carpenter explains that "50mM sodium phosphate containing 0.1M NaCl, [is] a buffer that has a relatively high ionic strength" where phosphate provides the buffering effect. Ex. 1018, 32. A "buffer of moderate ionic strength" is "10mM Tris, pH 8.5, 100 mM NaCl, l mM EDTA" (*id.*, 33), where Tris and EDTA are the compounds that provide the buffering effect at pH 8.5. Both the moderate and high ionic strength buffer solutions have the same NaCl concentrations (0.1 M = 100 mM), so the difference in their ionic strengths is from the buffering species not NaCl, because the NaCl contributes the same level of ionic strength (μ = 100 mM) to both systems.

84. Moreover, Wright is silent on adjusting ionic strength by modifying salt concentration. Wright does not provide any indication that high NaCl concentration was important to Wright's formulation. For example, an adenovirus formulation disclosed in Wright contains only 25 mM NaCl (Ex. 1007, 176), which is ten-fold below the 250 mM NaCl concentration the Petition alleges was obvious. Wright provides no indication that NaCl concentration or ionic strength should be

increased to prevent aggregation. Thus, it is my opinion that Wright, like Evans, Huang, and Mingozzi, fails to provide any indication that ionic strength is a results-effective variable for rAAV aggregation.

85. Wright further states that in addition to rAAV, "aggregation is a significant and not fully resolved issue for adenovirus vectors." Ex. 1007, 176. Thus, if solving the issue of viral particle aggregation was just "a matter of routine optimization" (Petition, 36), the problem of viral aggregation would not have persisted for years and produced multiple peer-reviewed publications, including Huang, Croyle, and Wright.

2. The Petition Does Not Consider Numerous Teachings in Evans that Would Have Led the POSA to Select Lower Salt Concentrations than 250 mM NaCl

86. As I explained above in Section VIII introducing my opinion for Ground 1, Evans does not address viral particle aggregation, and instead relates to instability due to radical oxidation mechanisms. The Petition also omits that Evans conducted stability testing only on formulations having viral particle concentrations ranging from 10^7 vp/mL to 10^{11} vp/ml, which is several orders of magnitude below the 10^{13} vp/ml upper limit of Evans' concentration range. Evans, 24:21-22 and 25:14-15 (Example 2), 25:31-32 and 26:3-4 (Example 3), 29:6 (Example 7), 30:14 (Example 9). As I explained above in Section VIII.A, this would have even been further below the claimed rAAV vector concentration range

exceeding 10^{13} vg/ml. Given the relationship between high concentrations of rAAV vectors and aggregation, the POSA would not have looked towards Evans as providing any guidance on this issue.

87. The Petition's argument that the POSA would have been motivated to "select the high end of the concentration ranges for NaCl and $MgCl_2$ in Evans' claim 5 composition" (Petition, 36) is inconsistent with the Evans' examples and stability testing. Evans would have led the POSA to much lower NaCl concentrations than 250 mM. In fact, Evans does not contemplate concentrations as high as 200 mM NaCl.

88. For example, Evans' formulations A105 and A104 differ only by their NaCl concentrations (150 mM NaCl in A104, 75 mM NaCl in A105) and the presence of 5% sucrose in A105 versus no sucrose in A104, as shown in the Table below. According to Evans, sucrose is a cryoprotectant (Evans, 11:10-11), so formulation A104 does not contain a cryoprotectant, whereas A105 does. Evans, 24:24-27.

Example	**Description**
A104	5mM Tris, 150 mM NaC1, 1 mM $MgCl_2$, 0.005% PS-80, pH 8.0
A105	5 mM Tris, 75 mM NaC1, 5% sucrose (w/v), 1 mM $MgCl_2$, 0.005% PS-80, pH 8.0

Evans, 22:1-2.

89. Evans shows, however, that these differences in formulation excipients had a dramatic effect on composition stability (Evans, FIG. 2) and infectivity (Evans, FIG. 3) after freeze-thaw cycles. Evans, 24:18-25:24 (Example 2). Figure 2 shows that the formulation with the higher NaCl concentration but no cryoprotectant, A104, lost a significant amount of material after a single freeze-thaw cycle, whereas A105, with a lower NaCl concentration but a cryoprotectant, lost much less, according to Evans' "Adenovirus Infectivity Assay." Evans, 21:21-23 ("The QPA assay is a procedure for the rapid quantitation of adenovirus infectivity based on the use of Q-PCR technology to quantitate accumulated adenoviral genomes 24 hours after infection of cells."). The results for A104 and A105 are highlighted in the annotated Figure 2 from Evans, below.

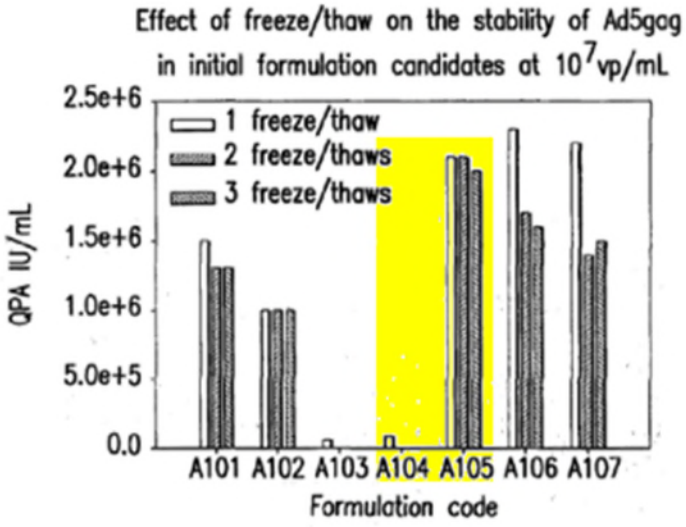


FIG.2

90. Figure 3 also demonstrates that A105 also maintained much higher infectivity than A104 after one freeze/thaw cycle according to Evans' "TCID50

Adenovirus Infectivity Assay." Evans, 21:15-20 ("The $TCID_{50}$ assay is a method for titrating the infectivity of adenovirus."). Thus, Evans' Example 2 indicates that a ***lower NaCl concentration***, but included cryoprotectant was better for ensuring viral particle stability.

