

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.
Petitioner

v.

GENZYME CORPORATION,
Patent Owner.

Case: IPR2025-01194
Patent No. 9,051,542

DECLARATION OF DR. MARTYN C. DAVIES

TABLE OF CONTENTS

	Page
I. Introduction	1
II. Qualifications.....	1
III. Materials Considered.....	6
IV. Summary of My Opinions.....	8
V. Legal Standard for Obviousness.....	9
VI. Background Scientific Concepts Relevant to the '542 Patent	12
A. Osmolarity and Tonicity.....	12
B. Ionic strength.....	16
C. Detection of Aggregates.....	18
VII. The '542 Patent.....	26
VIII. Ground 1: Claims 3-6 Would Not Have Been Obvious to the POSA in view of Wu and Konz	32
A. 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.....	32
1. The Petition Fails to Establish that Wu or Konz Would Have Led the POSA to a High Concentration rAAV Composition Having an Ionic Strength > 200 mM and a pH Buffer Having a pH between 7.5 and 8.0.....	35
a. The Petition Fails to Establish that Wu Discloses an Ionic Strength > 200 mM and a pH Buffer Having a pH between 7.5 and 8.0.....	35
i. Dr. Kay's Ionic Strength Analysis Makes Unsupported Assumptions About Residual NaCl in Wu's Composition	37
ii. Dr. Kay Makes Unsupported Assumptions About the pH in Wu's Composition.....	39
b. The POSA Would Not Have Been Motivated to Make an rAAV Composition with an Ionic Strength > 200 mM Based on Konz and Evans	41
i. Konz's Formulation Buffers All Have Ionic Strengths Far Below 200 mM.....	41

ii.	The Petition Omits that Evans Did Not Relate to High Concentration rAAV Compositions	45
iii.	Evans Would Have Led the POSA to Select Lower Salt Concentrations than 250 mM NaCl	45
c.	The POSA Would Not Have Considered Wu’s Electron Microscopy Analysis to Detect rAAV Aggregation	52
d.	Konz Analyzed Aggregation in Compositions Having Much Lower Viral Particle Concentrations than Claimed	54
2.	The Petition Does Not Consider that the POSA Would Have Avoided Developing an rAAV Vector Compositions Having High Salt Concentrations	55
a.	A POSA Would Have Understood that Formulations with High NaCl Concentrations Are Hypertonic and Can Cause Tissue Damage and Pain.....	55
b.	The Petition Fails to Address Prior Art Teaching High Salt Concentrations Promote Precipitation of rAAV Particles.....	58
c.	The Cited Background Art is Not Relevant to rAAV Compositions....	60
B.	The Petition Does Not Establish a Reasonable Expectation of Success in Developing Concentrated rAAV Compositions Without Significant Aggregation	62
1.	The Petition Does Not Consider the Unpredictability Caused by Minor Changes in Formulation Conditions	62
2.	Neither Konz/Evans Nor Wu Establish a Reasonable Expectation of Success	65
IX.	Ground 2: Claim 3 Would Not Have Been Obvious to the POSA In View of Wu, Konz, and Croyle	69
X.	Ground 3: Claims 3-6 Would Not Have Been Obvious to the POSA in view of Potter and Konz.....	69
A.	The Petition Fails to Establish a Motivation to Arrive at an Ionic Strength Greater than 200 mM	70
1.	Konz/Evans Would Not Motivate the POSA to Make an rAAV Composition with an Ionic Strength Greater than 200 mM	71
2.	Potter Would Not Motivate the POSA to Make the Claimed rAAV Compositions with an Ionic Strength Greater than 200 mM	71

a.	Potter’s rAAV Large-Scale Production Compositions	72
b.	Potter Does Not Provide Information the POSA Would Have Required to Determine the Ionic Strength of the National Reference Standard rAAV Compositions	74
3.	The POSA Would Not Understand Potter to Connect Ionic Strength and rAAV Aggregation.....	77
4.	The Petition Does Not Consider that the POSA Would Have Avoided Developing an rAAV Vector Compositions Having the High Salt Concentrations of Potter or Konz/Evans.....	80
B.	Ground 3 Fails to Establish a Reasonable Expectation that a High Ionic Strength rAAV Composition Could be Stored Without Significant Aggregation	83
XI.	Ground 4: Claim 3 Would Not Have Been Obvious to the POSA In View of Potter, Konz, and Croyle	84
XII.	Conclusion	85

I. INTRODUCTION

1. I submit this declaration in connection with Patent Owner's Preliminary Response in the *Inter Partes* Review IPR2025-01194 (the "IPR") of claims 3-6 ("challenged claims") 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, 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.

2. I previously submitted a declaration in connection with Patent Owner's Preliminary Responses in *Inter Partes* Review IPR2023-00608 and IPR2023-00609, which also challenged claims of the '542 patent. I understand that the Patent Trial and Appeal Board ("PTAB") declined to institute trial in both IPR2023-00608 and IPR2023-00609.

3. I am also submitting a declaration in connection with Patent Owner's Preliminary Responses in *Inter Partes* Review IPR2025-01195, which challenges claims of U.S. Patent No. 7,704,721, of which the '542 patent is a continuation.

II. QUALIFICATIONS

4. I have over 37 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 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.

5. 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.

6. 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.

7. From 1985 until I recently retired, I 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.

8. 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.

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

10. 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.

11. 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.

12. 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.

13. 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.

14. 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.

15. 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.

16. 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.

17. 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.

18. 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.

19. 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.

20. Attached as Exhibit 2024 is my curriculum vitae, which summarizes my background, credentials, and includes a list of my publications.

III. MATERIALS CONSIDERED

21. 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 the person having ordinary skill in the art (“POSA”), provided in the Petition and Dr. Kay’s declaration (Ex. 1005). Petition, 20-21; Ex. 1005, ¶ 166. I note that I qualify as a POSA under this definition. In addition to the Petition and its exhibits, including Dr. Kay’s declaration, I reviewed and considered the materials identified in the list below.

Exhibit No.	Description
2013	A.R. Kucernak et al., <i>Avoid Using Phosphate Buffered Saline (PBS) as an Electrolyte for Accurate OER Studies</i> , 9 ACS Energy Lett. 3939–3946 (2024)
2015	A.E. Smith, “Gene Therapy: Where Are We,” 354 The Lancet SI1 (July 1999)
2016	<i>Remington: The Science and Practice of Pharmacy</i> , Nineteenth Ed., Vol. I, Chapters 17, 18, 23, and 36 (1995).
2017	<i>Remington: The Science and Practice of Pharmacy</i> , Nineteenth Ed., Vol. II, Chapter 87(1995).
2018	Carpenter, J. F. & Manning, M.C. (Eds.), <i>Rational Design of Stable Protein Formulations: Theory and Practice</i> , Vol. 13 (2002).
2019	Gatlin, L., Chapter 17, “Formulation and Administration Techniques to Minimize Injection Pain and Tissue Damage Associated with Parenteral Products,” in <i>Injectable Drug Development: Techniques to Reduce Pain and Irritation</i> , (eds. P. Gupta and G. Brazeau), pp. 401-421 (1999).
2020	International Patent Pub. No. WO 99/41416 to Frei, et al.
2021	Croyle, et al., “Development of Formulations That Enhance Physical Stability of Viral Vectors for Gene Therapy,” <i>Gene Ther.</i> , 8:1281-1290 (2001).
2022	Adadevoh, K. et al., “A Short-Term Field Use and Shipping Stability Study of a Wild Type Ad5 Adenoviral Reference Material,” <i>BioProcessing</i> 1(2) 62-69 (2002).
2025	Ayache, J. et al., <i>Sample Preparation Handbook for Transmission Electron Microscopy</i> . (2010).

Exhibit No.	Description
2026	Arista-Romero, M. et al., “Towards a Quantitative Single Particle Characterization by Super Resolution Microscopy: From Virus Structures to Antivirals Design.” (2021).
2027	Chu, J. et al., “A bright cyan-excitable orange fluorescent protein facilitates dual-emission microscopy and enhances bioluminescence imaging in vivo.” <i>Nat Biotechnol.</i> 2016 July ; 34(7): 760–767 (2016).
2028	Aaron, K. et al., “Selection of Viral capsids and promoters affects the efficacy of rescue of Tmprss3-deficient cochlea.” <i>Molecular Therapy: Methods & Clinical Development</i> (2023).
2029	Jia, F. et al., “A nonviral minicircle vector for deriving human iPS cells” <i>Nature Methods</i> (2010).
2031	J.O. Konz et al. “ <i>Development of a Purification Process for Adenovirus: Controlling Virus Aggregation to Improve the Clearance of Host Cell DNA,</i> ” 21 <i>Biotechnol. Prog.</i> 466 (2005)
2032	Booy, F. P., et al. “Electron microscopy of influenza virus: a comparison of negatively stained and ice-embedded particles.” <i>Journal of molecular biology</i> 184.4 (1985): 667-676.

IV. SUMMARY OF MY OPINIONS

22. First, it is my opinion that Grounds 1 and 2, alleging that the challenged claims are obvious over Wu (Ex. 1007) and Konz (Ex. 1008), do not establish the obviousness of claims 3-6 of the '542 patent.

23. Second, it is my opinion that Grounds 3 and 4, alleging that the challenged claims are obvious over Potter (Ex. 1010) and Konz (Ex. 1008), do not establish the obviousness of claims 3-6 of the '542 patent.

V. LEGAL STANDARD FOR OBVIOUSNESS

24. I understand that at pre-institution stage of the proceeding the Petitioner has the burden of proving by a reasonable likelihood that the challenged claims are obvious.

25. 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.”

26. I have been informed that for a claimed invention to be considered obvious over a combination of prior art 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 claimed invention. 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(s) or to combine prior-

art reference teachings to arrive at the claimed invention.

27. 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.

28. I have been informed that for a combination of prior art 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.

29. 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.

30. 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 that 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 RELEVANT TO THE '542 PATENT

A. Osmolarity and Tonicity

31. The osmotic pressure of a solution is determined by the concentration of particles of solute (i.e., the dissolved compound) in that solution. Ex. 2016, 55. The osmotic pressure of an aqueous solution can be increased by adding a salt as a solute to that solution. A salt is made up of positively and negatively charged particles, cations and anions, respectively, bonded by their complimentary charges, i.e., ionic bonds. The most common salt in therapeutic compositions is sodium chloride (NaCl). When added to solution, NaCl dissociates into its cation, Na⁺, and anion, Cl⁻, so for every molecule of NaCl added to the solution there are two particles of solute present in the solution.

32. 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.*

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.*

33. Osmotic pressure can be measured in osmolarity, which is a measure of the number of particles of solute per liter of solution. *Id.*, 56. For an ionic compound, such as a salt containing cationic and anionic particles, osmolarity is determined by multiplying the molarity of the solute in solution by the number of ions into which it dissociates. *Id.*, 57-58.

34. 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. One mole (M) of NaCl becomes two osmoles (Osm) in solution. The osmolarity of salts of monovalent ions such as NaCl will be double the molarity in solution. *Id.*, 58. For example, a 100 mM NaCl solution will have an osmolarity of 200 mOsm as shown below:

$$100 \text{ mmol NaCl} \times 2 = 200 \text{ mOsm}$$

35. As another example, magnesium chloride (MgCl₂) dissociates in water into three particles of solute, one Mg²⁺ and two Cl⁻ ions, so its osmolarity is triple the molarity of MgCl₂. Thus, a 100 mM MgCl₂ solution will have an osmolarity of 300 mOsm as shown below.

$$100 \text{ mmol MgCl}_2 \times 3 = 300 \text{ mOsm}$$

36. 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.*, 55. Isotonicity, in the context of cells, occurs when a cell’s intracellular solute concentration is the same as the solute (e.g., NaCl) concentration of the environment surrounding the cell. A composition that is isosmotic with the tissue and cells it is in contact with is considered isotonic.

37. Serum osmolarity is about 300 mOsm, so a 300 mOsm salt solution is isotonic with serum. *Id.*, 57. “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.*, 55. In molarity, 0.9% NaCl is equal to ~150 mM NaCl. Ex. 1001, 5:15-16.

