UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.,

Petitioner

v.

GENZYME CORPORATION,

Patent Owner

U.S. Patent No. 9,051,542

"Compositions and Methods to Prevent AAV Vector Aggregation"

IPR2025-01194

DECLARATION OF MARK A. KAY, M.D., PH.D., IN SUPPORT OF PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 9,051,542

Sarepta Ex. 1005

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I, Mark Kay, M.D., Ph.D., hereby declare as follows.

1. I have been retained as an expert witness on behalf of Sarepta Therapeutics, Inc. ("Sarepta") in connection with the above-captioned request for *inter partes* review ("IPR"). I am being compensated for my time in connection with this IPR at my standard consulting rate, which is $1200 per hour.

2. I understand that Sarepta is petitioning for *inter partes* review of U.S. Patent No. 9,051,542 ("the '542 patent") (EX1001) and requests that the United States Patent and Trademark Office cancel claims 3-6 of the '542 patent as unpatentable. The following discussion and analysis provides my opinions as to why claims 3-6 would have been obvious to a person of ordinary skill in the art ("POSA").[1]

I. BACKGROUND AND QUALIFICATIONS

3. I am the Dennis Farrey Family Professor of Pediatrics and Genetics at Stanford University in Stanford, CA. I also hold an appointment as the Head of the

[1] I understand that, on June 15, 2023, Genzyme disclaimed claims 1 and 2 of the '542 patent. EX1019 (Statutory Disclaimer). Because claims 3-6 are dependent claims that depend from claims 1 and 2, I have considered claims 1 and 2 in my analysis of obviousness, as discussed further below.

Division of Human Gene Therapy in the Department of Pediatrics at Stanford University.

4. I received a B.Sc. degree in physical sciences from Michigan State University in 1980. I received my Ph.D. in developmental genetics from Case Western Reserve University in Ohio in 1986. I also received my M.D. from Case Western Reserve University in 1987. I then performed an internship and residency at Baylor College of Medicine in Houston, Texas from 1987 through 1990, and a medical genetics clinical fellowship and postdoctoral research on gene therapy for hepatic deficiencies at the Baylor College of Medicine from 1990 through 1993.

5. In 1993, I became an assistant professor in the Department of Medicine and the Markey Molecular Medicine Center at the University of Washington. In 1997, I was promoted to associate professor of Medicine with adjuncts in Pediatrics, Biochemistry and Pathology at the University of Washington. I moved to the Stanford University School of Medicine in 1998, where I became a professor in 2001 in the Departments of Pediatrics and Genetics.

6. For the past 30 years, I have led an active academic research program and collaborated with industry in various settings.

7. Over the course of my research career, I have published over 275 peer-reviewed articles and 5 book chapters. These publications cover a variety of topics

related to gene therapy; many of them are related to recombinant adeno-associated virus ("rAAV") vectors.

8. I have supervised or advised on clinical trials of gene therapy using rAAV:

- Phase I/II AAV-human factor IX mediated gene transfer into skeletal muscle 1998-1999 Co-PI; 1999-2001 Scientific Advisor;
- Phase I/II AAV-human factor IX mediated gene transfer into liver IND BB-9398 Holder 1/2001-1/2002; Scientific Advisor 2002-2005;
- Phase I/II AAV-2/8-human factor IX mediated gene transfer into the liver. Co-Investigator 2009-2013.

9. I was a scientific co-founder of Voyager Therapeutics (2013) and a co-founder of LogicBio Therapeutics (2014), both of which develop gene therapy for treating various human diseases using AAV vectors.

10. I am currently on or have served on the following editorial boards and/or served as the editor for the following publications: Gene Therapy; Human Gene Therapy; Molecular Therapy; Silence; and Nucleic Acid Therapeutics.

11. I have received a number of scientific awards throughout my education and career. In 2000, I received the E. Mead Johnson Award for Pediatric Researcher of the Year, and was elected as the National Hemophilia Foundation Researcher of the Year. In 2010, I became the elected member of the Association for American Physicians. In 2013, I received the Samuel Rosenthal Prize in Pediatrics and the Outstanding Investigator Award from the American Society of Gene and Cell

Therapy. In 2015, I received the Stanford OTL Outstanding Inventor Award. In 2020, I was elected to the National Academy of Inventors.

12. EX1006 is a copy of my *curriculum vitae* setting forth additional information concerning my background, credentials, publications, and awards.

II. MATERIALS CONSIDERED

13. In formulating my opinions, I considered all of the references cited in this Declaration, which are set out in the table below, including the following documents:

- U.S. Patent No. 9,051,542 ("the '542 patent") (EX1001);
- Wu *et al*., "A novel method for purification of recombinant adeno-associated virus vectors on a large scale," *Chinese Science Bulletin*, Vol. 46, 2001, 485-89 ("Wu") (EX1007);
- WO 03/097797 A1 (PCT/US03/15061), "Methods of Adenovirus Purification," International Publication Date Nov. 27, 2003 ("Konz") (EX1008);
- Croyle, *et al*. "Development of novel formulations that enhance adenoviral-mediated gene expression in the lung in vitro and in vivo," *Molecular Therapy* (2001): 22-28 ("Croyle") (EX1009);
- Potter *et al*., "Streamlined Large-Scale Production of Recombinant Adeno-Associated Virus (rAAV) Vectors," Methods in Enzymology, Vol. 346, 2002, 413-430 ("Potter") (EX1010).

Exhibit Number	Description
EX1001	U.S. Patent No. 9,051,542 ("the '542 patent")
EX1002	Prosecution history of U.S. Patent No. 9,051,542 ("the '542 prosecution history")
EX1003	U.S. Provisional Patent Application No. 60/575,997 (filed June 1, 2004) ("the '997 provisional")
EX1004	U.S. Provisional Patent Application No. 60/639,222 (filed Dec. 22, 2004) ("the '222 provisional")
EX1006	*Curriculum vitae* of Dr. Mark A. Kay ("Kay CV")
EX1007	Wu *et al*., "A novel method for purification of recombinant adeno-associated virus vectors on a large scale," *Chinese Science Bulletin*, Vol. 46, 2001, 485-89 ("Wu")
EX1008	WO 03/097797 A1 (PCT/US03/15061), "Methods of Adenovirus Purification," International Publication Date Nov. 27, 2003 ("Konz")
EX1009	Croyle *et al*., "Development of Novel Formulations That Enhance Adenoviral-Mediated Gene Expression in the Lung *in Vitro* and *in Vivo*, *Molecular Therapy* (2001): 22-28 ("Croyle")
EX1010	Potter *et al*., "Streamlined Large-Scale Production of Recombinant Adeno-Associated Virus (rAAV) Vectors," *Methods in Enzymology*, Vol. 346, 2002, 413-30 ("Potter")
EX1014	IPR2023-00608, Petition for *Inter Partes* Review of U.S. Patent No. 9,051,542 ("608 Petition")
EX1015	IPR2023-00609, Petition for *Inter Partes* Review of U.S. Patent No. 9,051,542 ("609 Petition")

Exhibit Number	Description
EX1016	IPR2023-00608, Petition for *Inter Partes* Review of U.S. Patent No. 9,051,542, Patent Owner's Prelimiary Response ("608 POPR")
EX1017	IPR2023-00608, Petition for *Inter Partes* Review of U.S. Patent No. 9,051,542, Decision Denying Institution ("608 Decision")
EX1018	IPR2023-00609, Petition for *Inter Partes* Review of U.S. Patent No. 9,051,542, Decision Denying Institution ("609 Decision")
EX1019	Patent Owner's Statutory Disclaimer ("Disclaimer")
EX1020	WO 01/66137 A1 (PCT/US01/07194), "Adenovirus Formulations," International Publication Date Sept. 13, 2001 ("Evans")
EX1021	Verma and Somia, "Gene therapy – promises, problems and prospects," *Nature*, Vol. 389, 1997 ("Verma")
EX1022	Clark *et al*., "Highly Purified Recombinant Adeno-Associated Virus Vectors Are Biologically Active and Free of Detectable Helper and Wild-Type Viruses," *Human Gene Therapy*, 10:1031-1039 (1999) ("Clark")
EX1023	Hermens *et al*., "Purification of Recombinant Adeno-Associated Virus by Iodixanol Gradient Ultracentrifugation Allows Rapid and Reproducible Preparation of Vector Stocks for Gene Transfer in the Nervous System," *Human Gene Therapy* 10:1885-1891 (1999) ("Hermens")
EX1024	Girod *et al*., "The VP1 capsid protein of adeno-associated virus type 2 is carrying a phospholipase A2 domain required for virus infectivity," *Journal of General Virology* 83.5 (2002): 973-978 ("Girod")

Exhibit Number	Description
EX1025	Salvetti *et al*., "Factors Influencing Recombinant Adeno-Associated Virus Production," *Human Gene Therapy*, 9:695-706 (1998) ("Salvetti")
EX1026	Hauswirth *et al*., "Production and Purification of Recombinant Adeno-Associated Virus," *Methods in Enzymology*, Vol. 316, 2000, pp. 743-61 ("Hauswirth")
EX1027	Grimm and Kay, "From Virus Evolution to Vector Revolution: Use of Naturally Occurring Serotypes of Adeno-Associated Virus (AAV) as Novel Vectors for Human Gene Therapy," *Current Gene Therapy*, 2003, 3, 281-304 ("Grimm and Kay")
EX1028	Xie *et al*., "Large-scale production, purification, and crystallization of wild-type adeno-associated virus-2," *J. Virol. Methods*, 122 (2004) 17-27 ("Xie")
EX1029	Tamayose *et al*., "A New Strategy for Large-Scale Preparation of High-Titer Recombinant Adeno-Associated Virus Vectors by Using Packaging Cell Lines and Sulfonated Cellulose Column Chromatography," *Human Gene Therapy*, 7:507-513 (1996) ("Tamayose")
EX1030	Floyd and Sharp, "Aggregation of Poliovirus and Reovirus by Dilution in Water," *Applied and Environmental Microbiology*, p. 159-167 (1977) ("Floyd I")
EX1031	Floyd and Sharp, "Viral Aggregation: Effects of Salts on the Aggregation of Poliovirus and Reovirus at Low pH," *Applied and Environmental Microbiology*, pp. 1084-1094 (1978) ("Floyd II")
EX1032	Floyd and Sharp, "Viral Aggregation: Buffer Effects in the Aggregation of Poliovirus and Reovirus at Low and High pH," *Applied and Environmental Microbiology*, pp. 395-401 (1979) ("Floyd III")

Exhibit Number	Description
EX1033	Kegel and van der Schoot, "Competing Hydrophobic and Screened-Coulomb Interactions in Hepatitis B Virus Capsid Assembly," *Biophysical Journal* (2004), 3905-3913 ("Kegel")
EX1034	Davidoff *et al*., "Purification of recombinant adeno-associated virus type 8 vectors by ion exchange chromatography generates clinical grade vector stock," *Journal of Virological Methods* (2004): 209-215 ("Davidoff")
EX1035	Dika *et al*., "Impact of Internal RNA on Aggregation and Electrokinetics of Viruses: Comparison between MS2 Phage and Corresponding Virus-Like Particles," *Applied and Environmental Microbiology* (2011): 4939-4948 ("Dika")
EX1036	De Sá Magalhães *et al*., "Quality assessment of virus-like particle: A new transmission electron microscopy approach," *Frontiers in Molecular Biosciences* (2022): 975054, ("De Sá Magalhães")
EX1037	Janc *et al*., "In-Depth Comparison of Adeno-Associated Virus Containing Fractions after CsCl Ultracentrifugation Gradient Separation," *Viruses* (2024): 1235 ("Janc")
EX1038	Dobnik *et al*., "Accurate Quantification and Characterization of Adeno-Associated Viral Vectors," *Frontiers in Microbiology* (2019): 1570 ("Dobnik")
EX1039	Stagg, *et al*. "Cryo-electron Microscopy of Adeno-Associated virus," *Chemical Reviews* 122.17 (2022): 14018-14054 ("Stagg")
EX1040	Hoggan *et al.*, "Studies of small DNA viruses found in various adenovirus preparations: physical, biological, and immunological characteristics," *Proceedings of the National Academy of Sciences* 55.6 (1966): 1467-1474 ("Hoggan")

Exhibit Number	Description
EX1041	Johnson and Bodily, "Effect of environmental pH on adenovirus-associated virus," *Proceedings of the Society for Experimental Biology and Medicine*, 150.3 (1975): 585-590 ("Johnson")
EX1042	Zolotukhin *et al*., "Recombinant adeno-associated virus purification using novel methods improves infectious titer and yield," *Gene Therapy* (1999): 973-985 ("Zolotukhin")
EX1043	Huang *et al*., "Aggregation of AAV Vectors, its Impact on Liver-directed Gene Transfer and Development of Vector Formulations to Prevent and Dissolve Aggregation and Enhance Gene Transfer Efficiency," *Molecular Therapy*, Vol. 1, No. 5, May 2000, S286 ("Huang")
EX1044	Qu *et al*., "Evidence That Ionic Interactions Are Involved in Concentration-Induced Aggregation of Recombinant Adeno-Associated Virus," *Molecular Therapy*, Vol. 7, No. 5, May 2003, S348 ("Qu")
EX1045	Wright *et al*., "Recombinant adeno-associated virus: Formulation challenges and strategies for a gene therapy vector," 8(2) *Current Opinion in Drug Discovery & Development* 2003: 174-178 ("Wright 2003")
EX1046	Wright *et al*., "Formulation Development for AAV2 Vectors: Identification of Excipients That Inhibit Vector Aggregation," *Molecular Therapy*, Vol. 9, Supp. 1, May 2004, S163 ("Wright 2004")
EX1047	Wright *et al*., "Identification of Factors that Contribute to Recombinant AAV2 Particle Aggregation and Methods to Prevent Its Occurrence during Vector Purification and Formulation," *Molecular Therapy*, 2005, pp. 171-78 ("Wright 2005")

Exhibit Number	Description
EX1048	Weichert *et al*., "Assaying for Structural Variation in the Parvovirus Capsid and Its Role in Infection," *Virology* 250, 106-117 (1998) ("Weichert")
EX1049	Okada *et al*., "Scalable Purification of Adeno-Associated Virus Serotype 1 (AAV1) and AAV8 Vectors, Using Dual Ion-Exchange Adsorptive Membranes," *Human Gene Therapy* 20.9 (2009): 1013-1021 ("Okada")
EX1050	Venkatakrishnan *et al*., "Structure and Dynamics of Adeno-Associated Virus Serotype 1 VP1-Unique N-Terminal Domain and Its Role in Capsid Trafficking," *Journal of Virology* 87.9 (2013): 4974-4984 ("Venkatakrishnan")
EX1051	Tibbetts and Giam, "In Vitro Association of Empty Adenovirus Capsids with Double-Stranded DNA," *Journal of Virology* 32.3 (1979): 995-1005 ("Tibbetts")
EX1052	Huyghe *et al*., "Purification of a Type 5 Recombinant Adenovirus Encoding Human p53 by Column Chromatography," *Human Gene Therapy* 6.11 (1995): 1403-1416 ("Huyghe")
EX1053	Roth and Jeltsch, "Biotin-Avidin Microplate Assay for the Quantitative Analysis of Enzymatic Methylation of DNA by DNA Methyltransferases," *Biol. Chem*., Vol. 381, pp. 269 – 272, March 2000 ("Roth")
EX1054	O'Riordan *et al*., "Scaleable chromatographic purification process for recombinant adeno-associated virus (rAAV)," *The Journal of Gene Medicine*, 2.6 (2000): 444-454 ("O'Riordan")
EX1055	Kreilgaard *et al*. "Effect of Tween 20 on Freeze-Thawing- and Agitation-Induced Aggregation of Recombinant Human Factor XIII," *Journal of Pharmaceutical Sciences* (1998): 1593-1603 ("Kreilgaard")

Exhibit Number	Description
EX1056	Auricchio *et al*., "Isolation of Highly Infectious and Pure Adeno-Associated Virus Type 2 Vectors with a Single-Step Gravity-Flow Column," *Human Gene Therapy* (2001): 71-76 ("Auricchio")
EX1057	*Phosphate-buffered saline,* COLD SPRING HARBOR LABORATORY PRESS (2006), https://cshprotocols.cshlp.org/content/2006/1/pdb.rec8247
EX1058	Kessler *et al*., "Gene delivery to skeletal muscle results in sustained expression and systemic delivery of a therapeutic protein," *Proceedings of the National Academy of Sciences* (1996): 14082-14087 ("Kessler")
EX1059	U.S. Patent Application Publication No. 2004/0209245 ("the '245 Publication")
EX1060	IPR2023-00608, Petition for Inter Partes Review of U.S. Patent No. 9,051,542, Declaration of Martyn C. Davies ("608 Davies Decl.")
EX1061	Claim Construction Order, *Genzyme Corp. v. Novartis Gene Therapies, Inc.*, C.A. No. 21-1736 (RGA), D.I. 268 (D. Del. Aug. 30, 2023) ("Claim Construction Order")
EX1062	Memorandum Opinion, *Genzyme Corp. v. Novartis Gene Therapies, Inc.*, C.A. No. 21-1736 (RGA), D.I. 263 (D. Del. Aug. 18, 2023) ("Claim Construction Opinion")
EX1063	Joint Claim Construction Brief, *Genzyme Corp. v. Novartis Gene Therapies, Inc.*, C.A. No. 21-1736 (RGA), D.I. 101 (D. Del. Jan. 13, 2023) ("Claim Construction Brief")
EX1064	Sommer *et al*., "Quantification of Adeno-Associated Virus Particles and Empty Capsids by Optical Density Measurement," *Molecular Therapy* (2003): 122-128 ("Sommer")

Exhibit Number	Description
EX1065	Yeung and Tufaro, "Virus Vectors for Gene Therapy of the Nervous System," in *Protocols for Neural Cell Culture*, 3d edition, Fedoroff and Richardson, eds., 2001, pp. 229-44 ("Yeung")
EX1066	Grimm *et al*. "Novel Tools for Production and Purification of Recombinant Adenoassociated Virus Vectors," *Human Gene Therapy* 9 (18) (1998): 2745-2760 ("Grimm")
EX1067	Grieger *et al*., "Production and characterization of adeno-associated viral vectors," *Nature Protocols* (2006), 1412-1428 ("Grieger")
EX1068	Schwartz, "Diafiltration for Desalting or Buffer Exchange," *BioProcess International*, May 2003, pp. 43-49 ("Schwartz")
EX1069	Hatano *et al*. "Immunogenic and Antigenic Properties of a Heptavalent High-Molecular-Weight O-Polysaccharide Vaccine Derived from Pseudomonas aeruginosa," *Infection and Immunity* (1994): 3608-3616 ("Hatano")
EX1070	Monahan *et al*., "Direct intramuscular injection with recombinant AAV vectors results in sustained expression in a dog model of hemophilia," *Gene Therapy* (1998): 40-49 ("Monahan")
EX1071	Oster, "The isoelectric points of some strains of tobacco mosaic virus," *J. Biol. Chem.* 190 (1951): 55-59 ("Oster")
EX1072	Konz *et al*., "Development of a Purification Process for Adenovirus: Controlling Vrius Aggregation to Improve the Clearance of Host Cell DNA," *Biotechnol. Prog*. 2005, 21, 466-472 ("Konz 2005")

Exhibit Number	Description
EX1073	Conway *et al*., "High-titer recombinant adeno-associated virus production utilizing a recombinant herpes simplex virus type I vector expressing AAV-2 Rep and Cap," *Gene Therapy* (1999): 986-993 ("Conway")
EX1074	Booth *et al*., "Transfection-free and scalable recombinant AAV vector production using HSV/AAV hybrids," *Gene Therapy* (2004): 829-837 ("Booth")
EX1075	Dufour *et al*., "Toxicity and Efficacy Evaluation of an Adeno-Associated Virus Vector Expressing Codon-Optimized RPGR Delivered by Subretinal Injection in a Canine Model of X-linked Retinitis Pigmentosa," *Human Gene Therapy* (2020): 253-267 ("Dufour")
EX1076	Flotte *et al*., "Phase 2 Clinical Trial of a Recombinant Adeno-Associated Viral Vector Expressing α1-Antitrypsin: Interim Results," *Human Gene Therapy* (2011): 1239-1247 ("Flotte")
EX1077	Keeler and Flotte, "Recombinant Adeno-Associated Virus Gene Therapy in Light of Luxturna (and Zolgensma and Glybera): Where Are We, and How Did We Get Here?" *Annual Review of Virology* (2019): 601-621 ("Keeler")
EX1078	Davidsson *et al*., "A comparison of AAV-vector production methods for gene therapy and preclinical assessment," *Scientific Reports* (2020): 21532 ("Davidsson")
EX1079	T.W. Graham Solomons, ORGANIC CHEMISTRY (5th ed. 1992) ("Solomons")
EX1080	Bates and Acree, "pH Values of Certain Phosphate-Chloride Mixtures and the Second Dissociation Constant of Phosphoric Acid From 0° to 60° C," *J. Res. Natl. Bur. Stand*. 30.2 (1943): 129-155 ("Bates")

Exhibit Number	Description
EX1081	Po and Senozan, "The Henderson-Hasselbalch Equation: Its History and Limitations," *Journal of Chemical Education* 78.11 (2001): 1499 ("Po")
EX1082	Green, "The Preparation of Acetate and Phosphate Buffer Solutions of Known pH and Ionic Strength," *Journal of the American Chemical Society* 55.6 (1933): 2331-2336 ("Green")
EX1083	CURRENT PROTOCOLS IN NUCLEIC ACID CHEMISTRY (2000) A.2A.1-A.2A.12 (2000) ("Current Protocols")

III. LEGAL STANDARDS – OBVIOUSNESS

14. In this section, I describe my understanding of certain legal standards relating to the issue of obviousness that I have been asked to consider for claims 3-6 of the '542 patent. These legal standards have been explained to me in connection with the preparation of this Declaration. I have applied these standards in my analysis, as described in the sections below.

15. I understand that a claim is obvious when the differences between the claim and the prior art are such that the claim as a whole would have been obvious to a POSA at the relevant time. It is my understanding that four factors are applied in determining whether a claim is unpatentable as obvious under 35 U.S.C. § 103: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at issue; (3) the level of ordinary skill in the art; and (4) objective

evidence indicating obviousness or non-obviousness – also referred to as "secondary considerations" – if present.

16. I understand that secondary considerations may include: (1) long felt but unmet need in the prior art that was satisfied by the invention of the patent; (2) commercial success or lack of commercial success of the subject matter claimed in the patent; (3) unexpected results achieved by the invention; (4) praise of the invention by others skilled in the art; (5) the taking of licenses under the patent by others; and (6) deliberate copying of the invention.

17. I understand that a claim can be obvious over a single reference in combination with the knowledge of a POSA, or based on the teachings in a combination of references. For obviousness based on a combination of prior art references, I understand that a POSA must have a motivation to combine the references. I understand that the prior art references themselves may provide a suggestion, motivation, or reason to combine. I further understand that a motivation to combine two or more prior art references need not be express, but may be based upon common sense or the knowledge available to a POSA.

18. In addition, I understand that a POSA must have a reasonable expectation of success in modifying or combining the prior art to arrive at the claimed invention. However, I understand that obviousness cannot be avoided merely because there is some degree of unpredictability in the art.

19. Finally, I understand that it is impermissible to evaluate obviousness from a hindsight perspective, using the teachings of the patent as a guide. However, I understand that a POSA is not an automaton, and is assumed to have a reasonable degree of creativity. As such, I understand that an analysis of obviousness may take account of the inferences and creative steps that a POSA would employ.

IV. SUMMARY OF INVALIDITY GROUNDS

20. The table below summarizes the invalidity grounds for claims 3-6 of the ’542 patent that I address in this declaration.

Ground	Claims	Description
1	3-6	Obvious in view of Wu and Konz
2	3	Obvious in view of Wu, Konz, and Croyle
3	3-6	Obvious in view of Potter and Konz
4	3	Obvious in view of Potter, Konz, and Croyle

21. I note that Wu, Potter, Konz, and Croyle were not considered by the Patent Office during prosecution. EX1001 (the ’542 patent) (“References Cited”); EX1002 (the ’542 Prosecution History).

22. Wu was published in March 2001, more than one year before the earliest priority date listed on the face of the ’542 patent, June 1, 2004. EX1001 (the ’542 patent); EX1007 (Wu).

23. Konz was filed on May 13, 2003, and published on November 27, 2003. EX1008 (Konz). Konz was therefore filed and published before June 1, 2004, the earliest priority date listed on the face of the '542 patent, and published more than one year before December 22, 2004, the filing date of the '222 provsional application. EX1001 (the '542 patent); EX1004 (the '222 provisional).

24. Croyle was published in July 2001, more than one year before the earliest priority date listed on the face of the '542 patent. EX1009 (Croyle); EX1001 (the '542 patent).

25. Potter was published in 2002, more than one year before the earliest priority date listed on the face of the '542 patent, June 1, 2004. EX1001 (the '542 patent); EX1010 (Potter).

26. As discussed further below (Section VIII.A.4), Potter was cited as a background reference, but not addressed by the Board, in two earlier IPR petitions brought by a different petitioner, Novartis, challenging the validity of claims 1, 2, 5, and 6 of the '542 patent – IPR2023-00608 and IPR2023-00609. EX1014 (608 Petition); EX1015 (609 Petition). Claim 3, which is one of the challenged claims here, was not challenged in either of the prior petitions. The PTAB denied institution of the Novartis petitions but did not address Potter in either decision denying institution. EX1017 (608 Decision); EX1018 (609 Decision). Notably, as I discuss in detail below (Section VIII), Patent Owner and its expert, Dr. Martyn Davies,

materially mischaracterized Potter and the state of the art as of 2004 in their preliminary response to the 608 Petition. EX1016 (608 POPR); EX1060 (608 Davies Decl.). They did not address Potter in their preliminary response to the 609 Petition.

V. TECHNICAL BACKGROUND AND STATE OF THE PRIOR ART

A. Gene Therapy

27. Gene therapy is the term used for putting corrective genetic material into cells to alleviate the symptoms of disease. *See, e.g.*, EX1021 (Verma), Abstract. Gene therapy requires the development of methods to place sufficient amounts of therapeutic DNA into the correct cell types to alleviate the symptoms of a particular disease. *See* EX1021 (Verma), p.1. The promise of gene therapy includes treating the symptoms of a variety of different genetic diseases, slowing tumor progression, and arresting the progress of neurodegenerative diseases. *See* EX1021 (Verma), p. 1. Gene therapy involves delivering therapeutic genetic material to somatic (non-reproductive) cells, for example, gene therapy delivered to the lung to treat cystic fibrosis, or gene therapy delivered to liver cells to treat haemophilia. *See* EX1021 (Verma), p. 1.

28. A number of different viruses have been studied as potential gene therapy vectors to deliver therapeutic DNA to target cells. *See* EX1021 (Verma),

pp. 1-3 (discussing retroviral vectors, lentiviral vectors, adenoviral vectors, and adeno-associated viral vectors).

B. Adeno-Associated Virus (AAV)

29. Adeno-associated virus (AAV) is a replication-defective human parvovirus that has been studied for decades as a possible gene therapy vector. *See, e.g.*, EX1022 (Clark), Abstract; EX1023 (Hermens), Abstract; EX1024 (Girod), p. 1.

30. Multiple features of AAV make the virus attractive as a vector for gene therapy, including its ability to infect a wide range of tissues, such as muscle, retina, and liver, and the fact that it does not cause any known human diseases. *See, e.g.*, EX1025 (Salvetti), p. 2; EX1026 (Hauswirth), pp. 1-2; EX1027 (Grimm and Kay), p. 1.

31. AAV has a linear, single-stranded DNA genome of about 4681 nucleotides, which is packaged into an icosahedral particle. *See* EX1024 (Girod), p. 1; EX1027 (Grimm and Kay), p. 3. The capsid is non-enveloped, and is about 20 nm in diameter. *See* EX1027 (Grimm and Kay), p. 3. The capsid consists of three structural proteins, VP1, VP2, and VP3, which are expressed from the same open reading frame by using alternative splicing and an atypical start codon. *See* EX1024 (Girod), pp. 1-2, Fig. 1A. The capsid proteins, VP1, VP2, and VP3, are produced in a 1:1:10 ratio. *See* EX1027 (Grimm and Kay), p. 3.

32. Recombinant AAVs ("rAAVs") are AAVs in which the AAV genes have been replaced by genes that are potentially therapeutic. EX1028 (Xie), pp. 1-2. AAVs occur naturally in a variety of different serotypes, each with its own specific genome sequence and physical properties. *See, e.g.*, EX1027 (Grimm and Kay), p. 2, Table 1. The first serotype that was studied was AAV2, and was observed to have some advantages and some drawbacks for use as a gene therapy vector. *See* EX1027 (Grimm and Kay), p. 1. Subsequently, other serotypes were identified and studied to determine whether they were free from some of the problems with AAV2. *See* EX1027 (Grimm and Kay), p. 2.

C. Purification and Production of rAAV

33. Since the 1990s, researchers have been working to develop methods to produce high titer, pure, large scale preparations of rAAV. *See, e.g.*, EX1029 (Tamayose). In general, these techniques involve three basic components: (1) an AAV vector plasmid containing a transgene expression cassette flanked by inverted terminal repeats ("ITRs"), which are AAV packaging signals; (2) AAV *rep* and *cap* genes, encoding Rep proteins for replication and encapsidation of the vector genome and capsid proteins to build the capsid shell; (3) adenoviral genes that provide helper functions for AAV to generate particles. EX1027 (Grimm and Kay), p. 6. These components are then delivered to "packaging" cells by transient transfection of the

cells with plasmids containing the three components listed above. *See* EX1027 (Grimm and Kay), p. 6.

34. Density gradient (CsCl) centrifugation and column chromatography have both been used to purify rAAV from crude packaging cell lysates. *See, e.g.*, EX1022 (Clark), p. 2; EX1027 (Grimm and Kay), p. 8. Columns including heparin columns and anion exchange columns have been used successfully for rAAV purification. *See, e.g.*, EX1027 (Grimm and Kay), p. 8.

35. It was known in the art that certain AAV purification methods, such as particular types of gradient purification, would remove empty capsids from the preparation, while others, such as column chromatography, would not. *See, e.g.*, EX1010 (Potter), pp. 14-17.

D. Viral Aggregation

36. A POSA at the relevant time would have been aware of the phenomenon of aggregation of AAV particles, for example during storage at 4°C or during dialysis, resulting in a loss of infectivity. *See, e.g.*, EX1023 (Hermens), p. 5. Hydrophobic interactions between capsid proteins were believed to cause viral particle aggregation. *See, e.g.*, EX1033 (Kegel), p. 7. It was known in particular that empty AAV capsids have a tendency to aggregate during dialysis. *See, e.g.*, EX1023 (Hermens), p. 6. In addition, the size of AAV aggregates was known to be concentration dependent – the higher the concentration, the larger the aggregates and

the less efficient the gene transfer. *See, e.g.*, EX1043 (Huang) (disclosing that when the rAAV vector titer reached 5-10 x 10^{13} genome copies ("GCs") per ml, gene transfer efficiency was 10-100 fold lower at the same dose as it was with the same rAAV vector at a titer of 1-5 x 10^{12} GCs/ml).

37. Factors that influence aggregation of viral particles, including the effects of ionic strength, pH, and the presence of ions such as Na^+ and multivalent ions such as Mg^{2+}, have been studied since at least the 1970s. *See, e.g.*, EX1030 (Floyd I); EX1031 (Floyd II); EX1032 (Floyd III); *see also* EX1041 (Johnson), p. 6 (finding that purified AAV particles aggregated at pH 7.2 and below, but that no aggregates were observed at pH 7.5); EX1046 (Wright 2004) (stating that divalent salts inhibit aggregation of AAV2 at a lower molar concentration than NaCl).

