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. 7,704,721

"Compositions and Methods to Prevent AAV Vector Aggregation"

IPR2025-01195

DECLARATION OF MARK A. KAY, M.D., PH.D., IN SUPPORT OF PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 7,704,721

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. 7,704,721 ("the '721 patent") (EX1001) and requests that the United States Patent and Trademark Office cancel claims 1-4, 6, 7, and 11 of the '721 patent as unpatentable. The following discussion and analysis provides my opinions as to why claims 1-4, 6, 7, and 11 would have been obvious to a person of ordinary skill in the art ("POSA").

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 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. EX1005 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. 7,704,721 ("the '721 patent") (EX1001);
- 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, 12:71-76 (2001) ("Auricchio");
- WO 03/097797 A1 (PCT/US03/15061), "Methods of Adenovirus Purification," International Publication Date Nov. 27, 2003 ("Konz");
- Potter *et al*., "Streamlined Large-Scale Production of Recombinant Adeno-Associated Virus (rAAV) Vectors," Methods in Enzymology, Vol. 346, 2002, 413-430 ("Potter").

Exhibit Number	Description
EX1001	U.S. Patent No. 7,704,721 ("the '721 patent")
EX1002	Prosecution history of U.S. Patent No. 7,704,721 ("the '721 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	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")

Exhibit Number	Description
EX1008	WO 03/097797 A1 (PCT/US03/15061), "Methods of Adenovirus Purification," International Publication Date Nov. 27, 2003 ("Konz")
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	U.S. Patent No. 9,051,542 ("the '542 patent")
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")
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")

Exhibit Number	Description
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")
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")

Exhibit Number	Description
EX1030	Floyd and Sharp, "Aggregation of Poliovirus and Reovirus by Dilution in Water," *Applied and Environmental Microbiology*, pp. 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")
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")

Exhibit Number	Description
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")
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," *Current Opinion in Drug Discovery & Development* 2003: 174-178 ("Wright 2003")

Exhibit Number	Description
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")
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")

Exhibit Number	Description
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")
EX1056	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")
EX1057	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")
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	*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 ("Claim Construction Order")

Exhibit Number	Description
EX1062	*Genzyme Corporation and Aventis Inc. v. Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation*, C.A. No. 21-1736 (RGA) (D. Del.), D.I. 263 ("Claim Construction Opinion")
EX1063	*Genzyme Corporation and Aventis Inc. v. Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation*, C.A. No. 21-1736 (RGA) (D. Del.), D.I. 101 ("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")
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")

Exhibit Number	Description
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")
EX1073	*Phosphate-buffered saline,* COLD SPRING HARBOR LABORATORY PRESS (2006), https://cshprotocols.cshlp.org/content/2006/1/pdb.rec8247 **Error! Hyperlink reference not valid.**
EX1074	Po and Senozan, "The Henderson-Hasselbalch Equation: Its History and Limitations," *Journal of Chemical Education* 78.11 (2001): 1499 ("Po")
EX1075	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")
EX1076	T.W. Graham Solomons, ORGANIC CHEMISTRY (5th ed. 1992) ("Solomons")
EX1077	CURRENT PROTOCOLS IN NUCLEIC ACID CHEMISTRY (2000) A.2A.1-A.2A.12 (2000) ("Current Protocols")
EX1078	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")

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 1-4, 6, 7, and 11 of the '721 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 1-4, 6, 7, and 11 of the ’721 patent that I address in this declaration.

Ground	Claims	Description
1	1-4, 6, 7, 11	Obvious in view of Auricchio and Konz
2	1-4, 6, 7, 11	Obvious in view of Potter and Konz

21. I note that Auricchio, Potter, and Konz were not considered by the Patent Office during prosecution. EX1001 ('721 patent) ("References Cited"); EX1002 ('721 prosecution history).

22. Auricchio was published in January 2001, more than one year before the earliest priority date listed on the face of the '721 patent, June 1, 2004. EX1001 ('721 patent); EX1007 (Auricchio).

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 '721 patent, and published more than one year before December 22, 2004, the filing date of the '222 provsional application. EX1001 ('721 patent).

24. Potter was published in 2002, more than one year before the earliest priority date listed on the face of the '721 patent, June 1, 2004. EX1001 ('721 patent); EX1009 (Potter).

25. 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 a related patent, U.S. Patent No. 9,051,542, a continuation

of the application that issued as the '721 patent. EX1010 ('542 patent); EX1014 (608 Petition); EX1015 (609 Petition). 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.C.4), Patent Owner and its expert, Dr. Martyn Davies, materially mischaracterized Potter and the state of the art as of 2004 in the preliminary response to the 608 Petition. EX1016 (608 POPR). Patent Owner did not address Potter in its preliminary response to the 609 Petition.

V. TECHNICAL BACKGROUND AND STATE OF THE PRIOR ART

A. Gene Therapy

26. 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.

27. 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)

28. 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), at Abstract; EX1023 (Hermens), Abstract; EX1024 (Girod), p. 1.

29. 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.

30. 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.

31. Recombinant AAVs ("rAAVs") are AAVs in which the AAV genes have been replaced by genes that are potentially therapeutic. EX1028 (Xie), at 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

32. 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.

33. Density gradient (CsCl, iodixanol) 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; EX1042 (Zolotukhin), p. 2, Figure 1. Columns including heparin columns and anion exchange columns have been used successfully for rAAV purification. *See, e.g.*, EX1027 (Grimm and Kay), p. 8.

34. 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.*, EX1009 (Potter), pp. 14-17.

D. Viral Aggregation

35. 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).

36. 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).

37. 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. *See* 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.

38. 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). *See* 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. *See* EX1030 (Floyd I), p. 2.

39. Floyd I also tested the effect of different cations, Na^{+}, Mg^{2+}, and Ca^{2+}, on viral aggregation. *See* 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. *See* 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. *See* EX1030 (Floyd I), p. 4. Dilution in $CaCl_2$, however, produced a different result. *See* 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. *See* EX1030 (Floyd I), p. 4.

40. Floyd I also examined the effect of pH on poliovirus aggregation. *See* EX1030 (Floyd I), p. 4. Low pH values (pH 5 and 3) produced substantial amounts of aggregation. *See* EX1030 (Floyd I), p. 162. Aggregation at low pH was found to be influenced by the ionic strength of the solution. *See* EX1030 (Floyd I), p. 5. In particular, addition of NaCl or $MgCl_2$ prevented this low pH aggregation at certain

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

41. 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. *See* EX1030 (Floyd I), p. 7.

42. As far as the effect of the ionic strength of the solution on viral particle aggregation, reovirus behaved similarly to poliovirus. *See* 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. *See* 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. *See* 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 $CaCl_2$. *See* EX1030 (Floyd I), p. 7.

43. Like poliovirus, reovirus aggregated at lower pH values of 5 and 3 (but did not aggregate at pH 6, unlike poliovirus). *See* 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 was also the concentration required to prevent poliovirus aggregation at these low pH values. *See* EX1030 (Floyd I), pp. 5, Table 2, 8.

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

45. 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.

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

47. 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.

48. 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.

49. 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), p. 2-3.

50. 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.

51. 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.

52. 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.

53. 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.

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

55. 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.

56. 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.

57. 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.

58. 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.

59. 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, Figs. 3B, 4B, 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.

60. 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, Figs. 3, 7.

61. 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.

62. 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.

63. 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. EX1032 (Floyd III), p. 6. 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.

64. 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.

65. 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).

66. 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

67. 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.

68. 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.

69. 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).

70. 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).

71. 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).

72. 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").

73. 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. *See* EX1045 (Wright 2003), p. 2.

74. 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

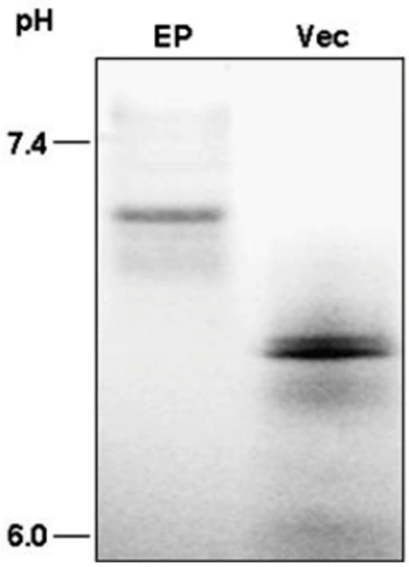
75. It was known in the art that empty parvovirus capsids have a different, higher isoelectric point from full capsids containing a viral genome. *See, e.g.*, EX1048 (Weichert), Abstract. Weichert studied the canine parvovirus (CPV), and found that the isoelectric point of CPV empty capsids was pH 5.3,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

76. 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.

77. 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).

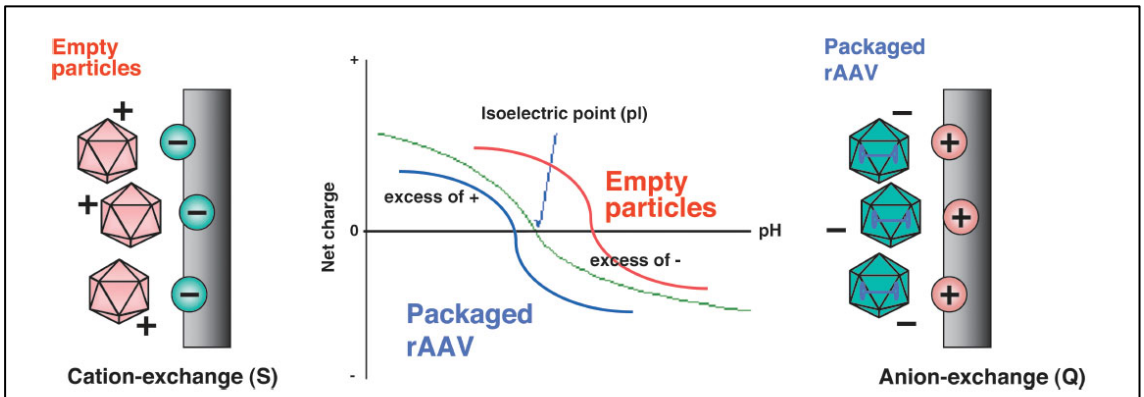
78. Notably, subsequent research confirmed the applicability to AAV of the CPV findings regarding pI of full and empty capsids, specifically the finding that empty capsids have a higher pI than full capsids. *See, e.g.*, EX1049 (Okada); EX1050 (Venkatakrishnan). Okada found that the isoelectric point 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.

79. 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.

80. 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. *See* 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. *See* EX1049 (Okada), p. 16, Supp. Fig. 1.

81. 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

82. 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 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").

83. 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)[1]); 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).

84. 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

85. 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; EX (Croyle), Abstract, p. 6. Non-ionic surfactants were known to be able to stabilize

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

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

86. 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)").[2]

87. 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. EX1056 (Croyle), Abstract, pp. 2, 6.

I. High Physical Titer rAAV Formulations

88. 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.*, EX1057 (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); EX1009 (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"); EX1007 (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

[2] 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).

89. As I discuss below (Section VIII), 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.*, EX1009 (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").

90. As I also discuss below (Section VIII.C.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 .

VI. THE ’721 PATENT

91. The ’721 patent is titled “Compositions and Methods to Prevent AAV Vector Aggregation.” EX1001 (’721 patent). The patent names John Fraser Wright and Guang Qu as inventors. EX1001 (’721 patent). The ’721 patent issued on April 27, 2010. EX1001 (’721 patent).

92. The ’721 patent is assigned to Genzyme Corporation. EX1001 (’721 patent).

A. The Claims

93. The challenged claims of the ’721 patent are directed to a method of preventing aggregation in a purified preparation of rAAV virions. EX1001 (’721 patent), 14:9-29, 33-40, 49-51.

94. I have reproduced the challenged claims in the table below.

Claim	Element
1 [pre]	A method of preventing aggregation of recombinant adeno-associated virus (rAAV) virions in a purified preparation of rAAV virions, comprising:
1[a]	1) providing a lysate comprising rAAV virions;
1[b]	2) purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, wherein said virions are purified; and
1[c]	3) adding one or more salts of multivalent ions selected from the group consisting of citrate, phosphate, sulfate and magnesium to said purified virions

Claim	Element
1[d]	to produce a preparation of virions with an ionic strength of at least 200 mM,
1[e]	wherein the concentration of rAAV virions in said preparation exceeds $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml;
1[f]	and wherein the pH of the purified preparation of rAAV virions is between 7.5 and 8.0.
2	The method of claim 1, further comprising treating said purified virions with a nuclease.
3	The method of claim 2, wherein the nuclease is an endonuclease from *Serratia marcescens*.
4	The method of claim 1, wherein the multivalent ion is citrate.
6	The method of claim 1, wherein, after addition of the one or more salts of multivalent ions, the average particle radius (Rh) of the virions in the preparation of virions is less than about 20 nm as measured by dynamic light scattering.
7	The method of claim 1, wherein, after addition of the one or more salts of multivalent ions, recovery of the virions is at least about 90% following filtration of the preparation of virions through a 0.22 μm filter.
11	The method of claim 2, further comprising diafiltering the purified rAAV virions to achieve an ionic strength of at least 200 mM.

95. As the table shows, claim 1 is an independent claim. EX1001 (’721 patent), 14:9-23. Claims 2-4, 6, 7, and 11 are dependent claims, which recite additional elements. EX1001 (’721 patent), 14:9-29, 33-40, 49-51.

B. The Specification

96. The specification of the ’721 patent discusses the effect of different buffers and methods of purification on aggregation of AAV2-FIX particles. EX1001 (’721 patent), Fig. 1B, Fig. 2, 4:11-29, 6:62-9:4; 10:18-11:49. “AAV2-FIX” vectors are AAV2 serotype viral vectors containing a human coagulation factor IX (“FIX”) transgene. EX1001 (’721 patent), 10:55-56. AAV2 is the only serotype tested in the ’721 patent. The specification discusses various methods to detect viral particle aggregation, including ultrafiltration and diafiltration, and dynamic light scattering. EX1001 (’721 patent), 11:51-13:3 (Examples 2 and 3).

97. The specification also discusses the effect of storage and freeze-thaw cycles on viral stability and activity, and methods of measuring virion infectivity. EX1001 (’721 patent), 9:5-10:15, 13:5-50 (Example 4). In particular, the specification describes the effects of storage and 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). EX1001 (’721 patent), 9:5-10:15, Table 3, 11:65-12:2.