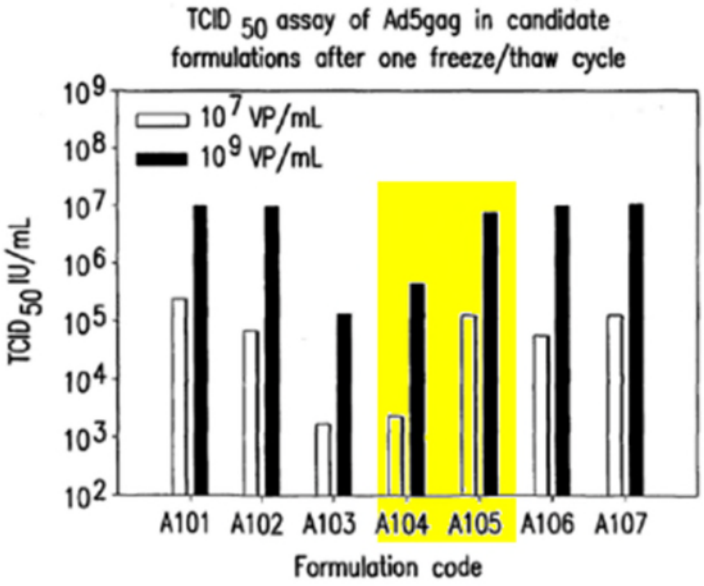


FIG.3

91. In the short-term stability study of Example 3, Evans compares the stability of Ad5gag in A102, A105, A106, and A107, at 10^7 and 10^9 vp/mL. *Id.*, 25:27-26:17. The table below shows that A102 has the highest NaCl concentration (150 mM) of the tested formulations, whereas A105 has a lower NaCl concentration (75 mM) and A106 and A107 do not contain NaCl.

Example	Description
A102	6mM phosphate, 150 mM NaC1, 10% glycerol (v/v), pH 7.2

A105	5 mM Tris, 75 mM NaC1, 5% sucrose (w/v), 1 mM $MgCl_2$, 0.005% PS-80, pH 8.0
A106	5 mM Tris, 14% sucrose (w/v), 1 mM $MgCl_2$, 0.005% PS-80, pH 8.0
A107	5 mM Tris, 8% sorbitol (w/v), 1 mM $MgCl_2$, 0.005% PS-80, pH 8.0

92. All four formulations contain a cryoprotectant, A102 (glycerol), A105 and A106 (sucrose), and A107 (sorbitol). Figure 6 of Evans, below, shows that of these formulations, A102, with the ***highest NaCl concentration*** of 150 mM, ***lost significantly more infectivity*** in 72 hours according to the Evans' QPA Adenovirus Infectivity Assay than A105, A106, and A107, as shown below with A102's results highlighted. *Id.* 26:3-17.

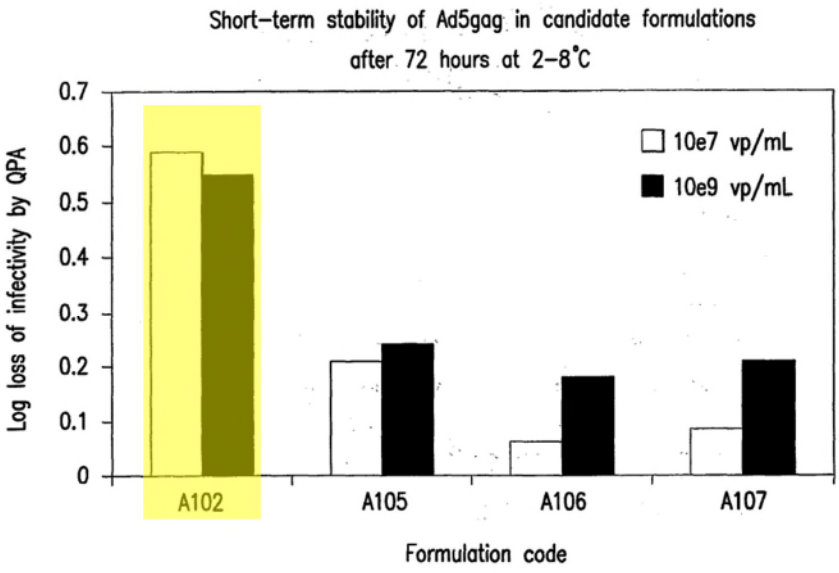


FIG.6

93. Example 10, which measured accelerated and real-time stability, confirmed that formulations with higher NaCl concentrations, A102-A104 (each

containing 150 mM NaCl), with A102 containing glycerol as cryoprotectant, and A103 and A104 not containing a cryoprotectant, were less stable than A105, which contained a lower concentration of NaCl (75 mM NaCl), but 5% sucrose (w/v) as a cryoprotectant. *Id.*, 30:17-25. The long-term stability study of Example 13 tested formulations A105, A113, A114, and A116-A121 (*id.*, 33:5-34:6), but none of these formulations contained more than 75 mM NaCl (*id.*, 22:2-25).

94. Evans' testing results also contradict the Petition's assertion that the POSA would have been motivated to select the "high end" $MgCl_2$ concentration of 5 mM to increase ionic strength. In studies that isolated the effect of $MgCl_2$ concentration on formulation stability by only varying $MgCl_2$ concentration, Evans' Example 5 shows that vector stability was maximized at 2 mM $MgCl_2$, whereas increasing the quantity of $MgCl_2$ to 5 mM led to poorer stability after two months storage than 1 or 2 mM $MgCl_2$. *Id.*, 28:1-11 (Example 5); Fig. 16.