38. The Floyd studies showed that dilution of viral particles can result in aggregation. *See, e.g.*, EX1030 (Floyd I), Abstract. Aggregation was found to depend on the composition of the diluting liquid. EX1030 (Floyd I), Abstract. For example, poliovirus and reovirus were found to aggregate when diluted 10-fold into distilled water from a stock solution of 0.05 M phosphate buffer, pH 7.2, plus 22 to 30% sucrose, where there was "minimal aggregation." EX1030 (Floyd I), Abstract. Reovirus also aggregated when diluted into phosphate-buffered saline ("PBS"). EX1030 (Floyd I), Abstract. The aggregation occurred upon dilution up to a point – neither virus aggregated when diluted 100-fold or more into water. EX1030 (Floyd

I), Abstract. At pH 7.2, the aggregation of poliovirus was reversible, while that of reovirus was not. EX1030 (Floyd I), Abstract. Both viruses aggregated upon dilution into buffers at pH 5 and 3, and poliovirus aggregated upon dilution into buffer at pH 6. EX1030 (Floyd I), Abstract. At these lower pH values, the aggregation of both viruses was reversible when the pH was returned to pH 7. EX1030 (Floyd I), Abstract. No aggregation was found at alkaline pH values. EX1030 (Floyd I), Abstract. Notably, aggregation of both viruses at low pH could be prevented by particular concentrations of sodium or magnesium ions. EX1030 (Floyd I), Abstract. Calcium ions produced aggregation of both viruses at 0.01 M. EX1030 (Floyd I), Abstract.

39. Ten-fold dilution of a poliovirus stock of 7×10^{11} particles/ml into PBS or 140 mM NaCl remained dispersed. EX1030 (Floyd I), p. 2. However, when the same preparation of poliovirus particles was diluted 10-fold into distilled water, the viral particles aggregated. EX1030 (Floyd I), p. 2. The presence and extent of aggregation were determined by electron microscopy. EX1030 (Floyd I), p. 3, Fig. 2. Floyd I notes that aggregation appeared to be produced as ionic strength decreased: "[t]hese aggregates were produced under the conditions of reduced ionic strength as revealed by the fact that there was rather a sharp cut-off level in ionic strength above which aggregation did not occur, and below which it did." EX1030 (Floyd I), p. 2. In particular, Floyd I found that the cutoff was about 10 mM for

phosphate buffer (ionic strength of 20 mM) and 60 mM saline (ionic strength 60 mM). EX1030 (Floyd I), p. 2. Floyd I found that increasing the ionic strength, for example by further dilution in PBS or 140 mM saline, led to dispersion of the aggregates. EX1030 (Floyd I), p. 2.

40. Floyd I also tested the effect of different cations, Na^{+}, Mg^{2+}, and Ca^{2+}, on viral aggregation. EX1030 (Floyd I), p. 4. They found that diluting poliovirus 10-fold (to 7 x 10^{10} particles/ml) in concentrations up to 5.0 M NaCl resulted in solutions of viral particles with only small amounts of aggregation. EX1030 (Floyd I), p. 4. For $MgCl_2$, a 10-fold dilution of poliovirus (to 7 x 10^{10} particles/ml) in concentrations up to 0.25 M similarly resulted in very little aggregation. EX1030 (Floyd I), p. 4. Dilution in $CaCl_2$, however, produced a different result. EX1030 (Floyd I), p. 4. Diluting poliovirus 10-fold (to 7 x 10^{10} particles/ml) in 0.001 M $CaCl_2$ did not produce aggregation, but dilution in 0.01 M $CaCl_2$ resulted in aggregation. EX1030 (Floyd I), p. 4.

41. Floyd I also examined the effect of pH on poliovirus aggregation. EX1030 (Floyd I), p. 4. Low pH values (pH 5 and 3) produced substantial amounts of aggregation. EX1030 (Floyd I), p. 4. Aggregation at low pH was found to be influenced by the ionic strength of the solution. EX1030 (Floyd I), p. 5. In particular, addition of NaCl or $MgCl_2$ prevented this low pH aggregation at certain concentrations. EX1030 (Floyd I), p. 5. For NaCl, at pH 3, 2.5 M NaCl was required

to prevent aggregation, while at pH 6, only 0.1 M NaCl was necessary. EX1030 (Floyd I), p. 5. For $MgCl_2$, 0.25 M was sufficient to prevent aggregation at pH 3 and pH 5, and 0.01 M was sufficient to prevent aggregation at pH 6. *See* EX1030 (Floyd I), Table 2. Poliovirus did not aggregate significantly in alkaline pH. EX1030 (Floyd I), p. 5. And aggregation at low pH was found to be reversible by raising the pH. EX1030 (Floyd I), p. 5.

42. For reovirus, viral particles aggregated when the stock solution was diluted, first into water at a 10-fold dilution, then allowed to stand at room temperature for two to three hours, and then further diluted 20-fold into PBS. *See* EX1030 (Floyd I), p. 6. The reovirus aggregation at the further 20-fold dilution contrasted with poliovirus, which did not aggregate at that dilution. *See* EX1030 (Floyd I), p. 6. Interestingly, after about 2 weeks of storage at 4°C to 6°C, reovirus particles failed to aggregate at these dilutions into PBS. EX1030 (Floyd I), p. 7.

43. As far as the effect of the ionic strength of the solution on viral particle aggregation, reovirus behaved similarly to poliovirus. EX1030 (Floyd I), p. 7. Reovirus did not aggregate significantly when diluted 10-fold to 5×10^{10} particles/ml in NaCl solutions up to 1.0 M. EX1030 (Floyd I), p. 7. Reovirus also did not aggregate significantly in solutions up to 0.25 M $MgCl_2$, when the reovirus particles were diluted 10-fold to 5×10^{10} particles/ml. EX1030 (Floyd I), p. 7. And just as poliovirus aggregated in $CaCl_2$, reovirus also aggregated when diluted 10-fold into

a 0.01 M $CaCl_2$ solution, but not into a more dilute, 0.001 M, solution of $CaCl_2$. EX1030 (Floyd I), p. 7.

44. Like poliovirus, reovirus aggregated at lower pH values of 5 and 3 (but did not aggregate at pH 6, unlike poliovirus). EX1030 (Floyd I), p. 7. Also like poliovirus, aggregation of reovirus particles at these low pH values was influenced by the ionic strength of the solution. *See* EX1030 (Floyd I), pp. 5, Table 2, 8. Both NaCl and $MgCl_2$ prevented reovirus aggregation at low pH. *See* EX1030 (Floyd I), pp. 5, Table 2, 8. Higher concentrations of NaCl were needed to prevent reovirus aggregation at pH 3 (> 1.0 M NaCl) than at pH 5 (0.6 M NaCl). *See* EX1030 (Floyd I), pp. 5, Table 2, 8. The same concentration of $MgCl_2$, however, was required to prevent reovirus aggregation at pH 5 as at pH 3 (0.25 M). *See* EX1030 (Floyd I), pp. 5, Table 2, 8. Notably, this concentration of $MgCl_2$ was also the concentration required to prevent poliovirus aggregation at these low pH values. *See* EX1030 (Floyd I), pp. 5, Table 2, 8.

45. As with poliovirus, reovirus aggregation was negligible at alkaline pH values. *See* EX1030 (Floyd I), p. 8.

46. Additional studies investigating the relationship between aggregation of poliovirus and reovirus and ionic strength showed that mono- and divalent cations generally decreased aggregation, with the divalent cations being much more effective than the monovalent cations. *See* EX1031 (Floyd II), Abstract.

47. Trivalent ions ($A1^{3+}$), in micromolar concentrations, caused aggregation beyond the aggregation that occurred at low pH alone. *See* EX1031 (Floyd II), Abstract.

48. Monovalent and divalent anions did not produce significant inhibition of viral aggregation. *See* EX1031 (Floyd II), Abstract. The inability of anions to inhibit aggregation was found irrespective of whether the overall charge on the virus particle was positive or negative, as determined by the relationship between the isoelectric point and the pH at which the tests were carried out. *See* EX1031 (Floyd II), Abstract.

49. Floyd II states: "The basic underlying mechanism which governs the aggregation of virus particles and their adsorption to other particulate matter involves the nature of (i) the soluble ionic groups with the virus in suspension (such as Na+, Cl-, etc.), (ii) the charged groups on the surface of the virus particle (the isoelectric point of the virus is the single most important overall reflection of these groups), and (iii) the resulting ionic double layer, which is a result of the interaction of the first two. The ionic double layer is quite markedly affected by the pH, ionic composition of the medium, and isoelectric point of the virus. Therefore, an examination of the effects of ionic species such as Na^{+}, Mg^{2+}, $C1^{-}$, Al^{3+}, as well as others, on the aggregation of virus particles induced by low pH should provide some

understanding of the nature of virus aggregation and adsorption." EX1031 (Floyd II), p. 2.

50. To study the effects of ionic strength on viral aggregation, Floyd II determined the isoelectric point of each virus. *See* EX1031 (Floyd II), p. 2. The isoelectric point (pI) of a virus is a measure of the pH at which the net electrical potential on the viral particle is neutral. *See* EX1032 (Floyd III), p. 5. The isoelectric point of poliovirus was found to be 8.3, and the isoelectric point of reovirus was found to be 3.9. *See* EX1031 (Floyd II), pp. 2-3.

51. Therefore, at values below pH 3.9, reovirus particles would have a net positive charge, and at pH values above 3.9, they would have a net negative charge. *See* EX1031 (Floyd II), p. 3. At pH values below pH 8.3, poliovirus particles would have a net positive charge, and at pH values above pH 8.3, poliovirus particles would have a net negative charge. *See* EX1031 (Floyd II), p. 3.

52. And notably, at pH 3 and pH 5, both viruses showed marked aggregation. *See* EX1031 (Floyd II), p. 3. Floyd II investigated the effects of salts on aggregation of both viruses at these low pH values. *See* EX1031 (Floyd II), pp. 3-4.

53. Floyd II tested the effect of increasing concentrations of $MgCl_2$ on both viruses at these low pH values. For poliovirus, at pH 5, low concentrations of $MgCl_2$ (0.02 M) enhanced aggregation, but as the $MgCl_2$ concentration was increased up to

1.0 M $MgCl_2$, aggregation was markedly inhibited. *See* EX1031 (Floyd II), pp. 4-5, Fig. 3A.

54. For reovirus, at pH 5, there was no initial enhancement of aggregation even at low concentrations of $MgCl_2$ (0.005 M), but as the concentration of $MgCl_2$ was increased, aggregation was gradually inhibited. *See* EX1031 (Floyd II), pp. 4-5, Fig. 3A.

55. Notably, at pH 5, reovirus has a net negative charge, while poliovirus has a net positive charge. *See* EX1031 (Floyd II), p. 3.

56. At pH 3, the effects of $MgCl_2$ on reovirus and poliovirus diverged much more substantially than they did at pH 5. *See* EX1031 (Floyd II), p. 5, Fig. 3B. At pH 3, at low concentrations of $MgCl_2$, poliovirus and reovirus did not aggregate. *See* EX1031 (Floyd II), p. 5, Fig. 3B. However, at pH 3 and concentrations of $MgCl_2$ above 0.4 M, reovirus reaggregated, while poliovirus did not. *See* EX1031 (Floyd II), p. 5, Fig. 3B.

57. The effects of $CaCl_2$ on poliovirus aggregation at low pH were similar to $MgCl_2$, except that the initial enhancement of aggregation at pH 5 in 0.02 M $CaCl_2$ was not as marked as with $MgCl_2$. *See* EX1031 (Floyd II), pp. 5-6, Fig. 4A. At pH 5, reovirus showed enhanced aggregation in the presence of 0.02 to 0.04 M $CaCl_2$ that was not observed with $MgCl_2$, but higher concentrations of $CaCl_2$ markedly inhibited aggregation. *See* EX1031 (Floyd II), pp. 5-6, Fig. 4A. At pH 3, reovirus

showed the same peak inhibition at 0.2 to 0.25 M $CaCl_2$ as with $MgCl_2$, and rapidly reaggregated at higher $CaCl_2$ concentrations. *See* EX1031 (Floyd II), pp. 5-6, Fig. 4B.

58. The effects of $AlCl_3$ on viral particle aggregation were complicated by the formation at pH 5 of an insoluble "floc" or substance composed of insoluble aluminum hydroxides. *See* EX1031 (Floyd II), pp. 6-7, Fig. 5. At pH 3, $AlCl_3$ did not inhibit poliovirus aggregation, but did inhibit reovirus aggregation at concentrations above 0.02 mM. *See* EX1031 (Floyd II), pp. 6-7, Fig. 5B.

59. Floyd II also investigated whether the cation or the anion of a salt played a larger role in inhibiting viral particle aggregation. *See* EX1031 (Floyd II), pp. 6-8. For reovirus at pH 5 (a pH where reovirus particles carry a net negative charge), significantly lower concentrations of the divalent Mg^{2+} ion than the monovalent Na^+ ion inhibited aggregation, regardless of whether the cations were in the sulfate or chloride form. *See* EX1031 (Floyd II), pp. 7-8, Fig. 6A.

60. At pH 3, a pH value at which reovirus particles carry a net positive charge, the divalent Mg^{2+} ion inhibited aggregation more effectively than the divalent SO_4^{2-} ion. *See* EX1031 (Floyd II), pp. 7-8, Fig. 6B. $MgSO_4$ produced a typical paraboloid curve of inhibition of aggregation (compared with $MgCl_2$ in Fig. 3B and $CaCl_2$ in Fig. 4B), whereas Na_2SO_4 at the same concentrations caused a slight but measurable increase in aggregation, and did not disrupt aggregates even when

the concentration was increased to 0.5 M. *See* EX1031 (Floyd II), pp. 5-8, Fig. 3B, Fig. 4B, Fig. 6B. NaCl produced a slight increase in single particles at pH 3, but the effects fell off after 0.5 M, and no further effect was noted when the NaCl concentration was increased to 1.0 M. *See* EX1031 (Floyd II), pp. 7-8, Fig. 6B.

61. Floyd II obtained similar results with poliovirus at pH 3, a pH value at which poliovirus is strongly positively charged. Nonetheless, the Mg^{2+} cation, either in the form of $MgSO_4$ (Fig. 7) or $MgCl_2$ (Fig. 3) was more effective in inhibiting aggregation than was the SO_4^{2-} anion, in the form of Na_2SO_4. *See* EX1031 (Floyd II), pp. 5, 7-8, Fig. 3, Fig. 7.

62. Floyd III summarizes the work of Floyd I and Floyd II as follows: "Previous work on the aggregation of viruses has established that the ionic composition of the medium plays a dominant role in determining the state of aggregation of the virus particles." EX1032 (Floyd III), p. 1. Floyd III further states: "Generally, viruses have been shown to remain dispersed in salt solutions of near physiological strength (0.14 M NaCl), but to aggregate in solutions of lowered ionic strength." EX1032 (Floyd III), p. 1.

63. Floyd III also describes the results obtained in their prior studies of the effects of adding salts at low pH, and the differing effects of cations in general, multivalent cations in particular, and anions: "Viral aggregation also occurs in buffers at low pH (4-6). The addition of salts at low pH can modify the aggregation

reaction, and the effects of a particular salt are strongly dependent upon two factors: (i) the cationic component of the salt as opposed to the anionic component, and (ii) the magnitude of the charge on the cationic component. Thus, Mg^{2+}, for example, is more effective in inhibiting aggregation of poliovirus at pH 3 in glycine buffer than is SO_4^{2-} or Na^{+}." EX1032 (Floyd III), p. 1.

64. Floyd III tested aggregation of poliovirus and reovirus in neutral and alkaline buffers. *See* EX1032 (Floyd III), Abstract, p. 6. Notably, neither poliovirus nor reovirus aggregated in neutral to alkaline buffers. *See* EX1032 (Floyd III), Abstract, p. 6. From pH 7 to pH 10.5, both poliovirus and reovirus did not aggregate (except in one particular buffer, borate buffer). *See* EX1032 (Floyd III), Abstract, p. 6. From pH 8 to pH 10.5, both viruses would maintain an overall negative potential, given their respective pIs. Not surprisingly, as with reovirus at pH 6 (a pH at which it maintains an overall negative potential), the anionic component of the buffer had little effect on the virus particles at these neutral and alkaline pH values. *See* EX1032 (Floyd III), p. 6.

65. Floyd III concluded that their results, "when correlated with the isoelectric point of the viruses (poliovirus at pH 8.2; reovirus at pH 3.9) indicated that both viruses aggregated strongly when their overall charge was positive, but only under certain circumstances when their overall charge was negative." EX1032 (Floyd III), Abstract.

66. Other researchers had also found that viral particles showed maximum aggregation at their isoelectric point. *See, e.g.*, EX1071 (Oster), pp. 1, 3, Table 1, 4 (finding that different strains of tobacco mosaic virus had different isoelectric points, and that each strain showed maximum aggregation at its isoelectric point).

67. Moreover, Oster found that salt rendered the viral particles more soluble and less prone to aggregate at the isoelectric point. EX1071 (Oster), p. 4.

E. rAAV Aggregation

68. It has been known since at least the 1960s that AAV forms aggregates. *See, e.g.*, EX1040 (Hoggan), p. 7. In particular, by 1976, it was known that AAV aggregates more at lower pH than at higher pH. *See* EX1041 (Johnson), pp. 2, 6.

69. Johnson found that at pH 7.5, the AAV virus particles occurred singly and were evenly distributed. *See* EX1041 (Johnson), p. 6. At pH 7.2 and all lower pHs tested, the particles were aggregated. *See* EX1041 (Johnson), p. 6. At pH 7.2, the aggregates were reproducibly smaller than at pH 7.0 and lower pH values. *See* EX1041 (Johnson), p. 6. In sum, "aggregates of virus were present at pH 7.2 and below, but at pH 7.5 no aggregates were seen." EX1041 (Johnson), p. 6.

70. It was also known that rAAV particles produced *in vitro* were prone to aggregation. *See, e.g.*, EX1042 (Zolotukhin), p. 3 (reporting finding that rAAV aggregated with proteins in the cell lysate). It was known also that rAAV aggregation was concentration-dependent. *See, e.g.*, EX1043 (Huang). It was

known, for example, that the higher the concentration of rAAV, the larger the aggregates formed, and the less efficient the gene transfer to target tissues. *See* EX1043 (Huang). In particular, when the rAAV vector titer reached 5-10 x 10^{13} genome copies ("GCs") per ml, gene transfer efficiency was 10-100 fold lower at the same dose as it was with the same rAAV vector at a titer of 1-5 x 10^{12} GCs/ml. *See* EX1043 (Huang).

71. It was also known that rAAV aggregation could result in reduced yield and undesirable effects after administration, including reduced efficacy and increased immunogenicity. *See* EX1044 (Qu); *see also* EX1047 (Wright 2005), p. 1. Researchers were actively working on developing formulations to inhibit rAAV aggregation at high vector concentrations. *See*, *e.g.*, EX1043 (Huang).

72. Electron microscopy was used at the time, and has been used since, to assess aggregation of AAV and other particles, and has been referred to as a "gold standard analytical method" for characterizing nanoparticles such as viral vectors. *See, e.g.*, EX1030 (Floyd I), *passim*; EX1031 (Floyd II), p. 5; EX1034 (Davidoff), p. 5 ("Further analysis of the purified rAAV-5 preparations by electron microscopy over multiple fields showed exclusively full, ~ 25 nm sized particles with the typical icosohedral structure that were evenly distributed and not clumped"); EX1036 (De Sá Magalhães), Abstract (stating that "[t]ransmission electron microscopy (TEM) is a gold standard analytical method for nanoparticle characterization and is playing a

valuable role in virus-like particle (VLP) characterization extending to other biological entities such as viral vectors"); EX1035 (Dika), pp. 3-4 (using electron microscopy to confirm dynamic light scattering data and evaluate particle aggregation); EX1037 (Janc), p. 3 (using electron microscopy to evaluate aggregation); EX1038 (Dobnik), Abstract, pp. 2, 9, Fig. 6 (discussing the benefits of combined use of molecular methods and electron microscopy for evaluating AAV particles, referring to their results as showing the "indispensibility" of electron microscopy, and using electron microscopy to evaluate aggregation); *see also* EX1039 (Stagg), Abstract (discussing the use of cryo-electron microscopy as an analytic tool for process development and production quality control of AAV vectors).

73. It was also known that aggregation could be assessed by dynamic light scattering and size-exclusion chromatography. *See, e.g.*, EX1008 (Konz), 48:11-15 (noting that dynamic light scattering results were "consistent with theoretical expecations"); EX1044 (Qu), S348. Some researchers also assessed aggregation by quantification of loss following 0.22 μm filtration. *See, e.g.*, EX1008 (Konz) 25:29-30, 36:24-27 (disclosing that "[n]o pressure build-up was seen during the sterile filtration which suggests a lack of aggregated virus"), 48:11-15, Table 12; EX1044 (Qu), S348. rAAV aggregation was known to be concentration dependent. *See, e.g.*, EX1043 (Huang) (stating, "However, we have recently found that, at high

concentrations, AAV virions form aggregates of different sizes in a range of different buffer systems and storage conditions. The size of aggregates appears to be concentration dependent"). Aggregation was also known to be influenced by the level of empty capsids present in the preparation. EX1044 (Qu) (stating, "Considerable variability in the concentration at which aggregation occurred was observed, which may be attributable to variability in the levels of empty capsids, and in levels of DNA and/or protein impurities in the vector preparations").

74. It was known in the art that AAV aggregation occurred in a time- and concentration-dependent manner in vector preparations at concentrations $\geq 10^{13}$ or 10^{14} capsid particles (cp)/ml. *See* EX1043 (Huang); EX1045 (Wright 2003), p. 2. It was also known that freeze-thaw cycling increased vector aggregation, and could lead to aggregation at concentrations substantially lower than 10^{14} cp/ml. EX1045 (Wright 2003), p. 2. Specifically, using dynamic light scattering, Wright observed that highly purified vector preparations at concentrations of 5×10^{13} cp/ml that were stable in a non-aggregated, monomeric state when stored at 2° to 8° C, could be induced to undergo some aggregation following a single freeze-thaw cycle to –20° C. EX1045 (Wright 2003), p. 2.

75. Wright also found that loss of rAAV following a 0.2-μm filtration step correlated with the extent of vector aggregation. *See* EX1045 (Wright 2003), p. 2.

F. Empty Capsids

76. It was known in the art that empty parvovirus capsids have a different, higher isoelectric point from full capsids containing a viral genome. *See, e.g.*, EX1048 (Weichert), Abstract. Weichert studied the canine parvovirus (CPV), and found that the isoelectric point of CPV empty capsids was pH 5.3, while that of full capsids was 0.3 pH more acidic (pI 5.0). EX1048 (Weichert), Abstract.

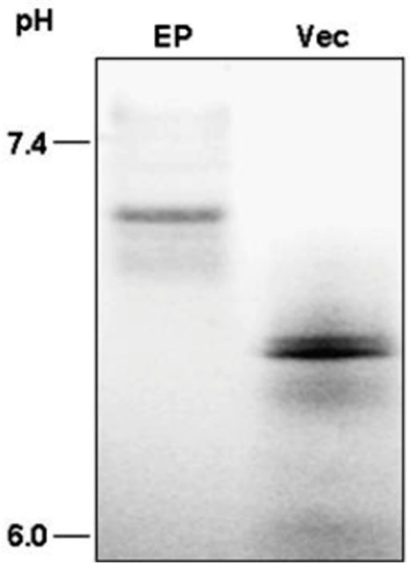
77. A POSA would have understood that, given that DNA is negatively charged, it is not surprising that full capsids would be, overall, more negatively charged than empty capsids, and therefore have a lower pI. In other words, more H^+ ions (a more acidic environment) would be required to neutralize the more negatively charged full capsids, lowering the pI relative to empty capsids.

78. Similarly, a POSA would have understood that, to the extent that cations such as Mg^{2+} are used to inhibit aggregation of rAAV particles (as Floyd II used them to inhibit aggregation of poliovirus and reovirus), at any given Mg^{2+} concentration at a pH above the pI of the viral capsids (where the capsids carry an overall net negative charge), aggregation of empty capsids (less negatively charged) will be inhibited less effectively than aggregation of full capsids (more negatively charged).

79. Notably, subsequent research confirmed the applicability to AAV of the CPV findings regarding pI of full and empty capsids, specifically the finding that

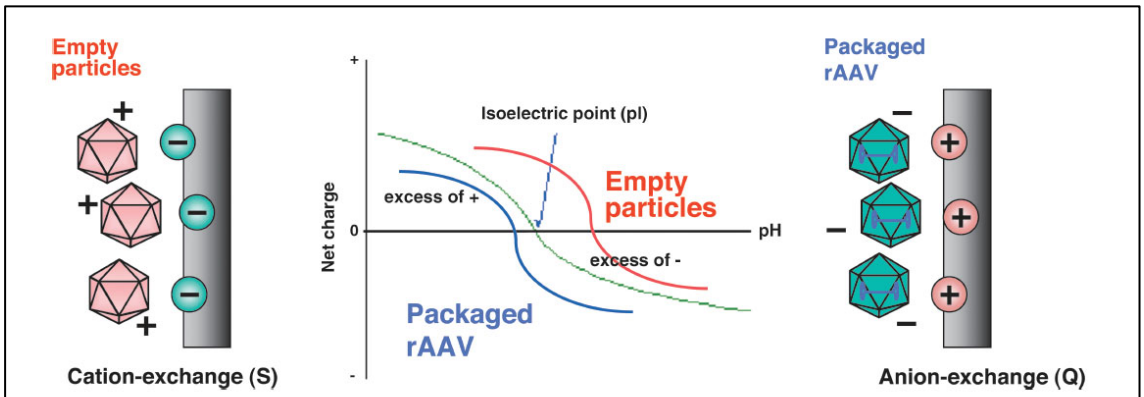
empty capsids have a higher pI than full capsids. *See, e.g.*, EX1049 (Okada); EX1050 (Venkatakrishnan). Okada found that the isoelectric point of empty AAV1 particles was "significantly higher than that of packaged virions." EX1049 (Okada), Abstract. Okada was focused on developing a protocol to purify rAAV that would remove the majority of the empty capsids in the preparation. *See* EX1049 (Okada), Abstract, p. 2.

80. Okada used isoelectric focusing (IEF), a technique that separates proteins in a pH gradient according to their isoelectric points (pI). *See* EX1049 (Okada), p. 3. As the samples containing empty and full AAV1 capsids moved through the gradient, they encountered a point where the pH was equal to their pI and they stopped migrating. *See* EX1049 (Okada), p. 5. Using this technique, Okada found that the pI of the empty particles was significantly higher than that of packaged virions, as shown in the figure below (where "EP" stands for "empty particle," and "Vec" stands for "packaged vector virions":



See EX1049 (Okada), p 5, Fig. 2e. From the gel, it appears that the isoelectric point of the empty particles was about 7.1, and that of the packaged vector virions was about 6.7.

81. The following figure from Okada's supplementary materials describes the isoelectric point of empty and packaged rAAV particles:



SUPPLEMENTARY FIG. 1. The pI is the specific pH at which the net charge of the protein is zero. The net charge of a virus is the sum of all the negative and positive charges of its amino acid side chains and amino- and carboxyl-termini along with the packaged DNA in the virion. We showed that pI of empty particles is higher than that of packaged virions. Since proteins are positively charged at pH values below their pI and negatively charged at pH values above their pI, the net positive charge of empty particles would be higher than that of packaged rAAV particles at a constant pH below the pI of empty particles. On the other hand, the net negative charge of packaged virions would be stronger than that of empty particles at a constant pH above the pI of packaged virions.

EX1049 (Okada), p. 16, Supp. Fig. 1. As the figure legend states, proteins are positively charged at pH values below their pI, and negatively charged at pH values above their pI. EX1049 (Okada), p. 16, Supp. Fig. 1. Given Okada's finding that empty rAAV particles have a higher pI than packaged rAAV particles, at a constant pH value below the pI of empty particles, the net positive charge on empty particles will be higher than that on packaged particles. Correspondingly, the net negative charge on packaged rAAV particles will be higher than that of empty particles at a constant pH above the pI of packaged rAAV particles. EX1049 (Okada), p. 16, Supp. Fig. 1.

82. Venkatakrishnan similarly found that empty AAV capsids have a higher pI value than packaged AAV particles. *See* EX1050 (Venkatakrishnan), pp. 5-6. Venkatakrishnan calculated a mean pI value of ~ 6.3 for empty capsids for a number of different AAV serotypes investigated (AAV1-AAV12). *See* EX1050 (Venkatakrishnan), pp. 5-6. This value was comparable to the previous experimentally determined value for AAV1 that Okada calculated. *See* EX1050 (Venkatakrishnan), p. 6; EX1049 (Okada), p. 5, Fig. 2e. Venkatakrishnan noted that the accepted pI value for DNA (nucleotides) from the literature is 5.0. *See* EX1050 (Venkatakrishnan), p. 6. Using this information, Venkatakrishnan calculated that the AAV capsids with packaged genomes (4.7 kb) had an average calculated pI value of 5.9, a difference of 0.4 compared to empty capsids. *See* EX1050

(Venkatakrishnan), p. 6. Notably, Okada found that empty particles had a pI value about 0.4 higher than packaged virions, essentially the same difference that Venkatakrishnan found. *See* EX1050 (Venkatakrishnan), p. 6; EX1049 (Okada), p. 5, Fig. 2e.

G. Nonencapsulated Nucleic Acids and Viral Particle Aggregation

83. It had been known in the art for decades that purified, empty adenoviral capsids have a "remarkable affinity" for DNA *in vitro*, and form stable complexes of multiple empty capsids per unencapsulated DNA molecule *in vitro* in low salt (<100 mM NaCl) conditions. *See, e.g.*, EX1051 (Tibbetts), Abstract, pp. 4-6, 8, 10. In addition, it had been known for decades that the formation of these empty capsid-DNA complexes could be inhibited by high salt (>100 mM NaCl) concentrations. EX1051 (Tibbetts), pp. 5-6, 8, 10. The unencapsulated DNA bound to empty capsids, furthermore, was shown to be as susceptible to digestion by nucleases as DNA free in solution. EX1051 (Tibbetts), p. 6. Empty polyoma particles similarly were known to bind to viral DNA *in vitro*. EX1051 (Tibbetts), p. 10 (stating, "Their results [polyoma] and ours may reflect a general property of empty capsid structures as intermediates in the assembly of DNA-containing animal viruses").

84. It was also known in the art that addition of nucleases during purification of viral particles such as adenovirus and rAAV degraded non-encapsulated DNA and otherwise contaminating nucleic acids. *See, e.g.*, EX1052

(Huyghe), p. 5 (finding that, for an adenovirus purfication, host cell, non-encapsulated, or incomplete adenoviral nucleic acids could be enzymatically degraded with the addition of nuclease (Benzonase)[2]); EX1054 (O'Riordan), pp. 4, 8 (for an rAAV purification, explaining that Benzonase (a nuclease) enzymatically degrades host cell, nonencapsidated or incomplete rAAV nucleic acids); EX1042 (Zolotukhin), p. 12 (stating that the purified viral stock was first treated with DNaseI to digest any contaminating unpackaged DNA).

85. A POSA at the relevant time would have understood that adding nucleases to purified viral particles to degrade non-encapsulated viral DNA, along with other contaminating nucleic acids, could reduce viral particle aggregation and enhance the stability of the purified viral particle preparation.