1. Aggregation as a Function of Excipient Concentration

98. 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. EX1001 ('721 patent), 6:5-18. In these experiments, vector aggregation is produced by dilution of vector preparations (5-fold in the case of the '721 patent) into neutral-buffered saline with low concentration buffer (20 mM sodium phosphate, pH 7.2). EX1001 ('721 patent), 6:5-18. The '721 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. EX1001 ('721 patent), 6:5-18. For screening, aggregation was measured by dynamic light scattering (DLS). EX1001 ('721 patent), 6:14-15. The results of these experiments are shown in Table 1. EX1001 ('721 patent), 6:20-42, Table 1. The '721 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. EX1001 ('721 patent), 6:43-60.

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

99. The '721 patent also discloses experiments to investigate AAV2 aggregation as a function of osmolarity and ionic strength. EX1001 ('721 patent),

6:60-7:45. The results of these experiments are shown in Figures 1A and 1B. EX1001 ('721 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. EX1001 ('721 patent), 7:66-8:1. As noted above, no other AAV serotypes were tested.

100. The specification states that the ionic strength of a solution is the primary factor affecting aggregation. EX1001 ('721 patent), 7:21-24. Ionic strength is a parameter that depends on solute concentration and charge valency of the ions in solution. EX1001 ('721 patent), 7:21-24. The specification discusses the calculation of the ionic strength (μ) of various buffer solutions, according to the following equation:

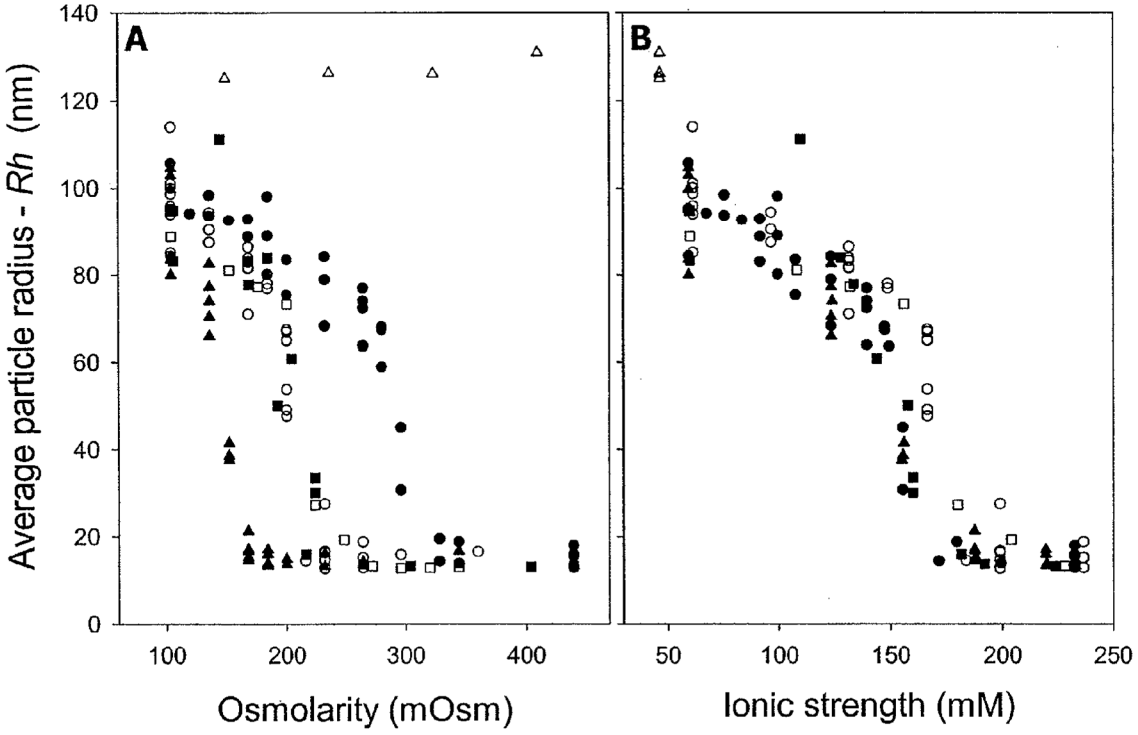
$$\mu = 1/2 \sum c_i z_i^2$$

where c_i is the molar concentration of each solute species and z_i is the charge on each solute species. EX1001 ('721 patent), 12:59-62. The ionic strength values in Figs. 1 and 2 were calculated "using all excipients present in the mixture (i.e. weighted: test diluent (80%) and starting vector formulation (20%))." EX1001 ('721 patent), 12:54-57.

101. 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. EX1001 ('721 patent), Fig. 1B, Fig. 2, 4:11-29,

6:62-9:4. 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." EX1001 ('721 patent), 9:25-27.

102. I have reproduced Fig. 1 of the '721 patent below.



EX1001 ('721 patent), Fig. 1.

103. The '721 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 ('721 patent), 4:11-20.

104. The '721 patent states that the vectors tested in the experiments shown in Figs. 1A and 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. EX1001 ('721 patent), 4:14-23, 11:46-49.

105. Like Floyd I, II, and III, which I have discussed above (Section V.D.), the '721 patent concludes that multivalent ions are more effective in preventing aggregation than univalent ions. EX1001 ('721 patent), 7:28-31.

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

106. In addition, the '721 patent discloses experiments to investigate AAV aggregation as a function of the method of AAV purification. EX1001 ('721 patent), 7:47-64. The results of these experiments are shown in Figure 2. EX1001 ('721 patent), Fig. 2, 7:47-64. These experiments were carried out on AAV2 vectors purified at analytical scale, and used DLS to measure aggregation. EX1001 ('721 patent), 7:66-8:1.

107. The specification describes four different methods of viral particle preparation, which were used to generate the data shown in Figure 2. EX1001 ('721 patent), 4:21-29, 7:46-63, 10:51-11:49 (Example 1). Method 1 involves purification of viral particles by a double CsCl (cesium chloride) gradient. EX1001 ('721

patent), 4:25-26, 7:47-63, 10:62-11:25 (Example 1). Method 2 involves purification by cation exchange chromatography. EX1001 ('721 patent), 4:26-27, 7:47-63, 11:26-30 (Example 1). Method 2 was also carried out using a nuclease digestion step following cation exchange chromatography. EX1001 ('721 patent), 4:27, 7:47-63, 11:31-34 (Example 1). Method 3 involves chromatography followed by a CsCl gradient. EX1001 ('721 patent), 4:28, 7:47-63, 11:35-38 (Example 1).

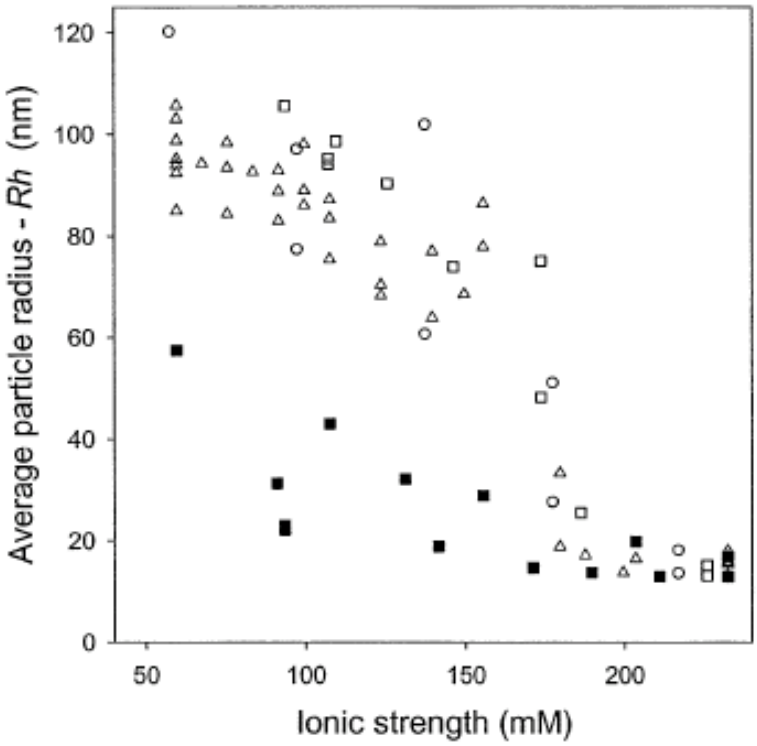
108. The specification states that vectors purified by Methods 1 and 3 do not contain empty capsids, while vectors purified by Method 2 contain empty capsids, ranging from 3-10 empty capsids per vector genome. EX1001 ('721 patent), 11:46-49 (Example 1).

109. 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), pp. 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).

110. The data presented in Figure 2 of the '721 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).

111. I have reproduced Fig. 2 of the '721 patent below:

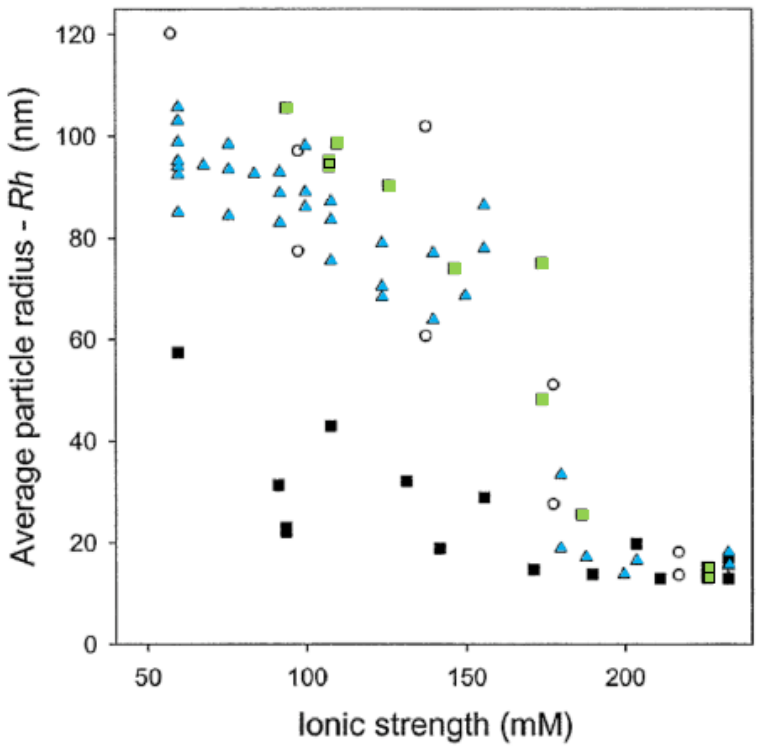


EX1001 ('721 patent), Fig. 2.

112. The '721 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 ('721 patent), 4:21-29.

113. 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. EX1001 ('721 patent), 4:21-29. The figure below is a modified version of Fig. 2 of the '721 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:



114. 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. EX1001 ('721 patent), 11:26-38.

115. 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.

116. 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

117. The '721 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. EX1001 ('721 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. EX1001 ('721 patent), 8:10-13. As I discussed above (Section VI.B.3.), AAV2 vectors purified by Method 1 would be essentially empty-capsid free. EX1001 ('721 patent), 11:46-49.

118. 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, pH7.5); and "Test Formulation 2" (TF2: 100 mM sodium citrate, 10 mM Tris, pH8.0). EX1001 ('721 patent), 8:1-9:4, 11:65-12:2.

119. Two different concentrated vector preparations were tested (2.5×10^{13} vg/ml, and 6.7×10^{13} vg/ml), and one preparation with added nuclease was also evaluated. EX1001 ('721 patent), 8:57-65, 12:3-30 (noting that these concentrations are "target[s]" and "assum[e] no vector loss").

120. 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. EX1001 (’721 patent), 8:8-9, 50-56. Results are shown in Table 2. EX1001 (’721 patent), 8:28-39, Table 2.

121. The ’721 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. EX1001 (’721 patent), 8:66-9:4.

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

122. The ’721 patent also discloses experiments investigating the effects of storage or freeze-thaw cycling on AAV2 vector titer. EX1001 (’721 patent), 9:6-10:15. In these assays, aggregation was measured by DLS, and the preparations were also examined for aggregation by visual inspection. EX1001 (’721 patent), 9:26-28, 50-55.

123. 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). EX1001 (’721 patent), 8:10-13, 9:21-25. Like the preparations in the preparative scale experiments, these AAV2 vector preparations were essentially empty-capsid free.

124. The TF2 preparation was also tested for infectious titer and transduction efficiency. EX1001 (’721 patent), 9:56-10:15, Fig. 3. The ’721 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. EX1001 (’721 patent), 9:28-10:15, Fig. 3, Table 3.

C. The Prosecution History

125. 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 (’721 prosecution history), pp. 83-90. The Examiner also issued a 35 U.S.C. § 103(a) rejection over several of these prior art references. EX1002 (’721 prosecution history), pp. 90-95. In response, the applicant amended the claims. EX1002 (’721 prosecution history), pp. 104-14. These amendments included adding the limitations that the claimed particles are recombinant AAV particles, that the preparation is purified, and that the purification involves ultracentrifugation and/or chromatography. EX1002 (’721 prosecution history), pp. 104-14.

126. The Examiner then issued additional 35 U.S.C. § 102(b) rejections and another § 103(a) rejection, after which the applicants amended the claims again. EX1002 (’721 prosecution history), pp. 120-39, 153-59.

127. The Examiner then issued another 35 USC § 103(a) rejection. EX1002 ('721 prosecution history), pp. 165-79. In response, the applicant amended the claims again. EX1002 ('721 prosecution history), pp. 186-95. This amendment added the limitation that the concentration of rAAV virions in the preparation exceeds 1 x 10^{13} vg/ml. EX1002 ('721 prosecution history), p. 187.

128. The Examiner then issued another 35 USC § 103(a) rejection, after which the applicant amended the claims again. EX1002 ('721 prosecution history), pp. 264-79; 296-305. This amendment added the limitation that the upper limit of the concentration of rAAV particles is 6.4 x 10^{13} vg/ml. EX1002 ('721 prosecution history), p. 297.

129. The Examiner then proposed an Examiner's Amendment, which was agreed to by the applicant. EX1002 ('721 prosecution history), pp. 318-22. The Examiner's Amendment added the limitations that salts of multivalent ions must be added, which are "selected from the group consisting of citrate, phosphate, sulfate and magnesium," and also that the pH of the purified preparation of rAAV virions must be between 7.5 and 8.0. EX1002 ('721 prosecution history), p. 321.

130. The primary prior art references at issue here – Auricchio, Konz, and Potter – were not before the USPTO during prosecution of the '721 patent. EX1001 ('721 patent); EX1002 ('721 prosecution history).