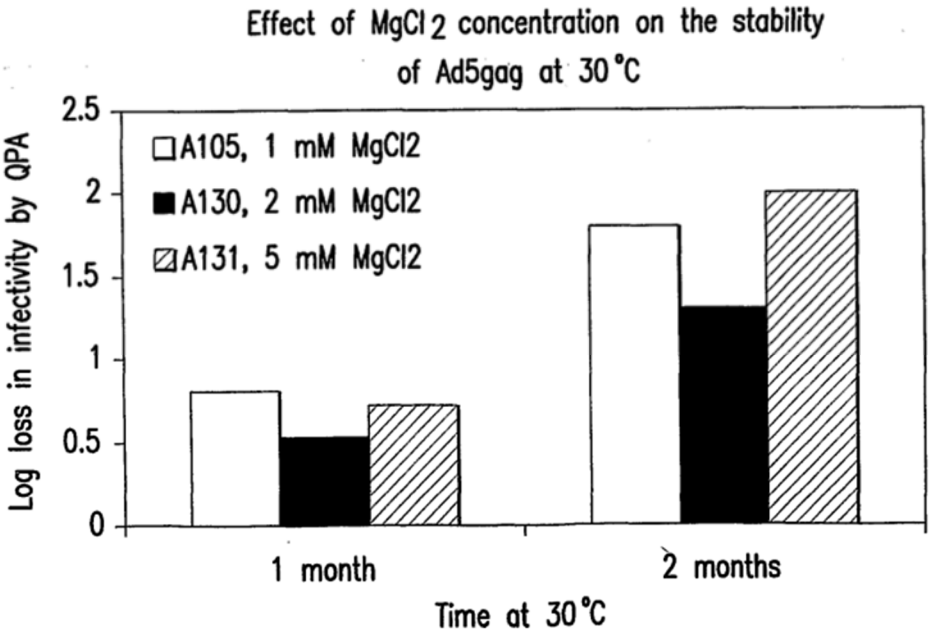


FIG.16

95. Evans' stability studies described above indicate the importance of including a cryoprotectant as part of an adenovirus composition. Evans, 9:10-12 (A "component which contributes to virus stabilization over large temperature ranges and for prolonged storage periods is a cryoprotectant, especially at concentrations amenable to human administration."). The POSA would have understood that both NaCl and the cryoprotectant contribute to a composition's osmolarity. Evans teaches that the concentrations of salt and cryoprotectant in a composition should ensure that osmolarity is "within an appropriate range." Evans, 10:25-27. ("[I]t will be apparent that the amount of a cryoprotectant, such as sucrose or sorbitol, will depend upon the amount of salt in the formulation in order for the total osmolarity of the solution to remain within an appropriate

range."). Thus, Evans teaches that to accommodate a desired cryoprotectant to maximize stability, while ensuring osmolarity remains within an appropriate range for parenteral administration, salt concentration within compositions should be decreased.

96. Thus, it is my opinion that the POSA considering the entirety of Evans's teachings would have been motivated to select salt concentrations far below Novartis's proposed 250 mM NaCl and 5 mM $MgCl_2$ concentrations, which does not support the Petition's motivation argument.

3. The Petition Does Not Consider that the POSA Would Have Avoided of Developing an rAAV Vector Composition that Deviates Significantly from Being Isotonic

97. As explained above in Section VI.A, the POSA would have avoided a high salt concentration that deviates significantly from isotonicity in view of the risks posed by tissue damage and pain associated with, for example, hypertonic compositions. But the salt concentration proposed in the Petition of 250 mM NaCl and 5 mM $MgCl_2$ deviates significantly from isotonicity. Petition, 35-37.

98. "For an isotonic product" the POSA would have understood that "often it is ***advantageous to keep the concentration of salt as low as possible***." Ex. 1018, 187. The Petition, however, ignores this motivation and goes the opposite way to argue for developing a high-concentration rAAV composition having Evans' maximum disclosed salt concentrations. Petition, 35-36. This even

contradicts Dr. Amiji's declaration, which emphasizes the importance of making an isotonic composition for injection to avoid tissue damage and pain. Amiji, ¶¶ 65, 131.

99. Dr. Amiji's declaration emphasizes that "[i]t was well known ***as of June 2004*** that it is preferrable to ***maintain the osmolarity*** of a pharmaceutical composition to be ***as close to isotonic as possible, especially for parenteral administration***, to reduce injection pain." Amiji, ¶ 65 (citing Ex.1008, 410-411; Ex.1021, 1525 ("Solutions to be administered subcutaneously ***require strict attention to tonicity*** adjustment otherwise irritation of the plentiful supply of nerve endings in this anatomical area would give rise to pronounced pain.")).

100. But in the section relating to the ionic strength claim limitation of claim 1 of the '542 patent (Amiji, ¶¶ 174-177), Dr. Amiji's declaration does not explain why the POSA would have deviated so significantly from an isotonic rAAV composition. This is vexing, because Dr. Amiji explains that "Evans taught that its virus formulations" should also be "useful for parenteral, and especially intramuscular, injection." Amiji, ¶ 65 (quoting Evans, 10:18-21). Indeed, it was established that the POSA would have sought to make intramuscular injections that did not deviate significantly from isotonic.

101. As explained above in Section VI.A, NaCl has two ions (i.e., 2 osmol), one cation (Na^+) and one anion (Cl^-), so a 250 mM NaCl solution would have an osmolarity of 500 mOsm:

250 mmol x 2 = 500 mOsm

$MgCl_2$ has three ions, a di-cation (Mg^{2+}) and two anions ($2Cl^-$), so a 5 mM $MgCl_2$ solution would have an osmolarity of 15 mOsm:

5 mmol x 3 = 15 mOsm

102. The Petition's proposed composition containing 250 mM NaCl and 5 mM $MgCl_2$ would have an osmolarity attributed to just these salts (*i.e.*, not considering the osmolarity contributions of other components of the composition, such as a buffer system) of 515 mOsm (500 mOsm attributed to NaCl and 15 mOsm attributed to $MgCl_2$). A 515 mOsm solution has much higher osmolarity than 300 mOsm serum, and so the POSA would have considered such a solution as hypertonic. Ex. 1020, 615. Once a composition is made hypertonic, there is no way to adjust its tonicity downward, in contrast to a hypotonic composition where more salt can be added to increase osmolarity. Ex. 1008, 411 ("Unfortunately, there is no formulation solution for a product that is hypertonic.").

103. There is no explanation in the Petition or Dr. Amiji's declaration for why the POSA would have violated the principle of "maintain[ing] the osmolarity of a pharmaceutical composition to be as close to isotonic as possible, especially

for parenteral administration." Amiji, ¶ 65. The hypertonic composition the Petition relies on Dr. Amij's declaration to propose would potentially cause "tissue damage and injection pain" (Amiji, ¶ 131 (quoting Ex. 1008, 401-402)), which is of particular concern for intramuscular injections, such as those in Evans. Evans, 10:18-21.

4. The Petition Does Not Consider that the Prior Art as a Whole Does Not Support Increasing Salt Concentration to Prevent Significant rAAV Aggregation

104. The Petition states that "the '542 patent admits that AAV2 vectors require elevated concentrations of salt to prevent aggregation" and that it was "known that high salt concentrations increase AAV2 vector solubility." Petition, 36 (citing Ex. 1001, 1:54-55; *see also* 4:67-5:2). But the Petition takes the '542 patent's statement out of context. In its next sentence, the '542 patent states—consistent with the discussions on isotonicity above in Sections VI.A and VIII.B.3—"[h]owever, optimal formulations for pre-clinical and clinical studies ***should be close to isotonic*** (280-400 mOsm), especially for *in vivo* administration of vector to sites where dilution of hypertonic solutions may be slow." Ex. 1001, 5:4-7. The Petition's proposed 515 mOsm composition based on Evans is significantly more hypertonic than the highest 400 mOsm value stated in the patent.