H. Use of Non-Ionic Surfactants to Inhibit Aggregation

86. It was known in the art that non-ionic surfactants inhibited aggregation of proteins in solution. *See, e.g.*, EX1055 (Kreilgaard). Non-ionic surfactants were used as excipients to stabilize protein formulations because they were known to prevent protein denaturation and aggregation. *See* EX1055 (Kreilgaard), p. 1; EX1056 (Croyle), Abstract, p. 6. Non-ionic surfactants were known to be able to

[2] A POSA would have understood that the commonly used nuclease Benzonase was an endonuclease from *Serratia marcescens*. EX1053 (Roth), p. 2.

stabilize proteins against stresses including freeze-thawing and agitation. *See* EX1055 (Kreilgaard), p. 1.

87. Konz discloses the use of non-ionic surfactants to inhibit aggregation of viral particles, and states that a POSA, with routine experimentation, would be able to select an appropriate non-ionic surfactant at an appropriate concentration to inhibit aggregation in a viral formulation. *See* EX1008 (Konz), 23:17-24:9 ("The presence of 0.1 % PS-80 in the buffers is critical to achieving low residual DNA levels in the product because it attenuates virus/DNA association and virus aggregation. It will be within the realm of routine experimentation for the artisan of ordinary skill to establish higher or lower detergent concentrations or alternative detergents which would be useful to promote dissociation of virus particles away from other virus as well as various cell contaminants. It is also within this same realm of experimentation that the artisan may choose an alternative detergent to the process buffer. 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)").[3]

88. Croyle discloses the use of the non-ionic surfactant Pluronic F68 in particular, both to inhibit viral particle aggregation and to improve transduction efficiency of difficult to transduce lung tissue. EX1009 (Croyle), Abstract, pp. 2, 6.

I. High Physical Titer rAAV Formulations

89. People of skill in the art as of 2004 routinely purified and concentrated rAAV particles to high physical titers, exceeding 1 x 10^{13} vg/ml. *See, e.g.*, EX1007 (Wu), Abstract, p. 4 (disclosing purified rAAV preparations with a physical titer of about 5 x 10^{13} vp/ml determined by dot blot hybridization); EX1010 (Potter), p. 9, Table II (disclosing purified rAAV preparations with a physical titer of about 1.12-1.46 x 10^{13} particles/ml determined by dot blot hybridization and real-time PCR); EX1058 (Kessler), p. 2 (stating that "[v]ector titer was determined by quantitative dot-blot hybridization of DNase-treated stocks and was routinely in the range of 10^{12}-10^{13} particles per ml"); EX1056 (Auricchio), pp. 2, 4, Table 2 (disclosing that the purified preparations were concentrated to a volume of 1.3 ml (1 ml + three 0.1 ml washes), and also disclosing a purified rAAV preparation with 1.4 x. 10^{13} genome

[3] I note that Konz refers to non-ionic surfactants as a type of "detergent" throughout.

copes per 1.3 ml, or 1.1 x 10^{13} genome copies per ml); EX1059 ('245 Publication), p. 5, Fig. 4 (showing a purified rAAV1 preparation with a physical titer of 1.14 x 10^{13} vg/ml).

90. As I discuss below (Sections VIII.A, VIII.D, X.A.2, XII.A.2), physical titers determined by methods such as dot blot hybridization or real-time PCR measure packaged genomes and therefore provide titers that are the same as vg/ml. These methods do not include empty capsids in the physical titers. *See, e.g.*, EX1010 (Potter), p. 17 (stating that "both titering assays used in this protocol (DBA [dot blot assay] and RTPA [real-time PCR assay]) are based on quantification of packaged genomes, rather than on the assay of assembled particles").

91. As I also discuss below (Section VIII.D.4), Patent Owner and Dr. Davies, in the Novartis IPR proceedings, mischaracterized the state of the prior art with regard to high physical titer preparations of purified rAAV particles, and, in particular, mischaracterized Potter. Patent Owner and Dr. Davies argued that, as of 2004, researchers in the field were not obtaining high physical titer rAAV preparations with a vg/ml concentration greater than 1 x 10^{13}. *See, e.g.*, EX1016 (608 POPR), pp. 47-48, 68-69 (stating that Potter disclosed a "low viral particle concentration" that was "several orders of magnitude below the claimed concentration exceeding 10^{13} vg/ml"); EX1060 (608 Davies Decl.), ¶ 121.

J. Stability of Purified rAAV Preparations During Storage

92. A POSA at the relevent time would have understood that these high titer, purified preparations of rAAV were stable during storage. *See, e.g.*, EX1007 (Wu), p. 4 (disclosing that their high physical titer preparation was stable for more than a month at 4°C); EX1008 (Konz), 30:13-20 (disclosing that their rAAV formulations were stable during storage at 4°C); EX1010 (Potter), p. 2 (disclosing that the goal of their rAAV purification process is to create an rAAV reference standard stock, aliquot it, and distribute it to various laboratories, requiring that the reference stock be stable during distribution).

VI. THE '542 PATENT

93. The '542 patent is titled "Compositions and Methods to Prevent AAV Vector Aggregation." EX1001 ('542 patent). The patent names John Fraser Wright and Guang Qu as inventors. EX1001 ('542 patent). The '542 patent issued on June 9, 2015. EX1001 ('542 patent).

94. The '542 patent is assigned to Genzyme Corporation. EX1001 ('542 patent).

A. The Claims

95. The challenged claims of the '542 patent are directed to compositions for the storage of purified rAAV vector particles "without significant aggregation." EX1001 ('542 patent), 14:15-41.

96. I have reproduced the challenged claims in the table below (claims 1 and 2 were statutorily disclaimed by Genzyme during the prior Novartis IPR proceedings but are reproduced below because claims 3-6 depend from them). EX1019 (Disclaimer).

Claim	Element
1 [pre]	A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles, comprising:
1[a]	purified, recombinant AAV vector particles at a concentration exceeding $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml;
1[b]	a pH buffer, wherein the pH of the composition is between 7.5 and 8.0; and
1[c]	excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate;
1[d]	wherein the ionic strength of the composition is greater than 200 mM,
1[e]	and wherein the purified AAV vector particles are stored in the composition without significant aggregation.
2	The composition of claim 1, further comprising ethylene oxide/propylene oxide block copolymer Pluronic® F68.
3	The composition of claim 2, wherein the Pluronic® F68 is present at a concentration of 0.001% (w/v).
4	The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate.

Claim	Element
5	The composition of claim 1, wherein the purified, recombinant AAV vector particles have an average particle radius (Rh) of less than about 20 nm as measured by dynamic light scattering.
6	The composition of claim 1, wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of said AAV vector particles through a 0.22 μm filter.

97. As the table shows, claim 1 is an independent claim. EX1001 ('542 patent), 14:15-26. Claims 2-6 are dependent claims, which recite additional elements. EX1001 ('542 patent), 14:27-41.

B. The Specification

98. The specification of the '542 patent discusses the effect of different buffers and methods of purification on aggregation of AAV2-FIX particles. *See* EX1001 ('542 patent), Fig. 1B, Fig. 2, 4:14-32, 6:63-9:4; 10:19-11:50. "AAV2-FIX" vectors are AAV2 serotype viral vectors containing a human coagulation factor IX ("FIX") transgene. *See* EX1001 ('542 patent), 10:56-57. AAV2 is the only serotype tested in the '542 patent. The specification discusses various methods to detect viral particle aggregation, including ultrafiltration and diafiltration, and dynamic light scattering. *See* EX1001 ('542 patent), 11:52-12:67.

99. The specification also discusses the effect of storage and freeze-thaw cycles on viral stability and activity, and methods of measuring virion infectivity.

See EX1001 (’542 patent), 9:5-10:15, 13:1-14:4. In particular, the specification describes the effects of freezing and thawing on aggregation of viral particles stored in three different buffers: 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); Test Formulation 2 (TF2) (100 mM sodium citrate, 10 mM Tris, pH 8.0). *See* EX1001 (’542 patent), 9:5-10:15, Table 3, 11:66-12:3.

1. Aggregation as a Function of Excipient Concentration

100. The specification, like Floyd I, II, and III discussed above, describes the use of “dilution stress” experiments to test the effects of different buffers on viral particle aggregation. *See* EX1001 (’542 patent), 6:5-18. In these experiments, vector aggregation is produced by dilution of vector preparations (5-fold in the case of the ’542 patent) into neutral-buffered saline with low concentration buffer (20 mM sodium phosphate, pH 7.2). *See* EX1001 (’542 patent), 6:5-18. The ’542 patent does not disclose the purification method used to prepare the AAV2 vectors tested for aggregation in these experiments. Excipients were screened to identify excipients that could prevent vector aggregation when included in the diluent, despite the dilution. *See* EX1001 (’542 patent), 6:5-60. For screening, aggregation was measured by DLS. *See* EX1001 (’542 patent), 6:14-15. The results of these experiments are shown in Table 1. *See* EX1001 (’542 patent), 6:21-44, Table 1. The ’542 patent concluded that charged excipients prevented aggregation when present

at sufficient concentrations, and that non-ionic surfactants such as Pluronic F68 had no effect on aggregation in these dilution stress experiments. *See* EX1001 ('542 patent), 6:43-60.

2. AAV2 Aggregation as a Function of Osmolarity and Ionic Strength

101. The '542 patent also discloses experiments to investigate AAV2 aggregation as a function of osmolarity and ionic strength. *See* EX1001 ('542 patent), 6:61-7:46. The results of these experiments are shown in Figures 1A and 1B. *See* EX1001 ('542 patent), Fig. 1A and Fig. 1B, 6:63-65. These experiments were carried out on AAV2 vectors that had been purified at analytical scale, and used DLS to measure aggregation. *See* EX1001 ('542 patent), 7:66-8:1. As noted above, no other AAV serotypes were tested.

102. The specification states that the ionic strength of a solution is the primary factor affecting aggregation. *See* EX1001 ('542 patent), 7:22-25. Ionic strength is a parameter that depends on solute concentration and charge valency of the ions in solution. *See* EX1001 ('542 patent). The specification discusses the calculation of the ionic strength (μ) of various buffer solutions, according to the following equation:

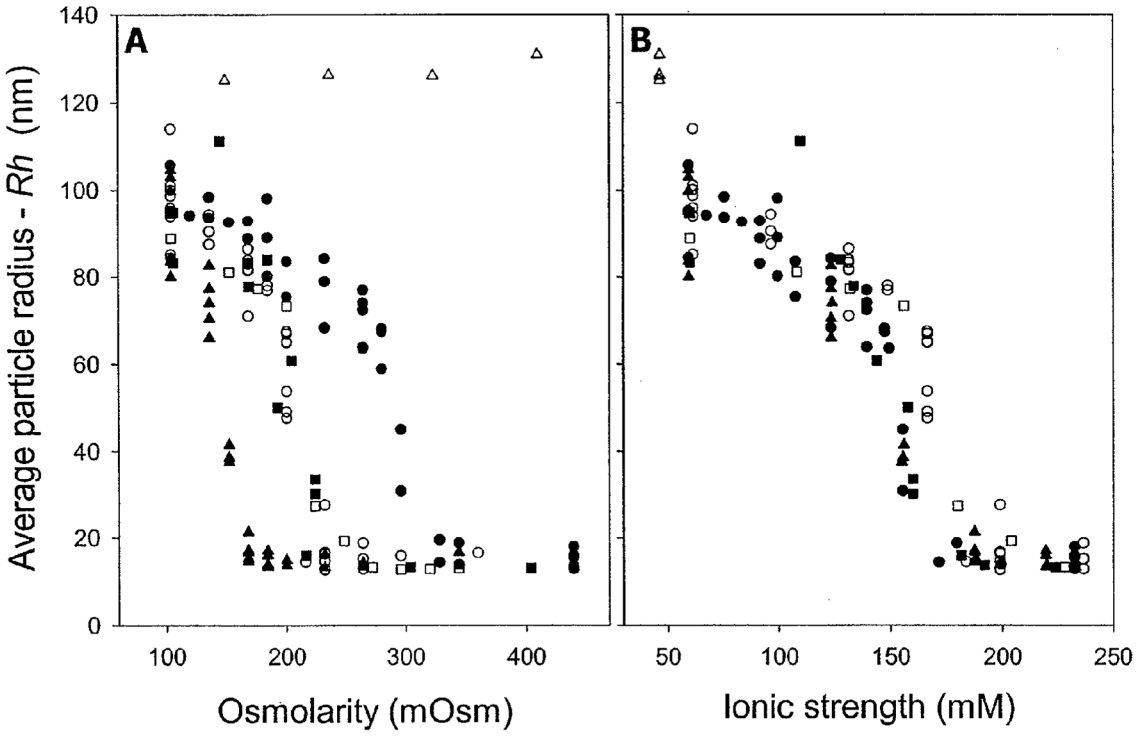
$$\mu = 1/2 \sum c_i z_i^2$$

where c_i is the molar concentration of each solute species and z_i is the charge on each solute species. *See* EX1001 ('542 patent), 12:57-59. The ionic strength values in

Fig. 1 and Fig. 2 were calculated "using all excipients present in the mixture (i.e. weighted: test diluent (80%) and starting vector formulation (20%))." *See* EX1001 ('542 patent), 12:52-55.

103. The specification describes a set of experiments in which the average particle radius (Rh) was determined at different ionic strengths of a buffer solution containing AAV2-FIX particles. *See* EX1001 ('542 patent), Fig. 1B, Fig. 2, 4:14-32, 6:63-9:4. The specification states that Rh is a measure of aggregation, stating that "Rh values >20 nm are deemed to indicate the occurrence of some level of aggregation." *See* EX1001 ('542 patent), 9:25-27.

104. I have reproduced Fig. 1 of the '542 patent below.



EX1001 ('542 patent), Fig. 1.

105. The ’542 patent describes Fig. 1 as follows: “FIGS. 1A and 1B present data showing aggregation of AAV2-FIX particles as a function of osmolarity (FIG. 1A) or ionic strength (FIG. 1B) for various buffer compositions. AAV2-FIX vectors are prepared by Method 2 of Example 1. Average 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. Excipients include sodium chloride (●), sodium citrate (○), sodium phosphate (■), sodium sulfate (□), magnesium sulfate (▲), and glycerol (Δ).” EX1001 (’542 patent), 4:14-23.

106. The ’542 patent states that the vectors tested in the experiments shown in Fig. 1A and Fig. 1B were prepared by Method 2 of Example 1, a method that produces large numbers of empty capsids, as I discuss in more detail below. *See* EX1001 (’542 patent), 4:14-23, 11:47-50.

107. Like Floyd I, II, and III, which I have discussed above (Section V.D.), the ’542 patent concludes that multivalent ions are more effective in preventing aggregation than univalent ions. *See* EX1001 (’542 patent), 7:29-32.

3. AAV2 Aggregation as a Function of the Method of AAV Purification

108. In addition, the ’542 patent discloses experiments to investigate AAV aggregation as a function of the method of AAV purification. *See* EX1001 (’542 patent), 7:47-64. The results of these experiments are shown in Figure 2. *See* EX1001 (’542 patent), Fig. 2, 7:47-64. These experiments were carried out on

AAV2 vectors purified at analytical scale, and used DLS to measure aggregation. *See* EX1001 ('542 patent), 7:66-8:1.

109. The specification describes four different methods of viral particle preparation, which were used to generate the data shown in Figure 2. *See* EX1001 ('542 patent), 4:24-32, 7:47-64, 10:52-11:50 (Example 1). Method 1 involves purification of viral particles by a double CsCl (cesium chloride) gradient. *See* EX1001 ('542 patent), 4:28-29, 7:47-64, 10:63-11:26 (Example 1). Method 2 involves purification by cation exchange chromatography. *See* EX1001 ('542 patent), 4:29-30, 7:47-64, 11:27-31 (Example 1). Method 2 was also carried out using a nuclease digestion step following cation exchange chromatography. *See* EX1001 ('542 patent), 4:30, 7:47-64, 11:32-35 (Example 1). Method 3 involves chromatography followed by a CsCl gradient. *See* EX1001 ('542 patent), 4:31-32, 7:47-64, 11:36-39 (Example 1).

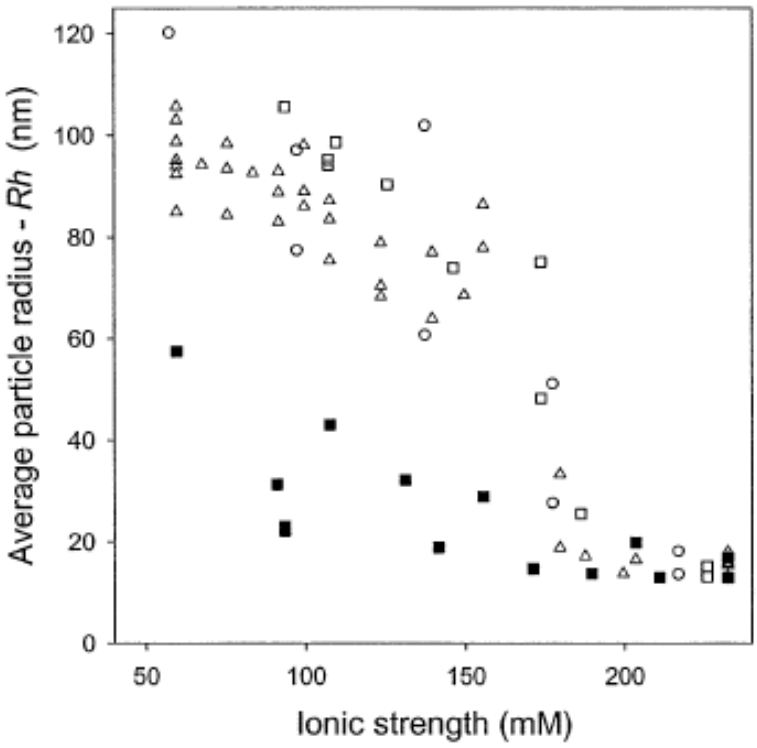
110. The specification states that vectors purified by Methods 1 and 3 do not contain empty capsids, while vectors purified by Method 2 contain empty capsids, ranging from 3-10 empty capsids per vector genome. EX1001 ('542 patent), 11:47-50 (Example 1).

111. A POSA at the time would have understood that empty rAAV capsids have a pI at a higher pH than filled capsids, as a result of the presence of negatively charged DNA in the filled capsids. *See, e.g.*, EX1049 (Okada), p. 5, 16, Fig. 2e,

Supp. Fig. 1; EX1050 (Venkatakrishnan), pp. 5-6. Given the difference in isoelectric points between filled and empty capsids, a POSA would have understood that empty and filled capsids would respond differently to the presence, for example, of positively charged ions in a buffer, when subjected to a dilution stress assay. Qu recognized that the aggregation behavior of AAV particles varied depending on the presence of empty capsids: "Considerable variability in the concentration at which aggregation occurred was observed, ***which may be attributable to variability in the levels of empty capsids***, and in levels of DNA and/or protein impurities in the vector preparations." EX1044 (Qu), S348 (emphasis added).

112. The data presented in Figure 2 of the '542 patent show that rAAV preparations containing empty capsids (Method 2 preparations) responded differently from preparations that did not contain empty capsids (Methods 1 and 3).

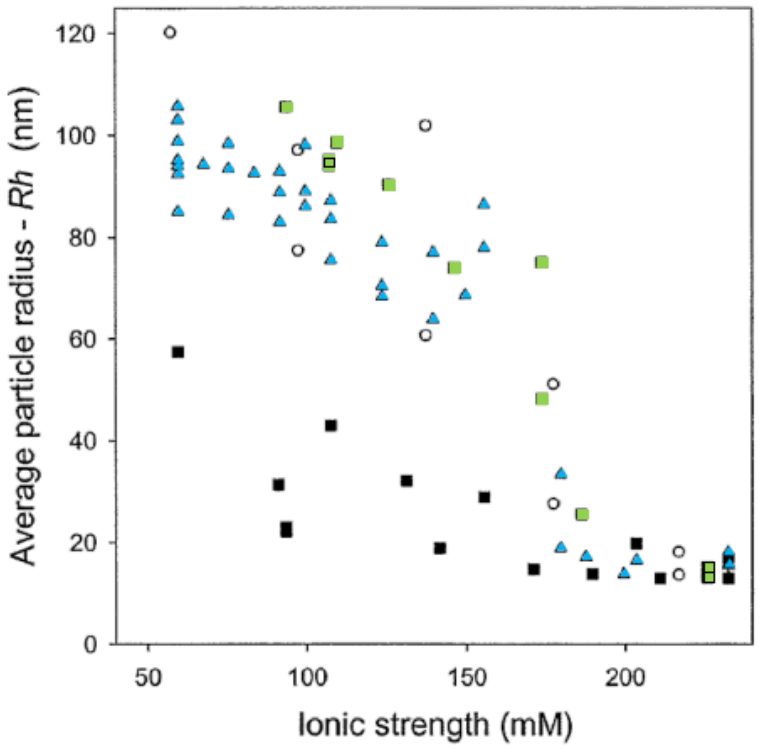
113. I have reproduced Fig. 2 of the '542 patent below:



EX1001 ('542 patent), Fig. 2.

114. The '542 patent describes Fig. 2 as follows: "FIG. 2 presents data on AAV2-FIX aggregation as a function of the method of purification. The average particle radius is measured by DLS following vector dilution in varying concentrations of sodium chloride buffered with 10 mM sodium phosphate at pH 7.5. Vectors are purified by Method 1 (double CsCl gradient) (○); Method 2 (cation exchange chromatography) (□); Method 2 plus nuclease digestion (■); or Method 3 (chromatography plus one CsCl gradient) (Δ). Purification Methods 1-3 are described in Example 1." EX1001 ('542 patent), 4:24-32.

115. The assays in Fig. 2 were carried out by diluting the various preparations of rAAV capsids with increasing concentrations of sodium chloride buffered with 10 mM sodium phosphate at pH 7.5, and measuring the aggregation in the resulting solutions. *See* EX1001 ('542 patent), 4:24-32. The figure below is a modified version of Fig. 2 of the '542 patent, which I have annotated to highlight the effect of the presence of empty capsids on the aggregation behavior of the viral particles prepared by Method 2 and Method 3. I have colored the open squares, indicating preparation by Method 2, in green, and the triangles, indicating preparation by Method 3, in blue:



116. As discussed above, the only difference between Method 2 (green) and Method 3 (blue) is that Method 3 involves an additional CsCl gradient purification step, after cation exchange chromatography. *See* EX1001 ('542 patent), 11:27-39.

117. As can be seen from the figure above, the curve delineated by the green squares (Method 2) is shifted to the right with respect to the curve delineated by the blue triangles (Method 3). For example, there are no green squares at or below 20 nm on the vertical axis at an ionic strength lower than about 230 mM on the horizontal axis. There are blue triangles, however, at or below 20 nm on the vertical axis at an ionic strength of about 180 mM on the horizontal axis.

118. The shift to the right of the green square curve indicates that viral particles prepared by Method 2 require a higher ionic strength solution of sodium chloride to achieve the same level of disaggregation than viral particles prepared by Method 3. In other words, the presence of empty viral capsids requires a higher ionic strength solution to prevent aggregation.

4. AAV2 Aggregation at Preparative Scale, After Concentration and Diafiltration

119. The '542 patent also discloses experiments carried out at a larger scale, investigating the effects of ionic strength and nuclease treatment on AAV2 vector aggregation in more concentrated preparations. *See* EX1001 ('542 patent), 7:65-9:4. In these experiments, AAV2 vectors purified by Method 1 (double CsCl gradient) were used as the starting material for diafilitration experiments. *See* EX1001 ('542

patent), 8:10-13. As I discussed above (Section VI.B.3.), AAV2 vectors purified by Method 1 would be essentially empty-capsid free. *See* EX1001 ('542 patent), 11:47-50.

120. In these experiments, the AAV2 capsids were diafiltered into three different buffers, "Control Formulation" (CF: 140 mM sodium chloride, 10 mM sodium phosphate, 5% sorbitol, pH 7.3); "Test Formulation 1" (TF1: 150 mM sodium phosphate, pH 7.5); and "Test Formulation 2" (TF2: 100 mM sodium citrate, 10 mM Tris, pH8.0). *See* EX1001 ('542 patent), 8:1-9:4, 11:66-12:3.

121. Two different concentrated vector preparations were tested (2.5 x 10^{13} vg/ml, and 6.7 x 10^{13} vg/ml), and one preparation at a concentration of 3.6 x 10^{13} vg/ml with added nuclease was also evaluated. *See* EX1001 ('542 patent), 8:57-65, 12:4-29 (noting that these concentrations are "target[s]" and "assum[e] no vector loss").

122. Aggregation was assessed by measuing vector recovery after filtration through a 0.22 μm filter and, in one case, by visual inspection using light microscopy. *See* EX1001 ('542 patent), 8:8-9, 50-56. Results are shown in Table 2. *See* EX1001 ('542 patent), 8:28-39.

123. The '542 patent concludes that the results in Table 2 demonstrate that various of the formulations tested increased the recovery of AAV2 vector particles

in these more concentrated vector preparations. *See* EX1001 ('542 patent), 8:66-9:4.

5. AAV2 Stability and Activity Following Storage or Freeze-Thaw Cycling

124. The '542 patent also discloses experiments investigating the effects of storage or freeze-thaw cycling on AAV2 vector titer. *See* EX1001 ('542 patent), 9:5-10:15. In these assays, aggregation was measured by DLS, and the preparations were also examined for aggregation by visual inspection. *See* EX1001 ('542 patent), 9:25-55, Table 3.

125. The AAV2 vectors used in these experiments were the concentrated vector preparations used in the preparative scale studies, which had been prepared using Method 1 (double CsCl gradient), and then diafiltered into one of three buffers (CF, TF1, or TF2). *See* EX1001 ('542 patent), 8:10-13, 9:21-25. Like the preparations in the preparative scale experiments, these AAV2 vector preparations were essentially empty-capsid free.

126. The TF2 preparation was also tested for infectious titer and transduction efficiency. *See* EX1001 ('542 patent), 9:56-10:15, Fig. 3. The '542 patent concludes that the TF2 formulation was the most successful at inhibiting aggregation and also that the formulation did not have a deleterious effect on vector infectivity or transduction efficiency. *See* EX1001 ('542 patent), 9:28-10:15, Table 3, Fig. 3.

C. The Prosecution History

127. During prosecution, the Examiner issued several 35 U.S.C. § 102(b) rejections over prior art disclosing viral preparations in high ionic strength buffers with multivalent ions. EX1002 ('542 prosecution history), pp. 82-94. The Examiner also issued a 35 U.S.C. § 103(a) rejection over several of these prior art references. EX1002 ('542 prosecution history), pp. 82-94. In response, the applicant amended the claims. EX1002 ('542 prosecution history), pp. 125-35. These amendments included adding the limitations that the claimed particles are "AAV" particles, and that "aggregation of the purified particles in the composition is prevented." EX1002 ('542 prosecution history), pp. 125-35.

128. The Examiner then issued another 35 U.S.C. § 102(b) rejection and another § 103(a) rejection, after which the applicant amended the claims again. EX1002 ('542 prosecution history), pp. 143-57, 164-74. This amendment specified the concentration of the purified AAV particles in the composition as "exceeding 1 x 10^{13} vg/ml up to 6.4 x 10^{13} vg/ml." EX1002 ('542 prosecution history), p. 166.

129. The Examiner then issued another 35 USC § 103(a) rejection. EX1002 ('542 prosecution history), pp. 181-92. In response, the applicant amended the claims again. EX1002 ('542 prosecution history), pp. 199-205. This amendment specified the pH of the composition as between 7.5 and 8.0. EX1002 ('542 prosecution history), p. 200.

130. The Examiner then issued another 35 USC § 103(a) rejection, after which the applicant amended the claims again. EX1002 ('542 prosecution history), pp. 212-25, 236-45. This amendment specified that the AAV particles are "recombinant" AAV particles. EX1002 ('542 prosecution history), p. 237.

131. The Examiner then rejected the claims again under 35 USC § 103(a) and proposed an Examiner's Amendment, which was agreed to by the applicant. EX1002 ('542 prosecution history), pp. 310-22, 325-28, 338-43. The Examiner's Amendment specified that the multivalent ions must be "selected from the group consisting of citrate, sulfate, magnesium, and phosphate," and also that the purified AAV particles must be "stored in the composition without significant aggregation." EX1002 ('542 prosecution history), p. 340.

132. The primary prior art references at issue here – Potter, Konz, and Croyle[4] – were not before the USPTO during prosecution of the '542 patent. EX1001 ('542 patent); EX1002 ('542 prosecution history).

[4] I note for the avoidance of confusion that a *different* reference by Croyle was cited during prosecution. *See, e.g.*, EX1001 ('542 patent), *passim* (citing Croyle *et al*., "Development of Formulations That Enhance Physical Stability of Viral Vectors for Gene Therapy." *Gene Therapy* 8(17): 1281-1290 (2001)).

133. Two IPR petitions, IPR2023-00608, and IPR2023-00609, were previously filed challenging claims 1, 2, 5, and 6 of the '542 patent. EX1014 (608 Petition), p. 11; EX1015 (609 Petition), p. 13. Claims 3 and 4, at issue here, were not at issue in these prior IPR proceedings. EX1014 (608 Petition), p. 11; EX1015 (609 Petition), p. 13. Neither petition relied on Wu, Potter, Konz, or Croyle as a primary reference. EX1014 (608 Petition), p. 11; EX1015 (609 Petition), p. 13. Potter was relied on as a background reference in the 608 petition and in the 609 petition. EX1014 (608 Petition), pp. 20, 22, and 63; EX1015 (609 Petition), pp. 22, 24. Wu, Konz,[5] and Croyle[6] were not raised by either party during the IPR proceedings.

134. In IPR2023-00608 (but not in IPR2023-00609), the Patent Owner briefly addressed Potter. EX1016 (608 POPR), pp. 47-48, 68-69. As I discuss

[5] I note for the avoidance of confusion that a *different* publication by Konz is cited in Patent Owner's preliminary response in IPR2023-00608. EX1016 (608 POPR), p. 10.

[6] Again, for the avoidance of confusion, the prior IPR proceedings cited the *different* Croyle reference that was cited during prosecution. *See supra* n. 4; EX1014 (608 Petition), *passim*; EX1015 (609 Petition), *passim*.

further below (Section VIII.A.4.), the Patent Owner incorrectly described Potter in the POPR.

135. In response to the filing of these petitions, the Patent Owner statutorily disclaimed claims 1 and 2. EX1019 (Disclaimer); EX1016 (608 POPR), p. 3.

136. The PTAB denied institution of both IPRs. EX1017 (608 Decision); EX1018 (609 Decision). The denial of institution decisions did not address Potter. EX1017 (608 Decision); EX1018 (609 Decision).

D. The Priority Date

137. The '542 patent claims priority to two provisional applications, 60/575,997, filed June 1, 2004, and 60/639,222, filed December 22, 2004. EX1001 ('542 patent); EX1003 ('997 provisional); EX1004 ('222 provisional). The '542 patent issued from U.S. Patent Application No. 12/661,553, filed on March 19, 2010, and is a continuation of U.S. Patent Application No. 11/141,996, which issued as the '721 patent. EX1001 ('542 patent).

138. In my opinion, the '542 patent is not entitled to the June 1, 2004 priority date of the earlier of the two provisionals, the '997 provisional, because the '997 provisional does not describe or enable compositions of viral particles that include empty capsids, or compositions of viral particles at the claimed pH range of 7.5-8.0.

139. The challenged claims of the '542 patent require a composition of purified rAAV particles, at a concentration between 1 x 10^{13} and 6.4 x 10^{13} vg/ml,

in a buffer with a pH between 7.5 and 8.0, containing citrate, sulfate, magnesium, or phosphate, and having an ionic strength greater than 200 mM. EX1001 (’542 patent), 14:15-41. The challenged claims recite that the purified AAV vector particles are stored in the claimed compositions “without significant aggregation.” EX1001 (’542 patent), 14:15-41.