131. Two IPR petitions were previously filed by Novartis challenging various claims of a related patent, the '542 patent. EX1014 (608 Petition), p. 11; EX1015 (609 Petition), p. 11. Neither petition relied on Auricchio, Potter, or Konz, as a primary reference. EX1014 (608 Petition), p. 13; 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. Auricchio and Konz[3] were not raised by either party during the IPR proceedings.

132. In IPR2023-00608 (but not in IPR2023-00609), Patent Owner briefly addressed Potter. EX1016 (608 POPR), pp. 47-48, 68-69. As I discuss further below (Section VIII.C.4.), Patent Owner and its expert, Dr. Davies, mischaracterized Potter in the POPR.

133. 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).

[3] 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.

D. The Priority Date

134. The ’721 patent claims priority to two provisional applications, 60/575,997, filed June 1, 2004, and 60/639,222, filed December 22, 2004. EX1001 (’721 patent); EX1003 (’997 provisional); EX1004 (’222 provisional). The ’721 patent issued from U.S. Patent Application No. 11/141,996, filed on June 1, 2005. EX1001 (’721 patent).

135. In my opinion, the challenged claims of the ’721 patent are not entitled to the June 1, 2004 priority date of the earlier of the two provisionals, the ’997 provisional for two reasons. First, the ’997 provisional does not sufficiently describe or enable methods of preventing aggregation of viral particles that include empty capsids. Second, the ’997 provisional does not sufficiently describe or enable methods wherein the pH of the purified preparation of rAAV virions is within the claimed pH range of 7.5-8.0.

136. The challenged claims of the ’721 patent recite a method of preventing aggregation of rAAV particles, in purified preparation 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, phosphate, sulfate, or magnesium multivatlent ions, and having an ionic strength greater than 200 mM. EX1001 (’721 patent), 14: 9-29, 33-40, 49-51.

137. It is my understanding that for the ’721 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 ’721 patent, meaning that it must describe and enable the full scope of the claims.

138. The challenged claims encompass purification methods that produce and retain empty capsids. Therefore, the ’997 provisional must describe and enable viral preparations purified by methods that retain empty capsids, such as column chromatography without a CsCl gradient to remove empty capsids. However, the ’997 provisional fails to disclose sufficient data to describe and enable the full scope of claims 1, 4, 6, and 7 in this regard.

139. Second, the ’997 provisional must describe and enable methods of preventing aggregation of rAAV purified preparations 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 all of the challenged claims.

140. 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 Purification Methods That Produce Empty Capsids

141. First, because the challenged claims encompass methods of purifying rAAV that produce empty capsids, the ’997 provisional must describe and enable such methods. EX1001 (’721 patent), 14:9-29, 33-40, 49-51.

142. Like the ’721 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 (’721 patent), 4:24-32, 7:47-64, 10:52-11:50, Fig. 2; EX1003 (’997 provisional), pp. 4-5, 7, 11, Appendix D.

143. I have put together the table below to show the corresponding AAV purification methods in the ’997 provisional and the ’721 patent:

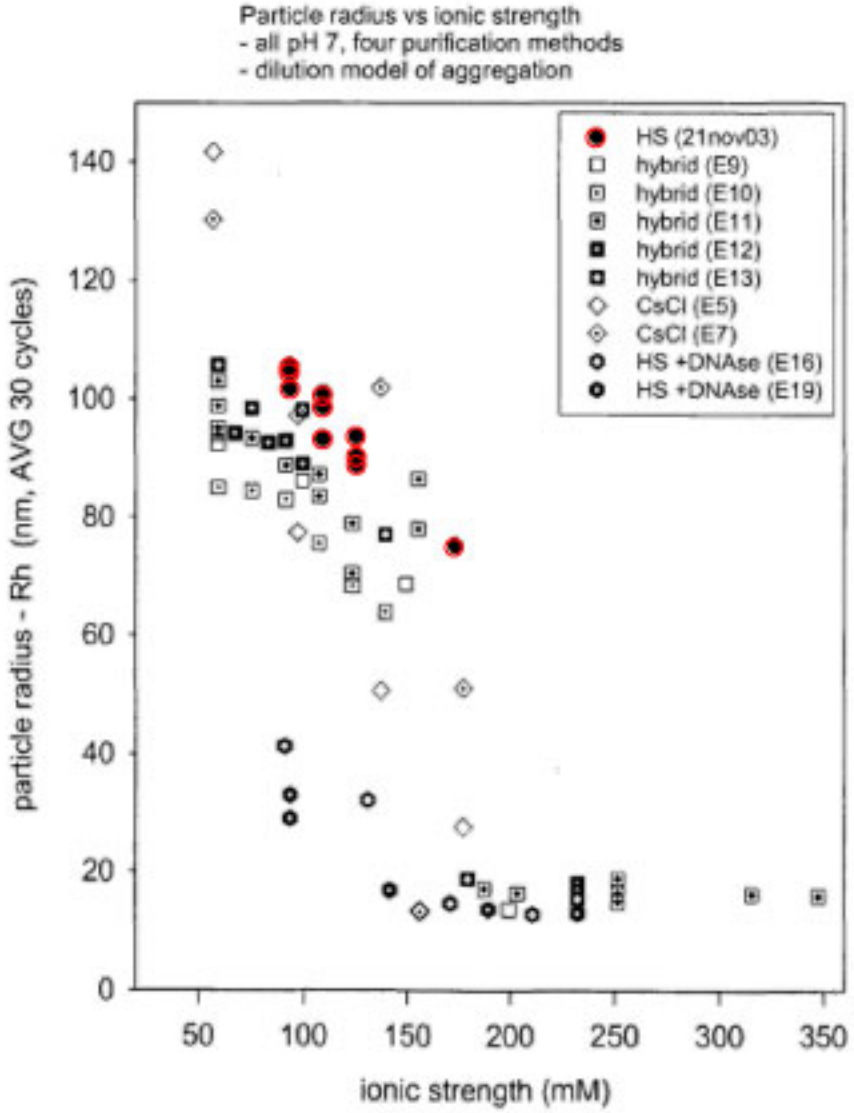
Method	**Empty Capsids**	**’997 Provisional**	**’721 Patent**
Double CsCl	N	Process 1, CsCl	Method 1
Column	Y	Process 2, HS	Method 2
Column + Nuclease	Y	HS + DNAse	Method 2 + nuclease digestion
Column + CsCl	N	Process 3, Hybrid	Method 3

144. The ’997 provisional, however, does not disclose ***any*** purification method that produces empty capsids without aggregation, other than purified preparations that have been treated with a nuclease. In fact, the only data in the ’997 provisional relating to a method that produces empty capsids 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 the

preparation to which DNAse had been added. *See* EX1003 ('997 provisional), p. 13.

145. 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



146. 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.

147. I discussed this phenomenon with regard to the experiments shown in Figure 2 of the '721 patent. *See* Section VI.B.3., *supra*. Therefore, the '997 provisional, like the '721 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.

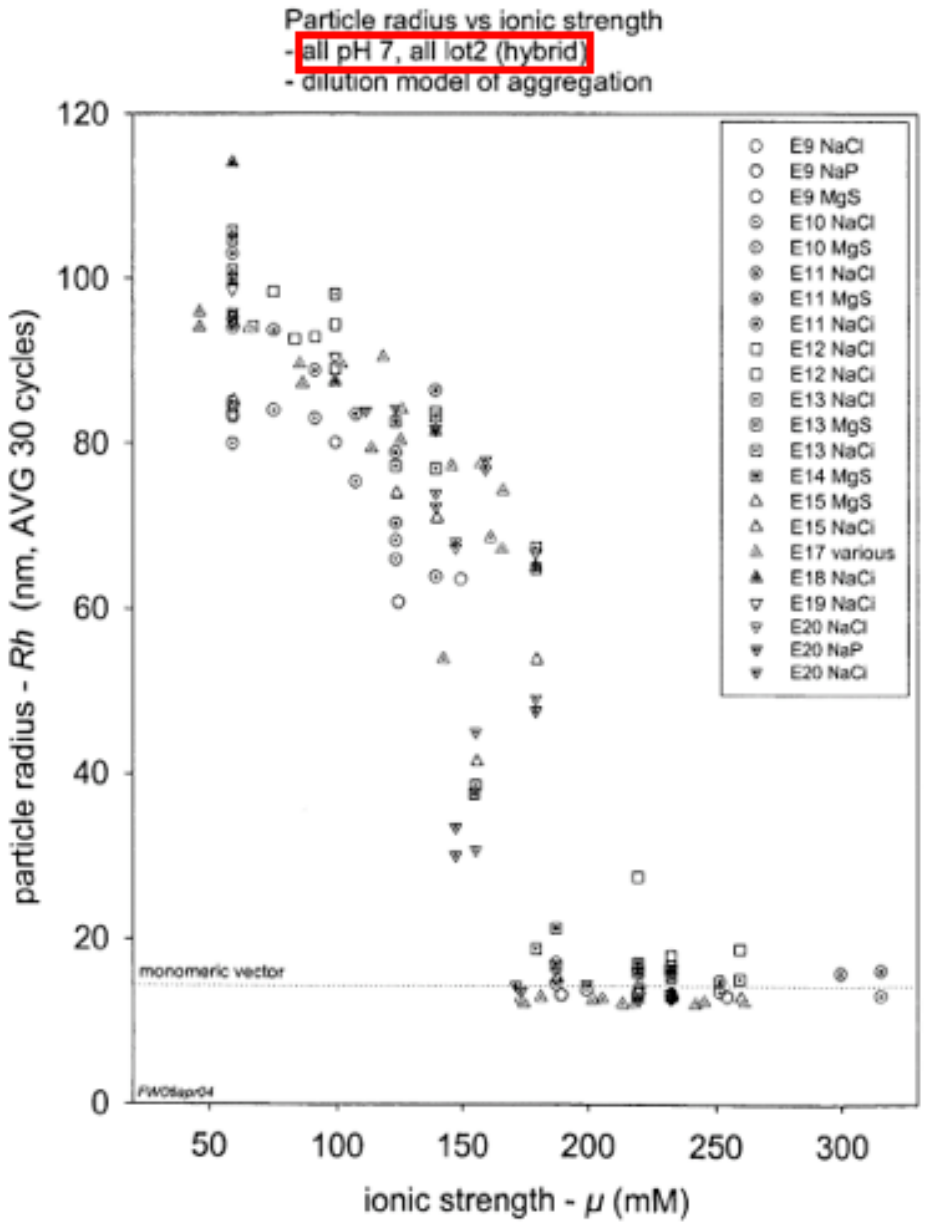
148. However, as can be seen above in the annotated version of Appendix D of the '997 provisional, there are no data points in this figure for the HS method at an ionic strength higher than about 175 mM. And all the data points for the HS method show an Rh value in the range of about 75 nm to about 105 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 ('721 patent), 1:34-35, 9:25-27, 12:48-50; EX1003 ('997 provisional), pp. 11-12, Appendices B and C.

149. Therefore, ***all*** the data points for the HS (empty capsid containing) method in Appendix D of the '997 provisional show aggregation, in the absence of

a nuclease. As a result, these data cannot provide support for the full scope of challenged claims 1, 4, 6, and 7 of the ’721 patent.

150. 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 B below:

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



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

151. Therefore, Appendix C (and similarly Appendix B) of the '997 provisional cannot provide written description or enablement support for the full

scope of the challenged claims, which encompass AAV preparations containing empty capsids.

152. Likewise, the concentration/diafiltration and freeze/thaw experiments disclosed in the '997 provisional were all carried out on AAV2 preparations purified by methods that remove empty capsids, so that these preparations were essentially empty capsid free.[4] EX1003 ('997 provisional), p. 3; *see also* EX1001 ('721 patent), 8:10-13, 9:21-24. These experiments, therefore, cannot provide written description or enablement support for the full scope of the challenged claims, which, as I have said, encompass methods that produce rAAV preparations containing empty capsids.

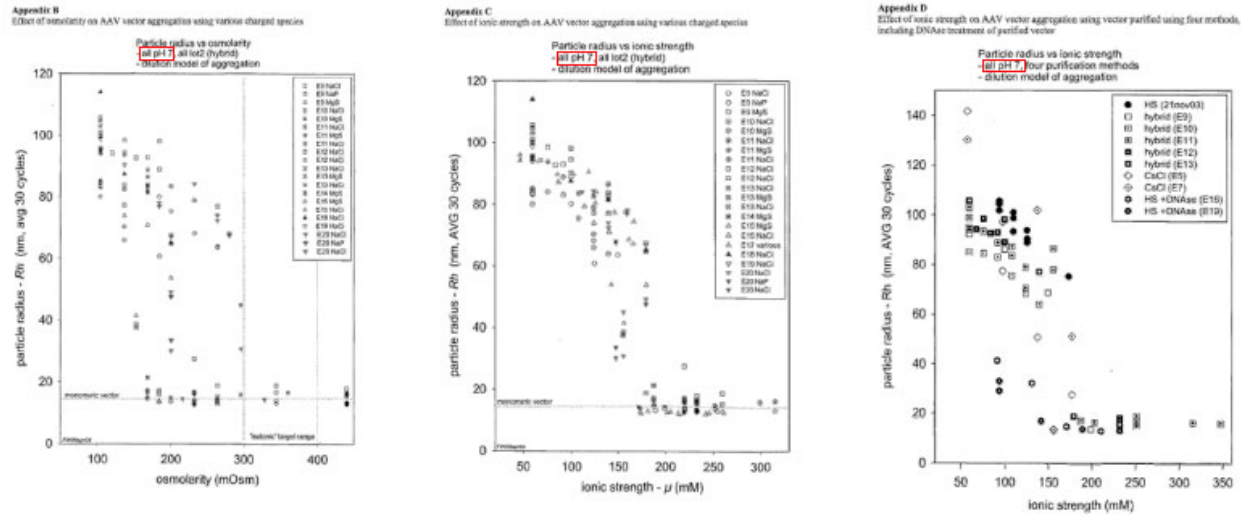
[4] The '997 provisional does not expressly disclose the purification method used to prepare the rAAV2 virions for the F/T screening experiments. EX1003 ('997 provisional), p. 5. Nonetheless, a POSA would have understood that the purification method appears to be the same empty capsid free method, Process 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).

153. For these reasons, challenged claims 1, 4, 6, and 7 of the ’721 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

154. 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.

155. I have reproduced below Appendices B, C, and D from the ’997 provisional, which 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.