38. In the timeframe of June 2004, the POSA would have sought to make therapeutic compositions that minimize deviation from isotonicity to avoid causing tissue damage and pain when administered. The POSA would have understood that “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. “For an

isotonic product, often it is advantageous to keep the concentration of salt as low as possible.” Ex. 2018, 197.

39. As of the effective date, the state of the art “emphasize[d] the two formulation parameters, pH and tonicity, that are usually associated with tissue damage and injection pain,” and “provide[d] a parenteral product development outline” that include[d] guidance on ways in which pH and tonicity “may be modified to minimize tissue damage and pain caused by a parenteral product.” Ex. 2019, 3-4; *see also* Ex. 2017, 4 (“Solutions to be administered subcutaneously require strict attention to tonicity adjustment.”)). 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. 2019, 13.

40. 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. 2018, 192. 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

41. 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:]

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

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. 2016, 5.

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

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

Id. Whereas for a 100 mM MgCl_2 solution, the ionic strength would be:

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

Id.

43. Because of the different valencies of Na^+ and Mg^{2+} , identical concentrations of NaCl and MgCl_2 dissolved in solution result in different ratios between osmolarities and ionic strength:

Salt (mM)	Ionic Strength (mM)	Osmolarity (mOsm)	Osmolarity/Ionic Strength (mOsm/mM)
NaCl, 100 mM	100 mM	200 mOsm	2.0 mOsm/mM

MgCl ₂ , 100 mM	300 mM	300 mOsm	1.0 mOsm/mM
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The table above illustrates how a higher ionic strength solution can be made using a multivalent ion-containing salt (e.g., MgCl₂) than a monovalent-ion containing salt (e.g., NaCl) without as significantly increasing osmolarity. The ratio of osmolarity to ionic strength is higher with a monovalent salt versus a multivalent salt. Therefore, higher ionic strength can be accomplished with a multivalent salt with less of an increase in osmolarity as compared to a monovalent salt.

44. 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 (H₃PO₄), which can dissociate into three species having different charges, including dihydrogen phosphate (H₂PO₄⁻), hydrogen phosphate (HPO₄²⁻), and phosphate (PO₄³⁻). *Id.*, 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, H₂PO₄⁻ and HPO₄²⁻ predominate.

45. 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.* 2018, 45. A “buffer of moderate ionic strength” is “10mM Tris, pH 8.5, 100 mM NaCl, 1 mM EDTA” (*id.*, 46), 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 attributable to the ionic particles from the amount of charged buffering species, not NaCl, because the NaCl contributes the same level of ionic strength (μ = 100 mM) to both systems.

C. Detection of Aggregates

46. As of 2004, one of the challenges facing the formulation of therapeutic recombinant adeno-associated viral vector (“rAAV”) compositions at concentrations sufficient to provide therapeutic benefit was their tendency to form aggregates. For example, administering rAAV vectors to patients for delivery to the central nervous system (“CNS”) requires “small volumes of highly concentrated vector.” Ex. 1001, 2:12-13. But rAAV particles tend to aggregate when stored. Aggregate-formation caused loss of rAAV infectivity, biodistribution issues, and adverse immune responses when administered. *Id.*, 2:15-39. Solving this problem was central to making gene therapies clinically effective, because “high doses of viral vector ... are usually required to achieve measurable gene transfer,” Ex. 2015, 4; *see also* Ex. 1047, 1; Ex. 1043, 1.

47. Very significant aggregation can be seen as particles within a solution by the naked eye. The absence of aggregates, however, when viewed by the naked

eye does not mean that there are no aggregates present. Ex. 1001, 9:50-52 (“Visual inspection of these samples reveals slight cloudiness, which is consistent with aggregation.”). 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. 1045, 1. 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. 2017, 26. 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 the 10,000 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.

48. Average particle radius is one measure of whether there is significant aggregation presence in a composition. An average radius of $R_h < 20$ nm indicates that there is effectively no aggregation whereas “ R_h values >20 nm are deemed to indicate the occurrence of some level of aggregation.” *Id.*, 9:26-27. The accurate

determination of the extent of rAAV aggregation in a composition requires analyzing the viral particles in that composition.

49. **DLS analysis.** 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. 1045, 1), 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.

50. 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. 2031, 6. Researchers 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.*, 9-10.

51. 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.*, 10. Even if the majority of viral particles remain monomeric in Lot B, the POSA would have understood that aggregates skewed the average particle diameter upward, because the studied aggregates had over four times the detected diameter as the known diameter of monomer particles. Indeed, “mass-weighted-mean aggregate contain[ed] roughly 15 particles,” showing a relatively small amount of formation can radically increase average particle diameter/radius. *Id.* Thus, the POSA would have understood that a composition with an average particle diameter (or radius) value close to the known monomer particle diameter (or radius) indicates a lack of significant aggregation.

52. **Filtration.** Another method to measure aggregation is to filter the composition through a filter that prevents larger aggregated particles from passing through and then determining the yield of particles in the filtrate versus the starting amount of particles. A higher yield of particles in the filtrate indicates that fewer aggregates formed.

53. Researchers determined the yield after “0.22 μm absolute filtration” (i.e., filtration that excluded particles with radii $>0.22 \mu\text{m}$) 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.*, 9-10. As noted prior to filtration, significant aggregation (~58% monomer) was present in Lot B, and following filtration the composition still contained only 77% monomer. These results confirm that a high percentage viral particle recovery following filtration of the AAV vector particle composition through a 0.22 µm filter is reflective of whether significant aggregation had occurred, as discussed further in the context of the ’542 patent.

54. ***Electron microscopy.*** The Petition and Dr. Kay assume lack of aggregation in prior art based on electron microscopy images. *See, e.g.*, Ex. 1005, ¶¶ 181, 243-251.

55. Electron microscopy is a way to “see” things smaller than what light microscopes can resolve by using a beam of electrons instead of light. Electrons have much shorter wavelengths than visible light, so electron beams can reveal fine details down to nanometers and even atoms. The electron beam is focused by electromagnetic lenses inside a vacuum and scanned or transmitted through the sample to form an image.

56. Wu (Ex. 1007) and Potter (Ex. 1010) used electron microscopy at high magnification to examine the structure of individual rAAV particles. For example, Wu used electron microscopy at sufficiently high magnification to “visualize rAAV-GFP particles” and qualitatively observe the presence of full and

intermediate rAAV particles, as well as empty capsids. Ex. 1007, 3, Fig. 3. In other words, Wu was using high magnification to visualize the nucleic acid, if any, inside each viral particle. *Id.* Likewise, Potter used electron microscopy with a “magnification factor [of] 49,500” to visualize empty, intermediate, and full capsids with respect to payload inside the viral particles. Ex. 1010, 16, Fig. 5.

57. I note that the image in Figure 3 of Wu focuses in on an exceedingly small number of particles (i.e., 100s of particles), which is a result of the required resolution to “see” inside very small virus particles. At this resolution, it is only possible to visualize a small number of particles, representing a tiny fraction of the viral particles in the total prepared composition. The presence or absence of aggregates can only be observed using lower magnification to visualize a much larger sample of viral particles (i.e., millions).

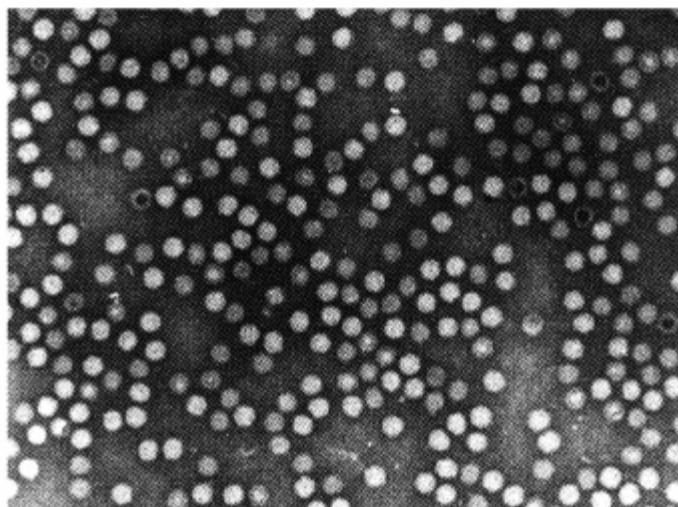


Fig. 3. Purified and concentrated recombinant adeno-associated virus particles visualized by electron microscopy after negative staining with uranyl acetate ($\times 35620$).

Ex. 1007, 3.

58. Detection of viral particle aggregates, however, was not the purpose of the studies in either Wu or Potter. Wu used electron microscopy to demonstrate how well its purification protocol separated full rAAV particles from empty and intermediately filled particles. *Id.* Potter used electron microscopy to “confirm the structure of infectious and noninfectious particles” identified as separate peaks in a HPLC chromatographic analysis. Ex. 1010, 17. Because both Potter and Wu focused their electron micrograph analyses at the particle level to assess their purification protocols, the published images focus on a small sample of particles, and are not probative of aggregation. Such images do not tell the POSA anything about whether there were aggregates in the composition.

59. Additionally, electron microscopy requires subjecting samples to special preparations that alter the chemical and physical environment surrounding viral particles. Wu, for example, explains its rAAV samples were “subjected to an electron microscope after negative staining with 2% uranyl acetate and then loading on 150 mesh nickel grid.” Ex. 1007, 2. Thus, the POSA would have understood that the rAAV particles in Wu were subjected to extreme conditions, namely, negative staining and exposure to heavy metals (i.e., nickel) that differ significantly from the conditions within an rAAV composition.

60. Exposing the viral particles to heavy metals, changes to pH, dehydration, and other preparatory techniques can modify the shape and morphology of the virus particles. *See* Ex. 2032 at 1 (“The validity of [viral structure] models is frequently brought into question as a result of the many possible artefacts arising intrinsically from the conventional techniques of electron microscopy.”); Ex. 2025; *see also* Ex. 2026. The physical artifacts introduced both by the sample preparation and the electron microscopy can be extensive, which can lead to irregular virus shapes and broken structures. These changes can disrupt viral particle interactions, including interactions that cause particles to aggregate. Ex. 2025, 2.

61. For these reasons, assessing viral aggregation using electron microscopy is not reliable in the manner in which Wu and Potter have undertaken

their use of the technique. The POSA interested in assessing aggregation would have used techniques such as DLS and filtration, which do not significantly change the rAAV composition, and can be used to assess the whole of the composition, not just a sample of it.

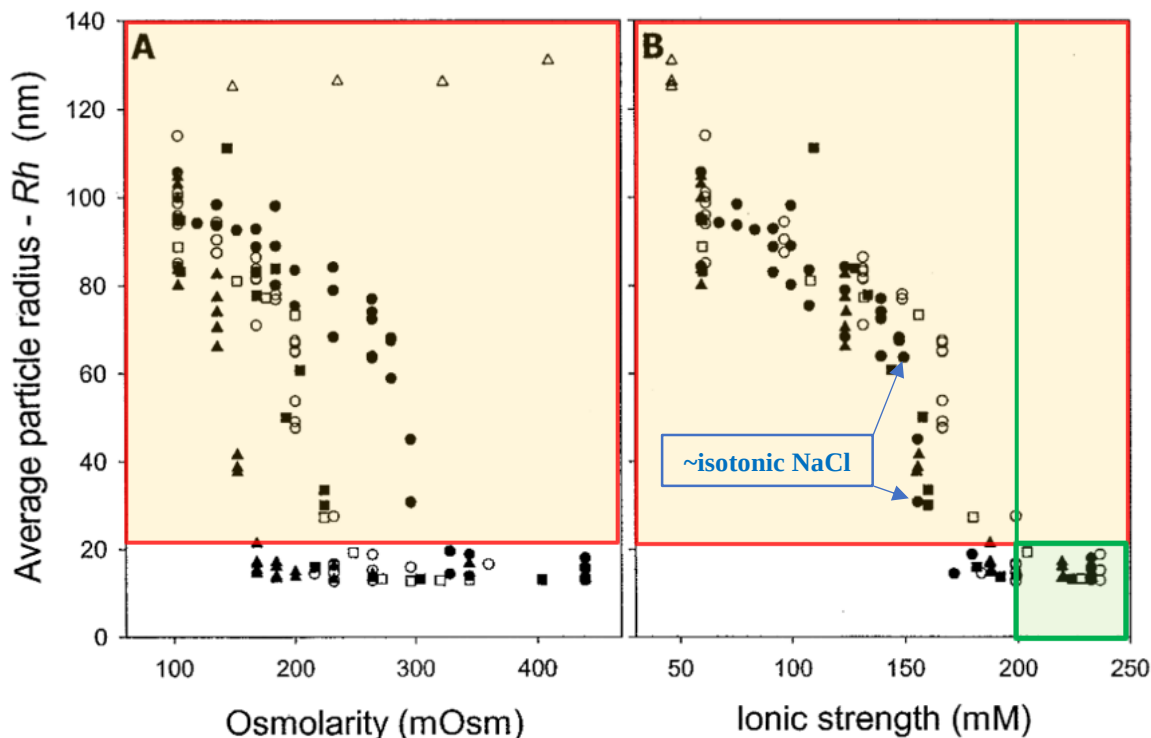
VII. THE '542 PATENT

62. The inventors studied vector aggregation in rAAV compositions comprising 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, 12:32-67 (Example 3), FIGS. 1A, 1B.