105. Regarding high salt concentrations and rAAV compositions, the '542 patent cites to the purification methods of Xie et al. (Ex. 2006). Ex. 1001, 1:52-55 ("Xie and coworkers similarly reported that at concentrations exceeding 0.1 mg/mL, AAV2 vectors require elevated concentrations of salt to prevent aggregation"). The AAV-2 concentration of 0.1 mg/ml reported in Xie corresponds to approximately 1.6 x 10^{13} particles/ml. rAAV genome particles have an average molecular weight of 3.746 x 10^3 kDa, and 0.1mg/ml is 6.022 x 10^{16} kDa/ml. Dividing 6.022 x 10^{16} kDa/ml by 3.746 x 10^3 kDa/particle results in a concentration of about 1.602 x 10^{13} particles/ml. The '542 patent also notes that 0.06 mg/ml of viral vector particles corresponds to approximately 10^{13} particles/ml. Ex. 1001, 1:58-60.

106. Xie stated that "[h]igh concentrations of AAV-2 in the ~3.3 M CsCl from ultracentrifugal purification remained mostly in solution at 4ºC, although there was some precipitation and adhesion to glass- and plastic-ware with time…." Ex. 2006, 22-23. The POSA would have understood that a 3.3M solution of CsCl would have an osmolarity of ~6,600 mOsm, which is so astronomically high that it would not have been considered relevant to human parenteral use. This level of salt concentration was applied for the purpose of viral particle purification, to separate components from a mixture and they would never be contemplated in the context of human parenteral administration. Moreover, even at this astronomically

high salt concentration, Xie still observed "some precipitation and adhesion to glass- and plastic-ware with time," suggesting that simply elevating salt concentration is not enough to prevent aggregation. *Id.*

107. Xie then stated that "[b]uffer exchange into even 0.25 M NaCl," which is 250 mM NaCl, the same concentration that the Petition states the POSA would have chosen in Evans (Petition, 35), "resulted in ***significant loss***." *Id.*, 23. Thus, Xie teaches that a salt concentration of 250 mM NaCl, the NaCl concentration that Novartis advocates for, was inadequate to prevent significant rAAV aggregation in Xie's solution.

108. The POSA would not have been motivated to simply increase the amount of salt in an rAAV composition. Based on Xie's teaching, which neither the Petition nor Dr. Amiji's declaration discusses despite its citation in the '542 patent, the POSA would have understood that only impracticably high salt concentrations reduced rAAV aggregation. Xie further teaches rAAV aggregation still occurred unabated at lower, but still high, NaCl concentrations. Thus, it is my opinion that the POSA would not have been motivated to increase the ionic strength of high-concentration rAAV compositions by simply adding more salt.

C. The Petition Does Not Establish a Reasonable Expectation of Success in Developing Concentrated rAAV Compositions Without Significant Aggregation

1. The Petition Does Not Consider the Unpredictability Caused by Minor Changes in Formulation Conditions

109. The Petition relies on what it alleges are similarities between viral vector and protein formulations to make its obviousness arguments. Petition, 8, 11, 28-29. The POSA would not have considered success in formulating one class of viral particle, such an adenovirus, with a different class of viral particle, such as an rAAV. One of the references that Dr. Amiji's declaration cites, Carpenter (Ex. 1018) goes as far to say that "[i]t can be assumed that ***most proteins will not exhibit sufficient stability in aqueous solution to allow a liquid formulation to be developed***." Ex. 1018, 188.

110. The Petition's reasonable expectation of success argument fails because the POSA would not have assumed that a method of stabilization for adenoviral particles would apply to rAAV particles, regardless of whether they are both types of viral vectors. The Petition stated that "distinctions between adenovirus and AAV lack meaningful differences with respect to 'proper conditions to prevent aggregation'" and "a POSA … would have reasonably expected Evans' compositions to provide similar, if not better stability for storing AAV particles." Petition, 29. The POSA understood, however, that "[e]very

protein and product has unique characteristics, some of which may cause difficulty in designing stable formulations." Ex. 1018, 134.

111. The POSA would not have considered Evans' stability studies to support a reasonable expectation of success. Petition, 38 (citing Amiji, ¶¶183-187). Because it was already known that high-concentration rAAV compositions resulted in significant aggregation, Evans' stability studies cited by Petitioners on compositions containing adenovirus particles present at lower viral genome concentrations than 10^{13} vg/ml would not have supported the POSA's reasonable expectation of success. Additionally, Evans' most stable compositions, which contained only 75 mM NaCl, had lower ionic strengths than 200 mM. Section VIII.B.2, above. Evans did not even mention the issue of aggregation.

112. Also as explained above in Section VIII.B.2, Evans further demonstrated that even for a single viral vector, changes in excipients can produce dramatic changes in stability. *See* Evans 24:18-25:24 (Example 2), FIGS. 2, 3 (comparing stabilities of formulations A104 and A105). Evans shows that the stability of viral vector compositions is sufficiently unpredictable such that the formulation conditions that might work for one type of viral vector (e.g., an adenovirus), does not predict what will work for another (e.g., rAAV).

2. The Petition Does Not Consider that the Prior Art Generally Taught that rAAV Aggregation Was Not Well Understood by POSAs

113. Evans does not contain any disclosure relating to particle aggregation. The POSA would not have considered the fact that "Evans teaches that its compositions 'show enhanced stability for longer periods of time at temperatures in the range of 2-8°C,' 'allowing for storage and eventual host administration of these liquid formulations over about a 1-2 year period'" as relevant to aggregation of high-concentration rAAV compositions. Petition, 39-40 (quoting Evans, 2:29-32, 4:21-25). Evans relates to lower concentrations of viral particles than claimed, with compositions having lower ionic strength. Additionally, as explained above in Section VIII.B.2, increasing the NaCl concentrations in Evans' compositions led to *less* stable compositions.