156. Notably, the challenged claims require a pH in the range of 7.5-8.0. EX1001 ('721 patent), 14:19-20. 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.

157. 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.

158. For these reasons, it is my opinion that the claims of the '721 patent are not entitled to the priority date of the '997 provisional. Thus, the earliest possible priority date for the '721 patent claims is the filing date of the '222 provisional – December 22, 2004.

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

159. 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.

160. I understand that the earliest priority applications listed on the face of the '721 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 ('721 patent). I also understand that the earliest non-provisional application

listed on the face of the ’721 patent is U.S. Application No. 11/141,996, filed on June 1, 2005. EX1001 (’721 patent).

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

162. I have been asked to consider the level of education and experience of a POSA for the ’721 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.

163. In my opinion, a POSA in the technical field of the ’721 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

164. As I discuss further below (Section VIII.D.4), the Patent Owner and its expert, Dr. Martyn Davies, mischaracterized the prior art and the state of the art in responding to one of the IPR Petitions challenging the related '542 patent. *See, e.g.*, EX1016 (608 POPR), pp. 47-48, 68-69; EX1060 (608 Davies Decl.), ¶¶ 121, 123-24, 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 (POPR 608), pp. 47-48, 68-69; EX1060 (Davies Decl. 608), ¶¶121, 123-24, 151-52.

165. Yet Potter (in addition to others, as discussed above in Section V.I) expressly discloses formulations that fall within this range. *See, e.g.*, EX1009 (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).[5]

[5] 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

A. Auricchio

166. Auricchio was published in January 2001, more than a year before the earliest possible priority date for the '721 patent (June 1, 2004), and is therefore 35 U.S.C. 102(b) prior art, irrespective of whether the '721 patent is entitled to the June 1, 2004 priority date. EX1007 (Auricchio).

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is a "vg/ml" concentration that does not include empty capsids. *See, e.g.*, EX1009 (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 assay, viral DNA is isolated," and that the viral physical titer was derived from amplification of the isolated viral DNA).

167. Auricchio discloses a "simple and scalable" method for purifying rAAV. *See, e.g.*, EX1007 (Auricchio), Overview Summary. Auricchio explains that there was a need for such a method for large scale rAAV purification that would not require a high level of manual and technical skills, and would result in reproducible, high titer, infectious, and pure rAAV. EX1007 (Auricchio), p. 1. Auricchio states that prior methods either resulted in impure, low infectious virus (*e.g.*, CsCl gradient centrifugation), or demanded a high level of manual and technical skills. EX1007 (Auricchio), p. 1, Abstract.

168. Auricchio explains that their method involves a single step column purification ("SSCP") of AAV2 by gravity flow based on the affinity of AAV2 to heparin. EX1007 (Auricchio), p. 1, Abstract.

169. Auricchio describes the method as follows:

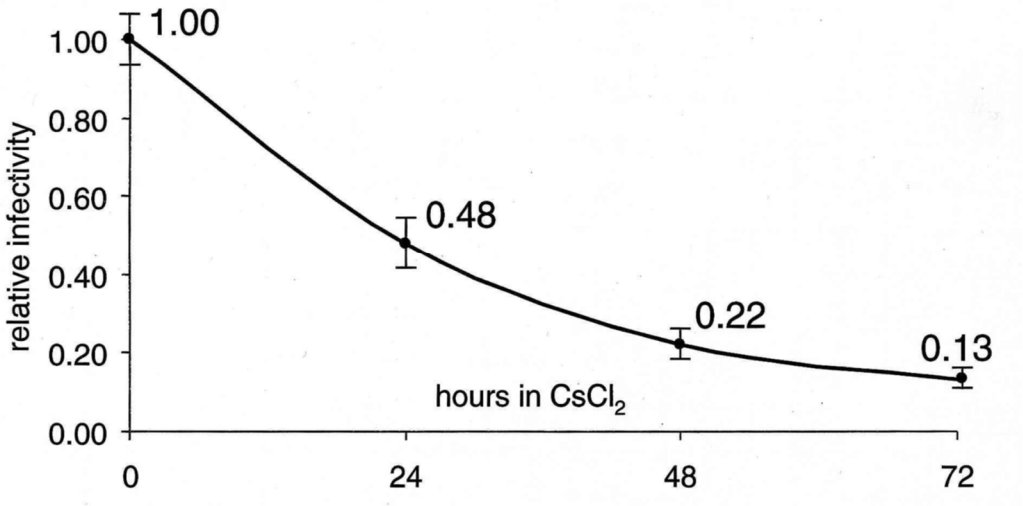
> In this study, we describe a single-step gravity-flow column purification protocol for AAV2, based on its affinity for heparin. This technique does not require expensive, special equipment or advanced skills, is highly reproducible, and comes close to HPLC-purified virus with respect to purity and infectivity. Briefly, the crude lysate containing the recombinant vector is directly applied to a preequilibrated heparin column. On binding of the virus to the matrix and after two washes, the vector is eluted by a change in buffer and concentrated.

EX1007 (Auricchio), p. 2.

170. As disclosed in the passage quoted above, the last step of Auricchio's method is concentration after elution using a high salt phosphate buffer. EX1007 (Auricchio), p. 2.

171. Auricchio teaches that purified rAAV2 is not stable in CsCl. EX1007 (Auricchio), pp. 2-3, 6, Fig. 1. Auricchio incubated 10^{10} genome copies of AAV2 CMV *lacZ* in either CsCl (1.4 g/ml) or in PBS for up to 72 hours at 4°C. EX1007 (Auricchio), pp. 2-3, Fig. 1.

172. Over the 72 hour period, the relative infectivity of the rAAV2 in CsCl steadily declined, as compared with that of the rAAV2 in PBS. EX1007 (Auricchio), pp. 2-3, Fig. 1, which I have reproduced below:



EX1007 (Auricchio), Fig. 1.

173. The SSCP method, which was therefore expressly designed to avoid the use of CsCl gradient purification, involved suspending cells contained in one 15-cm dish in 2.5 ml of Dulbecco's modified Eagles medium. EX1007 (Auricchio), pp. 2-

3. The cells were then frozen and thawed twice and then incubated with 0.1 mg each of DNase I and RNase A for 30 minutes at 37°C. EX1007 (Auricchio), p. 2.

174. After 15 minutes of centrifugation at 3000 rpm in a Sorvall RT6000D centrifuge at 4°C, the supernatant was transferred to a new tube and incubated with 0.5% (final concentration) deoxycholic acid for 30 minutes at 37°C, and then sequentially filtered through a 5 μm pore size filter. EX1007 (Auricchio), p. 2.

175. The cleared crude lysate was then applied on a heparin column prepared as follows: 8 ml of a heparin-agarose suspension was pipetted into a 2.5 cm diameter glass column. EX1007 (Auricchio), p. 2. After the agarose suspension solution flowed through, a filtration membrane was placed on top of the agarose bedding. EX1007 (Auricchio), p. 2.

176. The matrix was then equilibrated with 25 ml of phosphate-buffered saline (PBS), pH 7.4. EX1007 (Auricchio), p. 2.

177. The crude lysate was then applied to the column. EX1007 (Auricchio), p. 2. After all the lysate went through the column (which required several loadings), the matrix was washed twice with 25 ml of PBS, pH 7.4, plus 0.1 M NaCl (*i.e.*, PBS with a final concentration of 0.254 M NaCl). EX1007 (Auricchio), p. 2.

178. The rAAV was eluted with 15 ml of PBS, pH 7.4, plus 0.4 M NaCl. EX1007 (Auricchio), p. 2. The eluate was concentrated to about 1 ml with a

Millipore Biomax-100K NMWL filter device (UFV2BHK40) by centrifugation. EX1007 (Auricchio), p. 2.

179. The NaCl concentration was adjusted to physiological levels by refilling the filter device with PBS pH 7.4. EX1007 (Auricchio), p. 2. The membrane was then washed three times with 100 µl of PBS, pH 7.4, which was added to the main part of the recombinant AAV2. EX1007 (Auricchio), p. 2.

180. Auricchio further discloses that in two runs of this method, they obtained physical titers of 1.4×10^{13} genome copies, and 1.1×10^{13} genome copies. EX1007 (Auricchio), p. 4, Table 2. Genome copies were determined by real-time PCR, which a POSA would have recognized as a reliable method at the time for determining vector genomes. EX1007 (Auricchio), p. 2.

181. I note that, given that the rAAV2 was eluted in 15 mls of PBS, pH 7.4, plus 0.4 M NaCl, and then concentrated to 1 ml of this buffer, the concentration of these two runs was 1.4×10^{13} vg/ml, and 1.1×10^{13} vg/ml.

182. Auricchio touts the high titers obtained: "In these experiments, up to 1.4×10^{13} particles of AAV2 could be purified and concentrated using 8 ml of agarose-heparin suspension." EX1007 (Auricchio), p. 4.

183. Auricchio also touts the simplicity of its elution protocol, which it describes as a "simple buffer exchange to high salt," rather than a linear gradient. EX1007 (Auricchio), pp. 1 (Overview Summary), 5. For example, the Overview

Summary states regarding the washing and elution steps of the method: "The heparin-virus complex is then washed and AAV2 is finally eluted by a simple buffer exchange to high salt. We demonstrate that recombinant AAV2 purified by this simple and scalable method is reproducibly highly infectious *in vitro* and *in vivo*." EX1007 (Auricchio), p. 1 (Overview Summary).

184. A POSA would have understood from these disclosures that Auricchio's method included the steps up through elution and concentration in high salt, and that Auricchio's method produced rAAV2 with high physical and infectious titer. *See, e.g.*, EX1007 (Auricchio), p. 2 (describing the last steps of the method as "On binding of the virus to the matrix and after two washes, the vector is eluted by a change in buffer and concentrated"). Whether or not the concentrated rAAV2 preparation was subsequently subjected to an additional buffer exchange for the purpose of reducing the salt concentration or otherwise, the core steps of the method did not require any such additional buffer exchange.

185. Auricchio describes *in vitro* and *in vivo* experiments that showed the high infectivity of the concentrated rAAV2 preparation produced by Auricchio's method. EX1007 (Auricchio), pp. 2, 4-5.

186. Two different reporter constructs were used in Auricchio – GFP and LacZ. EX1007 (Auricchio), p. 2. Transducing units *in vitro* were assessed by limiting dilution of virus on 84/31 cells. EX1007 (Auricchio), p. 2. The number of

green fluorescent protein (GFP) positive cells was determined by fluorescence activated cell sorting (FACS). EX1007 (Auricchio), p. 2. The number of LacZ-positive cells was determined by X-Gal staining. EX1007 (Auricchio), p. 2.

187. Auricchio discloses that their SSCP method reproducibly produced pure, high physical and high infectious titer rAAV2. EX1007 (Auricchio), pp. 1 (Overview Summary), 3-5, Table 1, Table 2. I have reproduced Table 1 below.

TABLE 1. COMPARISON OF $CsCl_2$-, IODIXANOL-, AND SSCP-PURIFIED VECTORS: GENOME COPIES AND TRANSDUCING UNITS

Method	*GC*	*TU*	*Ratio GC/TU*	*Average GC yield*	*Average TU yield*	*Average GC/TU*
$CsCl_2$ 1	8.0×10^{12}	7.2×10^{10}	111			
$CsCl_2$ 2	6.6×10^{12}	1.8×10^{11}	37	7.3×10^{12}	3.9×10^{10}	74
Iodixanol 1	1.35×10^{12}	3.4×10^{10}	40	1.8×10^{12}	1.1×10^{11}	35
Iodixanol 2	2.2×10^{12}	7.5×10^{10}	30			
SSCP 1	6.24×10^{12}	6.0×10^{11}	10.4	5.7×10^{12}	5.5×10^{11}	8.4
SSCP 2	5.2×10^{12}	8.3×10^{11}	6.3			

Abbreviations: GC, genome copies; TU, transducing units.

EX1007 (Auricchio), p. 3, Table 1.

188. Table 1 shows data for rAAV2 containing the GFP reporter construct, purified by three different methods, CsCl,[6] iodixanol, and SSCP, with two runs for each method. EX1007 (Auricchio), p. 3, Table 1.

[6] Auricchio throughout refers to "$CsCl_2$." The correct notation for cesium chloride, however, is CsCl. *See, e.g.*, EX1042 (Zolotukhin), *passim*. I believe that a POSA at the time would have understood that the "$CsCl_2$" notation in Auricchio is a typographical error.

189. Table 1 shows genome copies (GC), transducing units (TU), and the ratio of GC/TU for each run of each purification method. EX1007 (Auricchio), p. 3, Table 1.

190. Table 1 also shows the average GC yield, average TU yield, and average GC/TU ratio for each pair of runs for each purification method. EX1007 (Auricchio), p. 3, Table 1.

191. A POSA would have understood that the GC/TU ratio is a measure of the infectivity of the viral preparation. The lower the ratio, the fewer the number of genome copies needed to transduce a given number of target cells, and the higher the infectivity of the virus.

192. As can be seen from Table 1, the average GC/TU ratio for the SSCP method was the lowest of the three different methods. EX1007 (Auricchio), p. 73, Table 1. Specifically, the average GC/TU for SSCP was 8.4, while that of CsCl was 74, and that of iodixanol was 35. EX1007 (Auricchio), p. 3, Table 1.

193. I note that the two CsCl runs produced substantially different GC/TU ratios that were used to calculate the average: the first CsCl run produced a GC/TU ratio of 111, while the second run produced a GC/TU ratio of only 37. EX1007 (Auricchio), p. 3, Table 1.

194. Nonetheless, even if the higher GC/TU ratio of 111 from the first run is disregarded, and only the lower GC/TU ratio of 37 is considered (which would bring

the average GC/TU ratio for CsCl in line with that of iodixanol), the average GC/TU ratio for SSCP would still be considerably lower than that of both CsCl and iodixanol.

195. In addition, the average GC yield for the SSCP preparations was higher than the average GC yield for the iodixanol preparations (5.7×10^{12} vs. 1.8×10^{12}). EX1007 (Auricchio), p. 3, Table 1.