63. 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.” *Id.*, 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 $R_h < 20$ nm indicate a lack of significant aggregation. *Id.*, 9:25-27.

64. In annotated Figures 1A and 1B below, the salts and excipients are denoted as follows: sodium chloride (●) (NaCl), sodium citrate (○) ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$),

sodium phosphate ($\blacksquare$) (Na_3PO_4), sodium sulfate ($\square$) (Na_2SO_4), magnesium sulfate ($\blacktriangle$) (MgSO_4), and glycerol (Δ) ($\text{C}_3\text{H}_8\text{O}_3$):



Id., FIGS. 1A, 1B (annotation added). In both Figures, compositions falling within the orange shaded portion of the plots exhibited significant aggregation ($R_h > 20$ nm), whereas compositions in the unshaded or green shaded portions of the plot did not ($R_h < 20$ nm).

65. 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 [$(\blacktriangle)$] prevented aggregation at >200 mOsm, whereas monovalent sodium chloride [$(\bullet)$] 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.

66. 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.*, 7:20-22. 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 (R_h) of less than about 20 nm as measured by dynamic light scattering.” *Id.*, 14:23-24, 14:35-37.

67. 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.

68. 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 strengths.” *Id.*, 5:8-10. 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.

69. 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-AAVC 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, pH 8.0).” *Id.*, 11:67-12:3. 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.

70. In Example 2, titled “Ultrafiltration and Diafiltration to Detect AAV Aggregation,” the exemplary formulations were filtered through a 0.22 µm filter. *Id.*, 8:1-9, 11:52-12:29. Table 2 summarizes the results of Experiments 1 and 2. *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)

71. As Table 2 shows, recoveries from TF1 and TF2, which have ionic strengths above 200 mM, exceeded 90% following filtration. Recovery from CF formulations, with ionic strengths of less than 200 mM, was only 77% and 59% for experiments 1 and 2, respectively.

72. 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								
Formu- lation	Particle radius - Rh (nm)							
	4° C.		-20° C.			-80° C.		
	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.

73. “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.

74. 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. GROUND 1: CLAIMS 3-6 WOULD NOT HAVE BEEN OBVIOUS TO THE POSA IN VIEW OF WU AND KONZ

75. The Petition and Dr. Kay's declaration fail to establish that the POSA would have had a reason to combine the teachings of Wu and Konz to develop the claimed high concentration rAAV compositions having an ionic strength of > 200 mM. The POSA also would not have had a reasonable expectation that the claimed compositions would not result in significant aggregation. For at least the reasons I set forth below, Wu and Konz would not lead the POSA to the claimed composition.

A. 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

76. The Petition and Dr. Kay's declaration do not provide a reason or motivation for why the POSA would develop a composition comprising a rAAV "concentration exceeding 1×10^{13} vg/ml," with an ionic strength "greater than

200mM,” “wherein the purified AAV vector particles are stored in the composition without significant aggregation.”

77. *First*, neither Wu nor Konz direct the POSA to make a composition having an ionic strength > 200 mM. Dr. Kay’s calculations concluding Wu’s preparation has an ionic strength > 200 mM rely on assumptions about Wu’s composition that are not in Wu, and that the POSA would never make. Konz’s formulation buffers contain rAAV concentrations well below 1×10^{13} vg/ml and ionic strengths well below 200 mM. Evans, which Konz incorporates by reference, studies compositions with viral particle concentrations well below 1×10^{13} vg/ml, and ionic strengths well below 200 mM. Moreover, Evans’ examples showed that lower ionic strength compositions were more stable. In totality, this evidence directed the POSA away from an ionic strength of > 200 mM.

78. *Second*, the Petition and Dr. Kay’s declaration fail to establish that the POSA would have recognized Wu and Konz to increase ionic strength for high concentration rAAV formulations so that they can be stored without significant aggregation. Konz’s aggregations analyses with DLS and filtration were conducted on compositions containing rAAV concentrations well below 1×10^{13} vg/ml. Konz’s formulation buffers had ionic strengths well below 200 mM. Evans did not even address aggregation. Wu’s electron microscopy analysis would not have been considered by the POSA to establish a lack of aggregation in Wu’s composition.

Wu used electron microscopy for a different aim and purpose—examining individual purified rAAV particles—and never mentions aggregation.

79. As I explain above (*see* § VI.C), the purpose of electron microscopy in Wu was to qualitatively observe the presence of full and intermediate rAAV particles, as well as empty capsids. Ex. 1007, 3, Fig. 3. At Wu’s high magnification it is only possible to visualize a small number of particles. The viral particles Wu analyzed represent a tiny fraction of the viral particles in the prepared composition. If there were aggregates in the composition they would almost certainly have been missed at this magnification. And while Konz looked at aggregation, it did not use electron microscopy. The two references simply do not go together and there would be no motivation to combine them.

80. *Third*, the Petition and Dr. Kay’s declaration fail to address the other factors that the POSA would have considered important for making a therapeutic rAAV composition. For example, the Petition and Dr. Kay’s declaration allege that the POSA would have formulated a composition with a high concentration of NaCl to achieve a high ionic strength. The Petition and Dr. Kay’s declaration ignore that high NaCl concentration solutions are hypertonic. When injected, hypertonic compositions cause pain and tissue damage. Additionally, the Petition and Dr Kay’s declaration ignore other teachings that viral particle compositions containing high concentrations of NaCl were less stable than lower NaCl concentration

solutions. The POSA's knowledge regarding the state of the art undercuts the motivation arguments in the Petition and Dr. Kay's declaration.

1. The Petition Fails to Establish that Wu or Konz Would Have Led the POSA to a High Concentration rAAV Composition Having an Ionic Strength > 200 mM and a pH Buffer Having a pH between 7.5 and 8.0

81. The Petition and Dr. Kay's declaration fail to establish that Wu even discloses a high rAAV concentration composition with an ionic strength over 200 mM and comprises a pH buffer having a pH between 7.5 and 8.0. The Petition and Dr. Kay's declaration fail to acknowledge that none of the exemplified compositions studied by Konz or Evans have an rAAV concentration close to 1×10^{13} vg/ml or an ionic strength close to 200 mM. Therefore, the Petition fails to establish that the disclosures of Konz and/or Evans provide a motivation to develop a composition having an ionic strength greater than 200 mM.

a. The Petition Fails to Establish that Wu Discloses an Ionic Strength > 200 mM and a pH Buffer Having a pH between 7.5 and 8.0

82. Wu describes a large-scale purification method for rAAV, using chloroform treatment, PEG/NaCl precipitation and chloroform extraction. *Id.*, 1. Wu explains that "purification and concentration of rAAV from crude cell lysate remains to be improved" and prior purification methods are "elaborate and time-consuming and often result[] in poor recovery." *Id.* The focus of Wu is high yield

of rAAV following purification. *Id.* Wu does not address the ionic strength of its purified compositions. *Id.*

83. The Petition, citing Dr. Kay, states that “Wu carried out electron microscopy analysis of the purified rAAV preparations” without finding evidence of aggregation. Petition, 22 (citing Ex. 1007, 3, Fig. 3; Ex. 1005, ¶ 181). The Petition, again citing Dr. Kay, further states that Wu’s “purified rAAV stock could be stored at 4°C for more than 1 month without significant decrease of infectious titer. *Id.* (citing Ex. 1007, 4; Ex. 1005, ¶ 183). As I explain below, I disagree that the electron microscopy analysis undertaken by Wu— for a different purpose— establishes a lack of aggregation.

84. But even setting that aside, the POSA would not have been able to discern the ionic strength of Wu’s composition from its disclosure. Thus, Wu does not provide sufficient information to determine whether its composition would meet the ionic strength requirement (>200 mM) of the challenged claims.

85. For the ionic strength claim element, the Petition cites Wu’s disclosure relating to viral particle resuspensions in PBS²⁺ buffer to argue that the POSA would understand Wu to meet the pH and ionic strength elements. *Id.*, 34-35 (citing Ex. 1007, 2). But the cited disclosure of resuspension in PBS²⁺ buffer does not provide any information about the pH, ionic strength, or other components in, or attributes of, the composition of the resuspended solutions. *Id.*

86. Regardless, Dr. Kay contends that the buffer Wu uses to resuspend its rAAV pellets would have resulted in an rAAV composition having an ionic strength of 209 mM or 212 mM. *Id.*, 35; Ex. 1005, ¶¶ 297-312. Dr. Kay alleges that two components contribute to these ionic strength values, PBS²⁺ buffer¹ (169.48 mM or 172.56 mM) and residual NaCl (40 mM) from a PEG/NaCl precipitation employed by Wu. Ex. 1005, ¶ 297. When summed, these values just exceed the claimed 200 mM ionic strength threshold. Wu, however, is silent on both the ionic strength of the PBS²⁺ buffer and amount of residual NaCl, if any, in its precipitated pellets that are resuspended in the buffer.

87. To reach these alleged ionic strength values, Dr. Kay undertakes a hindsight driven analysis to make several unsupported assumptions and then conducts a series of calculations based on those assumptions.

i. Dr. Kay’s Ionic Strength Analysis Makes Unsupported Assumptions About Residual NaCl in Wu’s Composition

88. For the 40 mM ionic strength contribution from residual NaCl, Dr. Kay assumes that “a POSA would have understood that there will be residual NaCl in the pellet that will be present when the pellet is resuspended in PBS²⁺ buffer.”

¹ Dr. Kay alleges that PBS²⁺ buffer contains a mixture of components, including Na₂HPO₄, NaCl, KCl, and KH₂PO₄. Ex. 1005, ¶301.

Id., ¶¶ 175, 309. Dr. Kay then speculates that “if only 0.20 ml of 1M NaCl were left in the tube when PBS²⁺ buffer was added to a final volume of 5 mls, then the concentration of NaCl in the final solution would be about 40 mM NaCl (1M NaCl diluted 0.2/5 or 1/25).” *Id.*, ¶¶ 176, 312.

89. Wu, however, does not state anything about residual NaCl in the pellet. Nor is there any basis for the POSA to assume that residual NaCl—if it existed at all—would be at a 40mM concentration. To show the impact on both Dr. Kay’s assumptions, I have calculated the ionic strength when altering the concentration of alleged residual NaCl.

90. Dr. Kay assumes that the volume of residual 1M NaCl is 0.2 mL. *Id.*, ¶ 311. If one were to accept the other assumptions that Dr. Kay has made (e.g., pH) and accept his calculations as accurate, which I am doing only for the purpose of this exercise, it is apparent that an assumed 0.20 mL of residual 1M NaCl was contrived because it is the volume that nudges the ionic strength of the final composition just above 200 mM.