114. Evans conducted stability testing only on formulations having viral particle concentrations ranging from 10^7 vp/mL to 10^{11} vp/ml, which is several orders of magnitude below the 10^{13} vp/ml upper limit of Evans' concentration range. Evans, 24:21-22 and 25:14-15 (Example 2), 25:31-32 and 26:3-4 (Example 3), 29:6 (Example 7)). Accounting for the likelihood that a significant percentage of the viral particles are empty capsids (*see* Section VIII.A, above) enlarges the gap between the viral particle concentrations of Evans' compositions and the claimed rAAV vector genome concentration exceeding 10^{13} vg/ml.

115. Regarding ionic strength and salt concentration, as explained above in Section VIII.B.2, none of the exemplary formulations in Evans had an ionic strength attributable to NaCl of more than 150 mM, and the most stable formulations contained 75 mM or less NaCl, which would have provided ionic strength values well below the 200 mM threshold of the claims. As explained above in Section VIII.B.2, Evans further demonstrated how small changes to excipients can dramatically change the stability of a viral vector composition. Evans 24:18-25:24 (Example 2), FIGS. 2, 3. Moreover, as explained below in Sections VIII.D-VIII.E, claims 5 and 6 require a measurable lack of significant aggregation for which Evans provides no information.

116. The other references that the Petition cites, including Huang and Mingozzi, also do not support a reasonable expectation of success. The Petition states that "based on Huang, the POSA would have reasonably expected that high-concentration AAV compositions (e.g., 5-10x10^{13} vg/ml) could be achieved and utilized for successful gene transfer" (Petition, 40) is contradicted by Huang itself. Huang, S286. The POSA would not have read Huang to support a reasonable expectation of success in preventing significant aggregation of rAAV particles. Huang, in fact, goes against a reasonable expectation of success.

117. For example, Huang explains that so much viral vector is lost due to aggregation that at a concentration of "5-10 x 10^{13} GCs/ml, ***gene transfer***

efficiency was 10-100 fold lower at the same dose as compared to the vector whose titer was 1-5 x 10^{12} GCs/ml." *Id.* Even though Huang alludes to a decrease in aggregate size under certain formulation conditions (*id.*), as explained above in Section VIII, the POSA would have understood that significant aggregation still occurred.

118. The POSA also would not have considered Mingozzi to support a reasonable expectation of success. Petition, 32-33 (citing Mingozzi, 10497-98). Neither the Petition nor Dr. Amiji's declaration provide any evidence that Mingozzi's formulations were stored. Mingozzi, in fact, does not state anything about the formulation of those tested compositions or whether aggregates formed.

119. The Petition states that "compositions capable of storing purified AAV vector particles at the claimed concentrations 'without significant aggregation' were described in Wright [Ex. 1007] and, therefore, cannot form the basis for patentability." Petition, 39-40. I disagree. Wright does not provide any information about the composition, including its pH, ionic strength, and whether there were multivalent ions present. Ex. 1007, 175 (stating that the formulations are based on "unpublished data"). There is nothing tying Wright's composition to the claimed composition.

120. Moreover, Wright devotes a whole section to viral vector aggregates, which starts out "[a]ggregation of AAV particles … constitutes a ***significant issue***

for vector formulation and stability" and that the "mechanism of vector ***aggregation is not well understood***." Ex. 1007, 175. Wright explains that "nature of the interparticle interactions that result in ***aggregation has not been well characterized***." *Id.*, 176. For adenovirus, such as the viral vector particles in Evans, the authors conclude in the final paragraph of the section, [a]ggregation is *a* ***significant and not fully resolved issue***." *Id.* Thus, if the problem of viral vector aggregation had been solved, Wright would not have included this detailed exposition of the many unknowns associated with viral vector aggregation.

121. The other references that the Petition cites, Liu (Ex. 1009) and Potter (Ex. 1011) do not support a reasonable expectation of success, because they are not relevant to the high-concentration rAAV compositions that are claimed. Petition, 37 (citing Ex. 1009 [00366], [00369], Table 15; Ex.1011, 417-419, 429). Both Liu and Potter, like Evans' examples, describe compositions containing viral particle concentrations several orders of magnitude below the claimed concentration exceeding 10^{13} vg/ml.

122. Liu's Table 15, cited by the Petition and Dr. Amiji's declaration, lists the "number of infectious particles per mL (FFU/mL)" over seven days of storage having a maximum value at Day 0 of 1.62×10^{10} FFU/ml. Ex. 1009, [00370] (Example 17, Table 15). Dr. Amiji's declaration calculates Liu's viral particle concentration as "3.24×10^{8} (viral particles) vp/ml" or several orders of magnitude

below the claimed concentration before even accounting for presence of empty capsids. Amiji, ¶ 285.

123. Dr. Amiji's declaration states that "Potter reported yields of 1.2-4.2 x 10^{12} total infectious particles, and 'characterize[s] the purified rAAV in terms of purity, infectivity, and packaged particle composition.'" Amiji, ¶ 139 (Ex. 1011, 413-14, 419). Neither the Petition nor Dr. Amiji's declaration translates Potter's total infectious particles into a viral genome concentration, because no volume is identified. The experiments that Dr. Amiji cites relate to pooled fractions from column chromatography experiments (Ex. 1011, 419), so the 10^{12} total infectious particles are likely in several milliliters of solution. Thus, the viral genome concentrations in Potter's cited experiments are likely significantly below 10^{13} vg/ml.

124. The POSA would not have considered Liu or Potter relevant to a reasonable expectation of success for the references in Ground 1. The POSA would have understood that "rAAV undergoes concentration-dependent aggregation" (Ex. 1007, 17) such that aggregation increases particularly at concentrations above 10^{13} vg/ml. Petition, 19, 32 (citing Ex. 1005, S286). Neither the Petition, nor Dr. Amiji's declaration explain how Liu's and Potter's compositions would have supported the POSA's reasonable expectation of success

for the claimed high-concentration rAAV composition, given the correlation between increasing concentration and worsening aggregation.

D. The Petition Does Not Provide Any Evidence that the Alleged Obvious Composition of Ground 1 Would Meet Claim 5's Average Particle Radius Requirement

125. For claim 5, the '542 patent explains that "[a]ggregation is assessed by DLS using undiluted samples, and Rh values >20 nm are deemed to indicate the occurrence of some level of aggregation." Ex. 1001, 9:25-27. The inventors analyzed the average radius of particles to determine whether significant aggregation was present in the composition. Ex. 1001, 4:61-5:25; FIGS. 1A, 1B, 2; 12:33-67.