196. Table 2 shows that the Table 1 results for SSCP were reproducible. EX1007 (Auricchio), p. 4, Table 2. I have reproduced Table 2 below:

TABLE 2. REPRODUCIBILITY OF VECTOR PREPARATIONS BY SSCP

Vector	*Size (kb)*	*Genome copies*	*Transducing units*	*Ratio*
AAV2 CMV *lacZ*	4.9	1.8×10^{12}	1.5×10^{10}	120
		2.7×10^{12}	2.1×10^{10}	144
		4.5×10^{12}	5.6×10^{10}	81
		1.4×10^{12}	1.1×10^{10}	129
AAV2 CMV eGFP	2.7	6.0×10^{12}	6.0×10^{11}	10
		3.6×10^{12}	3.1×10^{11}	12
		1.4×10^{13}	1.3×10^{12}	11
		1.1×10^{13}	1.4×10^{12}	8
AAV2 TBG *lacZ*	5.1	4.8×10^{12}	ND	ND
AAV2 TBG insulin	2.4	3.7×10^{12}	ND	ND
AAV2 TBG OTC	3.1	1.4×10^{12}	ND	ND

Abbreviations: ND, not determined; TBG, thyroxine-binding globulin promoter; OTC, ornithine transcarbamoylase.

EX1007 (Auricchio), p. 4, Table 2.

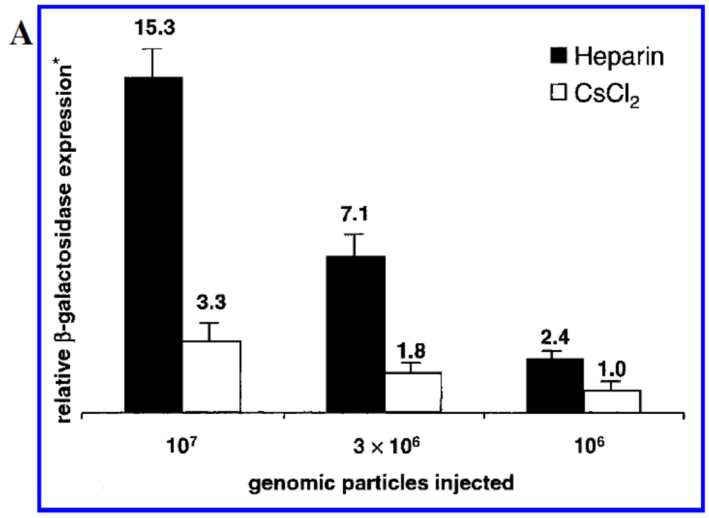
197. For example, Table 2 shows the GC, TU, and GC/TU ratio for four more runs where the SSCP method was used to purify the rAAV2 GFP construct. EX1007 (Auricchio), p. 4, Table 2. The GC/TU ratios for these four runs were 10, 12, 11, and 8. EX1007 (Auricchio), p. 4, Table 2. These ratios are consistent with one another and also with the GC/TU ratios reported in Table 1 for the SSCP method. EX1007 (Auricchio), pp. 3-4, Table 1, Table 2.

198. The GC yields shown in Table 2 for SSCP preparations of the rAAV2 GFP construct were even higher than those reported in Table 1, with two yields greater than 1 x 10^{13} GCs. EX1007 (Auricchio), p. 4, Table 2 (showing GC yields of 1.4 x 10^{13} and 1.1 x 10^{13}).

199. I note that for the rAAV2 *lacZ* construct, the GC/TU ratios were substantially higher, and the GC yields were lower, than those for the rAAV2 GFP construct. EX1007 (Auricchio), p. 4, Table 2.

200. A POSA would have understood that the *lacZ* construct was larger than GFP – as shown in Table 2, which gives the size of the *lacZ* construct as 4.9 kb, and that of the GFP construct as 2.7 kb. EX1007 (Auricchio), p. 4, Table 2. A POSA would have further understood that, in general, the larger the construct inserted into an AAV vector, the higher the GC/TU ratio, and the lower the infectivity will be.

201. *In vivo*, the *lacZ* construct (AAV2 CMV *lacZ*) was injected into the tibialis anterior muscles of immunocompetent C57BL/6 mice. EX1007 (Auricchio), pp. 2, 4-5, Fig. 3. Aurrichio discloses that rAAV2 *lacZ* purified by the SSCP method transduced muscle *in vivo* better than rAAV2 *lacZ* purified using the CsCl gradient purification method. EX1007 (Auricchio), pp. 4-5, Fig. 3. I have reproduced Figure 3A, showing these results, below:



EX1007 (Auricchio), p. 5, Fig. 3A.

B. Konz

202. 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 '721 patent if it cannot claim priority to the '997 provisional. EX1008 (Konz); EX1001 ('721 patent). Therefore, to the extent that the '721 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 '721 patent challenged claims.

203. Should the Patent Office determine that the '721 patent challenged claims are entitled to the priority date of the '997 provisional, then Konz is 102(e) prior art against the '721 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 '721 patent, June 1, 2004. EX1008 (Konz); EX1001 ('721 patent).

204. 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.

205. 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.

206. 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").

207. 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.

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

209. 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.

210. 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).

211. 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").

212. 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.

213. 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 preferably 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.

214. 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"); *see also* 54:8-9 ("Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties").[7]

215. 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

[7] I note that in IPR2023-00608 and IPR2023-00609, Genzyme's expert, Dr. Martyn 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 DNA. *See, e.g.*, Konz 2005, Abstract. Notably, Konz 2005 found that adding a high salt buffer (1M NaCl) to two process intermediates reversed aggregates. Konz 2005, p. 471. Konz 2005 further found that adding a non-ionic surfactant (Polysorbate-80) throughout the purification similarly inhibited aggregation. Konz 2005, p. 471.

enhanced storage stability include but are not limited to adenovirus, ***adeno-associated virus***, retroviruses, herpes virus, vaccinia virus, rotovirus, pox viruses") (emphasis added).

216. 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.

217. Konz discloses sterile filtration through a 0.22 μm filter as a means of assaying the extent of particle aggregation. EX1008 (Konz), 23:1-5, (Table 1), 25:29-30, 36:24-28 ("No pressure build-up was seen during the sterile filtration which suggests a lack of aggregated virus"); EX1069 (Hatano), p. 3. Konz discloses high yields from sterile filtration, 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).

218. Konz discloses the use of DLS to determine mean particle sizes, to assess aggregation. EX1008 (Konz), 30:13-30, 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, 30:19-30, 48:12-14.

219. A POSA would have understood that the high yield following sterile filtration (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. Potter

220. Potter was published in 2002, more than a year before the earliest possible priority date for the '721 patent (June 1, 2004), and is therefore 35 U.S.C. 102(b) prior art, irrespective of whether the '721 patent is entitled to the June 1, 2004 priority date. EX1009 (Potter); EX1001 ('721 patent).

221. Potter describes a method for the large scale production of rAAV vectors that they used to make a National Reference Standard (NRS) rAAV vector. EX1009 (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." EX1009 (Potter), p. 1.

222. Potter states that they had previously developed an rAAV purification protocol that resulted in higher yield and improved infectivity of particles. EX1009

(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. EX1009 (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. EX1009 (Potter), p. 1.

223. Potter sought to improve this protocol so that it could be used for large scale rAAV production. EX1009 (Potter), p. 1. They introduced new chromatography purification steps that eliminated the need for any centrifugation methods. EX1009 (Potter), p. 1. They characterized the purified rAAV in terms of purity, infectivity, and packaged particle composition. EX1009 (Potter), pp. 1-2.

224. 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. EX1009 (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. EX1009 (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. EX1009 (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. EX1009 (Potter), p. 2.

225. Potter describes the generation of the rAAV reference stock with the newly developed protocol. EX1009 (Potter), p. 2. The rAAV construct chosen for the National Vector Standard was pTR-UF5. EX1009 (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. EX1009 (Potter), p. 2. They appear to have used *rep* and *cap* genes from AAV2. EX1009 (Potter), p. 1 (citing EX1026 (Hauswirth), which, in turn, cites EX1066 (Grimm) (describing protocol for purification of rAAV2).

226. Potter sets out the steps of propagation of cells, transfection, and harvesting of transfected cells that they used. EX1009 (Potter), pp. 2-4.

1. Column Chromatography Purification

227. I have focused my analysis on Potter's protocol for purification of the rAAV vector obtained from the steps mentioned above. EX1009 (Potter), pp. 4-7.

228. 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. EX1009 (Potter), p. 4. Instead, Potter sought a method that would generate a lysate immediately usable for chromatography. EX1009 (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. EX1009 (Potter), pp. 4-5.

229. 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. EX1009 (Potter), pp. 5-7. All chromatography steps were carried out using an FPLC (fast protein liquid chromatography) system. EX1009 (Potter), p. 5.

230. 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. EX1009 (Potter), p. 5. Ten ml fractions were collected and the positive fractions determined by fluorescent cell assay (FCA). EX1009 (Potter), p. 5.

231. The pooled fractions from the first heparin column were adjusted to 1M NaCl, and then loaded onto the phenyl-Sepharose hydrophobic interaction column. EX1009 (Potter), p. 5. The virus eluted in the flow through, in about 100 ml volume. EX1009 (Potter), p. 5.

232. The eluate from the phenyl-sepharose column, which contained the sample, was concentrated on the Poros heparin affinity column. EX1009 (Potter),

p. 5. The phenyl Sepharose fraction was diluted to about 150 mM NaCl by addition of distilled water, about a 6-fold dilution. EX1009 (Potter), p. 5.

233. 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. EX1009 (Potter), pp. 5-7.

234. 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. EX1009 (Potter), p. 7, Table I.

235. The yield after each column chromatography step was estimated by titering the vector obtained after each step by FCA. EX1009 (Potter), p. 7, Table I.

2. Analytical Characterization of Purified rAAV

236. The purified rAAV obtained through these three column chromatography steps was characterized in four different, independent assays. EX1009 (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). EX1009 (Potter), pp. 7-12. Infectious titers were determined using an infectious center assay (ICA) and FCA, which scored for expression of GFP. EX1009 (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

EX1009 (Potter), p. 9, Table II. I discuss each of these assays in turn below.

237. 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." EX1009 (Potter), p. 17. Therefore, removal of empty capsids would have no effect on these titers. EX1009 (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

238. 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. EX1009 (Potter), pp. 9-10. The viral

DNA was purified by phenol/chloroform extraction, followed by chloroform extraction and ethanol precipitation. EX1009 (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). EX1009 (Potter), p. 10. The viral physical particles titer was calculated taking into account the dilution factor. EX1009 (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 x 10^{13} particles/ml, which is the same as a concentration of 1.12 x 10^{13} viral genomes/ml. EX1009 (Potter), pp. 9-10, Table II.

(b) RTPA

239. 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.

EX1009 (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. EX1009 (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. EX1009 (Potter), pp. 7-8, 12, Fig. 2. The viral titer of 7.3×10^7 was derived from these curves. EX1009 (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). EX1009 (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

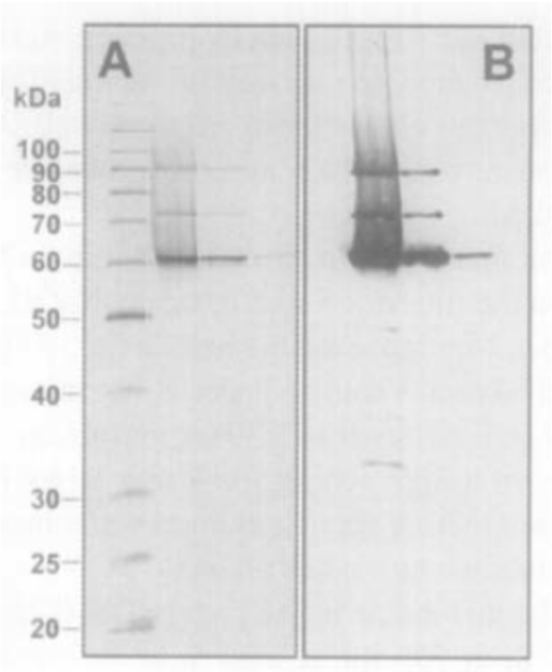
240. In addition to measuring the concentration of filled viral particles produced through the three column purification method, Potter also tested the ability of the purified virions to infect C12 cells, unpackage, and replicate. EX1009 (Potter), p. 12. C12 cells contain integrated wild-type AAV *rep* 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. EX1009 (Potter), p. 12. For FCA, C12 cells were plated and infected with the purified virus, along with helper adenovirus. EX1009 (Potter), p. 12. At 40 hours post-infection, cells were visually scored using a fluorescence microscope to monitor green fluorescence.

EX1009 (Potter), p. 12. Table II shows that the concentration of infectious particles derived from FCA was 2.16 x. 10^{12} infectious particles/ml. EX1009 (Potter), p. 9, Table II.

241. For ICA, the cells were fixed onto a membrane and probed with a random-primed, radioactive probe that hybridized to AAV DNA. EX1009 (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. EX1009 (Potter), p. 9, Table II.

(d) SDS-Gel Electrophoresis Analysis of Vector Purity

242. Further investigation of the purified rAAV was carried out using SDS-gel electrophoresis. EX1009 (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. EX1009 (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:



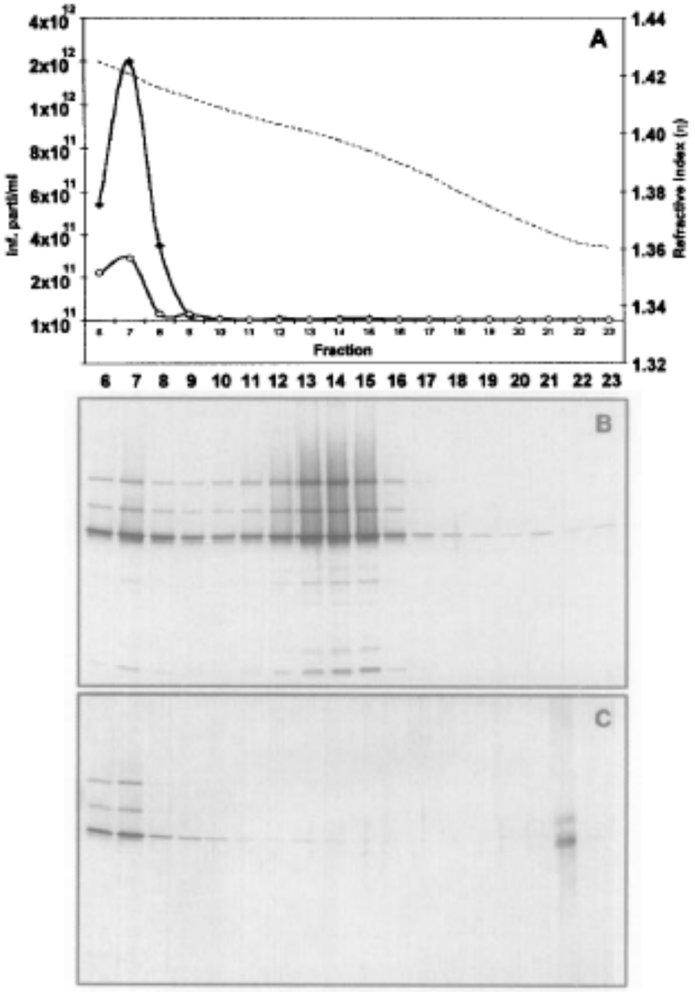
EX1009 (Potter), p. 14, Fig. 3.