Assumed Residual NaCl	0 mL	0.05 mL	0.10 mL	0.15 mL	0.20 mL
Residual NaCl Ionic Strength contribution	0 mM	10 mM	20 mM	30 mM	40 mM
Total Ionic Strength	169.48 mM	179.48 mM	189.48 mM	199.48 mM	209.48 mM

91. Dr. Kay provides no support, such as corroborating evidence, for choosing 0.20 mL residual 1M NaCl. There is simply no basis for this assumption, and there are reasons to assume that there was far less than 0.20 mL residual 1M NaCl remaining after the particles were pelleted. For example, if the residual volume of 1M NaCl was removed using a vacuum, which in my experience is a common technique to remove liquid from a viral or cell pellet, there would only be trace amounts of residual NaCl left in the pellet. Dr Kay only comes up with the unsupported assumption of 0.2 ml residual volume through hindsight driven by a need to meet the claimed 200 mM ionic strength threshold.

ii. Dr. Kay Makes Unsupported Assumptions About the pH in Wu's Composition

92. Wu does not provide a pH value for its resuspended solutions containing a PBS²⁺ buffer. To address the pH and the ionic strength elements of the claims, Dr. Kay speculates about the pH of Wu's composition. Dr. Kay's pH assumption is contradicted by the art he cites and his own publications relating to the use of PBS²⁺ buffer systems.

93. There are numerous references that use PBS²⁺ buffers having a pH outside the range of 7.5 and 8.0. The references that Dr. Kay cites for the pH value indicate that PBS²⁺ buffer is typically used at pH values below this range. Ex. 1005, ¶¶ 287, 303 n.16; Ex. 1057 ("Adjust the **pH to 7.4 (or 7.2, if required)**"); Ex. 1023, 2 ("After the supernatant had run through, the column was washed with 20

ml of phosphate-buffered saline (PBS, **pH 7.2**).”; Ex. 1065, 308 (“Dulbecco’s phosphate-buffered saline (PBS) with Ca^{2+} and Mg^{2+} , **pH 7.2**”); Ex. 1083, 7 (“Prepackaged PBS (**pH 7.4**)”). Additionally, Auricchio (Ex. 1056), relied on by Petitioner in IPR2025-01195 states “[t]he virus was then eluted with 15 ml of PBS, **pH 7.4**, plus 0.4 M NaCl.” Ex. 1056, 2.

94. And Dr. Kay himself is an author of multiple publications specifying a pH of PBS of less than pH 7.5. *E.g.*, Ex. 2027, 10 (“Proteins were desalted into phosphate-buffered saline (PBS) **pH 7.4** using Econo-Pac 10DG gravity flow chromatography columns (Bio-Rad).”); Ex. 2028, 12 (“Cryosections were prepared as described above. Tissues were washed in 0.1% Triton X-100 (in PBS) . . . for 5 min (cryosections) or 15 min (whole mounts) and then blocked with 5% donkey serum, 0.1% Triton-100, 1% bovine serum albumin, and 0.02% sodium azide (NaN_3) in PBS at **pH 7.4** for 1 h at room temperature.”); Ex. 2029, 4 (“All specimens were immediately placed on ice and were washed sequentially in serial dilutions of dilute Betadine, followed by two PBS (**pH 7.2**) washes of equal volume.”).

95. The POSA would not have assumed that the resuspension solution would be at pH 7.5-8.0. Thus, the Petition and Dr. Kay’s declaration have not established that a POSA would have understood Wu’s composition to have a “pH buffer, wherein the pH of the composition is between 7.5 and 8.0.”

96. Moreover, because Dr. Kay's ionic strength calculations depend on the pH of the buffer system, these calculations are not reliable. According to Dr. Kay's own calculations, the lower the pH of a PBS²⁺ buffered solution, the lower its ionic strength. Ex. 1005, ¶ 312 (showing a pH 7.5 PBS²⁺ solution has a lower ionic strength than a pH 8.0 PBS²⁺ solution). If Wu's solutions were at pH 7.2, for example, the ionic strength contribution of the PBS²⁺ buffer would be lower than the alleged 169.48 mM and 172.56 mM ionic strength values at pH 7.5 and pH 8.0.

Id.

b. The POSA Would Not Have Been Motivated to Make an rAAV Composition with an Ionic Strength > 200 mM Based on Konz and Evans

97. To reach the claimed ionic strength of greater than 200 mM, the Petition claims that the POSA would have made a composition with the maximum recited amounts of sodium chloride (NaCl), 250 mM disclosed in Evans, which Konz incorporates by reference. Petition, 36 (citing *id.*, ¶ 314). But that is not what the POSA would do. The POSA would have considered the totality of the teachings in Konz and Evans. Those teachings would have led the POSA to much lower NaCl concentrations, and consequently a much lower ionic strength.

i. Konz's Formulation Buffers All Have Ionic Strengths Far Below 200 mM

98. Konz describes a "process for purifying virus particles, especially recombinant adenovirus vector particles." Ex. 1008, Abstract. The focus of Konz is

purification of recombinant adenovirus, not rAAV, to reduce contaminating host cell DNA levels. *Id.* at 1:15-22.

99. Even at adenoviral concentrations well below the claimed rAAV concentration range (*infra* § VIII.A.1.d), Konz does not address how ionic strength impacts viral particle aggregation. Rather, Konz suggests that other parameters impact aggregation. Konz emphasizes that the presence of detergents, surfactants, and chromatography resins may prevent aggregation. Selected examples are reproduced here:

As mentioned above, anion exchange chromatography is extremely useful in such a process, depending upon careful resin selection and operating conditions (NaCl concentration), as well as inclusion of a detergent (e.g., PS-80) in the running buffers to inhibit aggregation during loading and elution.

Id., 6:20-23.

As an example, but in no way meant as a limitation, non-ionic surfactants which could potentially be used to inhibit aggregation in anion exchange and throughout the process include polyoxyethylene sorbitan fatty acid esters, including but not limited to Polysorbate-80 (Tween 80®) [as exemplified herein], Polysorbate-60 (Tween 60®), Polysorbate-40 (Tween 40®), and Polysorbate-20 (Tween 20®), polyoxyethylene alkyl ethers, including but not limited to Brij 58®, Brij 35®, as well as others such as Triton X-100®, Triton X-114®, NP40®, Span 85 and the Pluronic series of non-ionic surfactants (e.g. Pluronic 121).

Id., 24:1-9.

It is disclosed herein that a resin such as a Source 15Q resin (Amersham Biosciences) has a [sic] extremely high binding capacity for adenovirus which can be effectively utilized due to inhibition of aggregation.

Id., 24:9-12.

100. The Petition ignores that Konz discloses several formulation buffers, all of which contain a fraction of NaCl compared to the maximum 250 mM NaCl concentration in Evans. Immediately following Konz's reference to Evans (WO 0166137), Konz discloses a list of what it refers to as "formulation buffer[s]."

The particular diafiltration buffer chosen should be an appropriate formulation buffer (see WO 0166137) or a subset of the desired components. Examples include, but are not limited to (1) 5 mM Tris, 1 mM MgCl₂, **75 mM NaCl**, 5% sucrose, 0.005% PS-80, pH 8.0, (2) 10 mM Tris, 10 mM Histidine, 1 mM MgCl₂, **75 mM NaCl**, 5% sucrose, 0.005% PS-80, pH 7.4, (3) 5 mM Tris, 10 mM NaCl, 1 mM MgCl₂, 5% sucrose, 0.005% PS-80, pH 7.4, and (4) 10 mM Tris, 10 mM Histidine, 1 mM MgCl₂, **75 mM NaCl**, 5% sucrose, 0.02% PS-80, 0.1 mM EDTA, 0.5%(v/v) ethanol, pH 7.4.

Id., 25:20-27.

101. Each of Konz's formulation buffers contain only 75 mM NaCl. Therefore, the ionic strength contribution of the 75 mM NaCl in Konz's formulation buffers would be only 75 mM.

102. Konz also discloses other formulation buffers, none of which have an NaCl concentration exceeding 75 mM.

The AEX product will be diluted to *ca.* 1.1×10^{12} vp/mL (approximately 16 liters) and is then diafiltered using flux control against 5 volumes of formulation buffer (5 mM Tris, 1 mM MgCl₂, **75 mM NaCl**, 5% sucrose, 0.005% PS-80, pH 8.0).

Id., 30:16-19.

The batch was diafiltered into formulation buffer (5 mM Tris, **75 mM NaCl**, 1 mM MgCl₂, 5% sucrose, 0.005% PS-80, pH 8.0).

Id., 42:14-16.

The batch was diafiltered into buffer (5 mM Tris, **10 mM NaCl**, 1 mM MgCl₂, 5% sucrose, 0.005% PS-80, pH 7.4).

Id., 52:31-32.

103. Konz, therefore, specifically exemplifies formulation buffers having NaCl concentrations ranging from 10 mM to 75 mM, which contribute only 10 mM to 75 mM ionic strength to Konz's enumerated formulations. The other formulation buffer components do not increase this ionic strength significantly. Konz, consistent with the disclosure of Evans, teaches that lower ionic strengths give better stability and hence Konz's formulation buffers likewise have low ionic strengths.

ii. The Petition Omits that Evans Did Not Relate to High Concentration rAAV Compositions

104. Evans does not address viral particle aggregation, and instead relates to adenovirus 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. Ex. 1020, 24:22 and 25:13-14 (Example 2), 25:31-32 and 26:4 (Example 3), 29:6 (Example 7), 30:14 (Example 9). This is well below the claimed rAAV vector concentration range exceeding 10^{13} vg/ml. Given the relationship between high concentrations of rAAV vectors and aggregation (Ex. 1045, 2; Ex. 1043, 1), it is my opinion that the POSA would not have looked towards Evans as providing any guidance on this issue.

iii. Evans Would Have Led the POSA to Select Lower Salt Concentrations than 250 mM NaCl

105. The Petition's argument that the POSA would have been motivated to select the high end of the concentration ranges for NaCl and MgCl₂ in Evans' claim 5 composition (Petition, 36) is inconsistent with the Evans' examples and its stability testing. Evans would have led the POSA to much lower NaCl concentrations than 250 mM. In fact, Evans' examples do not have NaCl concentrations above 150 mM. Other ionic species in Evans' compositions, for

example MgCl_2 and Tris, are at such low concentrations that they do not contribute significantly to the total ionic strength of these compositions. At pH values of between 7.5-8.0, most of the tris buffer, which has a pK_a of 8.1, is not in an ionic state. At 5mM concentration, MgCl_2 would contribute 15 mM of ionic strength.

Supra VI.B.

106. 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. Ex. 1020, 11:10-11.

Formulation A104 does not contain a cryoprotectant. *Id.*, 24:24-27.

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

Id., 22:1-2.

107. Evans shows, however, that these differences in formulation excipients had a dramatic effect on composition stability (*id.*, FIG. 2) and infectivity (*id.*, FIG. 3) after freeze-thaw cycles. *Id.*, 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.” *Id.*, 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.

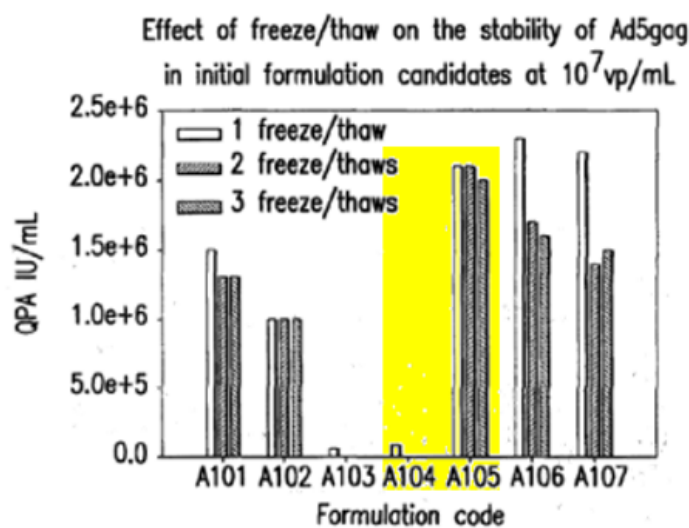


FIG.2

108. 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.” *Id.*, 21:15-20 (“The TCID₅₀ assay is a method for titrating the infectivity of adenovirus.”). Thus, Evans’ Example 2 indicates that a formulation containing a **lower NaCl concentration**, but including sucrose as a cryoprotectant, was better for ensuring viral particle stability.