126. As discussed above in Section VI.C, average particle radius is an effective way to determine aggregation. The presence of aggregates tends to skew the average particle radius because aggregates tend to be made of several particles. Ex. 2009, 470. Because the 20 nm threshold for average particle radius is close to the radius of monomeric rAAV, and because a small number of aggregates can substantially increase average particle radius, the "average particle radius (Rh) of less than about 20 nm" requirement is a meaningful indicator of a lack of significant aggregation. Indeed, average particle radius analysis allowed for the '542 patent's determination of the presence (or absence) of significant aggregation for formulations CF (ionic strength 160 mM), TF1 (ionic strength 310 mM), and

TF2 (ionic strength 510 mM), including after storage and freeze-thaw (F/T) cycles. Ex. 1001, 9:5-55 (Table 3).

127. The POSA would not have considered Liu's statement that adenovirus compositions showing "no signs of settling or precipitation" as relevant for claim 5's radius requirement. Petition, 43. As explained above in Section VI.C, visual detection methods can, at best, only detect particles that are orders of magnitude larger than the ~26 nm diameter rAAV particles and aggregates of viral particles. Ex. 1021, 1547 (explaining that "approximately 50 μm [50,000 nm] is the lower limit" for the naked eye "unless the Tyndall effect is used whereby particles as small as 10 μm [10,000 nm] can be seen by the light scattered from them."). Indeed, the largest average particle radius detected in the aggregation studies of the '542 patent was less than 140 nm (diameter = 280 nm) (Ex. 1001, Figs. 1A, 1B), is still magnitudes smaller than 10,000 nm the threshold for detection by the naked eye. Thus, Liu's visual methods used to assess "settling or precipitation" would have been understood to have been too insensitive to detect the presence of aggregates, and certainly much less sensitive than average particle size.

128. The Petition and Dr. Amiji's declaration do not provide any references or testing that would indicate that the claimed composition comprising "purified, recombinant AAV vector particles" would necessarily and inevitably have had "an average particle radius (Rh) of less than about 20 nm."

E. The Petition Does Not Provide Any Evidence that the Alleged Obvious Composition of Ground 1 Would Meet Claim 6's Post-Filtration Product Recovery Requirement

129. Claim 6 recites "recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of said AAV vector particles through a 0.22 μm filter." Ex. 1001, 14:38-41 (claim 6). Both claim 5 and claim 6, therefore, relate to specific quantitative limits on significant aggregate formation.

130. The Petition states that "[t]he '542 patent does not identify anything critical about the recited recovery rate" of claim 6. Petition, 44. That is not correct. Both the '542 patent and the art in the relevant time period of around June 2004 recognized the value of post-filtration recovery in assessing the presence of significant aggregation.

131. For claim 6, the '542 patent explains that the presence of aggregates can be measured through vector recovery following filtration through a 0.22 μM filter. Ex. 1001, 8:19-44. As discussed above in Section VI.C, determining post-filtration yield is a reliable indicator of significant aggregation. Ex. 2009, 470 (Table 3). Konz demonstrates that adenovirus compositions with significant aggregation, such as Lot B, when subjected to 0.22 μm filtration, gave a yield well below 90%. *Id.*

132. Similarly, as explained above in Section VII, the '542 patent's filtration experiments allowed for the comparison of aggregate formation for the formulations CF, TF1, and TF2 having different vector concentrations. As with particle radius determination, filtration recovery analysis allowed for the determination of the presence of significant aggregation for formulations CF (ionic strength 160 mM), TF1 (ionic strength 310 mM), and TF2 (ionic strength 510 mM). Ex. 1001, 8:19-56 (Table 6).

133. When the inventors assessed the effect of ionic strength on aggregation by measuring vector recovery after filtration through a 0.22 μm filter they found compositions having ionic strength greater than 200 mM surprisingly resulted in recoveries ***exceeding 90%***, while compositions having ionic strengths below this threshold exhibited recoveries below 80%. Ex. 1001, 8:1-10, 11:53-12:29 (Example 2). Table 2 summarizes the results alongside the ionic strength of each formulation. Ex. 1001, 8:19-44. The '542 patent explains that "[w]ithin the variability of the assays used, vector was recovered fully at both target concentrations using TF2, ***indicating that aggregation was prevented***." Ex. 1001, 8:44-46.

134. Because it was known that rAAV aggregates in compositions could lead to significant losses of infectivity along with deleterious biodistribution issues and adverse immune responses, the POSA understood it was critical to minimize

aggregation. Ex. 1001, 1:15-39. Thus, the >90% recovery exhibited in TF2 in both experiments 1 (93% recovery) and 2 (96% recovery) reflect a lack of aggregation, which is critical to the safety and efficacy of the claimed rAAV compositions.

IX. PETITION GROUND 3: CLAIMS 5 AND 6 WOULD NOT HAVE BEEN OBVIOUS TO THE POSA IN VIEW OF FREI, HUANG, AND MINGOZZI

A. The Petition Overlooks that the POSA Would Have Understood that Frei's Reported Vector Particle Concentrations Were Well Below Actual Vector Genome Concentrations

135. As discussed above for Ground 1 with respect to Evans, the Petition similarly does not provide any evidence or adequate explanation to support its contention that Frei discloses a composition comprising viral particles at "a concentration exceeding 1 x 10^{13} vg/ml up to 6.4 x 10^{13} vg/ml." Petition, 52. The Petition cites Frei's D-1 composition, which "has a virus concentration of '1.6 x 10^{13}particles/ml'" and contends "[p]rovided that >62.5% of the particles contain vector genomes, Frei's D-1 composition comprises viral vector particles exceeding 1x10^{13}vg/ml." Petition, 52 (citing Ex.1004, 22:31; Ex.1025, ¶¶223-224).

136. Dr. Amiji's declaration, however, indicates that the POSA ***would not have assumed*** that Frei's viral particle compositions were free of empty capsids. Dr. Amiji stated that "Wright [Ex. 1007] teaches that ≥ 10^{14} capsid particles

(cp)/ml corresponds to ≥ 10^{13} vg/ml)," indicating a ***10-fold excess in empty capsids***. Amiji, ¶ 119 (citing Ex. 1007, 176).

137. Lochrie states that "***more than 80% of AAV material*** created during rAAV production ***may be empty capsids***, and current column chromatography purification techniques do not separate packaged capsids from empty capsids." Lochrie, 4:20-23. Frei states its compositions "can be prepared during purification of the virus in a gel filtration chromatography column." Frei, 11:5-9. However, Lochrie states "column chromatography purification techniques d***o not separate packaged capsids from empty capsids***." Lochrie, 4:20-23.