140. It is my understanding that for the ’542 patent to obtain the benefit of the filing date of the ’997 provisional, the ’997 provisional must provide adequate support for the claims of the ’542 patent, meaning that it must describe and enable the full scope of the claims.

141. The challenged claims do not include any limitations regarding the purification method used to produce the viral particles in the claimed compositions. Therefore, the ’997 provisional must describe and enable viral particles produced by different purification methods. Specifically, the ’997 provisional must describe and enable viral preparations purified by methods that retain empty capsids, such as column chromatography. However, the ’997 provisional fails to disclose data sufficient to show that viral particles prepared by methods that retain empty capsids do not aggregate in the claimed compositions.

142. Second, the ’997 provisional must describe and enable compositions at a pH range of 7.5 to 8.0. But all of the dilution stress experiments testing for aggregation in the ’997 provisional (Appendix B, Appendix C, and Appendix D)

were carried out at pH 7.0. These data therefore do not provide any support for the pH range of 7.5-8.0 recited in the challenged claims.

143. For these reasons, set out in more detail below, it is my opinion that the challenged claims are not entitled to the priority date of the ’997 provisional.

1. The ’997 Provisional Does Not Adequately Support Claimed Compositions Containing Empty Capsids

144. First, because the ’542 claims encompass rAAV compositions that include empty capsids, the ’997 provisional must describe and enable compositions that include empty capsids without “significant aggregation.” EX1001 (’542 patent), 14:15-41.

145. Like the ’542 patent, the ’997 provisional investigated aggregation of AAV2 preparations purified by four different methods, in formulations having different ionic strengths: (1) double CsCl gradient; (2) column chromatography; (3) column chromatography plus nuclease; and (4) column chromatography plus CsCl gradient. EX1001 (’542 patent), 4:24-32, 7:47-64, 10:52-11:50, Fig. 2; EX1003 (’997 provisional), pp. 4-5, 7, 11, Appendix D.

146. I have put together the table below to show the corresponding AAV purification methods in the ’997 provisional and the ’542 patent:

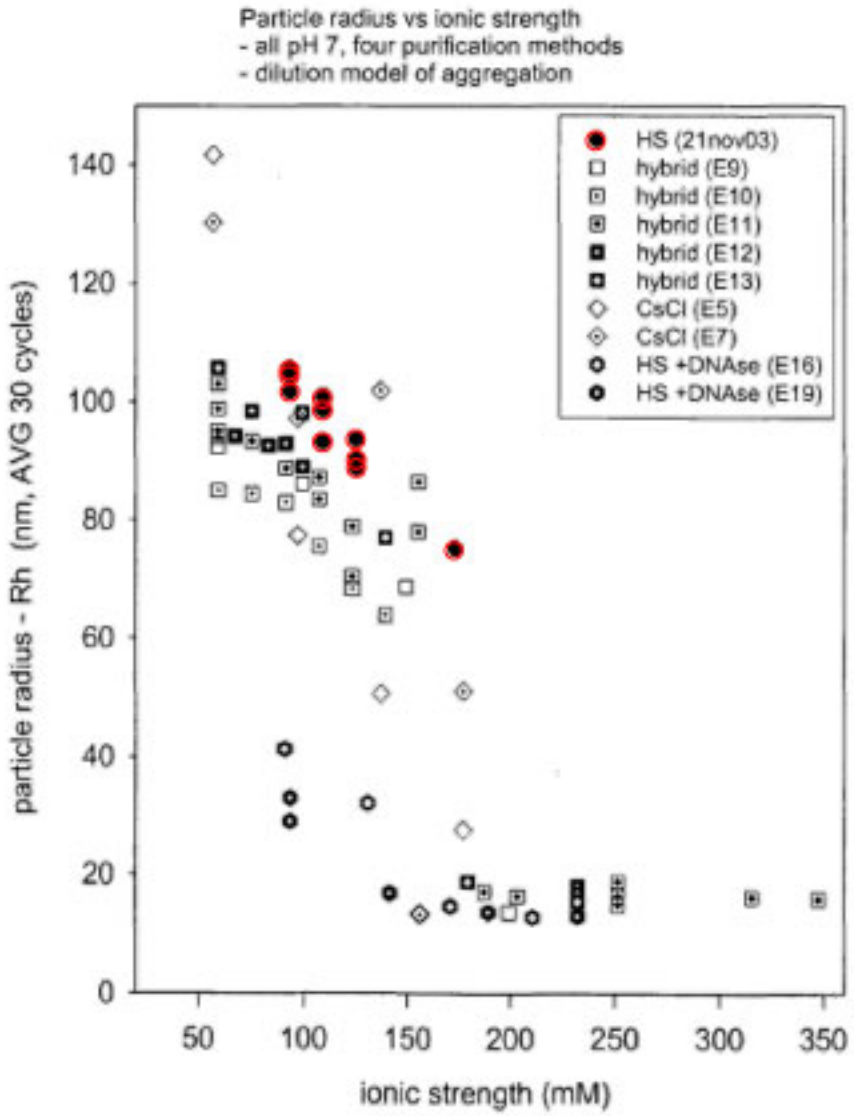
Method	Empty Capsids	’997 Provisional	’542 Patent
Double CsCl	N	Process 1, CsCl	Method 1
Column	Y	Process 2, HS	Method 2

Column + Nuclease	Y	HS + DNAse	Method 2 + nuclease digestion
Column + CsCl	N	Process 3, Hybrid	Method 3

147. The only data relating to a composition containing empty capsids in the '997 provisional are the dilution stress data shown in Appendix D (the "HS" and "HS + DNAse" formulations). EX1003 ('997 provisional), p. 13. And there are no data in this figure that show inhibition of aggregation in a preparation containing empty capsids (HS), other than possibly the preparation to which DNAse had been added. *See* EX1003 ('997 provisional), p. 13.

148. I have annotated Appendix D below by circling in red the solid circles that represent the AAV vectors purified by Process 2 (HS, column chromatography), containing empty capsids:

Appendix D
Effect of ionic strength on AAV vector aggregation using vector purified using four methods, including DNAse treatment of purified vector



149. Notably, the curve for Process 2 (HS) is shifted to the right with respect to the other purification methods. For example, at about 175 mM the Process 2 (HS) curve has a value of about 75 nm, while all other curves are at or below about 50 nm.

150. I discussed this phenomenon with regard to the experiments shown in Figure 2 of the ’542 patent. *See* Section VI.B.3., *supra*. Therefore, the ’997 provisional, like the ’542 patent, discloses experiments showing that higher ionic strength is necessary to prevent aggregation of preparations containing empty capsids than of preparations that are essentially empty capsid free.

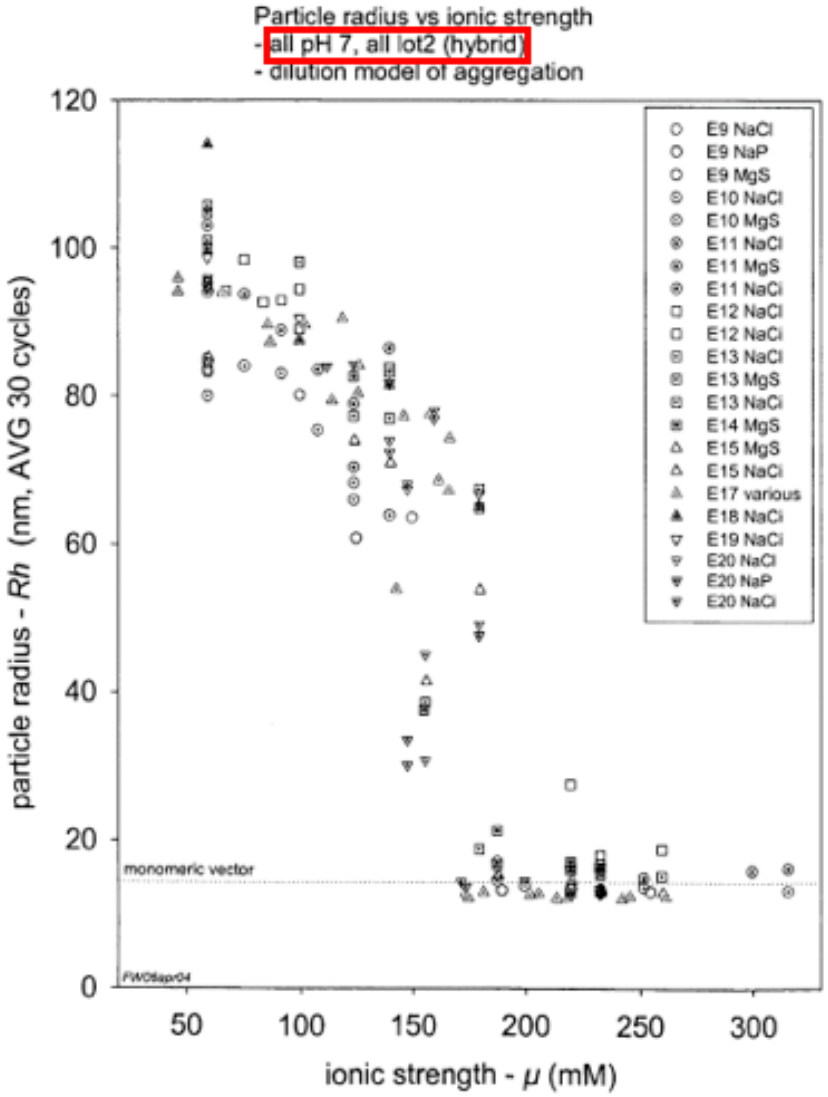
151. However, as can be seen above in the annotated version of Appendix D of the ’997 provisional, there are no data points in this figure for the HS method at an ionic strength higher than about 175 mM. And all the data points for the HS method show an Rh value in the range of about 75 nm to about 105 nm. Given that the average Rh value for monomeric AAV vector is about 14 nm according to the dotted line labeled “monomeric vector” in Appendix B and Appendix C, particles in these experiments with an Rh value in the range of 75-105 nm represent aggregates of multiple AAV vector particles. EX1001 (’542 patent), 1:37-38, 9:25-27, 12:46-48; EX1003 (’997 provisional), pp. 11-12, Appendices B and C.

152. Therefore, ***all*** the data points for the HS (empty capsid containing) method in Appendix D of the ’997 provisional show significant aggregation. As a result, these data cannot provide support for the full scope of the claims of the ’542 patent.

153. The headings on Appendices B and C in the ’997 provisional state that the dilution stress experiments disclosed in these figures were carried out on AAV

particles prepared using the "hybrid" method (column plus CsCl gradient, and essentially empty-capsid free). EX1003 ('997 provisional), pp. 11-12, Appendices B and C. I have placed a red box around this heading in the modified version of Appendix C below:

Appendix C
Effect of ionic strength on AAV vector aggregation using various charged species



EX1003 ('997 Provisional), Appendix C, *see also* Appendix B.

154. Therefore, Appendix C (and similarly Appendix B) of the ’997 provisional cannot provide written description or enablement support for the full scope of the ’542 patent claims, which encompass AAV preparations containing empty capsids.

155. The concentration/diafiltration and freeze/thaw experiments disclosed in the ’997 provisional were similarly all carried out on AAV2 preparations purified by methods that remove empty capsids, so that these preparations were essentially empty capsid free.[7] EX1003 (’997 provisional), p. 5; *see also* EX1001 (’542 patent), 8:10-13, 9:21-24. These experiments, therefore, cannot provide written description

[7] The ’997 provisional does not expressly disclose the purification method used to prepare the rAAV2 virions for the F/T screening experiments. EX1003 (’997 provisional), p. 5. Nonetheless, a POSA would have understood that the purification method appears to be the same empty capsid free method, Process 1b, used for the concentration/diafiltration experiments. EX1003 (’997 provisional), pp. 5, 7 (disclosing that the concentration/diafiltration experiments used three “finalist” formulations from the F/T screening experiments, and that the concentration/diafiltration experiments were carried out using particles prepared via Process 1b, a CsCl based purification).

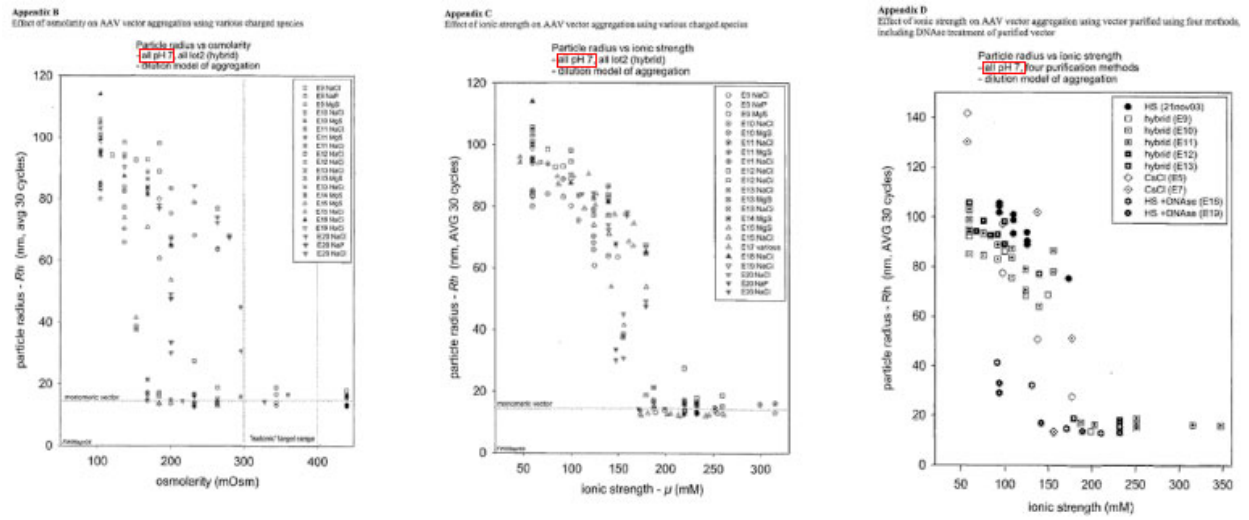
or enablement support for the full scope of the ’542 patent claims, which, as I have said, encompass formulations containing empty capsids.

156. Taken together, there is no evidence in the ’997 provisional that the inventors described or enabled the full scope of the challenged claims, which encompass preparations containing empty capsids. For this reason, the claims of the ’542 patent are not entitled to priority back to the filing date of the ’997 provisional.

2. The ’997 Provisional Does Not Adequately Support the Claimed pH Range

157. In addition, the ’997 provisional fails to provide written description and enablement support for the challenged claims because the dilution stress experiments testing for aggregation in the ’997 provisional were carried out at pH 7.0, rather than at the claimed pH range of 7.5-8.0. EX1003 (’997 provisional), pp. 11-13, Appendices B, C, and D.

158. I have reproduced below Appendices B, C, and D from the ’997 provisional that I have annotated by adding red boxes around the description of the pH for each figure, stating that all of these experiments were carried out at “pH 7”:



EX1003 (’997 Provisional), Appendices B, C, and D.

159. Notably, the challenged claims require a pH in the range of 7.5-8.0. EX1001 (’542 patent), 14:15-41. As a result, the dilution stress experiments in Appendices B, C, and D of the ’997 provisional at a reported pH of 7.0 provide no written description or enablement support for the challenged claims.

160. As discussed above, a POSA at the relevant time would have understood that aggregation is pH dependent, so that aggregation data obtained at pH 7.0 would not provide support for statements regarding aggregation at pH 7.5-8.0. *See supra*, Sections V.D, V.E, and V.F.

161. For these reasons, it is my opinion that the claims of the ’542 patent are not entitled to the priority date of the ’997 provisional. Thus, the earliest possible priority date for the ’542 patent claims is the filing date of the ’222 provisional – December 22, 2004.

VII. THE PERSON OF ORDINARY SKILL IN THE ART ("POSA")

162. I understand that a POSA is a hypothetical person who is presumed to be aware of all pertinent art, understands conventional wisdom in the art, and is a person of ordinary creativity.

163. I understand that the earliest priority applications listed on the face of the '542 patent are U.S. Provisional Patent Application Nos. 60/575,997, filed on June 1, 2004 (EX1003), and 60/639,222, filed on December 22, 2004 (EX1004). EX1001 ('542 patent). I also understand that the '542 patent issued from U.S. Patent Application No. 12/661,553, filed on March 19, 2010, and is a continuation of U.S. Patent Application No. 11/141,996, which issued as the '721 patent. EX1001 ('542 patent).

164. I have carried out my analysis of obviousness from the perspective of a POSA as of June 2004.

165. I have been asked to consider the level of education and experience of a POSA for the '542 patent. In evaluating the qualifications of a POSA, I have considered the following factors: (i) the types of problems encountered in the art, (ii) prior art solutions to those problems, (iii) the rapidity with which innovations are made, (iv) the sophistication of the technology, and (v) the educational level of active workers in the field. I have also relied on my experience working with and supervising others in the field of gene therapy formulation development.

166. In my opinion, a POSA in the technical field of the '542 patent would have had at least a Ph.D. in pharmaceutical sciences, biochemistry, molecular biology, genetics, or a related field and between one and four years of post-doctoral experience in the field of gene therapy, including development of viral vector formulations. Alternatively, a POSA would have had at least a Master's or Bachelor's Degree in pharmaceutical sciences, biochemistry, molecular biology, genetics, or a related field, with a corresponding number of additional years of experience in the field of gene therapy, including development of viral vector formulations.

VIII. THE PRIOR ART

167. As I discuss further below (Section VIII.D.4), Patent Owner and its expert, Dr. Davies, mischaracterized the prior art and the state of the art in responding to the Petition in IPR2023-00608. *See* EX1016 (608 POPR), pp. 47-48, 68-69; EX1060 (608 Davies Decl.), ¶¶ 121, 151-52. Specifically, Patent Owner and Dr. Davies incorrectly described the state of the art, and the Potter reference in particular, as disclosing viral formulations with concentrations "several orders of magnitude below the claimed concentration exceeding 10^{13} vg/ml." *See, e.g.*, EX1016 (608 POPR), pp. 47-48, 68-69; EX1060 (608 Davies Decl.), ¶ 121.

168. Yet Potter (in addition to others, as discussed above in Section V.I) expressly discloses formulations that fall within this range. *See, e.g.*, EX1010

(Potter), p. 9, Table II (disclosing a formulation with a physical titer of about 1.12 x 10^{13} vp/ml as measured by dot blot, and therefore the same as 1.12 x 10^{13} vg/ml, and a physical titer of 1.46 x 10^{13} vp/ml as measured by real-time PCR, and therefore the same as 1.46 x 10^{13} vg/ml).[8]

[8] A POSA at the time would have understood that the dot blot assay measures packaged viral genomes, not capsids (which could include empty particles lacking a viral genome), such that a concentration of "vp/ml" measured by the dot blot assay is a "vg/ml" concentration that does not include empty capsids. *See, e.g.*, EX1010 (Potter), p. 17. The dot blot assay involves extracting DNA from the AAV vector samples, and binding the DNA to nitrocellulose. *See, e.g.*, EX1064 (Sommer), p. 5 (describing the dot blot assay). A portion of the rAAV genome sequence is gel purified and radiolabeled with ^{32}P to create a radioactive probe. *See, e.g.*, EX1064 (Sommer), p. 5 (describing the dot blot assay). Then the hybridization signal from binding the probe to the vector sample hybridized to the nitrocellulose is compared to the signal from plasmid DNA standards bound to the same membrane. *See, e.g.*, EX1064 (Sommer), p. 5 (describing the dot blot assay). A POSA would similarly have understood that the real-time PCR assay, like the dot blot assay, measures viral genomes, not capsids. EX1010 (Potter), pp. 11-12 (stating that "[t]o perform the

A. Wu

169. Wu was published in March 2001, more than a year before the earliest possible priority date for the '542 patent (June 1, 2004), and is therefore 35 U.S.C. 102(b) prior art, irrespective of whether the '542 patent is entitled to the June 1, 2004 priority date. EX1007 (Wu).

170. Wu discloses a method for purifying rAAV on a large scale. *See, e.g.*, EX1007 (Wu), Abstract. Wu explains that there was a need for an efficient and cost-effective method for large scale rAAV purification. EX1007 (Wu), p. 4. Wu states that prior methods such as CsCl density gradient centrifugation are elaborate and time-consuming, and often result in poor recovery. EX1007 (Wu), p. 1.

171. Wu explains that their method was modeled on a classic method for isolation and purification of bacteriophage λ. EX1007 (Wu), p. 4. Wu describes their method as "simple, rapid, inexpensive, high recovery, practical and reproducible for large-scale rAAV preparation." EX1007 (Wu), p. 1.

172. To produce rAAV, Wu used a recombinant herpes simplex virus type 1 (rHSV-1) vector that possessed packaging functions (*rep* and *cap* genes) for rAAV

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assay, viral DNA is isolated," and that the viral physical titer was derived from amplification of the isolated viral DNA).

(HSV1-rc/ Δ UL2).[9] EX1007 (Wu), p. 1. They used this vector to infect an rAAV proviral cell line containing an integrated GFP expression cassette. EX1007 (Wu), p. 1.

173. Wu's purification method involves three steps: (1) chloroform treatment of cells containing rAAV; (2) PEG/NaCl precipitation by adding solid

[9] Use of recombinant HSV vectors to produce rAAV was a well known technique in the field at the time. *See, e.g.*, EX1073 (Conway), Abstract (describing the use of an rHSV construct to infect a proviral cell line with an integrated rAAV-GFP provirus to produce rAAV); EX1074 (Booth), Abstract (describing the use of rHSV vectors to produce rAAV). Furthermore, rHSV vectors have been used in the decades since Wu was published for rAAV production, including to produce rAAV vectors used in preclinical studies for Investigational New Drug applications, and for clinical trials. *See, e.g.*, EX1075 (Dufour), Abstract, p. 2 (describing preclinical studies for an rAAV gene therapy vector for patients with X-linked retinitis pigmentosa where the rAAV vectors were produced using an rHSV system); EX1076 (Flotte), Abstract, p. 2 (discussing production of rAAV using an HSV system for use in a clinical trial); EX1077 (Keeler), p. 7 (stating that the "rHSV-1 system has been used successfully to package rAAV vectors for both preclinical and clinical trials").

NaCl to a final concentration of 1 mol/L and then solid PEG8000 to a final concentration of 10% (*w*/*v*), resuspension of the precipitated rAAV particles in PBS^{2+}, and addition of DNase I and RNase to a final concentration of 1 μg/ml each; and (3) chloroform extraction and collection of the aqueous phase. EX1007 (Wu), pp. 6-7, Fig. 1.[10]

174. Wu teaches that treating the starting material (cells and media from five roller bottles) with chloroform accomplished three purposes: (1) inactivation of helper virus; (2) efficient and rapid cell lysis; and (3) denaturation and precipitation of large amounts of cellular proteins that could then be removed by centrifugation.[11] EX1007 (Wu) pp. 1, 4.

175. I note that after the PEG/NaCl precipitation, the precipitated rAAV particles were recovered by centrifugation at 11,000 r/m for 15 minutes at 4°C, the

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[10] PBS^{2+} is 1X PBS to which 0.01 volume 68 mM $CaCl_2$ and 0.01 volume 50 mM $MgCl_2$ have been added. *See, e.g.*, EX1065 (Yeung), p. 251.

[11] Other researchers have used Wu's chloroform purification method to purify rAAV vectors produced by methods other than infection with rHSV vectors. *See, e.g.*, EX1078 (Davidsson), p. 2 (disclosing a modified version of Wu's chloroform purification protocol used to purify rAAV vectors produced through triple transfection).

supernatant was discarded, and the pellets were then directly re-suspended in PBS^{2+} buffer with the final volume of 5 ml. EX1007 (Wu), p. 2, Fig. 1. In particular, Wu does not disclose any washing of the pellet before resuspension in PBS^{2+} buffer. As a result, given the high concentration of NaCl (1M) used in the precipitation, a POSA would have understood that there will be residual NaCl in the pellet that will be present when the pellet is resuspended in PBS^{2+} buffer.

176. For example (as I discuss in detail below, Section X.A.5), if only 0.20 ml of 1M NaCl were left in the tube when PBS^{2+} 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). That would bring the ionic strength of the final rAAV stock solution to greater than 200 mM.[12]

[12] This calculation is as follows (I set out the detailed calculation in Section X.A.5 below): assume 1X PBS has an ionic strength of about 165.94 mM at pH 7.5, and an ionic strength of about 169.02 mM at pH 8.0. *See* Section X.A.5, *infra*. Then PBS^{2+} has a higher ionic strength at both pH values, as the result of the presence of 0.68 mM $CaCl_2$ and 0.50 mM $MgCl_2$. These divalent ions raise the ionic strength by around 3.5 mM, bringing the ionic strength of the PBS^{2+} to about 169.48 mM at pH 7.5, and about 172.56 mM at pH 8.0. As a result, if the residual NaCl contributes

177. A POSA would also have understood that the NaCl will partition into the aqueous phase during the chloroform extraction in step 3 of the protocol. EX1007 (Wu), p. 2, Fig. 1. Wu states that after chloroform extraction, "[t]he aqueous phases containing the rAAV-GFP were collected and referred to as the purified rAAV-GFP stock." EX1007 (Wu), p. 2.

178. A POSA would further have understood that the pH of PBS is about 7.5 to 8.0, and therefore that the pH of PBS^{2+} is also in this range. *See, e.g.*, EX1065 (Yeung), pp. 257, 294.

179. Wu discloses that using this method, they could reproducibly obtain purified rAAV stocks with titers of around 5 x 10^{13} particles/ml. *See, e.g.*, EX1007 (Wu), Abstract, p. 4.

180. Wu further discloses that the physical titers of rAAV were obtained by dot blot hybridization. EX1007 (Wu), pp. 2-3. A POSA would have understood that because this assay quantifies only packaged genomes (not empty capsids), the concentrations measured using this assay are "vg/ml" concentrations. *See, e.g.*, EX1010 (Potter), p. 17; EX1064 (Sommer), p. 5 (describing dot blot assay). Therefore, Wu discloses purified rAAV stocks with titers of around 5 x 10^{13} vg/ml.

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at least 40 mM to the overall ionic strength, the total ionic strength would be greater than 200 mM at both pH values, as required by the challenged claims.

181. In addition, Wu carried out electron microscopy analysis of the purified rAAV preparations. EX1007 (Wu), p. 3, Fig. 3. Wu discloses that they visualized 150 mesh nickel grids, indicating that they examined a representative sample of the purified rAAV preparation. EX1007 (Wu), p. 2. Wu found that most of the AAV particles "appear[ed] full with few intermediates (fig. 3)" and that "[e]mpty particles were rarely seen." EX1007 (Wu), p. 3, Fig. 3. Wu stated, "The result indicates that the purified rAAV-GFP stock contains rAAV particles of high concentration and purity." EX1007 (Wu), p. 3.

182. I note that I do not see any evidence of aggregation of the purified rAAV particles in Fig. 3. EX1007 (Wu), p. 3, Fig. 3.

183. Moreover, Wu states that the "purified rAAV stock could be stored at 4°C for more than 1 month without significant decrease of infectious titer." EX1007 (Wu), p. 4.

184. Wu also states that "[f]urther steps should be taken to remove residual chloroform before the stocks are used in clinical trials." EX1007 (Wu), p. 4.

B. Konz

185. Konz is an international publication of a PCT application, filed in English and designating the United States. EX1008 (Konz). Konz was published on November 27, 2003, more than one year before the filing date of the '222 provisional, December 22, 2004, which would be the earliest possible priority date

on the face of the ’542 patent if it cannot claim priority to the ’997 provisional. EX1008 (Konz). Therefore, to the extent that the ’542 patent challenged claims are not entitled to the ’997 provisional date, at least for the reasons I have set out above (Section VI.D), Konz is 102(b) prior art against the ’542 patent challenged claims.

186. Should the Patent Office determine that the ’542 patent challenged claims are entitled to the priority date of the ’997 provisional, then Konz is 102(e) prior art against the ’542 patent challenged claims. Konz has an international filing date of May 13, 2003, which is more than a year earlier than the earliest possible priority date on the face of the ’542 patent, June 1, 2004. EX1008 (Konz).

187. Konz describes methods of purification of viral particles in light of a “need for large scale manufacture and purification of clinical-grade virus,” for applications including gene therapy. EX1008 (Konz), 1:25-27.

188. Konz explains: “In view of the increased popularity of these viral vectors and the ultimate need to prepare commercial scale quantities of either a viral based vaccine or gene therapy vehicle, it has become essential to devise economical and scalable methods of production and purification.” EX1008 (Konz), 2:23-26.

189. Specifically, “[t]he process relies on various combinations of cell lysis, detergent-based precipitation of host cell contaminants away from the virus, depth filtration or centrifugation, ultrafiltration, nuclease digestion and chromatography to robustly and economically produce highly purified product.” EX1008 (Konz),

Abstract; 1:15-19; *see also* 5:28-30 ("[T]he present invention relates to various methods of purifying viral particles, such as adenovirus, which is more economical and robust than known processes."), 15:33-16:2 ("The present invention thus relates to methodology which results in the purification of adenovirus vector particles from large scale production facilities which render commercially viable amounts of recovered virus as well as also showing excellent purity characteristics").

190. The process disclosed in Konz includes, but does not necessarily require, any particular process steps. EX1008 (Konz), 4:18-34, 5:1-6 ("The present invention relates to a process for purifying wild type or recombinant virus, especially wild type or recombinant adenovirus, wherein one or more steps of the exemplified procedure (see Table 1) are omitted. Such an omission may be utilized by the artisan on a mix and match basis in order to generate a complete protocol for purification of adenovirus which is qualitatively acceptable and is formulated at a concentration amenable to clinical and/or commercial applications"), 23:1-5, Table 1.

191. The process disclosed in Konz is applicable to purifying wild type or recombinant virus particles. EX1008 (Konz), 5:1-3.

192. Konz discloses that the methods set out are applicable to AAV, which is of "particular interest," because "purification using cation exchange chromatography has been demonstrated and large-scale production facilities may be

necessary if approved as a vector for either gene therapy or vaccine products." EX1008 (Konz), 14:24-29.

193. Konz discloses the use of high salt buffers with multivalent ions for the elution and stability of purified viral particles. *See, e.g.*, EX1008 (Konz), 22:20-22 (disclosing diafiltration into 50 mM HEPES, 2 mM $MgCl_2$, 1 M NaCl, pH 7.5, with or without detergent to prevent aggregation (such as 0.1% PS-80)), 27:1-6 (disclosing diafiltration into a buffer with 10mM phosphate and 0.5 M NaCl, pH 6.5 to 8.0), 29:18-20 (disclosing diafiltration into a HEPES buffer with 2mM $MgCl_2$, 1M NaCl, pH 7.5).

194. Konz discloses the use of nuclease during purification. EX1008 (Konz), 22:4-10 ("A nuclease treatment step can be contemplated at any point in the process, as long as residual nuclease content in the final product is acceptable to the application. . . . One useful manifestation of the process allows for nuclease treatment in the ultrafiltration apparatus after concentration").

195. Konz discloses methods to prevent aggregation of viral particles during purification. EX1008 (Konz), 6:20-23, 13:15-22, 22:18-22, 23:17-19, 24:1-12, 24:34-25:1, 30:8-12, 21-23, 34:15-35:17. In particular, Konz discloses the addition of "the Pluronic series of non-ionic surfactants" to "inhibit aggregation in anion exchange and throughout the process." EX1008 (Konz), 24:1-9.