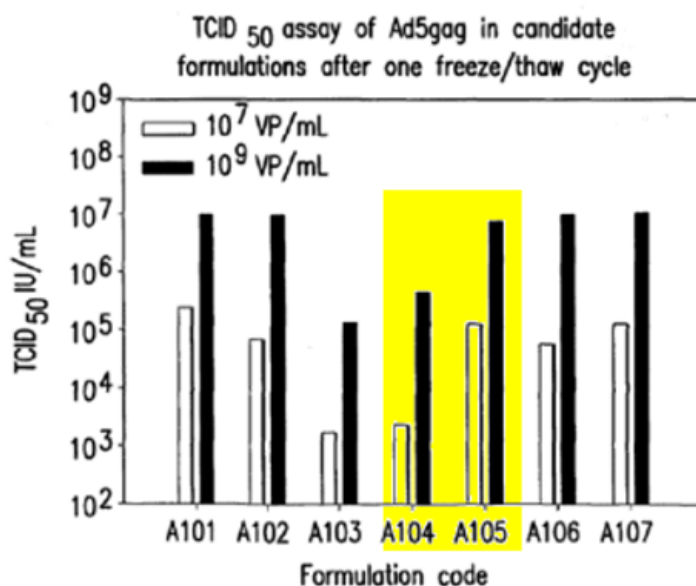


FIG.3

109. In the short-term stability study of Example 3, Evans compares the stability of Ad5gag in A102, A105, A106, and A107, at 10⁷ and 10⁹ 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 NaCl, 10% glycerol (v/v), pH 7.2
A105	5 mM Tris, 75 mM NaCl, 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

110. 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.

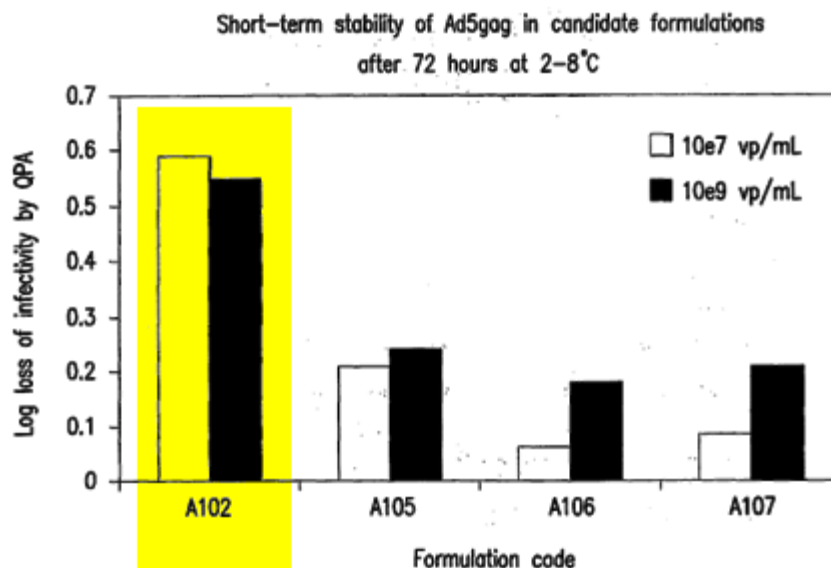
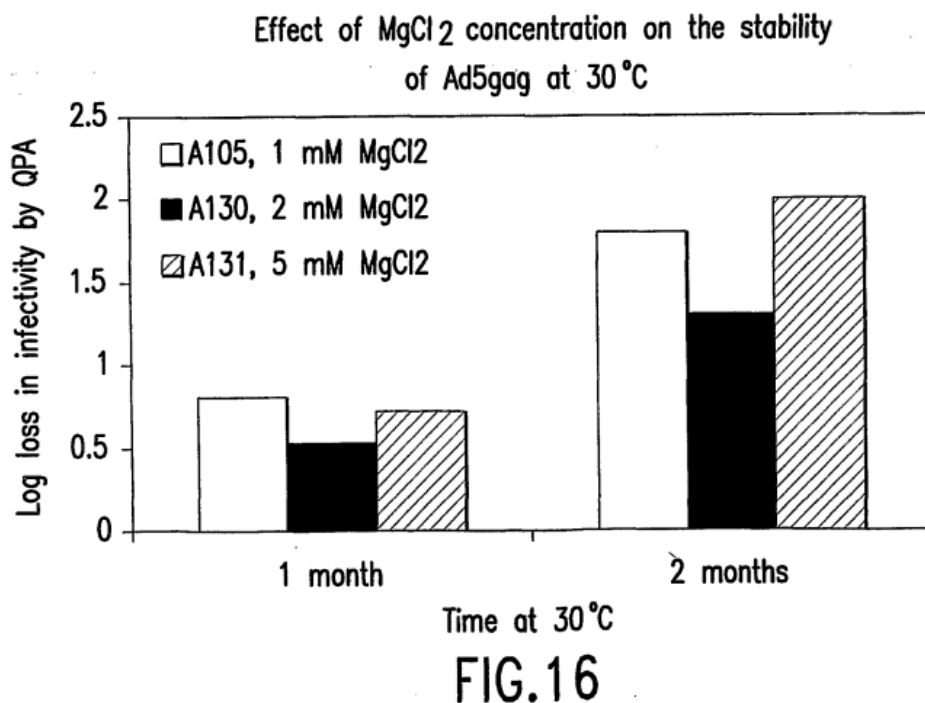


FIG.6

111. 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.*, 22:2-26).

112. Evans' testing results also contradict the Petition's assertion that the POSA would have been motivated to select the high end MgCl₂ concentration of 5 mM to increase ionic strength. EX1005, ¶¶ 313-314. In studies that isolated the effect of MgCl₂ concentration on formulation stability by varying MgCl₂ concentration, Evans' Example 5 shows that vector stability was maximized at 2 mM MgCl₂. Increasing the quantity of MgCl₂ to 5 mM, however, led to poorer stability after two months storage than 1 or 2 mM MgCl₂. *Id.*, 28:1-11 (Example 5); Fig. 16.



113. Evans’ stability studies described above indicate the importance of including a cryoprotectant as part of an adenovirus composition. *Id.*, 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.” *Id.*, 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.

114. Thus, the POSA considering the entirety of Evans’s teachings would have been motivated to select salt concentrations far below the Petition’s proposed 250 mM NaCl and 5 mM MgCl₂ concentrations. In my opinion, this undercuts the Petition’s motivation argument.

c. The POSA Would Not Have Considered Wu's Electron Microscopy Analysis to Detect rAAV Aggregation

115. The Petition and Dr. Kay's declaration focus on Wu's electron microscopy studies to show that its "rAAV stock formulation did not exhibit any observable aggregation." Petition, 3; *see also id.*, 22 (citing Ex. 1007, 3, Fig. 3). Wu, however, did not use electron microscopy to detect aggregates. Wu's focus is to provide a method of purifying rAAV particles in order to maximize the amount of full rAAV particles in a composition. Wu used electron microscopy to "visualize rAAV-GFP particles" and qualitatively observe the presence of full and intermediate rAAV particles, as well as empty capsids. Ex. 1007, 3.

116. To visualize the rAAV particles, Wu manipulated the sample by extracting an aliquot of the stock, then "negative staining" of that sample "with 2% uranyl acetate" to visualize the various particles species, followed by "loading on 150 mesh nickel grids." *Id.* The staining and mounting of the sample altered the composition's chemical environment from the stock composition.

117. Figure 3 demonstrates the aim of that study by permitting the POSA to see individual monomeric particles. But the POSA would not conclude the absence of aggregates in the composition from which the particles were extracted based on this level of magnification. Wu's silence on aggregation confirms that the authors were not attempting to use electron microscopy to detect rAAV aggregations.

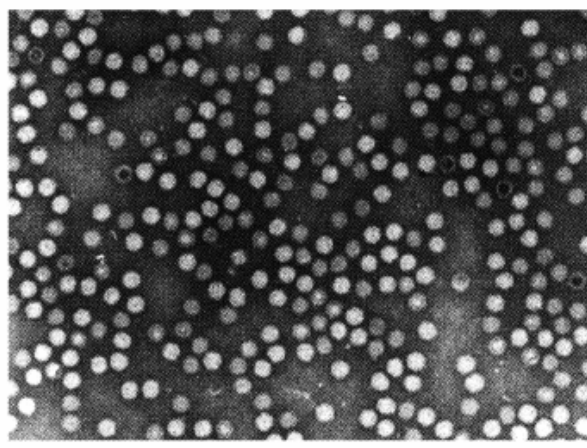


Fig. 3. Purified and concentrated recombinant adeno-associated virus particles visualized by electron microscopy after negative staining with uranyl acetate ($\times 35620$).

Id.

118. Even if the POSA were to know the conditions of Wu's stock compositions, Wu's electron microscopy studies would not have been considered probative of whether there were aggregates in that stock composition. The POSA would have looked to other known techniques such as DLS and filtration to study aggregation. These techniques permit the POSA to analyze the entire rAAV sample as present in the composition. This is demonstrated by the work of Konz (Ex. 1008), on which the Petition and Dr Kay's Declaration rely. It is important to note that when faced with the task of specifically studying the aggregation in rAAV compositions, Konz used DLS and filtration, not Electron Microscopy. See Ex. 1008, Example 9. Wu is simply not relevant to addressing the challenge of aggregation. Thus, the POSA would not have been motivated to use Wu's

composition (whatever its components) in an rAAV composition that minimizes aggregation.

d. Konz Analyzed Aggregation in Compositions Having Much Lower Viral Particle Concentrations than Claimed

119. Konz, like Wu, provides no connection between ionic strength and aggregation (or lack thereof) in high concentration rAAV compositions. The Petition states that “Konz also discloses methods to inhibit particle aggregation and promote stability during storage, including by reference to Evans.” Petition, 36. This ignores that Konz’s “sterile filtration and DLS” studies were conducted on *adenovirus* compositions having concentrations *magnitudes below* the claimed 1×10^{13} vp/ml threshold. *Id.*, 36-37 (citing Ex. 1008, 30:13-30 (The purified adenovirus product “will be diluted to *ca.* 1.1×10^{12} vp/mL”); 42:5-19 (“The product peak was diluted and adjusted to an adenovirus concentration of approximately 1.05×10^{12} vp/mL”), 48:4-21 (disclosing adenovirus concentrations ranging from 1.83×10^{10} vp/ml to 3.55×10^{12} vp/ml)). The POSA would not have considered these results to be relevant to the aggregation of higher concentration rAAV compositions.

120. Indeed, Konz’s adenoviral particle concentration would be understood by the POSA to include empty and partially filled capsids, in addition to particles with complete viral genomes. 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 Konz's most concentrated adenoviral compositions of $\sim 10^{12}$ vp/ml could have a viral genome concentration of only $\sim 10^{11}$ vg/ml, 100-fold lower than the claimed rAAV concentration of 1×10^{13} vg/ml. The POSA would have understood that rAAV particle aggregation was especially a problem at high rAAV concentrations (e.g., $>10^{13}$ vg/ml). Thus, the POSA would not have considered Konz's highest concentration adenoviral compositions of $\sim 10^{12}$ vp/ml relevant to overcoming the challenge of concentrated rAAV aggregation.

2. The Petition Does Not Consider that the POSA Would Have Avoided Developing an rAAV Vector Compositions Having High Salt Concentrations

121. The Petition and Dr. Kay fail to address the many reasons why the POSA would have been motivated not to use a high concentration of NaCl to achieve a high ionic strength. Prior art evidence not considered by the Petition indicated that high salt concentrations were undesirable for therapeutic rAAV compositions and were even observed to negatively impact rAAV stability.

a. A POSA Would Have Understood that Formulations with High NaCl Concentrations Are Hypertonic and Can Cause Tissue Damage and Pain

122. As of the effective filing date, a major concern that would have driven the development of therapeutic rAAV formulations was isotonicity. The POSA would have understood that "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. “For an isotonic product, often it is ***advantageous to keep the concentration of salt as low as possible.***” Ex. 2018, 197. The prior art “emphasize[d] 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.” Ex. 2019, 3-4; *see also* Ex. 2017, 4 (“Solutions to be administered subcutaneously ***require strict attention to tonicity*** adjustment.”)).