138. Thus, the Petition does not provide evidence that the POSA would have understood "that >62.5% of the particles contain vector genomes" in Frei's Example D-1.

B. The Petition Overlooks that Frei Teaches that Increased Salt Concentrations Pose a Threat to Viral Vector Stability

139. The Petition states that "Frei taught that '[p]referably, the salt is sodium chloride present in the amount of 0.6 to 10.0 mg/ml.'" Petition, 54 (citing Frei, 5:39-6:5). To reach the claimed ionic strength of greater than 200 mM, the Petition states that the POSA "would have been motivated to select the ***high end*** of Frei's concentration ranges, since Wright identified ionic strength as a condition that may affect vector aggregation." Petition, 55 (citing Ex.1007, 175).

140. The Petition further states that "Frei itself teaches that '[i]n addition to stabilizing the composition, sodium chloride may suppress the rate and extent of the appearance of by-products of fermentation, resulting in a more pharmaceutically elegant presentation that may have reduced antigenicity potential due to protein aggregates' and '[t]he addition of sodium chloride does not affect the pH of the formulation.'" Petition, 55 (citing Ex. 1004, 6:7-11). The POSA considering the quoted passage of Frei, would not have understood Frei to support maximizing NaCl concentration to prevent significant aggregation.

141. ***First***, Frei never ties the aggregation of viral particles to salt concentration (much less ionic strength).

142. ***Second***, to the extent that Frei ties the issue of viral vector stability to salt concentration, Frei emphasized that ***high*** salt concentration did not support improved stability, characterizing high salt concentration as detrimental to viral vector stability. Frei states that its vector concentration methods "must take into account the additional ***threat to stability posed by the high salt concentrations*** in the product eluted from the anion exchange column." Frei, 19:9-14; *see also id.*, 21:25-26 (referring to the "vulnerability of the DEAE pool — with its high salt concentration); 21:42-22:6 ("the methods of the present invention allow for greatly enhanced virus stability, despite the mechanical shear forces of concentrating the virus, and ***despite harsh conditions such as high salt levels*** in a DEAE pool.").

143. The Petition also leaves out the full context of Frei's discussion of preferred NaCl concentrations, which would not have led a POSA to the high end of Frei's disclosed NaCl concentration range. The Petition omits from its quotation of Frei (Petition, 54 (citing Frei, 5:39-6:5)), Frei's statement that NaCl concentration is "***more preferably*** in an amount of about 5.8 mg/ml," which is considerably below the upper limit of 10 mg/ml disclosed in Frei. Frei, 6:5-6:6. Indeed, Example D-1 of Frei—the exemplary formulation relied on by Novartis (Petition, 54-55)— contains an NaCl concentration of 100 mM, which is 5.8 mg/ml. Frei, 22:24-3. In fact, none of Frei's exemplary compositions containing particle concentrations exceeding 10^{13} particles/ml contain more than 100 mM NaCl. Frei, 22:15-23:20 (Examples D1-D3, S-1).

144. The Petition states that "Frei also demonstrated stability after short- and long-term storage (1 week to 12 months" for multiple adenoviral compositions, albeit at lower virus concentrations."). Petition, 57 (citing Frei, Tables 1-5). None of the cited examples contain NaCl concentrations near the level proposed in the Petition. Representative Formulation Examples 1-4 do not contain any NaCl, and Example 5 contains 5.8 mg/ml NaCl, all well below the 10 mg/ml NaCl concentration level maximum disclosed by Frei. Frei, 10:5-15:5. Thus, the POSA would have considered the overall teachings of Frei to not support maximizing NaCl concentration to prevent aggregation or improve stability.

145. In fact, none of Frei's studies would have identified the presence of aggregates. As described in Section VI.C above, the A_{320}/A_{260} absorbance was not understood by the POSA at the time to confirm the absence of aggregates in a composition. Thus, because Frei failed to disclose more reliable aggregate detection techniques, including average particle size measurement and product recovery following filtration, the POSA would suspect the formation of aggregates.

146. The Petition's citation to Wright (Ex. 1007) regarding the POSA's motivation to adjust "buffer ionic strength" does not support a POSA's motivation to increase the ionic strength of Frei's compositions. Petition, 55. As explained for Ground 1 in Section VIII.B.1 above, the POSA would not have understood Wright to teach or suggest adjusting ionic strength by adjusting NaCl and/or $MgCl_2$ concentrations as the Petition proposes. In fact, Wright emphasized that aggregation was "a significant issue for vector formulation and stability" that was "not well understood." Ex. 1007, 175.

147. The Petition states that "the '542 patent admits 'AAV2 vectors require elevated concentrations of salt to prevent aggregation.'" Petition, 55 (citing Ex. 1001, 1:54-55, 4:67-5:2). As explained for Ground 1 in Section VIII.B.4 above, however, the Petition does not consider the context of the '542 patent's statement. The reference that the '542 patent cites, Xie (Ex. 2006), stated that "[h]igh concentrations of AAV-2 in the ~3.3 M CsCl from ultracentrifugal purification

remained mostly in solution at 4ºC, although there was some precipitation and adhesion to glass- and plastic-ware with time…." Ex. 2006, 22-23. Thus, even for *molar* salt solutions of CsCl used for purification purposes, solutions that would be unsuitable as a formulation as having an astronomically high salt concentration, some AAV particle precipitation was observed. As discussed for Ground 1 in Section VIII.B.4 above, Xie further stated that a solution of "0.25 M NaCl [250 mM] resulted in ***significant loss***" of AAV. Ex. 2006, 22-23. Thus, Xie teaches that a salt concentration as high as 250 mM NaCl did not prevent rAAV aggregation.

C. The Petition Does Not Establish a Reasonable Expectation of Success in Developing Concentrated rAAV Compositions Without Significant Aggregation

1. The Petition Does Not Consider the Unpredictability Caused by Minor Changes in Formulation Conditions

148. The Petition relies on what it alleges are similarities between viral vector and protein formulations to argue that the adenovirus compositions of Frei would render the claimed rAAV compositions obvious. Petition, 8, 11, 51. Carpenter (Ex. 1018), which Dr. Amiji's declaration cites, states that "[i]t can be assumed that ***most proteins will not exhibit sufficient stability in aqueous solution to allow a liquid formulation to be developed***." Ex. 1018, 188. Thus, as explained for Ground 1 in Section VIII.C.1 above, the art is unpredictable and it is my opinion that the POSA would have been skeptical that a concentrated rAAV

composition would have been feasible particularly in view of the many accounts detailing rAAV aggregation issues.