196. Konz discloses high concentration preparations of viral particles, including the use of anion exchange resins with usable capacity demonstrated (but not necessarily limited) to 2.0 x 10^{13} vp/ml resin. EX1008 (Konz), 6:23-25, 7:30-32 (stating that preferred preparations of the invention contain viral particles at concentrations greater than 1 x 10^{12} vp/ml), 12:21-23, 34:18-21. Konz discloses that these high-capacity resins can be used to prepare high concentrations of viral particles in light of the disclosed methods to inhibit aggregation of the viral particles. EX1008 (Konz), 24:9-12, 30:11-12. Konz further discloses that their invention is an improvement over the prior art "industry norm," which involved low column loadings ("<1 x 10^{12} vp/ml resin"). EX1008 (Konz), 24:12-13.

197. Konz further discloses that "an appropriate formulation buffer (*e.g.*, *see* PCT publication WO 01/66137) can be used to maximize product stability." EX1008 (Konz), 22:15-16, 25:20-22 ("The particular diafiltration buffer chosen should be an appropriate formulation buffer (*see* WO 0166137) or a subset of the desired components"), 54:8-9 ("Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties").[13]

[13] I note that in IPR2023-00608 and IPR2023-00609, Genzyme's expert, Dr. Davies, cited Konz 2005, a paper that discusses a protocol for purifying adenovirus with the goal of controlling aggregation to improve the ability to remove host cell

198. Like Konz, WO 0166137 (Evans) states expressly that the disclosed formulations and methods apply to rAAV, in addition to adenovirus. EX1020 (Evans), 3:12-14 ("The recombinant viruses of the present invention which show enhanced storage stability include but are not limited to adenovirus, ***adeno-associated virus***, retroviruses, herpes virus, vaccinia virus, rotovirus, pox viruses") (emphasis added).

199. In particular, Konz discloses that higher pH buffers improve viral particle stability. EX1008 (Konz), 26:12. Konz discloses that after column chromatography, "the pH is increased to the formulation target through the addition of a high pH Tris buffer." EX1008 (Konz), 26:16-17. Konz discloses formulation buffers at pH 8.0. EX1008 (Konz), 25:23-24, 30:13-19, 42:14-15.

200. Konz discloses sterile filtration through a 0.22µm filter as a means of assaying the extent of particle aggregation after storage. EX1008 (Konz), 23:1-5, Table 1, 25:29-30, 36:24-27 ("No pressure build-up was seen during the sterile

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DNA. *See* EX1060 (Davies Decl. 608), ¶¶19, 46-52; EX1072 (Konz 2005), Abstract. Notably, Konz 2005 found that adding a high salt buffer (1M NaCl) to two process intermediates reversed aggregates. Konz 2005, p. 11. Konz 2005 further found that adding a non-ionic surfactant (Polysorbate-80) throughout the purification similarly inhibited aggregation. Konz 2005, p. 11.

filtration which suggests a lack of aggregated virus"); EX1069 (Hatano), p. 3. Konz discloses high yields from sterile filtration after storage, including yields above 90%. *See, e.g.*, EX1008 (Konz), 36:24-27, 37:1-6, Table 2 (showing 94% yield following sterile filtration); 48:1-21, Table 12 (Example 9) (showing a "600 Liter Scale Purification," with a sterile filtration step through a 0.22 μm filter (Example 9 refers back to Example 5, 42:18-19), and a 98% yield).

201. Konz discloses the use of DLS to determine mean particle sizes, to assess aggregation after storage. EX1008 (Konz), 30:19-20, 48:4-14. Using DLS, Konz found the mean particle size of an adenoviral preparation, which had been stored at 4°C and sterile filtered through a 0.22 μm filter, to be "123 nm, consistent with theoretical expectations," indicating that the particles were monomers and not aggregates. EX1008 (Konz), 25:29-30, 30:19-20, 48:12-14.

202. A POSA would have understood that the high yield following sterile filtration after storage (98%), indicates that sterile filtration had little if any effect on the preparation as far as removal of any aggregates, and therefore that the DLS result was representative of the preparation before sterile filtration. EX1008 (Konz) 48:11-21, Table 12.

C. Croyle

203. Croyle was published in 2001, more than a year before the earliest possible priority date for the '542 patent (June 1, 2004), and is therefore 35 U.S.C.

102(b) prior art, irrespective of whether the '542 patent is entitled to the June 1, 2004 priority date. EX1009 (Croyle).

204. Croyle describes a study directed towards developing an adenoviral formulation with enhanced cellular absorption of adenoviral vectors in the lung. EX1009 (Croyle), Abstract. Croyle tested various different formulations in vitro and in vivo. EX1009 (Croyle), Abstract, pp. 2-3.

205. Croyle found that addition of 0.001% Pluronic F68 alone to a formulation of adenovirus in PBS improved the transduction of lung cells in vivo from 15% to 72%. EX1009 (Croyle), p. 2.

206. Croyle determined that the formulation that was most successful at enhancing transduction of lung cells *in vitro* was a blended formulation, consisting of a 1:4 ratio of sucrose to mannitol with 0.001% Pluronic F68 in PBS. EX1009 (Croyle), p. 2. This formulation improved transduction to 100% of the cells in culture. EX1009 (Croyle), p. 2.

207. *In vivo*, addition of 0.001% Pluronic F68 to an adenovirus preparation improved transduction efficiency from 2000 pg β-gal/mg protein to 8265 pg β-gal/mg protein. EX1009 (Croyle), p. 3.

208. The blended formulation that was the most successful *in vitro* was also the best *in vivo*, increasing *in vivo* expression of the β-galactosidase reporter by 1 log. EX1009 (Croyle), p. 3. This formulation also enhanced the physical stability

of the virus. EX1009 (Croyle), p. 3. After storage in this formulation for 30 days at 4 C, titer dropped by 10%. EX1009 (Croyle), p. 3. After storage in PBS alone under the same conditions, titer dropped below detectable levels in five days. EX1009 (Croyle), p. 3.

209. This formulation also produced the highest level of gene expression in vivo in airway epithelium. EX1009 (Croyle), p. 4. Croyle did not test Pluronic F68 alone in this assay. EX1009 (Croyle), pp. 3-4.

210. Croyle explained that Pluronic F68 was selected as an excipient to test in the formulation based on its known properties, including its ability to inhibit aggregation of proteins (which would include viral capsids), in solution: "Pluronic F68 functions as a wetting agent in most formulations that promotes direct contact between an active ingredient and the lung epithelium and prevents aggregation of proteins in solution." EX1009 (Croyle), p. 6.

211. Significantly, Croyle found that addition of Pluronic F68 to the formulation successfully inhibited aggregation of the adenoviral particles, as determined by dynamic light scattering: "We saw this effect in our studies, ***as average particle size of a viral preparation fell from 163.2 ± 30.6 nm to 70.4 ± 6.2 nm (the size of a single viral particle) when Pluronic F68 was added to the formulation*** as determined by dynamic laser light scattering." EX1009 (Croyle), p. 6 (emphasis added).

D. Potter

212. Potter was published in 2002, more than a year before the earliest possible priority date for the '542 patent (June 1, 2004), and is therefore 35 U.S.C. 102(b) prior art, irrespective of whether the '542 patent is entitled to the June 1, 2004 priority date. EX1010 (Potter).

213. Potter describes a method for the large scale production of rAAV vectors that they used to make a National Reference Standard (NRS) rAAV vector. EX1010 (Potter), pp. 1-2. The disclosed method is "a preindustrial scale-up protocol" that allows "a modest facility to increase vector production at least 10- to 100-fold." EX1010 (Potter), p. 1.

214. Potter states that they had previously developed an rAAV purification protocol that resulted in higher yield and improved infectivity of particles. EX1010 (Potter), p. 1. Their prior method utilized a bulk purification of a crude lysate through an iodixanol step gradient followed by conventional heparin affinity or HPLC ion-exchange chromatography. EX1010 (Potter), p. 1. This protocol, although efficient and effective, was not readily amenable to large scale production of a clinical-grade vector because the iodixanol centrifugation step was rate limiting. EX1010 (Potter), p. 1.

215. Potter sought to improve this protocol so that it could be used for large scale rAAV production. EX1010 (Potter), p. 1. They introduced new

chromatography purification steps that eliminated the need for any centrifugation methods. EX1010 (Potter), p. 1. They characterized the purified rAAV in terms of purity, infectivity, and packaged particle composition. EX1010 (Potter), pp. 1-2.

216. Potter explains that there was a need for a national reference standard for rAAV to permit researchers to share preclinical data relating to the long-term potential risks for insertional mutagenesis and/or transmission of rAAV. EX1010 (Potter), p. 2. Potter states that members of the rAAV gene therapy community recognized that to pool preclinical data in a meaningful way, they needed to be able to discuss vector dosage, strength, and potency in equivalent titer units. EX1010 (Potter), p. 2. To facilitate this goal, they believed that a reference standard stock of rAAV with a precisely defined titer should be generated and made generally available to all members of the research community. EX1010 (Potter), p. 2. All users of this reference stock would essentially be able to calibrate their titering assays against a common standard, thus allowing each group to state their titers in units that were precisely understood by all. EX1010 (Potter), p. 2.

217. Potter describes the generation of the rAAV reference stock with the newly developed protocol. EX1010 (Potter), p. 2. The rAAV construct chosen for the National Vector Standard was pTR-UF5. EX1010 (Potter), p. 2. It contained a humanized *gfp* gene under the control of a CMV promoter and a *neo* gene under the control of a TK promoter. EX1010 (Potter), p. 2. They appear to have used *rep* and

cap genes from AAV2. EX1010 (Potter), p. 1 (citing EX1026 (Hauswirth), which, in turn, cites EX1066 (Grimm) (describing protocol for purification of rAAV2)).

218. Potter sets out the steps of propagation of cells, transfection, and harvesting of transfected cells that they used. EX1010 (Potter), pp. 2-4.

1. Column Chromatography Purification

219. I have focused my analysis on Potter's protocol for purification of the rAAV vector obtained from the steps mentioned above. EX1010 (Potter), pp. 4-7.

220. To generate crude lysate from the harvested cells, Potter chose not to use freeze-thaw cycles, which produce a lysate that requires further processing before being suitable for column chromatography. EX1010 (Potter), p. 4. Instead, Potter sought a method that would generate a lysate immediately usable for chromatography. EX1010 (Potter), p. 4. They used a microfluidics system that exerted forces of shear, impact, and cavitation (formation and collapse of bubbles in the fluid) on the cells as they passed through microchannels. EX1010 (Potter), pp. 4-5.

221. They then used three different column chromatography steps to purify and concentrate the crude lysate: Streamline Heparin affinity chromatography, phenyl-Sepharose hydrophobic interaction chromatography, and heparin affinity chromatography. EX1010 (Potter), pp. 5-7. All chromatography steps were carried

out using an FPLC (fast protein liquid chromatography) system. EX1010 (Potter), p. 5.

222. For the first chromatography step, the entire crude lysate was applied to the Streamline Heparin affinity column, the column was washed, and then the sample was eluted using PBS containing 0.5M NaCl. EX1010 (Potter), p. 5. Ten ml fractions were collected and the positive fractions determined by fluorescent cell assay (FCA). EX1010 (Potter), p. 5.

223. The pooled fractions from the first heparin column were adjusted to 1M NaCl, and then loaded onto the phenyl-Sepharose hydrophobic interaction column. EX1010 (Potter), p. 5. The virus eluted in the flow through, in about 100 ml volume. EX1010 (Potter), p. 5.

224. The eluate from the phenyl-sepharose column, which contained the sample, was concentrated on the Poros heparin affinity column. EX1010 (Potter), p. 5. The phenyl Sepharose fraction was diluted to about 150 mM NaCl by addition of distilled water, about a 6-fold dilution. EX1010 (Potter), p. 5.

225. The diluted sample (about 700 ml) was loaded onto the Poros heparin affinity column, washed, and eluted with PBS (phosphate-buffered saline) containing 0.5M NaCl. EX1010 (Potter), pp. 5-7.

226. One ml fractions were collected and analyzed by fluorescence cell assay (FCA), to find the fraction or fractions containing the majority of the rAAV-GFP vector. EX1010 (Potter), p. 7, Table I.

227. The yield after each column chromatography step was estimated by titering the vector obtained after each step by FCA. EX1010 (Potter), p. 7, Table I.

2. Analytical Characterization of Purified rAAV

228. The purified rAAV obtained through these three column chromatography steps was characterized in four different, independent assays. EX1010 (Potter), pp. 7-9. Physical particle titers were carried out with a dot-blot assay (DBA) and with a real-time polymerase chain reaction (PCR) assay (RTPA). EX1010 (Potter), pp. 7-12. Infectious titers were determined using an infectious center assay (ICA) and FCA, which scored for expression of GFP. EX1010 (Potter), pp. 7-9, 12-13. The results of these physical and infectious titer assays are shown in Table II, which I have reproduced below:

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

EX1010 (Potter), p. 9, Table II. I discuss each of these assays in turn below.

229. Notably, Potter states that the DBA and RTPA titering assays are based on "quantification of packaged genomes, rather than on the assay of assembled particles." EX1010 (Potter), p. 17. Therefore, removal of empty capsids would have no effect on these titers. EX1010 (Potter), p. 17. These titers therefore provide "vector genomes/ml" ("vg/ml") concentrations, despite the fact that they are referred to in Table II as "particles/ml." EX1010 (Potter), p. 9, Table II. As Potter explains, and as a person of ordinary skill would understand, the meaning of "particles/ml" in Potter's Table II is "packaged genomes/ml," which is the same as "vg/ml." *See, e.g.*, EX1067 (Grieger), p. 3, Table 2 (listing the "Unit Determination" for both the dot-blot assay and quantitative PCR as "Viral genome-containing particles/ml (vg/ml)").

(a) DBA

230. To prepare the viral DNA sample for the dot blot assay, the purified viral stock was first treated with DNase I, to digest any contaminating unpackaged DNA, and then treated with proteinase K. EX1010 (Potter), pp. 9-10. The viral DNA was purified by phenol/chloroform extraction, followed by chloroform extraction and ethanol precipitation. EX1010 (Potter), p. 10. The viral DNA samples, after a 10-fold dilution, were applied to the membrane and probed with a ^{32}P-labeled probe (which Potter says can be any fragment of the DNA in the rAAV cassette being titered). EX1010 (Potter), p. 10. The physical particle titer was calculated taking into account the dilution factor. EX1010 (Potter), p. 10. In this

way, the DBA assay measures the concentration of viral DNA (viral genomes/ml), and does not include empty capsids in the calculation of concentration. *See, e.g.*, EX1067 (Grieger), p. 3, Table 2 (listing the "Unit Determination" for both the dot-blot assay and quantitative PCR as "Viral genome-containing particles/ml (vg/ml)"). Table II shows that the viral titer obtained via this assay was 1.12×10^{13} particles/ml, which is the same as a concentration of 1.12×10^{13} viral genomes/ml. EX1010 (Potter), pp. 9-10, Table II.

(b) RTPA

231. Like the DBA, the RTPA used in Potter measures the concentration of viral DNA (vg/ml), and does not include empty capsids in the calculation of concentration. *See, e.g.*, EX1067 (Grieger), p. 3, Table 2 (listing the "Unit Determination" for both the dot-blot assay and quantitative PCR as "Viral genome-containing particles/ml (vg/ml)"). For the RTPA, the viral DNA was isolated by sequential treatment with DNase I and Proteinase K as it was for the DBA assay. EX1010 (Potter), p. 11. A fluorescently labeled probe that binds to the AAV genome was used to monitor the amount of viral genome DNA generated through the PCR reaction. EX1010 (Potter), p. 11. Graphs of the increase in reporter fluorescence vs. PCR cycle were derived for dilutions of standards, and of the test sample DNAs. EX1010 (Potter), pp. 7-8, 12, Fig. 2. The viral titer of 7.3×10^{7} was derived from these curves. EX1010 (Potter), p. 12. This value was corrected for the dilution

factor to arrive at a final value of 1.46 x. 10^{13} particles/ml (Table II). EX1010 (Potter), pp. 9, 12, Table II. Given that this assay measures viral DNA genomes to derive the final concentration, the RTPA, like the DBA, provides a concentration of viral genomes, or filled viral particles, per ml.

(c) FCA and ICA

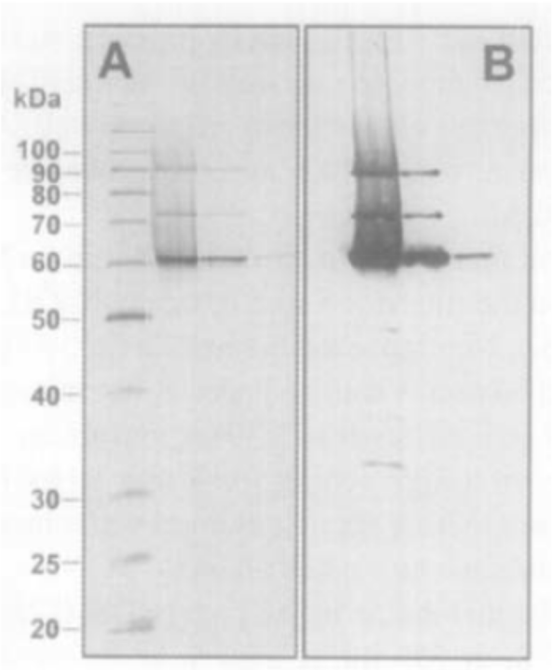
232. In addition to measuring the concentration of filled viral particles produced through the three column purification method, Potter also tested the ability of the purified virions to infect C12 cells, unpackage, and replicate. EX1010 (Potter), p. 12. C12 cells contain integrated wild-type AAV rep and cap genes. EX1009 (Potter), p. 7. Because the reporter gene contained GFP, the same cells were able to be used for both the ICA and FCA analyses. EX1010 (Potter), p. 12. For FCA, C12 cells were plated and infected with the purified virus, along with helper adenovirus. EX1010 (Potter), p. 12. At 40 hours post-infection, cells were visually scored using a fluorescence microscope to monitor green fluorescence. EX1010 (Potter), p. 12. Table II shows that the concentration of infectious particles derived from FCA was 2.16 x. 10^{12} infectious particles/ml. EX1010 (Potter), p. 9, Table II.

233. For ICA, the cells were fixed onto a membrane and probed with a random-primed, radioactive probe that hybridized to AAV DNA. EX1010 (Potter),

pp. 12-13. Table II shows that the concentration of infectious particles derived from ICA was 2.0 x. 10^{12} infectious particles/ml. EX1010 (Potter), p. 9, Table II.

(d) SDS-Gel Electrophoresis Analysis of Vector Purity

234. Further investigation of the purified rAAV was carried out using SDS-gel electrophoresis. EX1010 (Potter), p. 13. This technique denatures proteins as they are run on a gel. Tenfold serial dilutions were loaded into three separate wells, in duplicate. EX1010 (Potter), p. 13. Fig. 3A, reproduced below, showed three major bands representing rAAV capsid proteins VP1, VP2, and VP3; Fig. 3B, also reproduced below, a Western blot with anti-capsid antibodies, showed that the lower molecular weight bands were likely viral capsid proteolysis products:



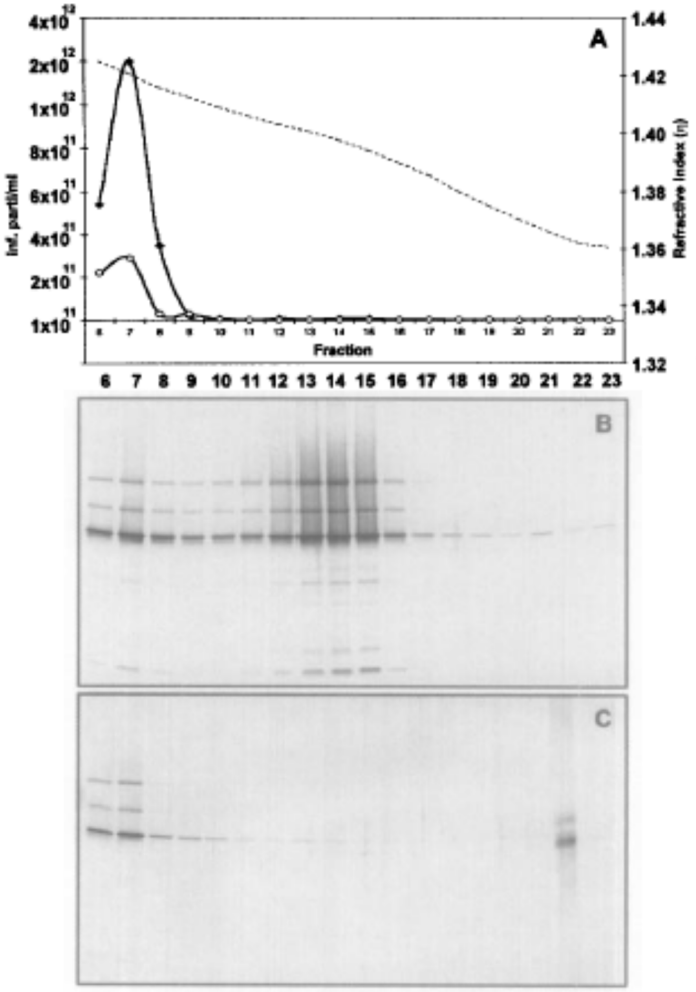
EX1010 (Potter), p. 14, Fig. 3.

235. The tenfold serial dilution experiment, showing dilution over a 100-fold range, demonstrated that the vector was 99.9% pure. EX1010 (Potter), p. 13.

(e) Empty Capsid (Density) Determination

236. To examine the density of viral particles, and investigate whether and to what extent empty capsids were present in the purified rAAV samples, the virus-containing fractions from the POROS column were pooled and analyzed in a continuous gradient of iodixanol. EX1010 (Potter), pp. 13-14. The gradient was formed by mixing virus with 30% iodixanol prepared in PBS containing 0.9 M $MgCl_2$. EX1010 (Potter), p. 14. The sample was centrifuged and then fractionated by puncturing the centrifuge tube at the bottom and collecting 1 ml fractions. EX1010 (Potter), p. 14.

237. Fractions were analyzed by FCA and by polyacrylamide gel electrophoresis, as shown below:



EX1010 (Potter), pp. 14-15, Fig. 4A and Fig. 4B.

238. Fig. 4A shows a curve with filled diamonds, representing the infectious titer of various iodixanol fractions purified by the three column method set out in Potter. EX1010 (Potter), p. 15, Fig. 4A. Fig. 4A also shows a curve with open ovals, representing the infectious titer of a previously purified rAAV preparation, purified by a method described in a prior publication, which was run over the same iodixanol

gradient as the rAAV preparation from Potter. EX1010 (Potter), p. 15, Fig. 4A. The dotted line shows a plot of refractive index of gradient fractions. EX1010 (Potter), p. 15, Fig. 4A.

239. Fig. 4B shows various fractions containing particles purified using Potter's method, eluted from the iodixanol gradient and loaded on an SDS PAGE protein gel. EX1010 (Potter), p. 15, Fig. 4B. The fractions containing the most rAAV were fractions 6-8 and fractions 13-15. The virus peak that banded in the gradient at a refractive index of about 1.425 η (fractions 6-8) contained most of the infectious virus, whereas the more prominent peak in the middle of the gradient (fractions 13-15) contained mostly empty, noninfectious particles. EX1010 (Potter), p. 15, Fig. 4A, Fig. 4B.

240. Fig. 4C shows various fractions containing the pre-purified viral particles, eluted from the iodixanol gradient and loaded onto an SDS-PAGE gel. EX1010 (Potter), pp. 14-15, Fig. 4A, Fig. 4C. Notably, this prior method would have separated out empty capsids in the first iodixanol step gradient used as part of the purification protocol, before being loaded on the analytical iodixanol gradient shown in Fig. 4A.

241. As expected, Fig. 4C shows that the empty particle peak (fractions 13-15) is no longer seen in the viral stock, given that it was separated away during the preceding iodixanol density step gradient purification. EX1010 (Potter), pp. 14-15,

Fig. 4A, Fig. 4C. The only peak that was seen, fractions 6-9, consisted of fully infectious particles, as judged by FCA (Fig. 4A, open circles). EX1010 (Potter), pp. 14-15, Fig. 4A, Fig. 4C. The additional bands seen in fraction 22 were contaminating cellular proteins. EX1010 (Potter), pp. 14-15, Fig. 4A, Fig. 4C.

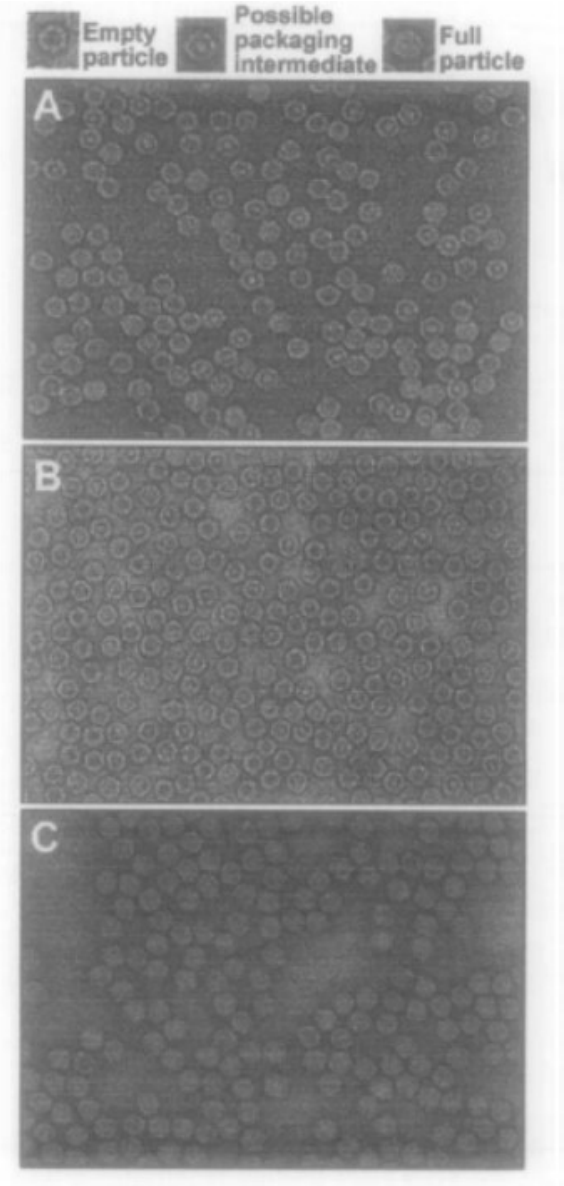
242. Potter explains that the presence of empty capsids in the purified preparations has no effect on the infectious particle titer, given that the DBA and RTPA quantify packaged genomes, rather than particles:

> It is worth noting that separation of full and empty particles does not improve the physical-to-infectious particle ratio of a given stock, ***since both titering assays used in this protocol (DBA and RTPA) are based on quantification of packaged genomes, rather than on the assay of assembled particles***. Removal of empty particles, however, improves the overall quality of a viral preparation by decreasing the capsid antigen burden of the stock and eliminating a competitor for cell surface receptors.

EX1010 (Potter), p. 17 (emphasis added).

3. Electron Microscopy of Purified rAAV

243. Electron microscopy was used to evaluate the rAAV purified per the preparative protocol set out in Potter involving the three column chromatographic steps, as shown below in Fig. 5A:



EX1010 (Potter), pp. 16-17, Fig. 5A.

244. The electron micrographs were taken at a magnification factor of 49,500, a standard electron microscopy magnification for viewing viral particles. EX1010 (Potter), p. 16, Fig. 5; EX1035 (Dika), p. 3, Fig. 1.

245. Potter describes the electron microscopy protocol they used as follows:

> Following chromatography, EM analysis of concentrated samples is performed as described below and shown in Fig. 5. The ***sample*** is prepared by placing 5 µl of purified virus stock on ***support films of Formvar/Carbon 400 mesh copper grids*** (Ted Pella, Inc.) for l min. Excess sample is removed by blotting with a filter paper. The sample is then stained with 5 µl of 2% uranyl acetate for 10 sec and excess stain is removed as described above.

EX1010 (Potter), p. 17 (emphasis added).

246. A POSA would have understood that because a "sample" is placed on multiple "grids," particles from each sample were visualized across multiple grids. Therefore, a POSA would have understood that the electron micrographs in Figure 5 of Potter were representative of particles on multiple grids. EX1010 (Potter), pp. 16-17, Fig. 5; *see also* EX1035 (Dika), p. 3 ("The size of the MS2 particles as obtained by DLS is supported by independent electron microscopy measurements (Fig. 1B) that confirm the presence of isolated viral particles all over the grid of observation"); EX1034 (Davidoff), pp. 3-4 ("For electron microscopy, aliquots of purified rAAV 5 and 8 were dropped onto 200 mesh electron microscopy grids, double coated with Formvar and thin carbon films. . . . The rAAV particles were visualized over a number of grids . . .").

247. The rAAV purified by the three successive chromatography steps appeared to consist of a mixture of full (uniformly stained) and empty (filled circle inside a particle) particles, as well as a possible packaging intermediate (open circle inside a particle). EX1010 (Potter), p. 16, Fig. 5A. I note that no aggregation was observed.

248. As I discussed above (Section V.E.), electron microscopy was used at the time, and has been used since, to assess aggregation of AAV and other particles, and has been referred to as a "gold standard analytical method" for characterizing nanoparticles such as viral vectors. *See, e.g.*, EX1034 (Davidoff), p. 5; EX1036 (De Sá Magalhães), Abstract; EX1035 (Dika), pp. 3-4; EX1037 (Janc), p. 3; EX1038 (Dobnik), Abstract, pp. 2, 9, Fig. 6.

249. To confirm the identification of certain particles as full, empty, or intermediates, fractions from the analytical iodixanol gradient were also analyzed by electron microscopy. EX1010 (Potter), pp. 16-17, Fig. 5B and Fig. 5C. Specifically, the fractions containing the largest numbers of particles from the iodixanol gradient were concentrated by POROS HPLC chromatography as described in Zolotukhin (EX1042 (Zolotukhin), pp. 8-9), and then analyzed by electron microscopy. EX1010 (Potter), pp. 16-17, Fig. 5B and Fig. 5C.

250. Pooled fractions 13-15 from the iodixanol gradient consisted essentially of empty particles and packaging intermediates that had the appearance shown in

Figure 5B. EX1010 (Potter), p. 16, Fig. 5B. Pooled fractions 6-8 from the iodixanol gradient consists predominantly of full particles that had the appearance shown in Figure 5C. EX1010 (Potter), p. 16, Fig. 5C.

251. Figures 5B and 5C confirm the interpretation of the particles seen in Fig. 5A as a mixture of full, empty, and intermediates.

4. Patent Owner and Its Expert, Dr. Davies, Incorrectly Characterized Potter in the 608 POPR

252. As I mentioned above (Section VI.C.), Novartis, in the prior IPR petitions challenging the '542 patent, relied on Potter as a background reference. EX1014 (608 Petition), at 20, 22, and 63; EX1015 (609 Petition), pp. 22, 24. Novartis described Potter as follows: "Potter likewise described 'an improved protocol adapted for large-scale production of a preclinical grade rAAV' in a high ionic strength (500mM NaCl) buffer 'consisting of three sequential chromatography purification steps resulting in highly purified (99.9% pure) and infectious (particle-to-infectivity ratios less than 10) vector preparations.'" EX1014 (608 Petition), p. 20 (citing Potter at 429 and also at 417-419); EX1015 (609 Petition), p. 22 (citing Potter at 429 and also at 417-419).

253. Novartis also stated: "Potter's 'improved protocol' for production of preclinical grade rAAV involved eluting and storing the stocks in a high ionic strength (500mM NaCl) buffer." EX1014 (608 Petition), p. 22 (citing Potter at 417-419); EX1015 (609 Petition), p. 24 (citing Potter at 417-419).

254. In response, Patent Owner addressed Potter in the 608 POPR (but did not discuss Potter in the 609 Patent Owner's Preliminary Response). EX1016 (608 POPR), pp. 47-48, 68-69. Patent Owner and Dr. Davies incorrectly described Potter in several different respects.