243. The tenfold serial dilution experiment, showing dilution over a 100-fold range, demonstrated that the vector was 99.9% pure. EX1009 (Potter), p. 13.

(e) Empty Capsid (Density) Determination

244. 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. EX1009 (Potter), pp. 13-14. The gradient was formed by mixing virus with 30% iodixanol prepared in PBS containing 0.9 M $MgCl_2$. EX1009 (Potter), p. 14. The sample was centrifuged and then fractionated by puncturing the centrifuge tube at the bottom and collecting 1 ml fractions. EX1009 (Potter), p. 14.

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



EX1009 (Potter), pp. 14-15, Fig. 4A and Fig. 4B.

246. 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. EX1009 (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. EX1009 (Potter), p. 427, Fig. 4A. The dotted line shows a plot of refractive index of gradient fractions. EX1009 (Potter), p. 427, Fig. 4A.

247. 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. EX1009 (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. EX1009 (Potter), p. 15, Fig. 4A, Fig. 4B.

248. Fig. 4C shows various fractions containing the pre-purified viral particles, eluted from the iodixanol gradient and loaded onto an SDS-PAGE gel. EX1009 (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.

249. 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. EX1009 (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). EX1009 (Potter), pp. 14-15, Fig. 4A, Fig. 4C. The additional bands seen in fraction 22 were contaminating cellular proteins. EX1009 (Potter), pp. 14-15, Fig. 4A, Fig. 4C.

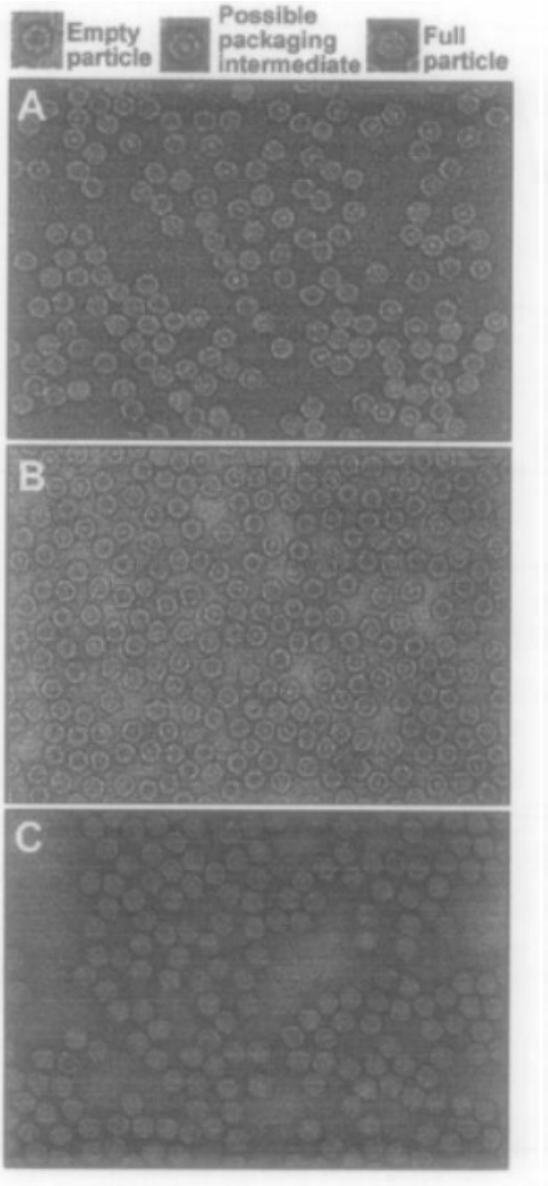
250. Potter explains that the presence of empty capsids in the purified preparations has no effect on the infectious particle titer, given that the DBA and RTPA quantify packaged genomes, rather than particles:

> 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.

EX1009 (Potter), p. 17 (emphasis added).

3. Electron Microscopy of Purified rAAV

251. 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:



EX1009 (Potter), pp. 16-17, Fig. 5A.

252. The electron micrographs were taken at a magnification factor of 49,500, a standard electron microscopy magnification for viewing viral particles. *See, e.g.*, EX1009 (Potter), p. 16, Fig. 5; EX1035 (Dika), p. 3, Fig. 1.

253. 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.

EX1009 (Potter), p. 17 (emphasis added).

254. 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. EX1009 (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 . . .").

255. 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). EX1009 (Potter), p. 16, Fig. 5A. I note that no aggregation was observed.

256. 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.

257. To confirm the identification of certain particles as full, empty, or intermediates, fractions from the analytical iodixanol gradient were also analyzed by electron microscopy. EX1009 (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. EX1009 (Potter), pp. 16-17, Fig. 5B and Fig. 5C.

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

Figure 5B. EX1009 (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. EX1009 (Potter), p. 16, Fig. 5C.

259. 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 Mischaracterized Potter in a Prior Proceeding

260. As I mentioned above (Section VI.C.), Novartis, in the prior IPR petitions challenging the related '542 patent, relied on Potter as a background reference. EX1014 (608 Petition), pp. 20, 22, and 63; EX1015 (609 Petition), at 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).

261. 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).

262. 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. The Patent Owner incorrectly described Potter in several different respects.

263. First, Patent Owner and Dr. Davies incorrectly characterized the concentration of the disclosed formulation in Potter. The 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."[8] EX1016 (608 POPR), pp. 47-48, 68-69; EX1060 (608 Davies Decl.), ¶¶121, 123-24, 151-52.

264. 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

[8] I note that the 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." EX1009 (Potter), p. 7, Table I. The Patent Owner, however, failed to address Table II, which discloses the concentrations of the final, concentrated reference standard stock. EX1009 (Potter), p. 9, Table II.

claims of the '721 patent. EX1009 (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, 123-24, 151-52.

265. 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.

266. 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.

267. 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 '721 patent does disclose such a "visual inspection" method.

268. As I discussed above (Sections VI.B.4. and VI.B.5.), the '721 patent assessed aggregation after concentration and diafiltration by visual inspection using light microscopy. EX1001 ('721 patent), 8:50-56 (finding "obvious amounts of visible material"); *see also* EX1001 ('721 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 '721 patent to assess aggregation after freeze/thaw cycling. EX1001 ('721 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 '721 patent, it is therefore particularly surprising, in my opinion, that the Patent Owner disparaged "visual inspection" techniques in the 608 POPR.

IX. CLAIM CONSTRUCTION

269. Challenged claims 1-4, 6, 7, and 11 of the '721 patent recite a method of preventing aggregation in a preparation of purified, rAAV virions. EX1001 ('721 patent), EX1001 ('721 patent), 14:9-29, 33-40, 49-51.

270. I have analyzed claims 1-4, 6, 7, and 11 according to their plain and ordinary meaning.

271. I note that several terms in the challenged claims are the same as terms in the related '542 patent that 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). It is my understanding that claim terms in patents derived from the same parent application, like the '721 and '542 patents, may be construed consistently across such patents. I have therefore considered the Court's constructions of the '542 patent in the Novartis litigation.

272. 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"	7	passing a liquid through a 0.22 μm filter to remove materials
"ionic strength"	1-4, 6, 7, 11	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"	1-4, 6, 7, 11	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"	1-4, 6, 7, 11	recombinant AAV virion or virus particles
"dynamic light scattering"	6	a technique in physics that can be used to determine a size distribution profile of small particles in suspension or polymers in solution
"purified"	1-4, 6, 7, 11	having been subjected to a purification procedure

273. I have considered the District Court's constructions in the earlier Novartis case regarding the '542 patent. My opinions do not change if the District Court's constructions above are applied to the challenged claims in the '721 patent, rather than the plain and ordinary meaning.

X. GROUND 1: CLAIMS 1-4, 6, 7, and 11 ARE OBVIOUS OVER AURICCHIO AND KONZ

274. In my opinion, claims 1-4, 6, 7, and 11 of the '721 patent are obvious over Auricchio and Konz.

275. A POSA would have been motivated to combine Auricchio with Konz because both Auricchio and Konz are directed towards efficient, cost-effective methods to produce pure, stable, high titer formulations of rAAV.

276. Auricchio touts the simplicity and reproducibility of its method, which involves the use of a single column. EX1007 (Auricchio), p. 1. Auricchio also touts

the high infectious titer, in addition to high physical titer, of the viral preparations produced using its method. EX1007 (Auricchio), pp. 1 (Overview and Summary), 4-6.

277. Auricchio also teaches that its method produces rAAV2 with greater stability during storage at 4°C than rAAV2 produced by a method using CsCl gradient purification. EX1007 (Auricchio), pp. 1 (Overview and Summary), 3, 6, Figure 1.

278. However, the method that Auricchio discloses to assess stability, determination of gene expression in infected cells, is time and labor intensive and not suited to large scale purification of rAAV. EX1007 (Auricchio), pp. 2-4, Figure 1.

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

280. Like Auricchio, Konz focuses on stability, which will help large scale production and distribution of rAAV preparations. *See* EX1008 (Konz), 22:15-16 ("an appropriate formulation buffer (e.g., see PCT publication WO 01/66137) can be used to maximize product stability").

281. Konz, including by reference to Evans, teaches higher pH formulation buffers, consistent with the knowledge in the art that rAAV, for example, did not aggregate at pH values greater than pH 7.5. *See, e.g.*, 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); EX1008 (Konz), 30:13-20 (disclosing a formulation buffer with pH 8.0), 42:12-17 (same); EX1020 (Evans), 11:31-12:4 (disclosing a high salt formulation buffer with pH 8.0), 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).

282. Konz is directed to methods of preventing aggregation, such as the use of non-ionic surfactants, and analytical techniques to evaluate the extent of aggregation and stability, such as dynamic light scattering and 0.22 μm filtration, which are more adaptable to scale than the infectivity assay disclosed in Auricchio. *See* EX1008 (Konz), 24:1-9, 25:29-30, 48:11-21, Table 12.

283. A POSA would therefore have been motivated to combine Auricchio's methods for simple, efficient, and cost-effective purification of rAAV with the analytical techniques, such as DLS and filtration using a 0.22 μm filter, in Konz to assess the stability of the viral preparation over time.

284. A POSA would have further understood that the purification methods of Auricchio, producing pure, high physical and high infectious titer rAAV, could be combined with the buffers and additional methods of Konz, by diafiltering Auricchio's 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) (discussing removal of residual solvents).

285. Moreover, a POSA would have understood that the methods of Auricchio produced a high physical and high infectious titer rAAV preparation in a high ionic strength buffer (PBS with 0.4 M NaCl), that includes phosphate, a multivalent ion. 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, at a pH in the claimed range of 7.5 to 8.0.

A. Claim 1

1. "A method of preventing aggregation of recombinant adeno-associated virus (rAAV) virions in a purified preparation of rAAV virions, comprising:"

286. Auricchio discloses a method of producing high physical and high infectious titer rAAV2. EX1007 (Auricchio), pp. 2-5.

287. Auricchio is directed to preventing aggregation of purified rAAV.

288. Auricchio teaches that its method produces rAAV2 with greater stability during storage at 4°C than rAAV2 produced by a method using CsCl gradient purification. EX1007 (Auricchio), pp. 1 (Overview and Summary), 3, 6, Figure 1.

289. Auricchio also discloses that its method produced good yields, high physical titer, and high infectious titer rAAV2 *in vitro* and *in vivo*.

290. These results would indicate to a POSA that the SSCP purified preparation did not contain large numbers of aggregates of viral particles. EX1007 (Auricchio), pp. 3-6.

291. Konz is expressly directed towards inhibition of viral particle aggregation during purification. For example, Konz teaches the addition of non-ionic surfactants to inhibit viral particle aggregation throughout the purification. *See, e.g.*, EX1008 (Konz), 23:17-24:12.

292. Konz also 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"). 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.

293. Konz discloses the application of analytical techniques demonstrating that the purified formulations were free of aggregation, such as filtration through a 0.22 μm filter and DLS. EX1008, 36:25-27 (stating that "no pressure build-up was seen during the sterile filtration which suggests a lack of aggregated virus"), 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).

294. Auricchio, in combination with Konz, thus meets this limitation of claim 1.

2. "providing a lysate comprising rAAV virions;"

295. Auricchio discloses creating a lysate as one of the earliest steps in the SSCP purification. *See, e.g.*, EX1007 (Auricchio), p. 2 (stating that "the crude lysate containing the recombinant vector is directly applied to a preequilibrated heparin column").

296. Konz similarly discloses creating a lysate as one of the earliest steps in the purification. *See, e.g.*, EX1008 (Konz), 27:23-25 (stating, "A first step in a purification process of the present invention is a host cell lysis step, which provides for maximum release of adenovirus particles from the cells as well as providing for an opportunity to potentially inactivate adventitious agents"), 27:25-28:4. Given the express teachings in Konz that its methods apply equally to rAAV purification, a POSA would understand Konz to disclose creating a lysate as one of the earliest steps in an rAAV purification. *See, e.g.*, EX1008 (Konz), 14:24-29.

297. Auricchio and Konz thus both meet this limitation of claim 1.

3. "purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, wherein said virions are purified; and"

298. Auricchio's SSCP method for purifying rAAV virions includes directly applying the lysate to a preequilibrated heparin column. *See, e.g.*, EX1007 (Auricchio), p. 2 (stating that "the crude lysate containing the recombinant vector is directly applied to a preequilibrated heparin column"). A POSA would have understood that a heparin column is a form of chromatography. *See, e.g.*, EX1042 (Zolotukhin), Abstract (referring to "heparin affinity chromatography").

299. Auricchio further analyzed the purity of the rAAV virions after elution from the heparin column and found it comparable to the purity of rAAV virions

produced by iodixanol/heparin and HPLC methods. *See, e.g.*, EX1007 (Auricchio), p. 4, Fig. 2.

300. Konz expressly discloses the use of column chromatography to purify rAAV. *See, e.g.*, EX1008 (Konz), 14:20-34 (discussing the use of anion or cation exchange chromatography to purify rAAV).