2. The Petition's Reasonable Expectation of Success Arguments Overlook Frei's Disclosure Regarding the Threat Posed by High Salt Concentration

149. The Petition alleges that "Frei taught that its salt-containing DEAE pool could be stored 'for >10 days at 2-10°C (thus allowing for subsequent steps of virus concentration and/or gel filtration to be performed on separate days with substantial flexibility across a 10 day period.'" Petition, 57 (citing Frei, 22:10-12). Frei, however, taught that its compositions were stable despite "the additional ***threat to stability posed by the high salt concentrations***…." Frei, 19:9-14. As explained above in Section IX.B, Frei's preferred NaCl concentrations for its compositions was significantly lower than the NaCl concentrations the Petition applies in its attempt to meet the 200 mM ionic strength claim requirement.

150. Additionally, contrary to the Petition's assertion, "Frei's light scattering data" would not have been understood by the POSA to "confirm[] its D-1 composition prevented aggregation" in its examples (Petition, 56). As explained above in Section VI.C, light scattering (A_{320}/A_{260}) is a much less sensitive technique that would not be able to detect the presence of significant aggregates to the degree particle radius determination or recovery by filtration through 0.22 μm filter. Ex. 2009, 469-70. Because Frei determines the presence of aggregates by established

techniques, such as average particle radius and product recovery following filtration, the POSA would have been concerned that aggregates were present in Frei's compositions.

151. The Petition states that the "POSA would have reasonably expected success in using the high ends of Frei's concentration ranges based on the teachings of Potter, Liu, Wright, and the admissions in the '542 patent." Petition 56; *see also id.* 57 n.8, 58. Wright does not provide any information about its composition, including its pH, ionic strength, and whether there are multivalent ions present. Section VIII.B.1, above (citing Ex. 1007, 175). Liu and Potter teach compositions containing viral particle concentrations well below the claimed amount exceeding 10^{13} vg/ml and fact that visual methods cannot accurately detect the presence of aggregates to the degree of the methods of claims 5 and 6. Section VI.C, above. The alleged "admissions in the '542 patent" the Petition cites, instead relate to purification compositions containing astronomically high concentrations of CsCl that the POSA would have considered irrelevant to parenteral compositions. Section VIII.B.4, above; Ex. 1001, 1:52-52 (citing Ex. 2006). The publication that the '542 patent cites, Xie (Ex. 2006), teaches that rAAV compositions containing high concentration NaCl resulted in significant aggregation. Section VIII.B.4 (citing Ex. 20026, 23). Moreover, as explained in Section IX.C.3 below, it is my opinion that the Petition does not provide any

evidence that an rAAV composition meeting requirements of claims 5 and 6 would necessarily result from the alleged obvious compositions.

3. Frei's Light Scattering (A_{320}/A_{260}) Data Does Not Indicate a Lack of Significant Aggregation as Claims 5 and 6 Require

152. Ground 3 raises similar arguments relating to the inherency issues as Ground 1's arguments. Petition, 59 (claim 5), 60 (claim 6). Additionally, Ground 3 also raises similar arguments that the POSA would have "reasonably expected success in minimizing particle size in view of Huang and Liu" as Ground 1's arguments. Petition, 60 (claim 5), 60-61 (claim 6). It is my opinion that these reasonable expectation arguments fail for the same reason as Ground 1. Sections VIII.D - VIII.E, above. Neither Liu or Potter relate to compositions containing rAAV concentrations exceeding 10^{13} vg/ml, and the methods of visual assessment would not detect significant aggregation to the degree of average particle size and product recovery following filtration. Section VIII.D, above.

153. The Petition further argues for claim 5 that "Frei's light scattering data shows that its D-1 composition contained monomeric particles." Petition, 59 (citing Ex. 1025, ¶¶ 258-259). Frei's compositions, however, are not predictive of the claimed compositions. Frei's compositions do not contain rAAV particles, the claimed rAAV concentration exceeding 10^{13} vg/ml (Section VIII.A, above), or an ionic strength greater than 200 mM required by claims 5 and 6 and certainly do not show that the elements of claims 5 and 6 necessarily and inevitably result from the

claimed compositions. Thus, it is my opinion that the POSA would not have considered Frei's results to reasonably predict the likely degree of aggregation in the claimed formulation, which have completely different formulation parameters than claimed.

154. Additionally, Frei's light scattering A_{320}/A_{260} assay data was insufficient to prove the absence of significant aggregation as required by claims 5 and 6. The light scattering techniques employed by Frei do not detect aggregates with near the level of accuracy as the average particle radius determination of claim 5 or the product recovery after filtration of claim 6. Section VI.C, above.

155. Konz's studies demonstrated that the A_{320}/A_{260} assay was not accurate for detection of the absence of significant aggregation. Ex. 2009, 469-70. For example, Konz's adenovirus composition Lot B gave A_{320}/A_{260} absorbance ratios of 0.29 and 0.23 (*id.*, 470, Table 3), which is within the monomer range according to Dr. Amiji's declaration. Amiji, ¶¶ 59, 100 249. Before and after filtration, however, Lot B contained only 58% and 77% monomer, respectively, which indicates significant aggregation. *Id.*, 470 (Table 3). Indeed, DLS analysis of particle diameters detected significant aggregation in the post-filtration composition. *Id.*, 470 (Figure 8).

156. The filtration of Lot B, which contained 58% monomer, through a 0.22 μm filter, resulted in a yield of 75%, well below the 90% recovery threshold

of claim 6. *Id.*, 470 (Table 3). The '542 patent's CF composition was similarly recovered in only 77% yield, which was an indicator of significant rAAV aggregation in that composition. Ex. 1001, 8:28-40 (Table 2). Thus, the fact that Frei's Example D-1 had a A_{320}/A_{260} ratio of 0.22 does not mean that there was no aggregation present.

157. Both the average particle radius analysis of claim 5 and the post-filtration product recovery analysis of claim 6 are much more accurate determination of the presence of significant aggregation than Frei's A_{320}/A_{260} absorbance ratio. Thus, it is my opinion that the Petition has not shown that the required parameters of claim 5 and 6 would necessarily have been achieved through the allegedly obvious variants of Frei's composition presented in Ground 3.

X. CONCLUSION

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and that these statements were made with 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.

Date: 15th June 2023

Dr. Martyn C. Davies