123. Serum osmolarity is about 300 mOsm. Ex. 2016, 57. “Physiological solutions with an osmotic pressure lower than that of body fluids, or of 0.9% [~150 mM] sodium chloride solution, are referred to commonly, as being hypotonic. Physiological solutions having a greater osmotic pressure are termed hypertonic.” *Id.*, 5; *see also* Ex. 1001, 5:15-16. A 300 mOsm NaCl solution is isosmotic with 300 mOsm serum, and thus isotonic.

124. The Petition’s obviousness argument relies on the POSA selecting the Evans buffer containing 250 mM NaCl and 5 mM MgCl₂. This buffer would have

an osmolarity of 515 mOsm (500 mOsm from NaCl and 15 mOsm from MgCl₂),² which is much higher than 300 mOsm serum, and is therefore hypertonic.

125. The POSA, concerned about the adverse effects of hypertonicity, including pain and tissue damage, would not have been motivated to use a buffer formulation with an osmolarity of 515 mOsm.

126. The Petition's motivation argument focusses on modifying Wu, which contains toxic chloroform, to make it suitable for therapeutic use by exchanging Wu's buffer with a buffer from Konz/Evans. Specifically, the Petition states that the "POSA would have been motivated to remove residual chloroform from Wu's rAAV stocks by exchanging Wu's buffer with a buffer in Konz (as incorporated via Evans)." Petition, 32; *see also id.*, 3 (discussing changing the formulation buffer in Wu's stocks to be suitable for clinical trials); Ex. 1007, 4 ("Further steps should be taken to remove residual chloroform before the stocks are used in clinical trials."). I agree that the Wu composition was not suitable for therapeutic use. For the reasons I provided above, I disagree that the POSA would have exchanged Wu's buffer with the Konz/Evans buffer in a manner that would have resulted in a composition having > 200 mM ionic strength.

² 250 mM NaCl x 2 = 500 mOsm; 5 mM MgCl₂ x 3 = 15 mOsm

127. Evans taught that its virus formulations should be made “useful for parenteral, and especially intramuscular, injection.” Ex. 1020, 10:18-21. The state of the art emphasized that injectable formulations should be isotonic, particularly for “intramuscular delivery,” Evans’ “especially preferred method” of delivery. *Id.*, 20:17-18. Thus, the POSA would not have been motivated to make rAAV compositions with Evans’ highest NaCl concentration because such formulation would be hypertonic.

128. The Petition and Dr. Kay do not explain why the POSA focused on making formulations suitable for clinical use nonetheless would have been motivated to substitute a hypertonic buffer inappropriate for such use. They fail to identify any reason the POSA would have violated the principle of maintaining isotonicity when the POSA would have considered such an issue central to formulation development.

b. The Petition Fails to Address Prior Art Teaching High Salt Concentrations Promote Precipitation of rAAV Particles

129. High salt concentration solutions were also observed to cause instability of rAAV particles. For example, Xie (Ex. 1028) explained that “[h]igh concentrations of AAV-2 in the ~3.3 M CsCl from ultracentrifugal purification remained mostly in solution at 4°C, although there was some precipitation and adhesion to glass- and plastic-ware with time....” Ex. 1028, 23. Thus, even for

molar salt solutions of CsCl having osmolarity of ~ 6600 mOsm that would be unsuitable as a parenteral formulation, AAV particle precipitation was observed. Moreover, Xie further stated that a solution of “0.25 M NaCl [250 mM] resulted in **significant loss**” of AAV. *Id.* Thus, Xie teaches that a salt concentration of 250 mM NaCl, the NaCl concentration that the Petition advocates for (Petition, 35-36), was observed as inadequate to prevent significant rAAV aggregation in Xie’s solution. Xie’s observation, published near the time of the effective filing date, contradicts the Petition’s motivation argument.

130. Xie, however, was not the only reference to suggest that high salt concentrations could lead to undesirable results for rAAV compositions. The Petition also disregards another reference. For example, WO 99/41416, published Aug. 19, 1999, to Frei et al., which was relied-upon prior art in IPR2023-00608, states that the POSA “must take into account the additional **threat to stability posed by the high salt concentrations.**” Ex. 2020, 19:9-14; *see also id.*, 18:19-24; 21:25-26; 21:42-22:6.

131. It is my opinion that the Petition and Dr. Kay’s declaration have failed to establish a motivation to make the claimed composition having an ionic strength greater than 200 mM.

c. The Cited Background Art is Not Relevant to rAAV Compositions

132. The “background, state of the art references” or “Floyd studies” (Exs. 1030-1032) cited in the Petition and Dr. Kay’s declaration relate to 1970s era research on disinfecting virus-containing “water passing through sewage or water treatment plants.” Ex. 1030, 1. The POSA looking to prepare an rAAV composition for therapeutic use would not have considered such a study as relevant to the POSA’s understanding of rAAV aggregation.

133. The goal of the Floyd study was to disinfect waterborne virus. Aggregation allowed viruses to survive and remain infective, whereas disrupting those aggregates allowed access by disinfecting agents, such as chloride and bromide ions. *Id.* Thus, “the disinfection of water supplies under conditions in which the virus is in the dispersed state may lead to a more nearly complete inactivation.” *Id.*, 8. In contrast, the POSA formulating an rAAV composition who would be seeking to maximize infectivity would not consider the Floyd studies, which focused on killing infectivity.

134. The POSA developing a therapeutic composition must consider several issues, including tonicity (*supra* §§ VI.A, VIII.A.2.a) and stability (*supra* § VIII.A.2.b). While Floyd sought to destabilize viral particles, the POSA would have sought to stabilize viral particles. Viral particle stability presents significant

challenges. *Infra* § VIII.B.1. Thus, the POSA would not have considered Floyd relevant to the development of a therapeutic composition.

135. The poliovirus and reovirus subjects of the Floyd study exhibited different pH-dependent behavior from rAAV particles. For example, “[i]n the pH region of 7 to 10.5, both poliovirus and reovirus showed ***no tendency to aggregate***.” EX1032, 6 (emphasis added). In contrast, researchers observed aggregation of rAAV particles at pH 8.0. EX1001, 1:65-2:8; EX1045, 3; EX2022, 3-4. Thus, a POSA would not have considered Floyd’s study of poliovirus and reovirus particles as relevant to rAAV particles. Additionally, the Floyd references studied viral aggregation on “[p]article concentrations of stocks [that] ranged from 10^{10} to 2×10^{12} particles/ml.” Ex. 1030, 1; Ex. 1005, ¶ 39. This is a significantly lower concentration than the 10^{13} vg/ml recited by the ’542 patent’s claims. The POSA understood that rAAV aggregation concentration dependent. Ex. 2021, 6 (“High concentrations of protein may induce aggregate formation.”); Ex. 1045, 2 (“rAAV undergoes concentration-dependent aggregation.”).

136. Dr. Kay acknowledged that aggregation led to significant losses in infectivity in high concentration rAAV compositions above 10^{13} vg/ml, such that high concentration compositions could be less infective than lower concentration compositions. Ex. 1005, ¶ 36 (citing Ex. 1043)

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

137. The Petition and Dr. Kay fail to establish that the POSA would have had reasonable expectation of success in achieving high concentrations of rAAV compositions without significant aggregation. In 2004, as remains the case today, there was significant unpredictability in formulating protein compositions, including viral vectors such as rAAV. None of the prior art cited (i.e., Konz, Evans and Wu) resolve that unpredictability or provide a reasonable expectation of success.

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

138. The Petition and Dr. Kay state that the “POSA would have chosen one of the Konz high ionic strength (250 mM NaCl) buffers, with a multivalent ion (MgCl₂), at a pH similar to that of Wu (about 7.4 or 7.5 to 8.0), and added a non-ionic surfactant, in accordance with Konz’s teachings.” Petition, 43 (citing *id.*, ¶ 358).

139. The Petition and Dr. Kay both conclude there is a reasonable expectation of success:

Given all of these steps to inhibit aggregation, given the starting point of Wu’s formulation where no aggregation was detected, and given Konz’s data showing greater than 90% yields and no aggregation per assessment by 0.22 µm sterile filtration after storage at 4°C, followed

by DLS, a POSA would have had a reasonable expectation of success in achieving the claimed combination – a high titer, high ionic strength formulation containing a multivalent ion and 0.001% Pluronic F68 without significant aggregation after storage, as measured by DLS and sterile filtration with a 0.22 µm filter.

Id.

140. But neither the Petition nor Dr. Kay explain what is meant by “[g]iven all of these steps to inhibit aggregation.” *Id.* As I have explained above, there is no teaching or suggestion in Wu or Konz/Evans that connects the ionic strength of the composition to the prevention of rAAV aggregation. *See supra* §§ VIII.A.1.a-VIII.A.1.b. Additionally, as I explained, the disclosures of Wu and Konz/Evans do not establish that high rAAV compositions did not aggregate. *See supra* VIII.A.1.c-VIII.A.1.d.

141. A POSA would not have assumed that substituting Wu’s buffer with Konz’s high ionic strength buffers would have resulted in a stable composition. For example, the prior art explained 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. 2018, 198. Carpenter further explains that “[e]very protein and product has unique characteristics, some of which may cause difficulty in designing stable formulations.” *Id.*, 118. Considering references that the Petition and Dr. Kay’s declaration cite, including Huang, Croyle, and Wright (2003), the POSA would have been skeptical that high-concentration rAAV aqueous

compositions that did not aggregate when stored would have been feasible. *Supra* § VIII.A.1.b.

142. Huang is a short abstract that explains “at high concentrations, AAV virions form aggregates of different sizes in a range of different buffer systems and storage conditions ...[p]articularly, when the vector titer reached $5\text{-}10 \times 10^{13}$ GC/ml.” Ex. 1043, 1. Huang then indicates that the aggregates impact the efficacy of gene transfer. *Id.*

143. Huang explains that so much viral vector is lost due to aggregation that at a concentration of “ $5\text{-}10 \times 10^{13}$ GCs/ml, gene transfer efficiency was 10-100 fold lower at the same dose as compared to the vector whose titer was $1\text{-}5 \times 10^{12}$ GCs/ml.” *Id.* Even though Huang alludes to a decrease in aggregate size under certain formulation conditions (*id.*), the POSA would have understood that significant aggregation still occurred.

144. Wright is a 2003 review article discussing in detail the continued challenges with rAAV formulation. Ex. 1045. Wright devotes a whole section to viral vector aggregates, which starts out stating that “[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. 1045, 2. Wright explains that “[t]he nature of the interparticle interactions that result in aggregation has not been well characterized.” *Id.*, 3. 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.

145. Croyle (Ex. 2021) is an article that describes the “efforts to address an issue that is critical to the success of any multicenter gene therapy clinical trial—maintenance of vector viability during shipping and storage at remote test sites.” Ex. 2021, 1. Croyle explains that for rAAV compositions, “[h]igh concentrations of protein may induce aggregate formation and precipitation upon freezing resulting in poor recovery of the product.” *Id.*, 6. Croyle does not mention ionic strength or provide any indications on how to solve the issue of aggregation.

2. Neither Konz/Evans Nor Wu Establish a Reasonable Expectation of Success

146. The Petition and Dr. Kay’s declaration rely on the disclosure of formulation buffers in Konz and Evans to arrive at the claimed ionic strength. The Petition states that “Konz also discloses methods to inhibit particle aggregation and promote stability during storage, including by reference to Evans.” Petition, 36. But Konz’s “sterile filtration and DLS” studies were carried out on adenovirus compositions having concentrations magnitudes below the claimed 1×10^{13} vg/ml threshold. *Id.*, 36-37 (citing Ex. 1008, 30:13-30 (The purified adenovirus product

“will be diluted to *ca.* 1.1×10^{12} vp/mL”); 42:5-19 (“The product peak was diluted and adjusted to an adenovirus concentration of approximately 1.05×10^{12} vp/mL”), 48:4-21 (disclosing adenovirus concentrations ranging from 1.83×10^{10} vp/ml to 3.55×10^{12} vp/ml)).

147. Konz provides no connection between ionic strength and aggregation in its composition. The POSA would not have considered Konz’s results to be predictive of aggregation of higher concentration rAAV compositions, which were known to present more significant aggregation issues. *Supra* § VIII.A.1.d. Moreover, Konz’s formulations buffer have much lower ionic strength than the claimed rAAV compositions require, and a POSA following Konz would have made a ~10-75 mM formulation buffer.