255. First, Patent Owner and Dr. Davies incorrectly characterized the concentration of the disclosed formulation in Potter. Patent Owner and Dr. Davies argued that the formulations disclosed in Potter "contain virus particle concentrations several orders of magnitude below the claimed concentration exceeding 10^{13} vg/ml."[14] EX1016 (608 POPR), pp. 47-48, 68-69; EX1060 (608 Davies Decl.), ¶ 121, *see also* ¶¶ 123-24, 151-52.

256. However, as I have discussed above, Potter actually disclosed formulations with concentrations of AAV particles (1.12×10^{13} viral genomes/ml and 1.46×10^{13} viral genomes/ml) that fall squarely within the range recited in the

[14] I note that Patent Owner discussed Table I of Potter, which discloses yields at each different column purification step from three representative vector runs of the purification process, shown as "Total inf. Particles" and "% yield." EX1010 (Potter), p. 7, Table I. Patent Owner, however, failed to address Table II, which discloses the concentrations of the final, concentrated reference standard stock. EX1010 (Potter), p. 9, Table II.

claims of the '542 patent. EX1010 (Potter), pp. 9-10, 12, Table II. Patent Owner and Dr. Davies therefore materially mischaracterized Potter in describing Potter's formulations as "several orders of magnitude below the claimed concentration exceeding 10^{13} vg/ml." EX1016 (608 POPR), pp. 47-48, 68-69; EX1060 (608 Davies Decl.), ¶ 121, *see also* ¶¶ 123-24, 151-52.

257. The Patent Owner also incorrectly characterized the analytical method, electron microscopy, that Potter used to assess aggregation of the purified AAV preparations. The Patent Owner stated that Potter was "unavailing" to show that a POSA would have had a reasonable expectation of success in making the claimed combination because "visual methods cannot accurately detect the presence of aggregates." EX1016 (608 POPR), pp. 68-69.

258. It is incorrect to describe electron microscopy, the analytical technique used in Potter, as a "visual method" that "cannot accurately detect the presence of aggregates." As I have explained above (Section V.E.), electron microscopy was commonly used in the art to assess aggregation of viral particles, including AAV, and was described as a "gold standard analytical method" for characterizing viral particles. *See* Section V.E., *supra*. It is simply wrong to say, as the Patent Owner did here, that electron microscopy "cannot accurately detect" AAV aggregates.

259. In my opinion, people of ordinary skill in the art would generally understand "visual methods" to mean methods such as visual inspection, or even

light microscopy, rather than electron microscopy. And I note that, while Potter does not use any technique that could be fairly described as "visual inspection" to assess the state of AAV aggregation, the '542 patent does disclose such a "visual inspection" method.

260. As I discussed above (Sections VI.B.4. and VI.B.5.), the '542 patent assessed aggregation after concentration and diafiltration by visual inspection using light microscopy. EX1001 ('542 patent), 8:50-56 (finding "obvious amounts of visible material"); *see also* EX1001 ('542 patent), 1:65-2:8 (discussing the use of visual inspection to assess aggregation for an adenovirus reference material in the prior art). "Visual inspection" was also used in the '542 patent to assess aggregation after freeze/thaw cycling. EX1001 ('542 patent), 9:50-52 (noting that "[v]isual inspection of these samples reveals slight cloudiness, which is consistent with aggregation"). Given these disclosures in the '542 patent, it is therefore particularly surprising, in my opinion, that the Patent Owner disparaged "visual inspection" techniques in the 608 POPR.

IX. CLAIM CONSTRUCTION

261. Challenged claims 3-6 of the '542 patent recite a composition of purified, recombinant AAV vector particles, where the AAV vector particles are stored "without significant aggregation." EX1001 ('542 patent), 14:15-41. The '542 patent does not define the degree to which aggregation is or is not "significant."

Likewise, I am not aware of a definition in the prosecution history of the '542 patent as to the term "significant aggregation." For purposes of my analysis, however, the construction of the term "significant aggregation" need not be determined. As discussed below, the prior art discloses formulations that do not show evidence of aggregation upon storage. Thus, the prior art formulations meet this element of the challenged claims, regardless of how it is construed.

262. I have analyzed the remaining terms recited in claims 3-6 according to their plain and ordinary meaning.

263. I note that several terms in the challenged claims were construed by the District Court in *Genzyme Corporation and Aventis Inc. v. Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation*, C.A. No. 21-1736 (RGA) (D. Del.), D.I. 268. EX1061 (Claim Construction Order). The District Court construed the following terms of the '542 patent:

Claim Term	Claim(s)	District Court's Construction
"filtration . . . through a 0.22 μm filter"	6	passing a liquid through a 0.22 μm filter to remove materials
"ionic strength"	3, 4, 5, 6	one half of the sum of the molar concentration of each solute species times the square of the charge on each species for all excipients present in the solution (calculated according to the equation: $\mu=\frac{1}{2}\Sigma c_i z_i^2$)
"multivalent ion"	3,4, 5, 6	an ionic species having a charge valency greater than one (whether positive or negative)

Claim Term	Claim(s)	District Court's Construction
"recombinant adeno-associated virus (AAV) vector particles" / "AAV vector particles" / "recombinant virus particles"	3, 4, 5, 6	recombinant AAV virion or virus particles
"dynamic light scattering"	5	a technique in physics that can be used to determine a size distribution profile of small particles in suspension or polymers in solution
"purified"	3, 4, 5, 6	having been subjected to a purification procedure
"significant aggregation"[15]	3, 4, 5, 6	plain and ordinary meaning
"storage" / "stored"	3, 4, 5, 6	maintenance in a frozen or non-frozen state

264. I have considered the District Court's constructions in the earlier Novartis case. My opinions do not change if the District Court's constructions above are applied to the challenged claims, rather than the plain and ordinary meaning.

[15] For this claim construction, the Court rejected Novartis's arguments that the term was indefinite, ruling instead that the term was defined by the additional limitations in claims 5 and 6, namely in relation to particle radius as measured by DLS, and product recovery after filtration through a 0.22 μm filter. EX1062 (Claim Construction Opinion), pp. 22-24. As a result, claims 5 and 6, consistent with the Court's ruling, should be interpreted to require that the formulation meet the limitations of particle radius and product recovery after storage.

X. GROUND 1: CLAIMS 3-6 ARE OBVIOUS OVER WU AND KONZ

265. In my opinion, claims 3-6 of the ’542 patent are obvious over Wu and Konz.

266. A POSA would have been motivated to combine Wu with Konz because both Wu and Konz are directed towards large scale production of concentrated, high titer formulations of rAAV. Wu states that in the disclosed methods, they eliminated the need to use CsCl gradients for purification, or column chromatography, to permit efficient, cost-effective large scale production. EX1007 (Wu), p. 1.

267. Nonetheless, Wu includes the analytical technique of electron microscopy, which is labor and time intensive and difficult to adapt to scale. EX1007 (Wu), p. 3, Figure 3.

268. In addition, Wu teaches that for clinical use, it is necessary to remove residual chloroform, and presumably replace the final buffer of the stock solution with a buffer appropriate for storage prior to clinical use. EX1007 (Wu), p. 4 (“Further steps should be taken to remove residual chloroform before the stocks are used in clinical trials”).

269. Konz similarly is directed to fulfilling a need for large scale manufacture and purification of clinical grade virus. EX1008 (Konz), 1:25-27.

270. Both Wu and Konz disclose the use of solvents to improve the efficiency of cell lysis and inactivate unwanted cellular components. EX1007 (Wu), p. 4 (discussing the use of chloroform to denature and precipitate "large amounts of cellular proteins"); EX1008 (Konz), 16:27-29, 27:23-27 (disclosing the addition of a solvent such as TNBP to the cell lysate "to increase the efficiency of inactivation of adventitious agents").

271. Konz is also directed to methods of preventing aggregation, such as the use of non-ionic surfactants, and analytical techniques to evaluate the extent of aggregation, such as dynamic light scattering and 0.22μm filtration, which are more adaptable to scale than the electron microscopy used in Wu. *See* EX1008 (Konz), 24:1-9, 25:29-30, 36:24-28 (noting that "[n]o pressure build-up was seen during the sterile filtration which suggests a lack of aggregated virus), 42:18-19, 48:11-21, Table 12.

272. Like Wu, Konz also focuses on stability during storage, which will help large scale production and distribution of rAAV preparations. *See* EX1008 (Konz), 22:15-16 (stating that "an appropriate formulation buffer (e.g., see PCT publication WO 01/66137) can be used to maximize product stability"); EX1007 (Wu), p. 4 (disclosing that the purified rAAV stock could be stored for more than a month at 4°C without significant decrease of infectious titer).

273. A POSA would therefore be motivated to combine Wu's methods for large scale purification of rAAV with the formulation buffers disclosed in Konz, and the additional improvements in Konz to streamline the production and make it even more adaptable to scale up.

274. A POSA would have further understood that the purification methods of Wu, producing pure, high titer rAAV, could be combined with the buffers and additional methods of Konz, by diafiltering Wu's final, purified rAAV preparation into one of Konz's formulation buffers, including those containing a non-ionic surfactant to prevent aggregation, and then analyzing the particles for aggregation according to Konz's methods of DLS and 0.22 μm filtration. A POSA at the time would have understood that diafiltration is a technique to exchange one buffer with another and that this technique would remove residual chloroform in addition to allowing the introduction of excipients such as a non-ionic surfactant. *See, e.g.*, EX1068 (Schwartz), p. 2 (discussing removal of residual solvents).

275. Moreover, a POSA would have understood that the methods of Wu produced a high titer rAAV preparation with no evidence of aggregation in a high ionic strength buffer (PBS^{2+} with additional NaCl), that includes several different multivalent ions (phosphate, Ca^{2+}, and Mg^{2+}), at about pH 7.4 or 7.5 to 8.0. Therefore a POSA would have been motivated to preserve these general characteristics in choosing one of the Konz buffers with the addition of a non-ionic

surfactant, for example, one from the Pluronic series, to inhibit aggregation even further.

276. As I discussed above, claims 1 and 2 were statutorily disclaimed in the prior IPR proceedings. EX1019 (Statutory Disclaimer). Nonetheless, I address these claims below because challenged claim 3 depends from claim 2 (which, in turn, depends from claim 1), and challenged claims 4, 5, and 6 depend from claim 1.

A. Claim 1

1. "A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles"

277. Wu discloses a composition for the storage of purified rAAV vector particles comprising purified, high titer rAAV particles, that is stable during storage for a month at 4°C. EX1007 (Wu), Abstract, pp. 3-4.

278. Konz discloses choosing formulation buffers to maximize product stability. *See, e.g.*, EX1008 (Konz), 22:15-16. Konz refers to a PCT publication (Evans), which it incorporates by reference. EX1008 (Konz), 22:15-16 (stating that "an appropriate formulation buffer (e.g., see PCT publication WO 01/66137 [Evans]) can be used to maximize product stability"), 25:20-22 (stating, "The particular diafiltration buffer chosen should be an appropriate formulation buffer (see WO 0166137 [Evans] or a subset of the desired components"); *see also* 54:8-9 (stating, "Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties").

279. Evans, in turn, states that "[e]specially preferred viral formulations disclosed herein are liquid adenovirus formulations, which show improved stability when stored in about the 2-8°C range while also being compatible with parenteral administration." EX1020 (Evans), 1:16-19, 20:19-24. Like Konz, Evans states expressly that the disclosed formulations and methods apply to rAAV, in addition to adenovirus. EX1020 (Evans), 3:12-14 ("The recombinant viruses of the present invention which show enhanced storage stability include but are not limited to adenovirus, ***adeno-associated virus***, retroviruses, herpes virus, vaccinia virus, rotovirus, pox viruses") (emphasis added).

280. Wu and Konz thus both meet this limitation of claim 1.

2. "purified, recombinant AAV particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4 x 10^{13} vg/ml"

281. Wu discloses purified, rAAV particles at concentrations exceeding 1 x 10^{13} vg/ml and less than 6.4 x 10^{13} vg/ml. Wu discloses concentrations of purified rAAV stock determined by dot blot that were about 5 x 10^{13} particles/ml. EX1007 (Wu), p. 4.

282. The notation "vg/ml" in the '542 claims would be understood by a POSA to mean "vector genomes/ml." *See, e.g.*, EX1001 ('542 patent), 10:2-4 ("After being stored for 45 days at 4° C. the preparation has a vector genome to infectious unit ratio (vg/IU) of 13 . . . ."). Expressing viral particle titer in terms of vector genomes per ml provides the number of filled capsids per ml of viral

preparation, meaning capsids that contain the viral genome. Empty capsids will not contain a "vector genome" and will not be counted in a concentration measured as vg/ml.

283. A POSA at the time would have understood that the notation "particles/mL," which is used in Wu, could mean "vg/ml," depending on the assay that was used to determine the viral concentration. If the assay determined the number of filled capsids, or viral genomes, then in that case the "particles/mL" notation would be the same as "vg/ml." If the assay, on the other hand, determined "viral particles," irrespective of whether they were empty particles or filled particles, then "vp/ml" would not have the same meaning as "vg/ml."

284. As I discuss above (*see* Section VIII.A), a POSA would have understood that Wu used the dot blot method, which provides a "vg/ml" concentration. This assay determines the number of packaged AAV genomes present per ml of the preparation. The AAV genome is a single stranded, DNA genome, and these assays determine the concentration of packaged DNA genomes in the preparation.

285. Therefore, Wu discloses "purified, recombinant AAV particles at a concentration exceeding 1×10^{13} vg/ml up to 6.4×10^{13} vg/ml."

286. Wu meets this limitation of claim 1.

3. "a pH buffer, wherein the pH of the composition is between 7.5 and 8.0"

287. The buffer used in Wu is PBS^{2+}, with additional NaCl, as discussed below. *See* EX1007 (Wu), p. 2; Section X.A.5. A POSA would have understood that the pH of PBS^{2+} varies depending on the exact preparation and conditions such as temperature, but is generally in the range of about 7.4 or 7.5 to 8.0. *See, e.g.*, EX1057 (Cold Spring Harbor Protocols); EX1023 (Hermens), pp. 2-3; EX1070 (Monahan), p. 9; EX1065 (Yeung), pp. 257, 294. Therefore, a POSA would have understood that Wu meets this limitation of the '542 patent claims.

288. In addition, Wu teaches that it is necessary to take further steps to remove residual chloroform from the the viral stocks before use in clinical trials. EX1007 (Wu), p. 4. Therefore, a POSA would have been motivated to exchange the buffer in the Wu viral stocks for a buffer that would preserve the stability of the stocks prior to use during clinical trials.

289. As I discussed above, Konz discloses that higher pH buffers improve viral particle stability. EX1008 (Konz), 26:12. Konz discloses that after column chromatography, "the pH is increased to the formulation target through the addition of a high pH Tris buffer." EX1008 (Konz), 26:16-17.

290. Konz incorporates Evans by reference, and Evans, in turn discloses formulation buffers that meet this pH limitation of the claims. *See, e.g.*, EX1020 (Evans), 11:13-21 (disclosing a formulation buffer in a pH range from pH 7.5 to pH

8.5, NaCl concentration above 250 mM, $MgCl_2$ from 0.1 mM to 10 mM, and a surfactant), 11:22-30 (disclosing a formulation buffer in a pH range from pH 7.5 to pH 8.5, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.1 mM to 5 mM, and a surfactant), 11:31-12:4 (disclosing a formulation buffer at pH 8.0, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.5 mM to 2.5 mM, and a surfactant), 14:15-28 (disclosing a formulation buffer at pH 8.0, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.5 mM to 2.5 mM, a surfactant, and a free radical inhibitor such as sodium citrate); *see also* 8:23-28 ("It will be known to one of skill in the art to provide virus formulations of the present invention in a physiologically acceptable buffer . . . within a pH range including but not limited to about 7.0 to about 9.0, preferably a pH range from about 7.5 to about 8.5"), 36:16-18 (claim 3) (reciting that "the buffer is selected from a group of buffers acceptable for human parenteral use, preferably a Tris buffer, at a pH from about 7.5 to about 8.5"), 41:9-11 (claim 36) (same). The pH range from 7.5 to 8.5 meets the limitation of the claims.

291. A POSA would have been motivated to exchange the buffer of Wu with a buffer in Konz (via incorporation of Evans), because both formulations have high ionic strengths, both have divalent ions, and both are in a similar pH range.

292. Wu, in combination with Konz, thus meets this limitation of claim 1.

4. "excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate"

293. Wu discloses a phosphate buffer (PBS^{2+}), also containing Mg^{2+} ions, which meets this limitation. *See* EX1007 (Wu), p. 2. A POSA would have understood "PBS^{2+}" to mean "phosphate buffered saline" with added $MgCl_2$. *See, e.g.*, EX1065 (Yeung), p. 251.

294. Konz incorporates Evans by reference, and Evans, in turn discloses formulation buffers that meet this limitation of the claims. *See, e.g.*, EX1020 (Evans), 11:13-21 (disclosing a formulation buffer in a pH range from pH 7.5 to pH 8.5, NaCl concentration above 250 mM, $MgCl_2$ from 0.1 mM to 10 mM, and a surfactant), 11:22-30 (disclosing a formulation buffer in a pH range from pH 7.5 to pH 8.5, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.1 mM to 5 mM, and a surfactant), 11:31-12:4 (disclosing a formulation buffer at pH 8.0, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.5 mM to 2.5 mM, and a surfactant), 14:15-28 (disclosing a formulation buffer at pH 8.0, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.5 mM to 2.5 mM, a surfactant, and a free radical inhibitor such as sodium citrate); *see also* 9:6-9 (stating, "An additional component which further stabilizes the added viral component comprise the addition of at least one salt of a divalent cation, including but not necessarily limited to $MgCl_2$, $CaCl_2$ and $MnCl_2$. The preferred divalent cations are $MgCl_2$ and $CaCl_2$ at a

concentration ranging from about 0.1 mM to about 5 mM."), 36:25-27 (claim 5) (stating, "wherein the divalent cation is selected from the group consisting of $MgCl_2$ and $CaCl_2$ in an amount from about 0.1 mM to about 5 mM"). The presence of $MgCl_2$ in this buffer meets this limitation of the claims.

295. A POSA would have been motivated to exchange the buffer of Wu with a buffer in Konz/Evans because both have high ionic strengths, both have divalent ions, and both are in a similar pH range. The Konz/Evans buffer, however, includes a non-ionic surfactant, which a POSA would have understood could be used to inhibit aggregation even further. A POSA would have understood that replacing the stock buffer of Wu with such a buffer from Konz, *e.g.*, by diafiltration, would have removed the residual chloroform, and been expected to maintain or enhance the stability of the rAAV stock solution during storage.

296. Wu and Konz thus both meet this limitation of claim 1.

5. "wherein the ionic strength of the composition is greater than 200mM"

297. The buffer disclosed in Wu contains 1X PBS^{2+}, and residual NaCl from the PEG/NaCl precipitation, given the lack of a washing step after pelleting and before resuspension in PBS^{2+}. EX1007 (Wu), p. 2; EX1065 (Yeung), pp. 250-51.

298. A POSA would have understood that the ionic strength of 1X PBS^{2+} is calculated as follows.

299. The components of 10X PBS are as follows, dissolved in water brought up to a volume of 1 liter: $Na_2HPO_4 \cdot H_2O$ (14.2 g) (0.089 moles, given molecular weight of 160 g); NaCl (80 g) (1.38 moles, given molecular weight of 58 g); KCl (2 g) (0.027 moles, given molecular weight of 74 g); KH_2PO_4 (2 g) (0.015 moles, given molecular weight of 136 g). EX1065 (Yeung), pp. 250-51; EX1079 (Solomons), pp. 4-5.

300. Therefore, the concentration of each component of 10X PBS is: $Na_2HPO_4 \cdot H_2O$ (89 mM); NaCl (1380 mM); KCl (27 mM); KH_2PO_4 (15 mM).

301. The concentration of each component of 1X PBS (a 1:10 dilution of 10X PBS) is therefore: $Na_2HPO_4 \cdot H_2O$ (8.9 mM); NaCl (138 mM); KCl (2.7 mM); KH_2PO_4 (1.5 mM).

302. The following equation is used to calculate ionic strength:

$$I = \frac{1}{2} \sum_{i=1}^{n} c_i z_i^2$$

where I is the ionic strength, c_i is the molar concentration of ion i (mol/L), and z_i is the charge on that ion.

303. Using this equation, the contribution to the ionic strength of 1X PBS of each component at pH 7.5 is: $H_2PO_4^-$ (1.735 mM); HPO_4^{2-} (13.86 mM); Na^+ [from

Na_2HPO_4] (8.9 mM); K^+ [from KH_2PO_4] (0.75 mM); NaCl (138 mM); KCl (2.7 mM).[16]

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[16] For the ionic strength calculations for Na_2HPO_4 and KH_2PO_4, the approximation was made that at a pH in the range of 7.4 to 8.0, $H_2PO_4^-$ and HPO_4^{2-} concentrations need to be considered, while the much smaller relative concentrations of H_3PO_4 and PO_4^{3-} do not. *See, e.g.*, EX1080 (Bates), pp. 3, 7. The pK_a of the equilbrium between $H_2PO_4^-$ and HPO_4^{2-} used for these calculations is 7.2. *See, e.g.*, EX1082 (Green), pp. 2-3; EX1083 (Current Protocols), p. 3, Table A.2A.2. The Henderson-Hasselbalch equation is used to calculate the concentrations of the $H_2PO_4^-$ and HPO_4^{2-} ions, given a pK_a of 7.2. *See, e.g.*, EX1081 (Po), p. 1 (stating that the Henderson-Hasselbalch equation is: $pH = pK_a + \log([A^-]/[HA])$).

The total concentration of HPO_4^{2-} ions from 8.9 mM Na_2HPO_4 and $H_2PO_4^-$ ions from 1.5 mM KH_2PO_4 is: 8.9 mM + 1.5 mM = 10.4 mM. According to Henderson-Hasselbalch, at pH 7.5:

$$pH = pKa + log\frac{[\mathrm{A}-]}{[\mathrm{HA}]}$$

$$7.5 = 7.2 + log\frac{[\mathrm{HPO42}-]}{[\mathrm{H2PO4}-]}$$

$$10^{7.5} = 10^{7.2}\ x\ \frac{[\mathrm{HPO42}-]}{[\mathrm{H2PO4}-]}$$

304. The total ionic strength of 1X PBS at pH 7.5 is therefore about 165.94 mM.

305. The components of 1X PBS^{2+} are: 1X PBS, 0.01 vol. 68 mM $CaCl_2$, and 0.01 vol. 50 mM $MgCl_2$. EX1065 (Yeung), pp. 250-51.

306. Therefore, 1X PBS^{2+} has additional contributions to the ionic strength from the added amounts of $CaCl_2$ and $MgCl_2$.

307. Specifically, 0.01 volume of 68 mM $CaCl_2$ is 0.68 mM $CaCl_2$, which contributes 2.04 mM to the ionic strength.[17]

308. And 0.01 volume of 50 mM $MgCl_2$ contributes 1.5 mM to the ionic strength.[18] Together, the added $CaCl_2$ and $MgCl_2$ add about 3.54 mM to the ionic

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$$10^{0.3} = 2 = \frac{[\mathrm{HPO42-}]}{[\mathrm{H2PO4-}]}$$

Also, we know that: $[HPO_4^{2-}] + [H_2PO_4^-] = 10.4$ mM. Therefore, given that the ratio of $[HPO_4^{2-}]$ to $[H_2PO_4^-]$ is 2:1, we know that $[HPO_4^{2-}] = 6.93$ mM, and $[H_2PO_4^-] = 3.47$ mM.

Therefore, at pH 7.5, the contribution to the total ionic strength of $[HPO_4^{2-}]$ is 1/2 [(6.93) x (4)] = 13.86 mM. The contribution to the total ionic strength of $[H_2PO_4^-]$ is 1/2 [(3.47) x (1)] = 1.735 mM.

[17] I ($CaCl_2$) = (1/2) [(0.68)(4) + (0.68)(2)(1)] = 2.04 mM.

[18] I ($MgCl_2$) = (1/2) [(0.5)(4) + (0.5)(2)(1)] = 1.5 mM.

strength. Therefore, at pH 7.5, the total ionic strength of 1X PBS^{2+} is about 169.48 mM.

309. At pH 8.0, the contribution to the ionic strength of 1X PBS of each component is: $H_2PO_4^-$ (0.71 mM); HPO_4^{2-} (17.96 mM); Na^+ [from Na_2HPO_4] (8.9 mM); K^+ [from KH_2PO_4] (0.75 mM); NaCl (138 mM); KCl (2.7 mM).[19] The total ionic strength of 1X PBS at pH 8.0 is therefore about 169.02 mM. For PBS^{2+}, adding in the contributions to the ionic strength from $CaCl_2$ and $MgCl_2$ (about 3.54 mM), the ionic strength becomes 172.56 mM at pH 8.0.

310. Given that, as I discussed above (Section VIII.A), Wu does not include any steps in which the rAAV pellet resulting from the PEG/NaCl precipitation is washed, there will be residual NaCl when the pellet is resuspended in PBS^{2+} buffer.

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[19] For a pH of 8.0, the Henderson-Hasselbalch equation becomes:

$$10^{8.0} = 10^{7.2}\, x\, \frac{[\mathrm{HPO42-}]}{[\mathrm{H2PO4-}]}$$

$$10^{0.8} = 6.3 = \frac{[\mathrm{HPO42-}]}{[\mathrm{H2PO4-}]}$$

Therefore, given that the ratio of $[HPO_4^{2-}]$ to $[H_2PO_4^-]$ is 6.3:1, we know that $[HPO_4^{2-}]$ = 8.98 mM, and $[H_2PO_4^-]$ = 1.42 mM. The contribution of $[HPO_4^{2-}]$ to the ionic strength is 1/2 (8.98)(4) = 17.96 mM. The contribution of $[H_2PO_4^-]$ to the ionic strength is 1/2 (1.42)(1) = 0.71 mM.

EX1007 (Wu), p. 2, Fig. 1. The final volume of the resuspension is 5 ml. EX1007 (Wu), p. 2.

311. In my opinion, it is likely that at least 0.20 mls of 1M NaCl remained after the supernatant was discarded, and therefore was present when the pellet was resuspended in PBS^{2+} for a final volume of 5 mls.

312. This residual 0.20 mls of 1M NaCl would contribute about 40 mM to the ionic strength of the PBS^{2+}solution,[20] resulting in a final ionic strength of about 209 mM (169.48 mM + 40 mM) at pH 7.5, and a final ionic strength of about 212 mM (172.56 mM + 40 mM), both of which meet the ionic strength limitation of the challenged claims.

313. Konz incorporates Evans by reference, and Evans, in turn discloses formulation buffers that meet this limitation of the claims. *See, e.g.*, EX1020 (Evans), 11:13-21 (disclosing a formulation buffer in a pH range from pH 7.5 to pH 8.5, NaCl concentration above 250 mM, $MgCl_2$ from 0.1 mM to 10 mM, and a surfactant), 11:22-30 (disclosing a formulation buffer in a pH range from pH 7.5 to pH 8.5, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.1 mM to 5 mM, and a surfactant), 11:31-12:4 (disclosing a formulation buffer at pH 8.0, NaCl

[20] 0.20 mls of 1M NaCl would contribute 0.20 mmol of NaCl to the final volume of 5 mls. And 0.20 mmol of NaCl in 5 mls gives a concentration of 40 mM.

concentration from 25 mM to 250 mM, $MgCl_2$ from 0.5 mM to 2.5 mM, and a surfactant), 14:15-28 (disclosing a formulation buffer at pH 8.0, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.5 mM to 2.5 mM, a surfactant, and a free radical inhibitor such as sodium citrate); *see also* 36:21-22 (claim 4) (reciting, "the salt is sodium chloride from about 25 mM to about 250 mM"), 41:14-15 (claim 37) (same).

314. Per the equation above, a buffer containing 250 mM NaCl would have an ionic strength of at least 250 mM, which is greater than the 200 mM limitation of the claims. Therefore, this Konz/Evans buffer meets this limitation of the claims.

315. A POSA would have been motivated to exchange the buffer of Wu with the buffer in Konz/Evans because both have high ionic strengths, both have multivalent ions, and both are in a similar pH range. The Konz/Evans buffer, however, includes a surfactant, which a POSA would have understood could be used to inhibit aggregation even further. And, as I discussed above, a POSA would have understood that a buffer exchange into a formulation buffer disclosed in Konz would remove residual chloroform from the Wu preparation, per Wu's teachings. EX1007 (Wu), p. 4.

316. Wu, in combination with Konz, thus meets this limitation of claim 1.

6. "and wherein the purified AAV vector particles are stored in the composition without significant aggregation"

317. Wu discloses an electron microscopy analysis of the purified rAAV particles. EX1007 (Wu), pp. 2-3, Fig. 3. There is no evidence of aggregation in this study. Moreover, Wu discloses that the rAAV purified stock was stored at 4°C for a month with no significant loss of infectious titer, indicating that the absence of aggregates was maintained during storage. EX1007 (Wu), p. 4. Wu therefore meets this limitation of the claims.

318. Konz also discloses methods to inhibit particle aggregation and promote stability, including by reference to Evans. *See, e.g.*, EX1008 (Konz), 23:17-19 ("The presence of 0.1% PS-80 in the buffers is critical to achieving low residual DNA levels in the product because it attenuates virus/DNA association and virus aggregation. It will be within the realm of routine experimentation for the artisan of ordinary skill to establish higher or lower detergent concentrations or alternative detergents which would be useful to promote dissociation of virus particles away from other virus as well as various cell contaminants"); EX1020 (Evans), 8:30-33 ("An additional aspect of the formulations of the present invention relates to a formulation which comprises a minimal amount of at least one non-ionic surfactant added to reduce adsorption to container surfaces as well as possible providing increased virus stabilization"); EX1008 (Konz) 48:11-21, Table 12 (showing DLS results indicating no aggregation, along with high yield (98%) for the sterile

filtration process step), 50:1-5, Table 14 (showing 100% yield for the sterile filtration process step), 51:5-10, Table 16 (showing 99% yield for the sterile filtration process step).

319. As discussed further below for claims 5 and 6, Konz teaches that sterile filtration is carried out after storage at 4ºC. *See, e.g.*, EX1008 (Konz), 30:13-30, 42:4-19, 48:4-5. Specifically, Konz discloses a protocol in which the purified product is diafiltered to introduce formulation buffer, stating that this step "is operated at approximately 4ºC and the product is held at 4ºC until sterile filtration." *See, e.g.*, EX1008 (Konz), 30:13-30, 42:4-19, 48:4-5.

320. Therefore, a POSA would have understood that in the examples in which the preparation of viral particles is found not to contain aggregation as assessed by sterile filtration or DLS, the sterile filtration and DLS have been carried out after storage in formulation buffer. Moreover, a POSA would have understood that the high yield following sterile filtration after storage (98%) indicates that sterile filtration had little if any effect on the preparation as far as removal of any aggregates, and therefore that the DLS result was representative of the preparation before sterile filtration. EX1008 (Konz) 48:11-21, Table 12.