301. Auricchio and Konz thus both meet this limitation of claim 1.

4. "adding one or more salts of multivalent ions selected from the group consisting of citrate, phosphate, sulfate and magnesium to said purified virions"

302. Auricchio discloses eluting the purified rAAV from the heparin column with a phosphate buffer (PBS). *See, e.g.*, EX1007 (Auricchio), p. 2.

303. Konz discloses diafiltering the virions after they have been purified on a chromatography column into an appropriate formulation buffer, such as those disclosed in Evans, as incorporated into Konz. *See, e.g.*, EX1008 (Konz), 22:15-17 (referencing formulation buffers disclosed in Evans "to maximize product stability"), 24:27-25:22 (again referencing formulation buffers disclosed in Evans to use for diafiltration of the virions purified on the chromatography column).

304. Evans, in turn discloses formulation buffers that include multivalent ions, in particular, magnesium ions. *See, e.g.*, EX1020 (Evans), 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.

305. Auricchio and Konz thus both meet this limitation of claim 1.

5. "to produce a preparation of virions with an ionic strength of at least 200 mM,"

306. The elution buffer disclosed in Auricchio contains PBS plus 0.4 M NaCl. EX1007 (Auricchio), p. 2; EX1065 (Yeung), pp. 250-51.

307. A POSA would have understood that the ionic strength of 1X PBS is calculated as follows.

308. 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; EX1076 (Solomons), pp. 4-5.

309. 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).

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

311. 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.

312. 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).[9]

[9] 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.*, EX1078 (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.*, EX1075 (Green), pp. 2-3; EX1077 (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.*, EX1074 (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:

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

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

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

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

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.

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

314. At pH 8.0, the contribution to the ionic strength of 1X PBS of each component is: $H2PO4^{-}$ (0.71 mM); $HPO4^{2-}$ (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). The total ionic strength of 1X PBS at pH 8.0 is therefore about 169.02 mM.

315. 0.4 M NaCl (400 mM) has an ionic strength of 400 mM. Therefore, PBS plus 0.4 M NaCl, at both pH 7.5 and pH 8.0, has an ionic strength of more than 550 mM, which meets this limitation of claim 1.

316. Konz incorporates Evans by reference, and Evans, in turn discloses formulation buffers that meet this limitation of the claims. *See, e.g.*, EX1020 (Evans), 11:31-12:4 (disclosing a formulation buffer at pH 8.0, NaCl concentration from 25 mM to 250 mM, MgCl2 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, MgCl2 from 0.5 mM to 2.5 mM, a surfactant, and a free radical inhibitor

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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.

such as sodium citrate); *see also* 36:21-22 (claim 4) (reciting that "the salt is sodium chloride from about 25 mM to about 250 mM"), 41:14-15 (claim 37) (same).

317. 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 buffer meets this limitation of the claims.

318. Auricchio and Konz thus both meet this limitation of claim 1.

6. "wherein the concentration of rAAV virions in said preparation exceeds $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml;"

319. Auricchio discloses purified rAAV particles at concentrations exceeding 1 x 10^{13} vg/ml and less than 6.4 x 10^{13} vg/ml. *See, e.g.*, EX1007 (Auricchio), pp. 2, 4, Table 2.

320. Specifically, Auricchio discloses numbers of genome copies obtained from various runs of the SSCP method. *See, e.g.*, EX1007 (Auricchio), p. 4, Table 2. Two of these runs produced more than 1 x 10^{13} genome copies of rAAV from a single run on an 8 ml heparin column. *See, e.g.*, EX1007 (Auricchio), p. 4, Table 2 (disclosing one run that produced 1.4 x 10^{13} genome copies, and one that produced 1.1 x 10^{13} genome copies, and noting that "[i]n these experiments up to 1.4 x 10^{13} particles of AAV2 could be purified and concentrated using 8 ml of agarose-heparin suspension").

321. Auricchio discloses that the genome copy numbers were obtained using real time PCR. *See* EX1007 (Auricchio), p. 2.

322. Auricchio further discloses that the purified rAAV was eluted from the heparin column with 15 mls of PBS with 0.4 M NaCl, and then concentrated to about 1 ml. *See* EX1007 (Auricchio), p. 2. Therefore, a POSA would have understood that the concentration of a single run that produced 1.4 x 10^{13} genome copies, and was concentrated into about 1 ml of buffer, would have a concentration of 1.4 x 10^{13} genome copies per ml.[10]

323. Auricchio therefore meets this limitation of claim 1.

7. "and wherein the pH of the purified preparation of rAAV virions is between 7.5 and 8.0."

324. In my opinion, the combination of Auricchio and Konz discloses this limitation.

325. Auricchio discloses eluting purified rAAV from the heparin column with a buffer at pH 7.4. *See, e.g.*, EX1007 (Auricchio), p. 2.

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[10] The notation "vg/ml" in the '721 claims would be understood by a POSA to mean "vector genomes / ml." *See, e.g.*, EX1001 ('721 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.

326. A POSA would have been motivated to combine Auricchio with Konz to improve the stability of the purified preparation and reduce aggregation even further using Konz's methods. Auricchio teaches improved stability of purified rAAV preparations through the avoidance of CsCl gradient ultracentrifugation in the purification. *See, e.g.*, EX1007 (Auricchio), pp. 2-4, Fig. 1.

327. Konz teaches improving the stability of purified viral preparations through the use of particular formulation buffers and through the addition of non-ionic surfactants to inhibit aggregation further. *See, e.g.*, EX1008 (Konz), 22:15-17 (referencing formulation buffers disclosed in Evans "to maximize product stability"), 24:27-25:22 (again referencing formulation buffers disclosed in Evans to use for diafiltration of the virions purified on the chromatography column), 24:1-25:1 (teaching the use of non-ionic surfactants throughout the purification process to inhibit aggregation).

328. Evans, in turn discloses formulation buffers that include non-ionic surfactants. *See, e.g.*, EX1020 (Evans), 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 non-ionic 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).

329. Konz also teaches that higher pH buffers improve the stability of the viral particles. *See, e.g.*, EX1008 (Konz), 26:10-17. These teachings are consistent with the knowledge in the prior art that rAAV did not aggregate at pH 7.5, but did aggregate at pH 7.2. 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).

330. A POSA would have understood that the methods of Auricchio produced a high physical and high infectious titer rAAV preparation in a high ionic strength buffer (PBS with 0.4 M NaCl), that includes phosphate, a multivalent ion. 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.

331. And given the teaching of Konz that higher pH buffers improve viral particle stability, a POSA would have selected a formulation buffer such as the buffer disclosed in Konz, by reference to Evans, at pH 8.0, with an NaCl concentration from 25 mM to 250 mM, an $MgCl_2$ concentration from 0.5 mM to 2.5 mM, and a non-ionic surfactant. EX1020 (Evans), 11:31-12:4, 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).

332. Therefore, the combination of Auricchio and Konz discloses this limitation of claim 1.

B. Claim 2: "The method of claim 1, further comprising treating said purified virions with a nuclease."

333. Konz discloses that 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." EX1008 (Konz), 22:4-6. In particular, Konz discloses that nuclease treatment can be applied during steps "downstream" of anion exchange chromatography. EX1008 (Konz), 6:5-7.

334. Konz further discloses that nuclease treatment leads to lower contaminating DNA levels in the final purified product. EX1008 (Konz), 21:14-16.

335. A POSA would therefore have been motivated to add low concentrations of nuclease downstream of anion exchange chromatography, at which point the virions would be "purified" away from the lysate, to inhibit aggregation resulting from unencapsulated viral DNA and otherwise contaminating nucleic acids.

336. Konz therefore meets the additional limitation of dependent claim 2.

C. Claim 3: "The method of claim 2, wherein the nuclease is an endonuclease from *Serratia marcescens*."

337. As discussed above, a POSA would have understood that the commonly used nuclease Benzonase was an endonuclease from *Serratia*

marcescens. EX1053 (Roth), 2. Konz expressly discloses the use of Benzonase. EX1008 (Konz), 22:2-3. A POSA would have understood that Benzonase was a nuclease commonly used for rAAV purification and would have been motivated to select Benzonase in particular. EX1042 (Zolotukhin), 11.

338. Konz therefore meets the additional limitation of dependent claim 3.

D. Claim 4: "The method of claim 1, wherein the multivalent ion is citrate."

339. Konz incorporates Evans by reference, and Evans, in turn, discloses adding a non-reducing free radical scavenger/chelator such as citrate to formulation buffers to maximize short and long term stability of viral preparations. EX1020 (Evans), 13:8-34. A POSA would therefore have been motivated to add citrate to the formulation buffer discussed above, with a pH of about 8.0, containing about 250 mM NaCl, and containing $MgCl_2$, as expressly disclosed in Evans. EX1020 (Evans), 11:31-12:4, 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).

340. Konz, by reference to Evans, therefore meets the additional limitation of dependent claim 4.

E. Claim 6: "The method of claim 1, wherein, after addition of the one or more salts of multivalent ions, the average particle radius (Rh) of the virions in the preparation of virions is less than about 20 nm as measured by dynamic light scattering."

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

342. Auricchio is directed towards an efficient purification method that produces stable, high physical and infectious titer rAAV preparations. Yet Auricchio does not disclose a simple, efficient, cost-effective method of assessing particle aggregation. The only method disclosed in Auricchio to assess the stability of a preparation of purified rAAV is transduction of cells in culture and observation of the relative infectivity of the preparation over 72 hours. *See, e.g.*, EX1007 (Auricchio), p. 3, Fig. 1.

343. A POSA would have understood that this assay is labor and time intensive and not suitable for large scale preparation of rAAV. A POSA would therefore have looked to Konz's more efficient analytical methods for assessing aggregation.

344. Konz discloses using DLS to evaluate particle aggregation in a final formulation of viral particles, which a POSA would have understood to include the salts of multivalent ions, as I discussed above (Section X.A.4). *See, e.g.*, EX1008 (Konz), 48:4-15 ("The mean particle size by Dynamic Light Scattering was 123 nm, consistent with theoretical expectations"). Konz used DLS to analyze the extent of

aggregation, and 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 to assay for aggregation of rAAV in a purified preparation. EX1008 (Konz), 14:24-29.

345. Auricchio, in combination with Konz, therefore meets the additional limitation of dependent claim 6.

F. Claim 7: "The method of claim 1, wherein, after addition of the one or more salts of multivalent ions, recovery of the virions is at least about 90% following filtration of the preparation of virions through a 0.22 μm filter."

346. In my opinion, the combination of Auricchio and Konz discloses the additional limitation of dependent claim 7.

347. Again, as I discussed above for claim 6, a POSA would have been motivated to combine Auricchio and Konz to apply Konz's more efficient methods to assess particle aggregation. Konz discloses the use of sterile filtration through a 0.22 μm filter as a method to assess aggregation. EX1008 (Konz), 36:25-27 (stating, "[n]o pressure build-up was seen during the sterile filtration which suggests a lack of aggregated virus").

348. Konz discloses sterile filtration of purified recombinant viral particles through a 0.22 μm filter with a recovery greater than 90% in a final formulation of

viral particles, which a POSA would have understood to include the salts of multivalent ions. *See, e.g.*, EX1008 (Konz), 30:27-30 (teaching the use of a 0.22 μm filter), 46:16-21, Table 12 (98% yield), 50:1-5, Table 14 (100% yield), 51:5-10, Table 16 (99% yield).

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 μm filter. EX1008 (Konz), 36:21-28. 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 cm2)." 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.

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. Therefore, Auricchio, in combination with Konz, meets the additional limitation of dependent claim 7.

G. Claim 11: "The method of claim 2, further comprising diafiltering the purified rAAV virions to achieve an ionic strength of at least 200 mM."

353. Konz discloses diafiltering purified rAAV virions into the final formulation buffer. EX1008 (Konz), 22:13-16. A POSA would have understood that diafiltration could be used for buffer exchange into the final formulation buffer, chosen to maximize product stability at high ionic strength with multivalent ions, at a pH in the 7.5 to 8.0 range.

354. Konz therefore meets the additional limitation of dependent claim 11.

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

355. A POSA would have had a reasonable expectation of success in combining Auricchio with Konz to arrive at the claimed purification method. The required techniques, 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.

356. Auricchio's purification method produced stable, high physical and infectious titer rAAV 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, producing yields greater than 90%, and DLS results indicating individual viral particles without aggregation.

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

358. Moreover, a POSA would have had a reasonable chance of success that this method of purifying rAAV would prevent aggregation. A POSA would have started with Auricchio's purification that produced stable, high physical and infectious titer rAAV preparation. And given the teachings of Auricchio to elute the purified rAAV from the heparin column in a high ionic strength buffer that contained a multivalent ion, the POSA would have sought to maintain these characteristics of the preparation in combining them with Konz.

359. Given Konz's teachings that higher pH buffers improved the stability of purified viral particle preparations, a POSA would have chosen one of the Konz high ionic strength (250 mM NaCl) buffers, with a multivalent ion (MgCl2), at a pH somewhat higher than Auricchio's (pH 8.0, for example), containing a non-ionic surfactant, in accordance with Konz's teachings. Given all these steps to inhibit

aggregation, given the starting point of Auricchio's formulation which was stable and had high physical and infectious titer, and given Konz's data showing greater than 90% yields and no aggregation per assessment by DLS, a POSA would have had a reasonable expectation of success in achieving the claimed combination – an rAAV method that prevented aggregation and produced a high titer, high ionic strength formulation containing a multivalent ion with a pH around 8.0.

I. Secondary Considerations Do Not Change the Conclusion of Obviousness

360. 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 '721 patent. For example, I am not aware of any commercial success attributable to a purification method meeting the limitations of the challenged claims.[11] Similarly, I am not aware of any licenses directed

[11] 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 '721 patent. There is no nexus between the commercial success of Elevidys® and the purification method recited in the challenged claims.

specifically to the ’721 patent or the subject matter recited in challenged claims 1-4, 6, 7, or 11.[12]

361. Finally, I am not aware of any unexpected results having a nexus to the claimed subject matter. The ’721 patent does not disclose unexpected properties of the claimed purification method. Effects of pH, multivalent ions, and ionic strength on viral particle aggregation had all been studied for decades before the ’721 patent and disclosed in prior art references such as Floyd I, II, and III. And purification methods that produced high titer rAAV formulations had been developed where aggregation was not present before the ’721 patent and disclosed in prior art references such as Auricchio. 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.