148. Evans, which is cited by Konz, presents similar issues as Konz. 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 both the 10^{13} vp/ml upper limit of Evans’ concentration range and the 1×10^{13} vg/ml threshold of the claims. *Supra* § VIII.A.1.b.ii (citing Ex. 1020, 24:22 and 25:13-14 (Example 2), 25:31-32 and 26:4 (Example 3), 29:6 (Example 7)).

149. Evans demonstrated that minor changes in excipients can produce dramatic changes in stability. As I explained above, 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." Ex. 1020, 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.").

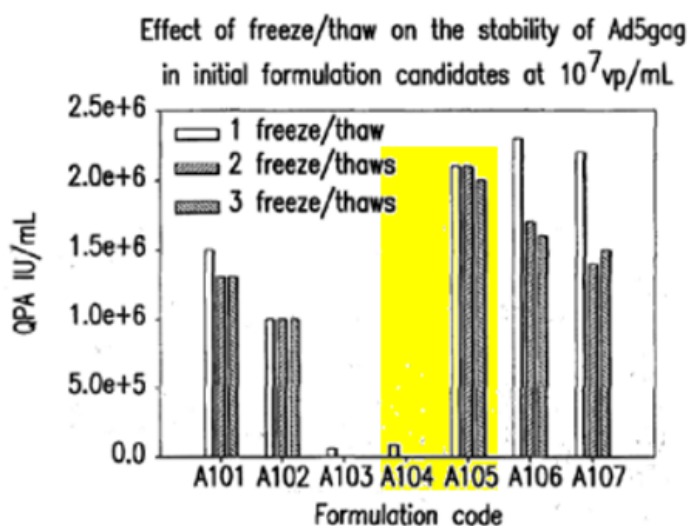


FIG.2

Supra §VIII.A.1.b.iii (citing *id.*, 24:18-25:24 (Example 2), FIGS. 2, 3) (comparing stabilities of formulations A104 and A105).

150. None of the exemplary formulations in Konz or Evans have a NaCl concentration greater than 150 mM NaCl. Konz does not address rAAV aggregation and discloses several formulation buffers, none of which have an NaCl

concentration exceeding 75 mM. *Supra* § VIII.A.1.b.i. In Evans, the most stable formulations contained 75 mM or less NaCl, with ionic strengths below 200 mM. *Supra* § VIII.A.1.b.iii. Evans nonetheless demonstrated that even small changes to excipients can dramatically change the stability of a viral vector composition, with the highest NaCl concentration buffer resulting in the poorest stability results. *Id.* (citing Ex. 1020, 24:18-25:24 (Example 2), FIGS. 2, 3).

151. Wu does not even disclose basic information about the pH, ionic strength, or composition of its resuspended solutions; the Petition relies on speculative assumptions made by Dr. Kay. *Supra* § VIII.A.1.a. Because of this lack of information, the POSA would not have been able to discern what components of Wu's formulation may contribute to Wu's storage data. Petition, 22 (citing Ex. 1007, 4; Ex. 1005, ¶ 183). The POSA would not have considered Wu's electron microscopy analysis as measuring the existence or the extent of aggregation in Wu's stock solution, as it was undertaken for a completely different objective. *Supra* § VIII.A.1.c. Therefore, Wu lacks the disclosure regarding both ionic strength and aggregation that the POSA would need to determine whether elevated ionic strength decreased aggregation.

152. There are no disclosures in Wu, Konz, or Evans that would have supplied the POSA with a reasonable expectation that compositions comprising

rAAV at concentrations $>10^{13}$ vg/ml and having an ionic strength greater than 200 mM would not result in significant aggregation.

IX. GROUND 2: CLAIM 3 WOULD NOT HAVE BEEN OBVIOUS TO THE POSA IN VIEW OF WU, KONZ, AND CROYLE

153. Claim 3 requires, *inter alia*, the same combination of elements that the Petition fails to render obvious through Wu and Konz in Ground 1. The Petition cites Croyle (Ex. 1009) solely to address the Pluronic F68 claim requirements in claim 2 and 3. Petition, 45-49.

154. For the reasons I set forth above, the addition of Croyle does not overcome the deficiencies of the Petition for the other elements of claim 3. Thus, it is my opinion that the Petition's Ground 2 fails for claim 3 for the same reasons Ground 1 fails for claims 3-6. *Supra* § VIII.

X. GROUND 3: CLAIMS 3-6 WOULD NOT HAVE BEEN OBVIOUS TO THE POSA IN VIEW OF POTTER AND KONZ

155. The Petition and Dr. Kay's declaration fail to establish both a motivation to combine the teachings of Potter and Konz to develop the high concentration rAAV compositions and a reasonable expectation of success in preventing aggregation using high ionic strength. For at least the reasons I set forth below, Potter and Konz would not lead the POSA to the claimed composition.

A. The Petition Fails to Establish a Motivation to Arrive at an Ionic Strength Greater than 200 mM

156. For similar reasons as discussed above for Ground 1, the combination of Potter and Konz also fails to establish any motivation for the POSA to prepare the claimed rAAV compositions having greater than 200 mM ionic strength. For Ground 3, the Petition and Dr. Kay's declaration rely on the same disclosure in Konz/Evans that, in combination with Wu, failed to establish that the POSA would have been motivated to make the claimed high ionic strength compositions. Potter does not make up for any of the deficiencies in Wu.

157. *First*, the Petition and Dr. Kay's declaration fail to establish that Potter's National Reference Standard rAAV composition has an ionic strength > 200mM. *Second*, Potter does not address rAAV aggregation. The POSA would not have concluded, as the Petition and Dr. Kay contend, that Potter's electron microscopy studies had any relevance to rAAV aggregation within a high concentration rAAV composition. Like Wu, Potter is focused on the purification and characterization of individual monomeric particles. *Third*, the POSA would have had reasons to avoid making the high NaCl concentrations that the Petition and Dr. Kay's declaration advocate would have been obvious.

158. As a result of these deficiencies, neither Potter nor Konz provide the POSA with any reason to make rAAV compositions with an ionic strength greater than 200 mM.

1. Konz/Evans Would Not Motivate the POSA to Make an rAAV Composition with an Ionic Strength Greater than 200 mM

159. For the ionic strength element, the Petition and Dr. Kay rely on Konz's incorporation of Evans by reference. Petition, 54-55 (citing Ex. 1005, ¶¶ 420-422). The Petition makes the same arguments for Ground 3 as in Ground 1, and thus the Petition fails to establish motivation for the same reasons as described for Ground 1. *Supra* §§ VIII.A, VIII.A.1.b, VIII.A.1.d, VIII.2.

160. Konz discloses several formulation buffers, all of which contain a fraction of NaCl (10-75 mM) compared to the maximum 250 mM NaCl concentration in Evans. *Supra* § VIII.A.1.b.i. The Petition disregards the totality of Evans' teachings that would have directed the POSA to lower salt concentrations, and therefore, lower ionic strength rAAV compositions for multiple reasons. *Supra* § VIII.A.1.b.ii-iii.

161. Konz and Evans, considered in their totality, directed the POSA to rAAV compositions having ionic strength values much lower than 200 mM. *Supra* §§ VIII.A.1.b, VIII.A.1.d.

2. Potter Would Not Motivate the POSA to Make the Claimed rAAV Compositions with an Ionic Strength Greater than 200 mM

162. The Petition and Dr. Kay fail to establish that the POSA would have considered Potter to disclose the claimed rAAV compositions with an ionic

strength greater than 200 mM. The Petition cites to Potter’s “National Reference Standard rAAV.” Petition, 51-52; Ex. 1005, ¶¶ 397-403 (citing Ex. 1010, Table II). Potter, however, does not provide specifics as to ionic strength or even to the components of the National Reference Standard rAAV composition.

a. Potter’s rAAV Large-Scale Production Compositions

163. Potter is a book chapter addressing large-scale production of rAAV vectors. Potter describes protocols used to (1) propagate and transfect cells that produce the rAAV; (2) purify rAAV vector; and (3) characterize rAAV. Ex. 1010. Table I estimates the yield of the vector obtained following each step of the purification protocol (i.e., heparin affinity chromatography, HIC phenyl Sepharose chromatography, and heparin affinity chromatography). *Id.*, 7. The material tested in Table 1 are from “three representative vector runs, each consisting of 10 pooled cell factories.” *Id.* Potter states that 1 ml fractions were collected and “each purification stage is titered by [Fluorescent Cell Assay].” *Id.* Thus, Table I sets forth information relevant to the yields that could be achieved using the cell culture and purification methods Potter discusses.

TABLE I
SUMMARY OF rAAV-UF5 PURIFICATION

Run	Crude lysate		Streamline heparin affinity chromatography		HIC phenyl sepharose chromatography		Heparin affinity chromatography	
	Total inf. particles	% yield	Total inf. particles	% yield	Total inf. particles	% yield	Total inf. particles	% yield
1	4.8×10^{12}	100	2.7×10^{12}	56	2.3×10^{12}	48	1.3×10^{12}	27
2	9.6×10^{12}	100	1.8×10^{13}	186	1×10^{13}	104	4.2×10^{12}	43
3	3.6×10^{12}	100	1.2×10^{12}	33	1.3×10^{12}	36	1.2×10^{12}	33

Id.

164. The rAAV product yields are provided in “total inf[ectious] particles,” but Potter does not disclose the rAAV concentration in the eluted rAAV solution. Potter states that the final step results in several “[one] ml fractions . . . collected” of the eluted rAAV solution, but does not state how many total fractions of the purified product are collected. *Id.*, 4-7.

165. It is not possible to determine the concentration of rAAV particles in each 1 ml fraction without knowing the total volume of the collected solutions. For example, if ten one milliliter fractions were pooled to result in the highest yielding run of 4.2×10^{12} infectious particles, each fraction would have a concentration of only 4.3×10^{11} infectious particles/ml, roughly **100X less** than the claimed concentration of 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml. Even if only one ml fraction

were collected, a concentration of $1.2\text{-}4.2 \times 10^{12}$ infectious particles per ml is about **10X less** than the claimed concentration.

166. The POSA would have understood, however, that multiple one milliliter fractions were likely pooled. *See id.*, 16, Fig. 5 (Electron micrograph of virus pooled in fractions 13-15 of iodixanol gradient (Fig. 4B)). The preparation includes empty particles and packaging intermediates. Therefore, the concentrations for each run would have likely been even lower than 10^{13} vg/ml, respectively, for runs 1-3. The POSA would have understood, therefore, that the concentrations in Potter's National Reference Standard composition are considerably higher than the pooled fractions from Table 1. Potter, however, does not explain how it arrives at its National Reference Standard rAAV composition, what components are present in that composition, or the conditions of that composition. A POSA would not assume that the National Reference Standard rAAV composition had the same buffer system as the large scale production batch pooled fractions.

b. Potter Does Not Provide Information the POSA Would Have Required to Determine the Ionic Strength of the National Reference Standard rAAV Compositions

167. The Petition and Dr. Kay's declaration are critical of my opinions regarding Potter in the IPRs filed by Novartis, IPR2025-00608 and IPR2025-00609. Petition, 15-16; Ex. 1005, ¶¶ 252-260. They claim that I failed to recognize

the compositions of Table II of Potter, which are directed to the National Reference Standard composition. This criticism fails to recognize that I was asked to respond to the opinions of Novartis's expert, Dr. Mansoor Amiji. Dr. Amiji did not rely on the compositions of Potter, Table II, to support the now rejected obviousness grounds in the Novartis Petitions. *See* Exs. 1014, 1015.

168. For ionic strength information, the Petition and Dr. Kay cite to the same rAAV composition resulting from the elution of a heparin affinity chromatography purification step that contains 0.5 M (500 mM) NaCl that the Novartis petitions cited. Petition, 53; Ex. 1005, ¶¶ 413-414; Ex. 1010, 7. But for rAAV concentration, the Petition and Dr. Kay point to a different composition in Potter, the National Reference Standard composition, in Table II. *Id.* The Petition and Dr. Kay, however, never explain why the POSA would have understood the National Reference Standard rAAV composition to be the same as the chromatographic purification elution. In fact, based on the information disclosed in Potter, the POSA would not have assumed that the rAAV composition eluted from the heparin affinity column and the National Reference Standard rAAV composition had the same buffer system.