321. Wu, in combination with Konz, thus meets this limitation of claim 1.

B. Claim 2: "The composition of claim 1, further comprising ethylene oxide/propylene oxide block copolymer Pluronic® F68"

322. In my opinion, the combination of Wu and Konz discloses the additional limitation of dependent claim 2.

323. Konz, including by reference to Evans, discloses formulations containing Pluronic non-ionic surfactants. *See, e.g.*, EX1008 (Konz), 23:17-24:9 ("The presence of 0.1 % PS-80 in the buffers is critical to achieving low residual DNA levels in the product because it attenuates virus/DNA association and virus aggregation. It will be within the realm of routine experimentation for the artisan of ordinary skill to establish higher or lower detergent concentrations or alternative detergents which would be useful to promote dissociation of virus particles away from other virus as well as various cell contaminants. It is also within this same realm of experimentation that the artisan may choose an alternative detergent to the process buffer. 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 . . . the Pluronic series of non-ionic surfactants (e.g., Pluronic 121)"; EX1020 (Evans), 8:30-9:5 ("An additional aspect of the formulations of the present invention relates to a formulation which comprises a minimal amount of at least one non-ionic surfactant added to reduce adsorption to container surfaces as well as possibly providing increased virus stabilization. Non-

ionic surfactants for use in the formulations of the present invention include but are not limited to . . . the Pluronic series of non-ionic surfactants (e.g., Pluronic 121)").

324. As Konz states, a POSA at the time would have selected the most appropriate non-ionic surfactant from the Pluronic series, which included Pluronic F68.

325. Therefore Wu, in combination with Konz, meets the additional limitation of dependent claim 2.

C. Claim 3: "The composition of claim 2, wherein the Pluronic® F68 is present at a concentration of 0.001% (w/v)"

326. In my opinion, the combination of Wu and Konz discloses the additional limitation of dependent claim 3.

327. Konz, by reference to Evans, discloses formulation buffers where the non-ionic surfactant is present at a concentration of 0.001% w/v. *See, e.g.*, EX1020 (Evans), 11:13-21 ("In a particular embodiment of the present invention the formulation is buffered with Tris to a range from about pH 7.5 to about pH 8.5; sucrose is added within a range upwards of a weight to volume percentage of 10, depending upon the salt concentration; the salt being NaCl which is added at concentration within a range of upwards of 250 mM NaCl, complementing the sucrose concentration such that total osmolarity ranges from about 200 mOs/L to about 800 mOs/L; the divalent cation is $MgCl_2$ in a range from about 0.1 mM to about 10 mM, and the surfactant is either Polysorbate-80 at a concentration from

about 0.001% to about 1% or Polysorbate-40 at a concentration from about 0.001% to about 1%").

328. As Konz explains, a POSA would have known to select the appropriate detergent and how to choose the appropriate concentration of detergent for a given formulation. *See, e.g.*, EX1008 (Konz), 23:19-24:1 ("It will be within the realm of routine experimentation for the artisan of ordinary skill to establish higher or lower detergent concentrations or alternative detergents which would be useful to promote dissociation of virus particles away from other virus as well as various cell contaminants. It is also within this same realm of experimentation that the artisan may choose an alternative detergent to the process buffer.").

329. Therefore, Wu, in combination with Konz, meets the additional limitation of dependent claim 3.

D. Claim 4: "The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate."

330. Konz incorporates Evans by reference, and Evans, in turn, discloses adding a non-reducing free radical scavenger/chelator such as sodium citrate to formulation buffers to maximize short and long term stability of viral preparations. EX1020 (Evans), 13:8-19. Evans discloses adding 100 mM citrate to enhance stability. EX1020 (Evans), 15:29-31.

331. A POSA would have understood that high ionic strength inhibits aggregation of viral particles, particularly at a high physical titer, and therefore would have been motivated to add citrate to the formulation buffer at a 100 mM concentration.

332. A POSA, furthermore, as discussed above, would have been motivated to select a formulation buffer similar to the buffer in Wu that successfully inhibited aggregation and maintained stability. A POSA, therefore, would have chosen the buffer disclosed in Evans with a pH of 8.0, containing NaCl at a concentration of about 250 mM, in addition to $MgCl_2$ and sodium citrate. EX1020 (Evans), 14:15-28. This buffer contains Tris in a range up to 7.5 mM, which a POSA would have understood to provide similar buffering capacity as 10 mM Tris to achieve and maintain the desired pH.

333. Konz, by reference to Evans, therefore meets the additional limitations of claim 4.

E. Claim 5: "The composition of claim 1, wherein the purified, recombinant AAV vector particles have an average particle radius (Rh) of less than about 20 nm as measured by dynamic light scattering"

334. In my opinion, the combination of Wu and Konz discloses the additional limitation of dependent claim 5.

335. Konz discloses using DLS to evaluate particle aggregation. *See, e.g.*, EX1008 (Konz), 48:12-14 (stating, "The mean particle size by Dynamic Light

Scattering was 123 nm, consistent with theoretical expecations"). Konz found that the mean particle size was as expected for individual particles that were not aggregated. Given that Konz states expressly that its teachings are applicable to rAAV, a POSA would have understood to use DLS to evaluate whether the particle size of rAAV was as expected for individual AAV particles. EX1008 (Konz), 14:27-29.

336. Konz further teaches carrying out DLS to assess aggregation after storage. EX1008 (Konz), 30:19-20, 48:4-14.

337. Konz teaches that sterile filtration is carried out after storage at 4ºC. *See, e.g.*, EX1008 (Konz), 30:13-30. Specifically, Konz discloses a protocol in which the purified product is diafiltered to introduce formulation buffer, stating that this step "is operated at approximately 4ºC and the product is held at 4ºC until sterile filtration." *See, e.g.*, EX1008 (Konz), 30:13-30.

338. Example 5 of Konz discloses a protocol involving diafiltering the purified batch of viral particles into formulation buffer, followed by sterile filtration with a 0.22 micron filter. Specifically, Example 5 states that sterile filtration was carried out with a Millipore Millipak-20 filter (100 cm^2)." EX1008 (Konz) 42:18-19. A Millipore Millipak-20 filter is a 0.22 μm filter. *See, e.g.*, EX1008 (Konz), 25:29-30; EX1069 (Hatano), p. 3. The yield from the sterile filtration step in Example 5 was 98%. EX1008 (Konz), 43:1-5, Table 6.

339. In Example 9, Konz states that the "process specifics used are similar to those described in Example 5 . . . ." EX1008 (Konz), 48:4-5. The yield from the sterile filtration step in Example 9 was 98%. EX1008 (Konz), 48:15-21, Table 12.

340. Example 9 further discloses analysis of the "final product" by DLS, which a POSA would have understood to mean the product after sterile filtration, and therefore after storage at 4°C. EX1008 (Konz), 48:11-14. In addition, in Example 9, the DLS analysis is discussed after the yields for the various process steps, including sterile filtration, further confirming that DLS was carried out after sterile filtration and therefore after storage. EX1008 (Konz), 48:11-15.

341. A POSA would have understood that the high yield following sterile filtration after storage (98%), indicates that sterile filtration had little if any effect on the preparation as far as removal of any aggregates, and therefore that the DLS result was representative of the preparation before sterile filtration. EX1008 (Konz) 48:11-21, Table 12.

342. A POSA would have understood from the teachings of Konz discussed above that in these examples, the ultrafiltration retentate was stored at 4°C before sterile filtration. *See, e.g.*, EX1008 (Konz), 30:13-30. Therefore, the yields greater than 90% for the sterile filtration step reported in Examples 2, 5, and 9 were obtained after storage in formulation buffer at 4°C.

343. Wu, in combination with Konz, therefore meets the additional limitation of dependent claim 5.

F. Claim 6: "The composition of claim 1, wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of said AAV vector particles through a 0.22 um filter"

344. In my opinion, the combination of Wu and Konz discloses the additional limitation of dependent claim 6.

345. Konz discloses using sterile filtration of purified recombinant viral particles through a 0.22 μm filter, with a recovery greater than 90%. *See, e.g.*, EX1008 (Konz), 25:29-30, 48:15-21, Table 12 (98% yield), 50:1-5, Table 14 (100% yield), 51:6-10, Table 16 (99% yield).

346. Konz teaches that sterile filtration is carried out after storage at 4ºC. *See, e.g.*, EX1008 (Konz), 30:13-30. Specifically, Konz discloses a protocol in which the purified product is diafiltered to introduce formulation buffer, stating that this step "is operated at approximately 4ºC and the product is held at 4ºC until sterile filtration." *See, e.g.*, EX1008 (Konz), 30:13-30.

347. Konz further teaches that the sterile filtration may be carried out using a 0.22 micron filter: "Sterile filtration may be added, as per Table 1, to eliminate bioburden. The final retentate will be filtered through a 0.22 micron modified polyvinylidene fluoride (PVDF) membrane . . . ." *See, e.g.*, EX1008 (Konz), 25:29-30, 30:27-30.

348. Therefore, Konz discloses storing the purified viral particles in formulation buffer at 4ºC, and then sterile filtering the composition through a 0.22 µm filter. *See also* EX1008 (Konz), 26:2-20 ("The product can be held frozen or at approximately 4°C for subsequent formulation and filling. An additional optional step downstream in the process is the inclusion of an orthogonal purification step in order to clear any remaining impurities and/or other agents. . . . The product can then be sterile filtered as before").

349. Example 2 of Konz discloses a protocol involving diafiltering the purified formulation, exchanging the virus into the formulation buffer using five diafiltration volumes. EX1008 (Konz), 36:21-28. After diafiltration, the product was sterile filtered with a 0.22 micron filter. EX1008 (Konz), 36:21-28. Example 2 states that "[n]o pressure build-up was seen during the sterile filtration which suggests a lack of aggregated virus." EX1008 (Konz), 36:25-27. Notably, as shown in Table 2, the yield from the sterile filtration step was 94%. EX1008 (Konz), 37:1-6, Table 2.

350. Example 5 of Konz also discloses a protocol involving diafiltering the purified batch of viral particles into formulation buffer, followed by sterile filtration with a 0.22 µm filter. Specifically, Example 5 states that sterile filtration was carried out with a Millipore Millipak-20 filter (100 cm^2). EX1008 (Konz) 42:18-19. A Millipore Millipak-20 filter is a 0.22 µm filter. *See, e.g.*, EX1008 (Konz), 25:29-30;

EX1069 (Hatano), p. 3. The yield from the sterile filtration step in Example 5 was 98%. EX1008 (Konz), 43:1-6, Table 6.

351. In Example 9, Konz states that the "process specifics used are similar to those described in Example 5 . . . ." EX1008 (Konz), 48:4-5. The yield from the sterile filtration step in Example 9 was 98%. EX1008 (Konz), 48:16-21, Table 12.

352. A POSA would have understood from the teachings of Konz discussed above that in these examples, the ultrafiltration retentate was stored at 4°C before sterile filtration. *See, e.g.*, EX1008 (Konz), 30:13-30. Therefore, the yields greater than 90% for the sterile filtration step reported in Examples 2, 5, and 9 were obtained after storage in formulation buffer at 4°C.

353. Therefore, Wu, in combination with Konz, meets the additional limitation of dependent claim 6.

G. A POSA Would Have Had a Reasonable Expectation of Success in Making the Claimed Combination

354. A POSA would have had a reasonable expectation of success in combining Wu with Konz to arrive at the claimed combination. The techniques required to make the claimed combination, namely, diafiltration, sterile filtration, and the use of DLS, were well known to people of skill in the art at the time and would have required nothing more than routine experimentation.

355. Wu's methods produced high titer rAAV that did not aggregate in a high ionic strength buffer containing multivalent ions. Konz teaches the addition of

a non-ionic surfactant to high salt buffers containing multivalent ions to decrease the probability of aggregation further, along with the use of sterile filtration and DLS to evaluate aggregation after storage, producing yields greater than 90%, and DLS results indicating individual viral particles without aggregation.

356. A POSA, using nothing more than routine experimentation, would have been able to complete Wu's preparation of high titer rAAV, then diafilter the preparation into one of the Konz high salt buffers containing a non-ionic surfactant such as Pluronic F68. Also, using nothing more than routine experimentation, after diafiltration, a POSA would have been able to sterile filter the preparation after storage, determine the yield, and also apply DLS to determine whether aggregation was present.

357. Moreover, a POSA would have a reasonable chance of success that the rAAV preparation would be without significant aggregation after storage. A POSA would have started with Wu's high titer rAAV preparation that did not aggregate, as indicated by the electron microscopic analysis and the fact that after storage for a month at 4°C there was no significant decrease of infectious titer, and then take further measures to ensure no aggregation. Specifically, given the teachings of Wu that a high titer rAAV preparation showed no aggregation in a high ionic strength buffer with multivalent ions, at a pH around 7.4 or 7.5 to 8.0, the POSA would have

sought to maintain these characteristics of the preparation in combining them with Konz.

358. The POSA would have chosen one of the Konz high ionic strength (250 mM NaCl) buffers, with a multivalent ion ($MgCl_2$), 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. Given all 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 DLS after storage at 4ºC, 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.

H. Secondary Considerations Do Not Change the Conclusion of Obviousness

359. For evidence of "secondary considerations" to be informative of obviousness, I understand that there must be a "nexus" or link between the alleged secondary consideration and the subject matter recited in the Asserted Claims. I am not aware of any secondary considerations of non-obviousness with the required nexus to the claims of the '542 patent. For example, I am not aware of any commercial success attributable to a formulation meeting the limitations of the

challenged claims.[21] Similarly, I am not aware of any licenses directed specifically to the '542 patent or the subject matter recited in challenged claims 3-6.[22]

360. Finally, I am not aware of any unexpected results having a nexus to the claimed subject matter. The '542 patent does not disclose unexpected properties of the claimed formulation. Effects of pH, multivalent ions, and ionic strength on viral particle aggregation had all been studied for decades before the '542 patent and disclosed in prior art references such as Floyd I, II, and III. And high titer rAAV formulations had been developed where aggregation was not present before the '542 patent and disclosed in prior art references such as Wu. The use of techniques such

[21] If Patent Owner attempts to rely on the commercial success of Sarepta's gene therapy treatment for Duchenne muscular dystrophy – Elevidys® – there is no nexus to the challenged claims of the '542 patent. There is no nexus between the commercial success of Elevidys® and the formulation recited in the challenged claims.

[22] If Patent Owner attempts to rely on any license to Novartis in the earlier case brought by Genzyme, I understand that any such license was executed in connection with the settlement of litigation and involved at least one other patent in addition to the '542 patent. Thus, there is no nexus between any Novartis license and the formulation recited in the challenged claims.

as DLS and sterile filtration using 0.22 μm filters for preparation of viral formulations had been disclosed in prior art references such as Konz.

361. To the extent Patent Owner attempts to raise secondary considerations that have only a marginal nexus, if any, to claims 3-6 of the '542 patent, such evidence of secondary considerations should not outweigh the compelling evidence of obviousness, discussed above. Thus, secondary considerations do not alter my opinion that claims 3-6 of the '542 patent are obvious over the combination of Wu and Konz.

XI. GROUND 2: CLAIM 3 IS OBVIOUS OVER THE COMBINATION OF WU, KONZ, AND CROYLE

362. In my opinion, dependent claim 3 is also obvious over the combination of Wu, Konz, and Croyle.

363. A POSA would have been motivated to combine Wu and Konz for the reasons I set out above regarding Ground 1. As I discussed above regarding Ground 1, the combination of Wu and Konz meets all the limitations of challenged claim 1.

364. A POSA would have been motivated to combine Wu and Konz with Croyle because Croyle discloses the use of a non-ionic surfactant, 0.001% Pluronic F68, not only to inhibit aggregation of a viral formulation but also to improve gene transfer and expression of a viral vector in a difficult to reach tissue. EX1009 (Croyle), Abstract, pp. 2-4, 6.

365. Croyle disclosed that addition of 0.001% Pluronic F68 alone to an adenoviral preparation substantially improved transduction of lung cells *in vivo* and *in vitro*. EX1009 (Croyle), pp. 2-3. When 0.001% Pluronic F68 was added to other excipients in a blended formulation, the best results of any formulation tested were obtained for in vitro transduction, in vivo transduction, and gene expression in lung cells and tissue. EX1009 (Croyle), pp. 2-4.

366. In addition, Croyle discloses that addition of 0.001% Pluronic F68 to the formulation completely inhibited aggregation of adenoviral particles, as determined by dynamic light scattering: "[the] ***average particle size of a viral preparation fell from 163.2 ± 30.6 nm to 70.4 ± 6.2 nm (the size of a single viral particle) when Pluronic F68 was added to the formulation*** as determined by dynamic laser light scattering." EX1009 (Croyle), p. 6 (emphasis added).

367. Given that Wu and Konz are directed to high physical titer preparations of viral particles without aggregation, that Konz teaches that its methods are applciable to rAAV formulations in addition to adenoviral formulations, and that Konz discloses the use of non-ionic surfactants such as the Pluronic series of surfactants to inhibit aggregation, a POSA would have been motivated to select 0.001% Pluronic F68 based on the disclosures of Croyle, to add to a high titer rAAV formulation to inhibit aggregation and perhaps also to improve transduction and expression of the viral vector.

A. Claim 2: "The composition of claim 1, further comprising ethylene oxide/propylene oxide block copolymer Pluronic® F68"

368. In my opinion, the combination of Wu, Konz, and Croyle discloses the additional limitation of dependent claim 2.

369. For the reasons I set out above for Ground 1, Wu and Konz disclose all the limitations of claim 1. As I discussed above, Wu and Konz together disclose high titer, high ionic strength preparations of viral particles containing multivalent ions and a non-ionic surfactant, including a surfactant from the Pluronic series of non-ionic surfactants to inhibit viral aggregation. As I further discussed above, Wu and Konz together disclose that analysis of the viral preparations in formulation buffer after storage showed no aggregation.

370. Croyle discloses specifically the use of Pluronic F68 non-ionic surfactant to inhibit viral particle aggregation.

371. Therefore, the combination of Wu, Konz, and Croyle discloses the additional limitation of dependent claim 2.

B. Claim 3: "The composition of claim 2, wherein the Pluronic® F68 is present at a concentration of 0.001% (w/v)"

372. In my opinion, the combination of Wu, Konz, and Croyle discloses the additional limitation of dependent claim 3.

373. Croyle discloses the use of 0.001% Pluronic F68 non-ionic surfactant to inhibit viral particle aggregation.

374. A POSA would have understood that the disclosure in Croyle of "0.001% Pluronic F68" refers to 0.001% "w/v" Pluronic F68.

C. A POSA Would Have Had a Reasonable Expectation of Success in Making the Claimed Combination

375. A POSA would have had a reasonable expectation of success in combining Wu and Konz with Croyle to arrive at the claimed combination. The techniques required to combine Wu and Konz to make the claimed combination, namely, diafiltration, sterile filtration, and the use of DLS, were well known to people of skill in the art at the time and would have required nothing more than routine experimentation. To combine Croyle with Wu and Konz requires only the addition of 0.001% Pluronic F68, which is clearly within the skill of a POSA at the relevant time.

376. Wu's methods produced high titer rAAV that did not aggregate in a high ionic strength buffer containing multivalent ions. Konz teaches the addition of a non-ionic surfactant to high salt buffers containing multivalent ions to decrease the probability of aggregation further, along with the use of sterile filtration and DLS to evaluate aggregation after storage.

377. A POSA, using nothing more than routine experimentation, would have been able to complete Wu's preparation of high titer rAAV, then diafilter the preparation into one of the Konz high salt buffers containing a non-ionic surfactant such as Pluronic F68. Also, using nothing more than routine experimentation, after

diafiltration, a POSA would have been able to sterile filter the preparation and also apply DLS to determine whether aggregation was present after storage.

378. Moreover, a POSA would have a reasonable chance of success that the rAAV preparation would be without significant aggregation after storage. A POSA would have started with Wu's high titer rAAV preparation that did not aggregate, per Wu's electron microscopy analysis and stability during storage, and then take further measures to ensure no aggregation. Specifically, given the teachings of Wu that a high titer rAAV preparation showed no aggregation in a high ionic strength buffer with multivalent ions, at a pH around 7.4 to 8.0, a POSA would have sought to maintain these characteristics of the preparation in combining them with Konz.

379. A POSA would have chosen one of the Konz high ionic strength (250 mM NaCl) buffers, with a multivalent ion ($MgCl_2$), at a pH similar to that of Wu (7.4 to 8.0), and added a non-ionic surfactant, in accordance with Konz's teachings. In accordance with Croyle's teachings that addition of 0.001% Pluronic F68 completely inhibited aggregation of a formulation of viral particles, a POSA would have selected 0.001% Pluronic F68 from the non-ionic surfactants disclosed in Konz.

380. As discussed above, Konz discloses purified preparations in formulation buffer that, after storage, produced yields greater than 90% after sterile filtration using a 0.22 micron filter, with no evidence of aggregation as evaluated by

DLS. *See* Sections X.E-F, *supra*. A POSA would have understood from Croyle that addition of Pluronic F68 to the formulation buffer in Konz would have further decreased the chance of aggregation.

381. Given all these steps to inhibit aggregation, given the starting point of Wu's formulation where no aggregation was detected, and given Konz's results, 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.

D. Secondary Considerations Do Not Change the Conclusion of Obviousness

382. For evidence of "secondary considerations" to be informative of obviousness, I understand that there must be a "nexus" or link between the alleged secondary consideration and the subject matter recited in the Asserted Claims. I am not aware of any secondary considerations of non-obviousness with the required nexus to the claims of the '542 patent. For example, I am not aware of any commercial success attributable to a formulation meeting the limitations of

dependent claim 3.[23] Similarly, I am not aware of any licenses directed specifically to the '542 patent or the subject matter recited in dependent claim 3.[24]

383. Finally, I am not aware of any unexpected results having a nexus to the claimed subject matter. The '542 patent does not disclose unexpected properties of the claimed formulation. Effects of pH, multivalent ions, ionic strength, and non-ionic surfactants on protein and viral particle aggregation had all been studied for decades before the '542 patent and disclosed in prior art references such as Floyd I, II, and III, Kreilgaard, Konz, and Croyle. And high titer, high ionic strength rAAV formulations containing multivalent ions had been developed where aggregation was not present before the '542 patent and disclosed in prior art references such as Wu.

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[23] If Patent Owner attempts to rely on the commercial success of Sarepta's gene therapy treatment for Duchenne muscular dystrophy – Elevidys® – there is no nexus to the challenged claims of the '542 patent. There is no nexus between the commercial success of Elevidys® and the formulation recited in challenged claim 3.

[24] If Patent Owner attempts to rely on any license to Novartis in the earlier case brought by Genzyme, I understand that any such license was executed in connection with the settlement of litigation and involved at least one other patent in addition to the '542 patent. Thus, there is no nexus between any Novartis license and the formulation recited in challenged claim 3.

The use of techniques such as DLS and sterile filtration using 0.22 μm filters for preparation of viral formulations had been disclosed in prior art references such as Konz and Croyle.

384. To the extent Patent Owner attempts to raise secondary considerations that have only a marginal nexus, if any, to claim 3 of the '542 patent, such evidence of secondary considerations should not outweigh the compelling evidence of obviousness, discussed above. Thus, secondary considerations do not alter my opinion that claim 3 of the '542 patent would have been obvious over the combination of Wu, Konz, and Croyle.

XII. GROUND 3: CLAIMS 3-6 ARE OBVIOUS OVER POTTER AND KONZ

385. In my opinion, claims 3-6 of the '542 patent are obvious over Potter and Konz.

386. A POSA would have been motivated to combine Potter with Konz because both Potter and Konz are directed towards large scale production of concentrated, high titer formulations of rAAV that are stable during storage. EX1010 (Potter), p. 2; EX1008 (Konz), 1:25-27, 22:15-16 (stating that "an appropriate formulation buffer (e.g., see PCT publication WO 01/66137) can be used to maximize product stability"), 25:20-22, 30:19-20.

387. Potter states that in the disclosed methods, they eliminated the need for any centrifugation steps to permit efficient large scale production. EX1010 (Potter),

p. 413. Nonetheless, Potter includes the analytical technique of electron microscopy, which is labor and time intensive and difficult to adapt to scale. EX1010 (Potter), pp. 16-17, Fig. 5.

388. Konz is also directed to methods of preventing aggregation, such as the use of non-ionic surfactants, and analytical techniques to evaluate the extent of aggregation, such as dynamic light scattering and 0.22 μm filtration, that are more adaptable to scale than the electron microscopy used in Potter. *See*, *e.g.*, EX1008 (Konz), 24:1-9, 48:11-21, Table 12.

389. A POSA would therefore be motivated to combine Potter's methods for large scale purification of rAAV with the additional improvements in Konz to streamline the production and make it even more adaptable to scale up.

390. A POSA would have further understood that the purification methods of Potter, producing pure, high titer rAAV, could be combined with the buffers and additional methods of Konz, by diafiltering Potter's final, purified rAAV preparation into one of Konz's formulation buffers, including those containing Pluronic non-ionic surfactant to prevent aggregation, and then analyzing the particles for aggregation according to Konz's methods of DLS and 0.22μ filtration. A POSA at the time would have understood that diafiltration is a technique to exchange one buffer with another. *See, e.g.*, EX1068 (Schwartz).

391. Moreover, a POSA would have understood that the methods of Potter produced a high titer rAAV preparation with no evidence of aggregation in a high ionic strength buffer (0.5M NaCl), with a multivalent ion (phosphate), around pH 7.4 or 7.5 to 8.0. Therefore a POSA would have been motivated to preserve these general characteristics in choosing one of the Konz buffers with the addition of a non-ionic surfactant to inhibit aggregation even further.

392. As I discussed above, claims 1 and 2 were statutorily disclaimed. EX1019 (Disclaimer). Nonetheless, I address these claims below because challenged claim 3 depends from claim 2 (which, in turn, depends from claim 1), and challenged claims 5 and 6 depend from claim 1.

A. Claim 1

1. "A composition for the storage of purified, recombinant adeno-associated virus (AAV) vector particles"

393. Potter discloses a composition for the storage of purified rAAV vector particles comprising a "reference standard stock of rAAV with a precisely defined titer." EX1010 (Potter), p. 2. This reference standard would be aliquoted into a large number of individual user vials, validated as a reference standard among a handful of rAAV laboratories, and then transferred to an appropriate distribution service. EX1010 (Potter), p. 2. This process, of creating the standard, aliquoting it, validating it at a handful of laboratories, and then transferring to a distribution service for distributing among a large number of rAAV laboratories, requires storing

the rAAV particles and maintaining their titer during storage. Otherwise, these aliquoted preparations of the standard would vary from the original stock, nullifying the entire purpose of creating a reference standard.

394. Konz discloses choosing formulation buffers to maximize product stability. *See, e.g.*, EX1008 (Konz), 22:15-16. Konz refers to a PCT publication (Evans), which it incorporates by reference. EX1008 (Konz), 22:15-16 (stating, "an appropriate formulation buffer (e.g., see PCT publication WO 01/66137 [Evans]) can be used to maximize product stability"), 25:20-22 ("The particular diafiltration buffer chosen should be an appropriate formulation buffer (see WO 0166137 [Evans] or a subset of the desired components"); *see also* 54:8-9 ("Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties").

395. Evans, in turn, states that "[e]specially preferred viral formulations disclosed herein are liquid adenovirus formulations, which show improved stability when stored in about the 2-8°C range while also being compatible with parenteral administration." EX1020 (Evans), 1:16-19, 20:19-24. Like Konz, Evans states expressly that the disclosed formulations and methods apply to rAAV, in addition to adenovirus. EX1020 (Evans), 3:12-14 ("The recombinant viruses of the present invention which show enhanced storage stability include but are not limited to

adenovirus, ***adeno-associated virus***, retroviruses, herpes virus, vaccinia virus, rotovirus, pox viruses") (emphasis added).

396. Potter, in combination with Konz, therefore meets this limitation of claim 1.

2. "purified, recombinant AAV particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4 x 10^{13} vg/ml"

397. Potter discloses purified, rAAV particles at concentrations exceeding 1 x 10^{13} vg/ml and less than 6.4 x 10^{13} vg/ml. Table II discloses titers of the rAAV reference standard obtained through Potter's purification process. EX1010 (Potter), p. 9, Table II. Two of the titers, obtained by a dot blot assay and by a real-time PCR assay, are disclosed in Table II as "1.12 x 10^{13} part/ml" and "1.46 x 10^{13} part/ml." EX1010 (Potter), p. 9, Table II.

398. The notation "vg/ml" in the '542 claims would have been understood by a POSA to mean "vector genomes / ml." *See, e.g.*, EX1001 ('542 patent), 10:2-4 ("After being stored for 45 days at 4° C. the preparation has a vector genome to infectious unit ratio (vg/IU) of 13 . . . ."). Expressing viral particle titer in terms of vector genomes per ml provides the number of filled capsids per ml of viral preparation, meaning capsids that contain the viral genome. Empty capsids will not contain a "vector genome" and will not be counted in a concentration measured as vg/ml.

399. A POSA at the time would have understood that the notation "vp/ml," which is used in Potter, could mean "vg/ml," depending on the assay that was used to determine the viral concentration. If the assay determined the number of filled capsids, or viral genomes, then in that case the "vp/ml" notation would be the same as "vg/ml." If the assay, on the other hand, determined "viral particles," irrespective of whether they were empty particles or filled particles, then "vp/ml" would not have the same meaning as "vg/ml."

400. As I discuss above (*see* Section VIII.D), a POSA would have understood that Potter used two assays, the DBA and the RTPA, that both provide a "vg/ml" concentration. Both of these assays determine the number of AAV genomes present per ml of the preparation. The AAV genome is a single stranded, DNA genome, and these assays determine the concentration of DNA genomes in the preparation.

401. Potter determined these vg/ml titers for the purified rAAV particles after the third column chromatography purification step of the procedure, the "national reference standard rAAV." *See* EX1010 (Potter), pp. 7-9, Table II. Potter found that the DBA and RTPA titers were very similar to one another, 1.12×10^{13} and 1.46×10^{13}, which provides confidence in both meaurements. *See* EX1010 (Potter), pp. 7-9, Table II. I have reproduced Table II below:

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

EX1010 (Potter), p. 9, Table II.

402. Both of these titers meet the claimed concentration range of the '542 patent. Therefore, Potter discloses "purified, recombinant AAV particles at a concentration exceeding 1 x 10^{13} vg/ml up to 6.4 x 10^{13} vg/ml."

403. Potter meets this limitation of claim 1.

3. "a pH buffer, wherein the pH of the composition is between 7.5 and 8.0"

404. The buffer used in Potter is PBS with 0.5M NaCl. *See* EX1010 (Potter), p. 7 (stating that the "virus is eluted with PBS containing 0.5M NaCl"). A POSA would have understood that the pH of PBS varies depending on the exact preparation and conditions such as temperature, but is generally in the range of approximately 7.4 or 7.5 to 8.0. *See, e.g.*, EX1057 (Cold Spring Harbor Protocols); EX1023 (Hermens), pp. 2-3; EX1070 (Monahan), p. 9; EX1065 (Yeung), pp. 257, 294. Therefore, a POSA would have understood that Potter meets this limitation of the '542 patent claims.

405. As I discussed above, Konz discloses that higher pH buffers improve viral particle stability. EX1008 (Konz), 26:12. Konz discloses that after column chromatography, "the pH is increased to the formulation target through the addition of a high pH Tris buffer." EX1008 (Konz), 26:16-17.

406. Konz incorporates Evans by reference, and Evans, in turn discloses formulation buffers that meet this pH limitation of the claims. *See, e.g.*, EX1020 (Evans), 11:13-21 (disclosing a formulation buffer in a pH range from pH 7.5 to pH 8.5, NaCl concentration above 250 mM, $MgCl_2$ from 0.1 mM to 10 mM, and a surfactant), 11:22-30 (disclosing a formulation buffer in a pH range from pH 7.5 to pH 8.5, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.1 mM to 5 mM, and a surfactant), 11:31-12:4 (disclosing a formulation buffer at pH 8.0, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.5 mM to 2.5 mM, and a surfactant), 14:15-28 (disclosing a formulation buffer at pH 8.0, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.5 mM to 2.5 mM, a surfactant, and a free radical inhibitor such as sodium citrate); *see also* 8:23-28 ("It will be known to one of skill in the art to provide virus formulations of the present invention in a physiologically acceptable buffer . . . within a pH range including but not limited to about 7.0 to about 9.0, preferably a pH range from about 7.5 to about 8.5"), 36:16-18 (claim 3) (reciting that "the buffer is selected from a group of buffers acceptable for human parenteral use, preferably a Tris buffer, at a pH from about 7.5 to about

8.5"), 41:9-11 (claim 36) (same). The pH range from 7.5 to 8.5 meets the limitation of the claims.