169. Table II relates to the comparison of four different methods of vector characterization: (1) dot blot; (2) Real-time PCR; (3) Infectious Center Assay; and (4) Fluorescent Cell Assay. Ex. 1010, 9. To evaluate these four methods, Potter

uses the National Reference Standard as the material being characterized. *Id.* Potter does not describe how the rAAV material in the National Reference Standard is prepared or what formulation/composition is used to make the compositions. For example, Potter does not provide specifics on ionic strength, pH, or ingredients. Potter also does not state that the rAAV material in the National Reference Standard composition was prepared by the same methods as the material in Table I.

TABLE II
PHYSICAL AND INFECTIOUS TITERS OF NATIONAL REFERENCE STANDARD rAAV
AS DETERMINED BY FOUR ASSAYS

	Dot blot	Real-time PCR	ICA	FCA
NRS rAAV titer	1.12×10^{13} part/ml	1.46×10^{13} part/ml	2.0×10^{12} infect.part/ml	2.16×10^{12} infect.part/ml

Id., 7.

170. Because Potter is silent on how it arrives at the rAAV in its National Reference Standard composition, or what components are present in that composition, the POSA would not infer from Potter that the eluted rAAV composition was the same as the National Reference Standard rAAV. Potter is focused on the purification of particles rather than the development of the final composition. I have not found anything in Potter or Dr. Kay's declaration that sheds light on what the composition of Potter's National Reference Standard rAAV composition is. There is no evidence connecting the elution buffer for the

chromatographic purification on page 7 of Potter with the National Reference Standard rAAV composition on page 9. Dr. Kay's declaration speculates they are the same, but a POSA would require more information, especially given the radically different rAAV concentrations in the two compositions.

171. Thus, the Petition and Dr. Kay's declaration fail to establish that the POSA would have considered Potter to disclose the claimed rAAV compositions with an ionic strength greater than 200 mM.

3. The POSA Would Not Understand Potter to Connect Ionic Strength and rAAV Aggregation

172. As I explained for Ground 1, the Petition fails to identify where Konz connects ionic strength with rAAV aggregation or infectivity. *Supra* § VIII.A.1.b.i. The Petition, likewise, fails to identify where Potter connects ionic strength with aggregation of high concentration rAAV or identify any correlation in the prior art between ionic strength and any other advantageous property, such as rAAV infectivity.

173. The Petition states that "Potter discloses an electron microscopy analysis of the purified rAAV particles ... There is no evidence of aggregation in this study." Petition, 54 (citing Ex. 1010, 16-17, Fig. 5; Ex. 1005, ¶ 419). But Potter never addresses rAAV aggregation in its purified rAAV particles or connects the alleged lack of aggregation with the ionic strength of the storage composition. The Petition claims that Potter's stock compositions are stored in a

buffer containing a 0.5 M NaCl, which would have an ionic strength of 500 mM. *Id.*, 53 (citing Ex. 1010, 7).

174. The composition that is cited for the no significant aggregation element is not the National Reference Standard. Instead, it is a composition that has undergone further chemical and chromatographic transformation, so the particles being analyzed are in a completely different environment from the environment of the composition. *Id.*, 54 (citing Ex. 1010, 16-17, Fig. 5).

175. The Petition alleges that “Potter discloses electron microscopy studies showing that the rAAV stock formulation did not exhibit any observable aggregation.” *Id.*, 6; *see also id.*, 17, 54 (citing Ex. 1010, 16-17, Fig. 5). But as I explained for Wu, the POSA would not have considered electron microscopy useful for detecting aggregates in a rAAV composition, particularly at the magnification used in Potter. *Supra* § VIII.A.1.c.

176. Like Wu, Potter did not use electron microscopy to detect aggregates. Potter used electron microscopy to “confirm the structure of infectious and noninfectious particles.” Ex. 1010, 17. Potter’s electron micrograph images are at far too high a magnification to indicate anything about the degree of aggregation in Potter’s overall composition. Potter’s images show only several hundred particles. The POSA, therefore, would have understood Potter’s use of electron micrographs

to detect full, partial, and empty rAAV particles, not aggregation. *Id.*, 16-17, Fig.

5. Indeed, Potter is silent on aggregates.

177. As with Wu, to visualize the rAAV particles, Potter conducted several steps that drastically changed the surrounding environment of those particles, and this makes it impossible to assess particle aggregation.

178. **First**, “[t]o confirm the structure of infectious and noninfectious particles [by electron microscopy] two peaks from the continuous iodixanol gradient are concentrated by POROS HPLC chromatography.” *Id.*, 17. Iodixanol is an iodine-containing non-ionic radiocontrast agent. Potter explains that a “continuous gradient of iodixanol ... is formed by mixing virus with 30% iodixanol prepared in PBS containing 0.9 M MgCl₂. The sample is transferred into a quick-sealing tube, placed in a Ti-70 rotor, and spun at 70,000 rpm for 8 hr at 15°. After centrifugation the sample is fractionated by puncturing the tube at the bottom and collecting 1 ml fractions.” *Id.*, 14. Then that sample further undergoes POROS chromatography. *Id.*, 17.

179. **Second**, “[f]ollowing chromatography, [electron microscopy] analysis of concentrated samples is performed ... by placing 5 µl of purified virus stock on support films of Formvar/Carbon 400 mesh copper grids ... for 1 min” *Id.*

180. **Third**, “[t]he sample is sample is then stained with 5 µl of 2% uranyl acetate for 10 sec[onds].” *Id.*

181. The separation, isolation, staining, and mounting of the sample significantly altered the composition's chemical environment from the stock composition. The conditions under which electron microscopy was conducted are not representative of the solution from which the sample was drawn, and therefore not useful for determining whether Potter's rAAV composition underwent significant aggregation. Potter's silence on aggregation further confirms that the authors were not attempting to use electron microscopy to detect rAAV aggregations. Neither the Petition nor Dr. Kay address this issue.

182. Accordingly, even if the POSA were to know the compositions and conditions of Potter's stock composition, Potter's electron microscopy studies would not have been useful detecting whether there was aggregation in that stock composition. Thus, the POSA would not have been motivated to use Potter's composition (whatever its components) in rAAV compositions that minimize aggregation.

4. The Petition Does Not Consider that the POSA Would Have Avoided Developing an rAAV Vector Compositions Having the High Salt Concentrations of Potter or Konz/Evans

183. As explained above, the Petition and Dr. Kay's declaration fail to address the many reasons why the POSA would have been deterred from using a high concentration of NaCl to achieve a high ionic strength. *Supra* § VIII.A.2.

184. The POSA would have avoided developing compositions with high osmolarity because such compositions were understood to cause tissue damage and pain upon injection due to hypertonicity. *Supra* § VIII.A.2.a. The Petition, however, argues that Potter's 0.5M NaCl solutions renders the claimed ionic strength element obvious. Petition, 53. The POSA would have avoided making a therapeutic composition with 0.5 M NaCl because that composition would be extremely hypertonic. Each Na⁺ and Cl⁻ ion contributes a unit of osmolarity. *Supra* § VIII.A.2.a. Therefore, a composition with 0.5 M (500 mM) NaCl concentration would have an osmolarity of 1000 mOsm—over three times the osmolarity of an isotonic solution. The POSA would not have considered Potter's high NaCl composition as viable for use in a therapeutic

185. The Evans buffer cited by the Petition and Dr. Kay's declaration contains 250 mM NaCl and 5 mM MgCl₂, giving it an osmolarity of 515 mOsm (500 mOsm from NaCl and 15 mOsm from MgCl₂). This osmolarity is much higher than 300 mOsm serum, and therefore this composition would be hypertonic. *Supra* § VIII.A.2.a. The POSA, concerned about the adverse effects of hypertonicity, including pain and tissue damage, would not have been motivated to use a buffer formulation with an osmolarity of 1000 mOsm or 515 mOsm.

186. High salt concentration solutions were also observed to cause instability of rAAV particles. *Supra* § VIII.A.2.b. Xie stated that a solution of

“0.25 M NaCl [250 mM] resulted in **significant loss**” of AAV. Ex. 1028, 23. Xie, however, was not the only reference to suggest that high salt concentrations could lead to undesirable results for rAAV compositions. For example, WO 99/41416, published Aug. 19, 1999, to Frei et al. (Ex. 2020), which was relied-upon prior art in IPR2023-00608, states that the POSA “must take into account the additional **threat to stability posed by the high salt concentrations.**” Ex. 2020, 19:9-14; *see also id.*, 18:19-24; 21:25-26; 21:42-22:6.

187. The Petition and Dr. Kay’s declaration rely on the 500 mM NaCl composition in Potter and the 250 mM NaCl solution in Konz/Evans to argue that the claimed ionic strength would have been obvious. But Potter’s 500 mM NaCl concentration doubles the NaCl concentration observed by Xie to result in aggregation, while the cited NaCl concentration in Konz/Evans is the same as Xie’s concentration. The POSA’s background knowledge and Evans’ examples demonstrated that lower NaCl concentration viral particle compositions were more stable than higher NaCl concentration compositions (*supra* § VIII.A.2.b) and would have deterred the POSA from making high ionic strength compositions. The POPR would have considered the background references identified by Dr. Kay’s declaration irrelevant to the development of rAAV compositions. *Supra* § VIII.A.1.2.c

B. Ground 3 Fails to Establish a Reasonable Expectation that a High Ionic Strength rAAV Composition Could be Stored Without Significant Aggregation

188. For the same reasons explained for Ground 1, the Petition fails to establish a reasonable expectation, based on Potter and Konz, that the claimed compositions having an ionic strength greater than 200 mM could have been stored without significant aggregation. *Supra* § VIII.B. The POSA would have found it unpredictable to develop high concentration rAAV compositions that remain stable without significant aggregation. *Supra* § VIII.B.1. As I explained, Konz's aggregation and stability studies did not address the unique challenges of formulating **high concentration** rAAV compositions that do not exhibit significant aggregation when stored. *Supra* § VIII.B.2.

189. The Petition and Dr. Kay's declaration fail to establish that Potter's stock solution has an ionic strength of greater than 200 mM. *Supra* § X.A.2. Potter does not even disclose basic information about the pH, ionic strength, or composition of stock solution; the Petition merely speculates, without supporting evidence, that the buffer system in the stock solution is the same as used for purification. *Supra* § X.A.2.b.

190. Because of this lack of information, the POSA would not have been able to discern what components of Potter's formulation may contribute to Wu's storage data. Petition, 29 (citing Ex. 1010, 17; Ex. 1005, ¶¶ 243-46). Further, the

POSA would not have considered Potter's electron microscopy analysis as accurately measuring the extent of aggregation in Potter's stock solution. *Supra* § X.A.3. In my opinion, Potter lacks any disclosure regarding both ionic strength and aggregation that the POSA would need to determine whether elevated ionic strength decreased aggregation.

191. Thus, the Petition and Dr. Kay fail to point to any disclosure in Potter, Konz, or Evans that would have supplied the POSA with a reasonable expectation that compositions comprising rAAV particles at concentrations $>10^{13}$ vg/ml and an ionic strength greater than 200 mM would not result in significant aggregation.

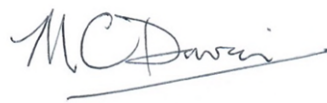
XI. GROUND 4: CLAIM 3 WOULD NOT HAVE BEEN OBVIOUS TO THE POSA IN VIEW OF POTTER, KONZ, AND CROYLE

192. Claim 3 requires, *inter alia*, the same combination of elements that the Petition fails to render obvious through Wu and Konz in Ground 1. The Petition cites Croyle (Ex. 1009) solely to address the Pluronic F68 claim requirements in claim 2 and 3. Petition, 60-64. For the reasons I set forth above, the addition of Croyle does not overcome the deficiencies of the Petition for the other elements of claim 3. Thus, Ground 4 fails for claim 3 for the same reasons Ground 1 fails for claims 3-6. *Supra* § X.

XII. 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: October 8, 2025

A handwritten signature in blue ink, appearing to read "MCDavies", is written above a horizontal line.

Dr. Martyn C. Davies