407. A POSA would have been motivated to exchange the buffer of Potter with a buffer in Evans, as referenced in Konz, because both formulations have high NaCl concentrations, both have divalent ions, and both are in a similar pH range. The Konz/Evans buffer, however, includes a surfactant, which a POSA would have understood could be used to inhibit aggregation even further.

408. Potter, in combination with Konz, therefore meets this limitation of claim 1.

4. "excipients comprising one or more multivalent ions selected from the group consisting of citrate, sulfate, magnesium, and phosphate"

409. Potter discloses a phosphate buffer (PBS), which meets this limitation. *See* EX1010 (Potter), p. 7 (stating that the "virus is eluted with PBS containing 0.5M NaCl"). A POSA would have understood "PBS" to mean "phosphate buffered saline."

410. Konz incorporates Evans by reference, and Evans, in turn discloses formulation buffers that meet this limitation of the claims. *See, e.g.*, EX1020 (Evans), 11:13-21 (disclosing a formulation buffer in a pH range from pH 7.5 to pH 8.5, NaCl concentration above 250 mM, $MgCl_2$ from 0.1 mM to 10 mM, and a surfactant), 11:22-30 (disclosing a formulation buffer in a pH range from pH 7.5 to

pH 8.5, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.1 mM to 5 mM, and a surfactant), 11:31-12:4 (disclosing a formulation buffer at pH 8.0, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.5 mM to 2.5 mM, and a surfactant), 14:15-28 (disclosing a formulation buffer at pH 8.0, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.5 mM to 2.5 mM, a surfactant, and a free radical inhibitor such as sodium citrate); *see also* 9:6-9 ("An additional component which further stabilizes the added viral component comprise the addition of at least one salt of a divalent cation, including but not necessarily limited to $MgCl_2$, $CaCl_2$ and $MnCl_2$. The preferred divalent cations are $MgCl_2$ and $CaCl_2$ at a concentration ranging from about 0.1 mM to about 5 mM"), 36:25-27 (claim 5) (reciting, "wherein the divalent cation is selected from the group consisting of $MgCl_2$ and $CaCl_2$ in an amount from about 0.1 mM to about 5 mM"). The presence of $MgCl_2$ in this buffer meets this limitation of the claims.

411. A POSA would have been motivated to exchange the buffer of Potter with a buffer in Konz/Evans because both have high NaCl concentrations, both have divalent ions, and both are in a similar pH range. The Konz/Evans buffer, however, includes a surfactant, which a POSA would have understood could be used to inhibit aggregation even further.

412. Potter, in combination with Konz, therefore meets this limitation of claim 1.

5. "wherein the ionic strength of the composition is greater than 200mM"

413. The buffer disclosed in Potter contains 0.5M NaCl. EX1010 (Potter), p. 7. The ionic strength of that solution, which does not take into account additions to the ionic strength from the phosphate ions in the buffer, is greater than 200 mM. The following equation is used to calculate ionic strength:

$$I = \frac{1}{2} \sum_{i=1}^{n} c_i z_i^2$$

where I is the ionic strength, c_i is the molar concentration of ion i (mol/L), and z_i is the charge on that ion. So, to calculate the ionic strength of NaCl in Potter's buffer, the equation would be:

$$I = \frac{1}{2} [(0.5M)(1)^2 + (0.5M)(-1)^2] = 0.5M = 500\ mM$$

414. Because 500 mM is greater than 200mM, the buffer disclosed in Potter meets this limitation of the challenged claims.

415. Konz incorporates Evans by reference, and Evans, in turn discloses formulation buffers that meet this limitation of the claims. *See, e.g.*, EX1020 (Evans), 11:13-21 (disclosing a formulation buffer in a pH range from pH 7.5 to pH 8.5, NaCl concentration above 250 mM, $MgCl_2$ from 0.1 mM to 10 mM, and a surfactant), 11:22-30 (disclosing a formulation buffer in a pH range from pH 7.5 to pH 8.5, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.1 mM to 5 mM, and a surfactant), 11:31-12:4 (disclosing a formulation buffer at pH 8.0, NaCl

concentration from 25 mM to 250 mM, $MgCl_2$ from 0.5 mM to 2.5 mM, and a surfactant), 14:15-28 (disclosing a formulation buffer at pH 8.0, NaCl concentration from 25 mM to 250 mM, $MgCl_2$ from 0.5 mM to 2.5 mM, a surfactant, and a free radical inhibitor such as sodium citrate); *see also* 36:21-22 (claim 4) (reciting, "the salt is sodium chloride from about 25 mM to about 250 mM"), 41:14-15 (claim 37) (same).

416. Per the equation above, a buffer containing 250 mM NaCl would have an ionic strength of at least 250 mM, which is greater than the 200 mM limitation of the claims. Therefore, this Konz/Evans buffer meets this limitation of the claims.

417. A POSA would have been motivated to exchange the buffer of Potter with the buffer in Konz/Evans because both have high NaCl concentrations, both have divalent ions, and both are in a similar pH range. The Konz/Evans buffer, however, includes a surfactant, which a POSA would have understood could be used to inhibit aggregation even further.

418. Potter, in combination with Konz, therefore meets this limitation of claim 1.

6. "and wherein the purified AAV vector particles are stored in the composition without significant aggregation"

419. Potter discloses an electron microscopy analysis of the purified rAAV particles. EX1010 (Potter), pp. 16-17, Fig. 5. There is no evidence of aggregation in this study. Potter therefore meets this limitation of the claims.

420. Konz also discloses methods to inhibit particle aggregation, including by reference to Evans. *See, e.g.*, EX1008 (Konz), 23:17-19 ("The presence of 0.1% PS-80 in the buffers is critical to achieving low residual DNA levels in the product because it attenuates virus/DNA association and virus aggregation. It will be within the realm of routine experimentation for the artisan of ordinary skill to establish higher or lower detergent concentrations or alternative detergents which would be useful to promote dissociation of virus particles away from other virus as well as various cell contaminants"); EX1020 (Evans), 8:30-33 ("An additional aspect of the formulations of the present invention relates to a formulation which comprises a minimal amount of at least one non-ionic surfactant added to reduce adsorption to container surfaces as well as possible providing increased virus stabilization"); EX1008 (Konz), 48:11-21, Table 12 (showing DLS results indicating no aggregation, along with high yield (98%) for the sterile filtration process step), 50:1-5, Table 14 (showing 100% yield for the sterile filtration process step), 51:5-10, Table 16 (showing 99% yield for the sterile filtration process step).

421. As discussed further below for claims 5 and 6, Konz teaches that sterile filtration is carried out after storage at 4ºC. *See, e.g.*, EX1008 (Konz), 30:13-30, 42:4-19, 48:4-5. Specifically, Konz discloses a protocol in which the purified product is diafiltered to introduce formulation buffer, stating that this step "is

operated at approximately 4ºC and the product is held at 4ºC until sterile filtration." *See, e.g.*, EX1008 (Konz), 30:13-30, 42:4-19, 48:4-5.

422. Therefore, a POSA would have understood that in the examples in which the preparation of viral particles is found not to contain aggregation as assessed by sterile filtration or DLS, the sterile filtration and DLS have been carried out after storage in formulation buffer. Moreover, a POSA would have understood that the high yield following sterile filtration after storage (98%) indicates that sterile filtration had little if any effect on the preparation as far as removal of any aggregates, and therefore that the DLS result was representative of the preparation before sterile filtration. EX1008 (Konz), 36:24-27, 48:11-21, Table 12.

423. Potter, in combination with Konz, therefore meets this limitation of claim 1.

B. Claim 2: "The composition of claim 1, further comprising ethylene oxide/propylene oxide block copolymer Pluronic® F68"

424. In my opinion, the combination of Potter and Konz discloses the additional limitation of dependent claim 2.

425. Konz discloses formulations containing Pluronic non-ionic surfactants, including by reference to Evans. *See, e.g.*, EX1008 (Konz), 23:17-24:9 ("The presence of 0.1% PS-80 in the buffers is critical to achieving low residual DNA levels in the product because it attenuates virus/DNA association and virus aggregation. It will be within the realm of routine experimentation for the artisan of

ordinary skill to establish higher or lower detergent concentrations or alternative detergents which would be useful to promote dissociation of virus particles away from other virus as well as various cell contaminants. It is also within this same realm of experimentation that the artisan may choose an alternative detergent to the process buffer. 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 . . . the Pluronic series of non-ionic surfactants (e.g., Pluronic 121)"; EX1020 (Evans), 8:30-9:5 ("An additional aspect of the formulations of the present invention relates to a formulation which comprises a minimal amount of at least one non-ionic surfactant added to reduce adsorption to container surfaces as well as possibly providing increased virus stabilization. Non-ionic surfactants for use in the formulations of the present invention include but are not limited to . . . the Pluronic series of non-ionic surfactants (e.g., Pluronic 121)").

426. As Konz states, a POSA at the time would have selected the most appropriate non-ionic surfactant from the Pluronic series, which included Pluronic F68.

427. Therefore Potter, in combination with Konz, meets the additional limitation of dependent claim 2.

C. Claim 3: "The composition of claim 2, wherein the Pluronic® F68 is present at a concentration of 0.001% (w/v)"

428. In my opinion, the combination of Potter and Konz discloses the additional limitation of dependent claim 3.

429. Konz, by reference to Evans, discloses formulation buffers where the non-ionic surfactant is present at a concentration of 0.001% w/v. *See, e.g.*, EX1020 (Evans), 11:13-21 ("In a particular embodiment of the present invention the formulation is buffered with Tris to a range from about pH 7.5 to about pH 8.5; sucrose is added within a range upwards of a weight to volume percentage of 10, depending upon the salt concentration; the salt being NaCl which is added at concentration within a range of upwards of 250 mM NaCl, complementing the sucrose concentration such that total osmolarity ranges from about 200 mOs/L to about 800 mOs/L; the divalent cation is $MgCl_2$ in a range from about 0.1 mM to about 10 mM, and the surfactant is either Polysorbate-80 at a concentration from about 0.001% to about 1% or Polysorbate-40 at a concentration from about 0.001% to about 1%").

430. As Konz explains, a POSA would have known to select the appropriate detergent and how to choose the appropriate concentration of detergent for a given formulation. *See, e.g.*, EX1008 (Konz), 23:19-24:1 ("It will be within the realm of routine experimentation for the artisan of ordinary skill to establish higher or lower

detergent concentrations or alternative detergents which would be useful to promote dissociation of virus particles away from other virus as well as various cell contaminants. It is also within this same realm of experimentation that the artisan may choose an alternative detergent to the process buffer.").

431. Therefore, Potter, in combination with Konz, meets the additional limitation of dependent claim 3.

D. Claim 4: "The composition of claim 1, wherein the pH buffer is 10 mM Tris, pH 8.0 and the excipients comprise 100 mM sodium citrate."

432. Konz incorporates Evans by reference, and Evans, in turn, discloses adding a non-reducing free radical scavenger/chelator such as sodium citrate to formulation buffers to maximize short and long term stability of viral preparations. EX1020 (Evans), 13:8-19. Evans discloses adding 100 mM citrate to enhance stability. EX1020 (Evans), 15:29-31.

433. A POSA would have understood that high ionic strength inhibits aggregation of viral particles, particularly at a high physical titer, and therefore would have been motivated to add citrate to the formulation buffer at a 100 mM concentration.

434. A POSA, furthermore, as discussed above, would have been motivated to select a formulation buffer similar to the buffer in Potter that successfully inhibited aggregation and maintained stability. A POSA, therefore, would have chosen the

buffer disclosed in Evans with a pH of 8.0, containing NaCl at a concentration of about 250 mM, in addition to $MgCl_2$ and sodium citrate. EX1020 (Evans), 14:15-28. This buffer contains Tris in a range up to 7.5 mM, which a POSA would have understood to provide similar buffering capacity as 10 mM Tris to achieve and maintain the desired pH.

435. Konz, by reference to Evans, therefore meets the additional limitations of claim 4.

E. Claim 5: "The composition of claim 1, wherein the purified, recombinant AAV vector particles have an average particle radius (Rh) of less than about 20 nm as measured by dynamic light scattering"

436. In my opinion, the combination of Potter and Konz discloses the additional limitation of dependent claim 5.

437. Konz discloses using DLS to evaluate particle aggregation. *See, e.g.*, EX1008 (Konz), 48:4-15 (stating, "The mean particle size by Dynamic Light Scattering was 123 nm, consistent with theoretical expectations"). Konz found that the mean particle size was as expected for individual particles that were not aggregated. Given that Konz states expressly that its teachings are applicable to rAAV, a POSA would have understood to use DLS to evaluate whether the particle size of rAAV was as expected for individual AAV particles. EX1008 (Konz), 14:17-29.

438. Konz further teaches carrying out DLS to assess aggregation after storage. EX1008 (Konz), 30:19-20, 48:4-14.

439. Konz teaches that sterile filtration is carried out after storage at 4ºC. *See, e.g.*, EX1008 (Konz), 30:13-30. Specifically, Konz discloses a protocol in which the purified product is diafiltered to introduce formulation buffer, stating that this step "is operated at approximately 4ºC and the product is held at 4ºC until sterile filtration." *See, e.g.*, EX1008 (Konz), 30:13-30.

440. Example 5 of Konz discloses a protocol involving diafiltering the purified batch of viral particles into formulation buffer, followed by sterile filtration with a 0.22 micron filter. Specifically, Example 5 states that sterile filtration was carried out with a Millipore Millipak-20 filter (100 cm^2)." EX1008 (Konz) 42:18-19. A Millipore Millipak-20 filter is a 0.22 μm filter. *See, e.g.*, EX1008 (Konz), 25:29-30; EX1069 (Hatano), p. 3. The yield from the sterile filtration step in Example 5 was 98%. EX1008 (Konz), 43:1-5, Table 6.

441. In Example 9, Konz states that the "process specifics used are similar to those described in Example 5 . . . ." EX1008 (Konz), 48:4-5. The yield from the sterile filtration step in Example 9 was 98%. EX1008 (Konz), 48:15-21, Table 12.

442. Example 9 further discloses analysis of the "final product" by DLS, which a POSA would have understood to mean the product after sterile filtration, and therefore after storage at 4°C. EX1008 (Konz), 48:11-14. In addition, in

Example 9, the DLS analysis is discussed after the yields for the various process steps, including sterile filtration, further confirming that DLS was carried out after sterile filtration and therefore after storage. EX1008 (Konz), 48:11-14.

443. A POSA would have understood that the high yield following sterile filtration after storage (98%), indicates that sterile filtration had little if any effect on the preparation as far as removal of any aggregates, and therefore that the DLS result was representative of the preparation before sterile filtration. EX1008 (Konz) 48:11-21, Table 12.

444. A POSA would have understood from the teachings of Konz discussed above that in these examples, the ultrafiltration retentate was stored at 4°C before sterile filtration. *See, e.g.*, EX1008 (Konz), 30:13-30. Therefore, the yields greater than 90% for the sterile filtration step reported in Examples 2, 5, and 9 were obtained after storage in formulation buffer at 4°C.

445. Potter, in combination with Konz, therefore meets the additional limitation of dependent claim 5.

F. Claim 6: "The composition of claim 1, wherein recovery of the purified, recombinant virus particles is at least about 90% following filtration of the composition of said AAV vector particles through a 0.22 um filter"

446. In my opinion, the combination of Potter and Konz discloses the additional limitation of dependent claim 6.

447. Konz discloses using sterile filtration of purified recombinant viral particles through a 0.22 μm filter, with a recovery greater than 90%. *See, e.g.*, EX1008 (Konz), 25:29-30, 48:15-21, Table 12 (98% yield), 50:1-5, Table 14 (100% yield), 51:6-10, Table 16 (99% yield).

448. Konz teaches that sterile filtration is carried out after storage at 4ºC. *See, e.g.*, EX1008 (Konz), 30:13-30. Specifically, Konz discloses a protocol in which the purified product is diafiltered to introduce formulation buffer, stating that this step "is operated at approximately 4ºC and the product is held at 4ºC until sterile filtration." *See, e.g.*, EX1008 (Konz), 30:13-30.

449. Konz further teaches that the sterile filtration may be carried out using a 0.22 micron filter: "Sterile filtration may be added, as per Table 1, to eliminate bioburden. The final retentate will be filtered through a 0.22 micron modified polyvinylidene fluoride (PVDF) membrane . . . ." *See, e.g.*, EX1008 (Konz), 25:29-30, 30:27-30.

450. Therefore, Konz discloses storing the purified viral particles in formulation buffer at 4ºC, and then sterile filtering the composition through a 0.22 μm filter. *See also* EX1008 (Konz), 26:2-20 ("The product can be held frozen or at approximately 4°C for subsequent formulation and filling. An additional optional step downstream in the process is the inclusion of an orthogonal purification step in

order to clear any remaining impurities and/or other agents. . . . The product can then be sterile filtered as before").

451. Example 2 of Konz discloses a protocol involving diafiltering the purified formulation, exchanging the virus into the formulation buffer using five diafiltration volumes. EX1008 (Konz), 36:21-28. After diafiltration, the product was sterile filtered with a 0.22 micron filter. EX1008 (Konz), 36:21-28. Example 2 states that "[n]o pressure build-up was seen during the sterile filtration which suggests a lack of aggregated virus." EX1008 (Konz), 36:25-27. Notably, as shown in Table 2, the yield from the sterile filtration step was 94%. EX1008 (Konz), 37:1-6, Table 2.

452. Example 5 of Konz also discloses a protocol involving diafiltering the purified batch of viral particles into formulation buffer, followed by sterile filtration with a 0.22 μm filter. Specifically, Example 5 states that sterile filtration was carried out with a Millipore Millipak-20 filter (100 cm^2). EX1008 (Konz) 42:18-19. A Millipore Millipak-20 filter is a 0.22 μm filter. *See, e.g.*, EX1008 (Konz), 25:29-30; EX1069 (Hatano), p. 3. The yield from the sterile filtration step in Example 5 was 98%. EX1008 (Konz), 43:1-6, Table 6.

453. In Example 9, Konz states that the "process specifics used are similar to those described in Example 5 . . . ." EX1008 (Konz), 48:4-5. The yield from the sterile filtration step in Example 9 was 98%. EX1008 (Konz), 48:16-21, Table 12.

454. A POSA would have understood from the teachings of Konz discussed above that in these examples, the ultrafiltration retentate was stored at 4°C before sterile filtration. *See, e.g.*, EX1008 (Konz), 30:13-30. Therefore, the yields greater than 90% for the sterile filtration step reported in Examples 2, 5, and 9 were obtained after storage in formulation buffer at 4°C.

455. Therefore, Potter, in combination with Konz, meets the additional limitation of dependent claim 6.

G. A POSA Would Have Had a Reasonable Expectation of Success in Making the Claimed Combination

456. A POSA would have had a reasonable expectation of success in combining Potter with Konz to arrive at the claimed combination. The techniques required to make the claimed combination, namely, diafiltration, sterile filtration, and the use of DLS, were well known to people of skill in the art at the time and would have required nothing more than routine experimentation.

457. Potter's methods produced high titer rAAV that did not aggregate in a high salt buffer containing a multivalent ion. Konz teaches the addition of a non-ionic surfactant to high salt buffers containing multivalent ions to decrease the probability of aggregation further, along with the use of sterile filtration and DLS to evaluate aggregation after storage, producing yields greater than 90%, and DLS results indicating individual viral particles without aggregation.

458. A POSA, using nothing more than routine experimentation, would have been able to complete Potter's preparation of high titer rAAV, then diafilter the preparation into one of the Konz high salt buffers containing a non-ionic surfactant such as Pluronic F68. Also, using nothing more than routine experimentation, after diafiltration, a POSA would have been able to sterile filter the preparation after storage, determine the yield, and also apply DLS to determine whether aggregation was present.

459. Moreover, a POSA would have a reasonable chance of success that the rAAV preparation would be without significant aggregation after storage. The POSA would have started with Potter's high titer rAAV preparation that did not aggregate, per Potter's electron microscopy analysis, and then take further measures to ensure no aggregation. Specifically, given the teachings of Potter that a high titer rAAV preparation showed no aggregation in a high ionic strength (0.5M NaCl) buffer with a multivalent ion (phosphate), at a pH around 7.4 or 7.5 to 8.0, the POSA would have sought to maintain these characteristics of the preparation in combining them with Konz.

460. A POSA would have chosen one of the Konz high ionic strength (250 mM NaCl) buffers, with a multivalent ion ($MgCl_2$), at a pH similar to that of Potter (7.4 or 7.5 to 8.0), and added a non-ionic surfactant, in accordance with Konz's teachings. Given all these steps to inhibit aggregation, given the starting point of

Potter's formulation where no aggregation was detected, and given Konz's data showing greater than 90% yields and no aggregation per assessment by DLS after storage at 4ºC, 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.

H. Secondary Considerations Do Not Change the Conclusion of Obviousness

461. For evidence of "secondary considerations" to be informative of obviousness, I understand that there must be a "nexus" or link between the alleged secondary consideration and the subject matter recited in the Asserted Claims. I am not aware of any secondary considerations of non-obviousness with the required nexus to the claims of the '542 patent. For example, I am not aware of any commercial success attributable to a formulation meeting the limitations of the

challenged claims.[25] Similarly, I am not aware of any licenses directed specifically to the '542 patent or the subject matter recited in challenged claims 3-6.[26]

462. Finally, I am not aware of any unexpected results having a nexus to the claimed subject matter. The '542 patent does not disclose unexpected properties of the claimed formulation. Effects of pH, multivalent ions, and ionic strength on viral particle aggregation had all been studied for decades before the '542 patent and disclosed in prior art references such as Floyd I, II, and III. And high titer rAAV formulations had been developed where aggregation was not present before the '542 patent and disclosed in prior art references such as Potter. The use of techniques

[25] If Patent Owner attempts to rely on the commercial success of Sarepta's gene therapy treatment for Duchenne muscular dystrophy – Elevidys® – there is no nexus to the challenged claims of the '542 patent. There is no nexus between the commercial success of Elevidys® and the formulation recited in the challenged claims.

[26] If Patent Owner attempts to rely on any license to Novartis in the earlier case brought by Genzyme, I understand that any such license was executed in connection with the settlement of litigation and involved at least one other patent in addition to the '542 patent. Thus, there is no nexus between any Novartis license and the formulation recited in the challenged claims.

such as DLS and sterile filtration using 0.22 μm filters for preparation of viral formulations had been disclosed in prior art references such as Konz.

463. To the extent Patent Owner attempts to raise secondary considerations that have only a marginal nexus, if any, to claims 3-6 of the '542 patent, such evidence of secondary considerations should not outweigh the compelling evidence of obviousness, discussed above. Thus, secondary considerations do not alter my opinion that claims 3-6 of the '542 patent are obvious over the combination of Potter and Konz.

XIII. GROUND 4: CLAIM 3 IS OBVIOUS OVER THE COMBINATION OF POTTER, KONZ, AND CROYLE

464. In my opinion, dependent claim 3 is also obvious over the combination of Potter, Konz, and Croyle.

465. A POSA would have been motivated to combine Potter and Konz for the reasons I set out above regarding Ground 3. As I discussed above regarding Ground 3, the combination of Potter and Konz meets all the limitations of challenged claim 1.

466. A POSA would have been motivated to combine Potter and Konz with Croyle because Croyle discloses the use of a non-ionic surfactant, 0.001% Pluronic F68, not only to inhibit aggregation of a viral formulation but also to improve gene transfer and expression of a viral vector in a difficult to reach tissue. EX1009 (Croyle), Abstract, pp. 2-4, 6.

467. Croyle disclosed that addition of 0.001% Pluronic F68 alone to an adenoviral preparation substantially improved transduction of lung cells in vivo and in vitro. EX1009 (Croyle), pp. 2-3. When 0.001% Pluronic F68 was added to other excipients in a blended formulation, the best results of any formulation tested were obtained for in vitro transduction, in vivo transduction, and gene expression in lung cells and tissue. EX1009 (Croyle), pp. 2-4.

468. In addition, Croyle discloses that addition of 0.001% Pluronic F68 to the formulation completely inhibited aggregation of adenoviral particles, as determined by dynamic light scattering: "[the] ***average particle size of a viral preparation fell from 163.2 ± 30.6 nm to 70.4 ± 6.2 nm (the size of a single viral particle) when Pluronic F68 was added to the formulation*** as determined by dynamic laser light scattering." EX1009 (Croyle), p. 6 (emphasis added).

469. Given that Potter and Konz are directed to high titer preparations of viral particles without aggregation, and that Konz discloses the use of non-ionic surfactants such as the Pluronic series of surfactants to inhibit aggregation, a POSA would have been motivated to select 0.001% Pluronic F68 based on the disclosures of Croyle, to add to a high titer rAAV formulation to inhibit aggregation and perhaps also to improve transduction and expression of the viral vector.

A. Claim 2: "The composition of claim 1, further comprising ethylene oxide/propylene oxide block copolymer Pluronic® F68"

470. In my opinion, the combination of Potter, Konz, and Croyle discloses the additional limitation of dependent claim 2.

471. For the reasons I set out above for Ground 3, Potter and Konz disclose all the limitations of Claim 1. As I discussed above, Potter and Konz together disclose high titer, high ionic strength preparations of viral particles containing multivalent ions and a non-ionic surfactant, including a surfactant from the Pluronic series of non-ionic surfactants to inhibit viral aggregation. As I further discussed above, Potter and Konz together disclose that analysis of the viral preparations in formulation buffer after storage showed no aggregation.

472. Croyle discloses specifically the use of Pluronic F68 non-ionic surfactant to inhibit viral particle aggregation.

473. Therefore, the combination of Potter, Konz, and Croyle discloses the additional limitation of dependent claim 2.

B. Claim 3: "The composition of claim 2, wherein the Pluronic® F68 is present at a concentration of 0.001% (w/v)"

474. In my opinion, the combination of Potter, Konz, and Croyle discloses the additional limitation of dependent claim 3.

475. Croyle discloses the use of 0.001% Pluronic F68 non-ionic surfactant to inhibit viral particle aggregation.

476. A POSA would have understood that the disclosure in Croyle of "0.001% Pluronic F68" refers to 0.001% "w/v" Pluronic F68.

C. A POSA Would Have Had a Reasonable Expectation of Success in Making the Claimed Combination

477. A POSA would have had a reasonable expectation of success in combining Potter and Konz with Coyle to arrive at the claimed combination. The techniques required to combine Potter and Konz to make the claimed combination, namely, diafiltration, sterile filtration, and the use of DLS, were well known to people of skill in the art at the time and would have required nothing more than routine experimentation. To combine Croyle with Potter and Konz requires only the addition of 0.001% Pluronic F68, which is clearly within the skill of a POSA at the relevant time.

478. Potter's methods produced high titer rAAV that did not aggregate in a high salt buffer containing a multivalent ion. Konz teaches the addition of a non-ionic surfactant to high salt buffers containing multivalent ions to decrease the probability of aggregation further, along with the use of sterile filtration and DLS to evaluate aggregation after storage.

479. A POSA, using nothing more than routine experimentation, would have been able to complete Potter's preparation of high titer rAAV, then diafilter the preparation into one of the Konz high salt buffers containing a non-ionic surfactant such as Pluronic F68. Also, using nothing more than routine experimentation, after

diafiltration, a POSA would have been able to sterile filter the preparation and also apply DLS to determine whether aggregation was present after storage.

480. Moreover, a POSA would have a reasonable chance of success that the rAAV preparation would be without significant aggregation after storage. A POSA would have started with Potter's high titer rAAV preparation that did not aggregate, per Potter's electron microscopy analysis, and then take further measures to ensure no aggregation. Specifically, given the teachings of Potter that a high titer rAAV preparation showed no aggregation in a high ionic strength (0.5M NaCl) buffer with a multivalent ion (phosphate), at a pH around 7.4 or 7.5 to 8.0, a POSA would have sought to maintain these characteristics of the preparation in combining them with Konz.

481. A POSA would have chosen one of the Konz high ionic strength (250 mM NaCl) buffers, with a multivalent ion ($MgCl_2$), at a pH similar to that of Potter (7.5), and added a non-ionic surfactant, in accordance with Konz's teachings. In accordance with Croyle's teachings that addition of 0.001% Pluronic F68 completely inhibited aggregation of a formulation of viral particles, a POSA would have selected 0.001% Pluronic F68 from the non-ionic surfactants disclosed in Konz.

482. As discussed above, Konz discloses purified preparations in formulation buffer that, after storage, produced yields greater than 90% after sterile

filtration using a 0.22 μm filter, with no evidence of aggregation as evaluated by DLS. *See* Section XII.E-XII.F, *supra*. A POSA would have understood from Croyle that addition of Pluronic F68 to the formulation buffer in Konz would have further decreased the chance of aggregation.

483. Given all these steps to inhibit aggregation, given the starting point of Potter's formulation where no aggregation was detected, and given Konz's results, 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.

D. Secondary Considerations Do Not Change the Conclusion of Obviousness

484. For evidence of "secondary considerations" to be informative of obviousness, I understand that there must be a "nexus" or link between the alleged secondary consideration and the subject matter recited in the Asserted Claims. I am not aware of any secondary considerations of non-obviousness with the required nexus to the claims of the '542 patent. For example, I am not aware of any commercial success attributable to a formulation meeting the limitations of

dependent claim 3.[27] Similarly, I am not aware of any licenses directed specifically to the '542 patent or the subject matter recited in dependent claim 3.[28]

485. Finally, I am not aware of any unexpected results having a nexus to the claimed subject matter. The '542 patent does not disclose unexpected properties of the claimed formulation. Effects of pH, multivalent ions, ionic strength, and non-ionic surfactants on protein and viral particle aggregation had all been studied for decades before the '542 patent and disclosed in prior art references such as Floyd I, II, and III, Kreilgaard, Konz, and Croyle. And high titer, high ionic strength rAAV formulations containing multivalent ions had been developed where aggregation was not present before the '542 patent and disclosed in prior art references such as Potter.

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[27] If Patent Owner attempts to rely on the commercial success of Sarepta's gene therapy treatment for Duchenne muscular dystrophy – Elevidys® – there is no nexus to the challenged claims of the '542 patent. There is no nexus between the commercial success of Elevidys® and the formulation recited in challenged claim 3.

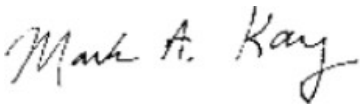
[28] If Patent Owner attempts to rely on any license to Novartis in the earlier case brought by Genzyme, I understand that any such license was executed in connection with the settlement of litigation and involved at least one other patent in addition to the '542 patent. Thus, there is no nexus between any Novartis license and the formulation recited in challenged claim 3.

The use of techniques such as DLS and sterile filtration using 0.22 μm filters for preparation of viral formulations had been disclosed in prior art references such as Konz and Croyle.

486. To the extent Patent Owner attempts to raise secondary considerations that have only a marginal nexus, if any, to claim 3 of the ’542 patent, such evidence of secondary considerations should not outweigh the compelling evidence of obviousness, discussed above. Thus, secondary considerations do not alter my opinion that claim 3 of the ’542 patent would have been obvious over the combination of Potter, Konz, and Croyle.

I hereby 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 further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Respectfully submitted,



Mark A. Kay, M.D., Ph.D.

Date: June 26, 2025