362. To the extent Patent Owner attempts to raise secondary considerations that have only a marginal nexus, if any, to claims 1-4, 6, 7, or 11 of the ’721 patent,

[12] 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 ’721 patent. Thus, there is no nexus between any Novartis license and the purification method recited in the challenged claims.

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 1-4, 6, 7, and 11 of the ’721 patent are obvious over the combination of Auricchio and Konz.

XI. GROUND 2: CLAIMS 1-4, 6, 7, and 11 ARE OBVIOUS OVER POTTER AND KONZ

363. In my opinion, claims 1-4, 6, 7, and 11 of the ’721 patent are obvious over Potter and Konz.

364. A POSA would have been motivated to combine Potter with Konz because both Potter and Konz are directed towards efficient, cost-effective methods suitable for large scale production of pure, stable, high titer formulations of rAAV. EX1009 (Potter), p. 2; EX1008 (Konz), 1:25-27.

365. Potter states that in the disclosed methods, they eliminated the need for any centrifugation steps to permit efficient large scale production. EX1009 (Potter), p. 1. Potter uses the analytical technique of electron microscopy to analyze the purified rAAV purifications, demonstrating no evidence of aggregation. However, the technique of electron microscopy is labor and time intensive and difficult to adapt to scale. EX1009 (Potter), pp. 16-17, Fig. 5.

366. 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.

367. A POSA would therefore have been 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. A POSA would further have been motivated to combine Potter's methods with those of Konz because Konz discloses the use of non-ionic surfactants to inhibit aggregation even further.

368. 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 non-ionic surfactants to inhibit aggregation even further, 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. *See, e.g*., EX1068 (Schwartz).

369. 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 (0.5 M 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.

A. Claim 1

1. "A method of preventing aggregation of recombinant adeno-associated virus (rAAV) virions in a purified preparation of rAAV virions, comprising:"

370. Potter discloses a method for purifying rAAV vector particles to create a "reference standard stock of rAAV with a precisely defined titer." EX1009 (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. EX1009 (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. Otherwise, these aliquoted preparations of the standard would vary from the original stock, an outcome that would be contrary to the entire purpose of creating a reference standard.

371. Potter, moreover, discloses electron microscopic analysis of the purified rAAV preparation. See EX1009 (Potter), pp. 16-17, Fig. 5. The analysis shows no evidence of aggregation in the purified rAAV preparation. Potter is

therefore directed towards a method of purifying rAAV that results in a stable preparation without aggregation.

372. Konz, similarly, is directed towards developing a purification method that results in a stable rAAV formulation without aggregation. For example, Konz teaches the addition of non-ionic surfactants to inhibit viral particle aggregation throughout the purification. *See, e.g.*, EX1008 (Konz), 23:17-24 ("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").

373. Konz discloses the application of analytical techniques demonstrating that the purified formulations were free of aggregation, such as filtration through a 0.22 μm filter and DLS. *See, e.g.*, EX1008 (Konz), 36:25-27 (stating that "no pressure build-up was seen during the sterile filtration which suggests a lack of aggregated virus"), 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).

374. Konz also discloses formulation buffers that will maximize product stability. *See, e.g.*, EX1008 (Konz), 22:15-16. Konz also incorporates Evans 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").

375. 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.

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

2. "providing a lysate comprising rAAV virions;"

377. Potter discloses creating a lysate as one of the earliest steps in the rAAV purification. *See, e.g.*, EX1009 (Potter), pp. 4-5 (disclosing the protocol for "Generation of Crude Lysate" as the first step of "Purification of rAAV vector").

378. Konz similarly discloses creating a lysate as one of the earliest steps in the purification. *See, e.g.*, EX1008 (Konz), 27:23-25 (stating, "A first step in a purification process of the present invention is a host cell lysis step, which provides

for maximum release of adenovirus particles from the cells as well as providing for an opportunity to potentially inactivate adventitious agents"), 27:25-28:4. Given the express teachings in Konz that its methods apply equally to rAAV purification, a POSA would understand Konz to disclose creating a lysate as one of the earliest steps in an rAAV purification. *See, e.g.*, EX1008 (Konz), 14:24-29.

379. Potter and Konz thus both meet this limitation of claim 1.

3. "purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, wherein said virions are purified; and"

380. Potter discloses the use of column chromatography to purify rAAV virions from the lysate. *See, e.g.*, EX1009 (Potter), pp. 5-7. Specifically, Potter's purification protocol involves the use of three different column chromatography steps: Streamline Heparin affinity chromatography, phenyl-sepharose hydrophobic interaction chromatography, and heparin affinity chromatography. *See, e.g.*, EX1009 (Potter), pp. 5-7, Figure 1.

381. Figure 1 of Potter shows the increasing purity of the rAAV preparation after each successive column chromatography step. *See, e.g.*, EX1009 (Potter), p. 6, Fig. 1.

382. Konz expressly discloses the use of column chromatography to purify rAAV. *See, e.g.*, EX1008 (Konz), 14:20-34 (discussing the use of anion or cation exchange chromatography to purify rAAV).

383. Potter and Konz thus both meet this limitation of claim 1.

4. "adding one or more salts of multivalent ions selected from the group consisting of citrate, phosphate, sulfate and magnesium to said purified virions"

384. Potter discloses a phosphate buffer (PBS) used to elute purified rAAV from the heparin affinity chromatography column (the third column chromatography purification step). *See* EX1009 (Potter), p. 7 (stating that the "virus is eluted with PBS containing 0.5 M NaCl"). A POSA would have understood "PBS" to mean "phosphate buffered saline," and would further have understood PBS to involve addition of phosphate salts. *See, e.g.*, EX1065 (Yeung), pp. 250-51. Potter therefore meets this limitation of claim 1.

385. Konz discloses diafiltering the virions after they have been purified on a chromatography column into an appropriate formulation buffer, such as those disclosed in Evans, as incorporated into Konz. *See, e.g.*, EX1008 (Konz), 22:15-17 (referencing formulation buffers disclosed in Evans "to maximize product stability"), 24:27-25:22 (again referencing formulation buffers disclosed in Evans to use for diafiltration of the virions purified on the chromatography column).

386. Evans, in turn discloses formulation buffers that include multivalent ions, in particular, magnesium ions. *See, e.g.*, EX1020 (Evans), 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.

387. Potter and Konz thus both meet this limitation of claim 1.

5. "to produce a preparation of virions with an ionic strength of at least 200 mM,"

388. The buffer disclosed in Potter contains 0.5 M NaCl. EX1009 (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:

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

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

390. Konz incorporates Evans by reference, and Evans, in turn discloses formulation buffers that meet this limitation of the claims. *See, e.g.*, EX1020 (Evans), 11:31-12:4 (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).

391. 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 buffer meets this limitation of the claims.

392. Potter and Konz thus both meet this limitation of claim 1.

6. "wherein the concentration of rAAV virions in said preparation exceeds $1x10^{13}$ vg/ml up to $6.4x10^{13}$ vg/ml;"

393. Potter discloses purified, rAAV particles at concentrations exceeding 1×10^{13} vg/ml and less than 6.4×10^{13} vg/ml. Table II discloses titers of the rAAV reference standard obtained through Potter's purification process. EX1009 (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." EX1009 (Potter), p. 9, Table II.

394. The notation "vg/ml" in the '721 claims would have been understood by a POSA to mean "vector genomes / ml." *See, e.g.*, EX1001 ('721 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.

395. 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."

396. As I discuss above (*see* Section VIII.C.2), 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.

397. 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* EX1009 (Potter), pp. 7-9, Table II. Potter found that the DBA and RTPA titers were very similar to one another, 1.12 x 10^{13} and 1.46 x 10^{13}, which provides confidence in both meaurements. *See* EX1009 (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

EX1009 (Potter), p. 19, Table II.

398. Both of these titers meet the claimed concentration range of the '721 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."

399. Potter thus meets this limitation of claim 1.

7. "and wherein the pH of the purified preparation of rAAV virions is between 7.5 and 8.0."

400. The buffer used in Potter is PBS with 0.5 M NaCl. *See* EX1009 (Potter), p. 7 (stating, "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.*, EX1073 (Cold Spring Harbor Protocols); EX1065 (Yeung), pp. 257, 294; EX1023 (Hermens), pp. 2-3; EX1070 (Monahan), p. 9. Therefore, a POSA would have understood that Potter meets this limitation of the '721 patent claims.

401. 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.

402. 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), 11:31-12:4 (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 (stating, "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, "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).

403. The formulation buffer with pH 8.0 disclosed in Konz meets this limitation of the claims.

404. A POSA would have been motivated to exchange the buffer of Potter with a buffer in Evans, as referenced in Konz, to improve viral particle stability even further. Specifically, the Konz/Evans buffer includes a surfactant, which a POSA would have understood would inhibit aggregation even further.

405. A POSA would have selected a buffer in Konz similar to that in Potter, given the success of Potter's purification method in producing high titer, non-aggregated, pure rAAV. Specifically, a POSA would have selected a buffer with high NaCl concentration, divalent ions, in a similar pH range. The teachings of Konz that higher pH buffers improve viral particle stability would have motivated a POSA to select a buffer with pH around 8.0, similar to the buffer in Potter.

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

B. Claim 2: "The method of claim 1, further comprising treating said purified virions with a nuclease."

407. Konz discloses that 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." EX1008 (Konz), 22:4-6. In particular, Konz discloses that nuclease treatment can be applied during steps "downstream" of anion exchange chromatography. EX1008 (Konz), 6:5-7.

408. Konz further discloses that nuclease treatment leads to lower contaminating DNA levels in the final purified product. EX1008 (Konz), 21:14-16.

409. A POSA would therefore have been motivated to add low concentrations of nuclease downstream of anion exchange chromatography, at which point the virions would be "purified" away from the lysate, to inhibit aggregation resulting from unencapsulated viral DNA and otherwise contaminating nucleic acids.

410. Konz therefore meets the additional limitation of dependent claim 2.

C. Claim 3: "The method of claim 2, wherein the nuclease is an endonuclease from *Serratia marcescens*."

411. A POSA would have understood that the commonly used nuclease Benzonase was an endonuclease from *Serratia marcescens*. EX1053 (Roth), 2. Konz expressly discloses the use of Benzonase. EX1008 (Konz), 22:2-3. A POSA would have understood that Benzonase was a nuclease commonly used for rAAV

purification and would have been motivated to select Benzonase in particular. EX1042 (Zolotukhin), 11.

412. Konz therefore meets the additional limitation of dependent claim 3.

D. Claim 4: "The method of claim 1, wherein the multivalent ion is citrate."

413. Konz incorporates Evans by reference, and Evans, in turn, discloses adding a non-reducing free radical scavenger/chelator such as citrate to formulation buffers to maximize short and long term stability of viral preparations. EX1020 (Evans), 13:8-34. A POSA would therefore have been motivated to add citrate to the formulation buffer discussed above, with a pH of about 8.0, containing about 250 mM NaCl, and containing $MgCl_2$, as expressly disclosed in Evans. EX1020 (Evans), 11:31-12:4, 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).

414. Konz, by reference to Evans, therefore meets the additional limitation of dependent claim 4.

E. Claim 6: "The method of claim 1, wherein, after addition of the one or more salts of multivalent ions, the average particle radius (Rh) of the virions in the preparation of virions is less than about 20 nm as measured by dynamic light scattering."

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

416. Potter is directed towards an efficient purification method that produces stable, high physical titer rAAV preparations that show no evidence of aggregation when analyzed using electron microscopy. *See, e.g.*, EX1009 (Potter), pp. 16-17, Fig. 5. Electron microscopy, however, is a time and labor intensive analytical method to assess aggregation of a purified rAAV preparation. A POSA would therefore have combined the more efficient analytical methods to assess aggregation disclosed in Konz with Potter's purification protocol.

417. Konz discloses using DLS to evaluate particle aggregation in a final formulation of viral particles, which a POSA would have understood to include the salts of multivalent ions. *See, e.g*., EX1008 (Konz), 48:4-15 ("The mean particle size by Dynamic Light Scattering was 123 nm, consistent with theoretical expectations"). Konz used DLS to analyze the extent of aggregation, and 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), 14:24-29.

418. Potter, in combination with Konz, therefore meets the additional limitation of dependent claim 6.

F. Claim 7: "The method of claim 1, wherein, after addition of the one or more salts of multivalent ions, recovery of the virions is at least about 90% following filtration of the preparation of virions through a 0.22 μm filter."

419. In my opinion, the combination of Potter and Konz discloses the additional limitation of dependent claim 7.

420. As I discussed above in relation to dependent claim 6, Potter is directed towards an efficient purification method that produces stable, high physical titer rAAV preparations that show no evidence of aggregation when analyzed using electron microscopy. *See, e.g.*, EX1009 (Potter), pp. 16-17, Figure 5. Electron microscopy, however, is a time and labor intensive analytical method to assess aggregation of a purified rAAV preparation. A POSA would therefore have combined the more efficient analytical methods to assess aggregation disclosed in Konz with Potter's purification protocol.

421. Konz discloses the use of sterile filtration through a 0.22 μm filter as a method to assess aggregation in a final formulation of viral particles, which a POSA would have understood to include the salts of multivalent ions. EX1008 (Konz), 36:25-27 (stating, "[n]o pressure build-up was seen during the sterile filtration which suggests a lack of aggregated virus").

422. 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), 30:27-30 (teaching the use of a 0.22 μm filter), 48:16-21, Table 12 (98% yield), 50:1-5, Table 14 (100% yield), 51:5-10, Table 16 (99% yield).

423. 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 μm filter. EX1008 (Konz), 36:21-28. Notably, as shown in Table 2, the yield from the sterile filtration step was 94%. EX1008 (Konz), 37:1-6, Table 2.

424. 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 cm2)." 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.

425. 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.

426. Therefore, Potter, in combination with Konz, meets the additional limitation of dependent claim 7.

G. Claim 11: "The method of claim 2, further comprising diafiltering the purified rAAV virions to achieve an ionic strength of at least 200 mM."

427. Konz discloses diafiltering purified rAAV virions into the final formulation buffer. EX1008 (Konz), 22:13-16. A POSA would have understood that diafiltration could be used for buffer exchange into the final formulation buffer, chosen to maximize product stability at high ionic strength with multivalent ions, at a pH in the 7.5 to 8.0 range.

428. Konz therefore meets the additional limitation of dependent claim 11.

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

429. A POSA would have had a reasonable expectation of success in combining Potter with Konz to arrive at the claimed purification method. The required techniques, 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.

430. Potter's purification method produced high physical 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, producing yields greater than 90%, and DLS results indicating individual viral particles without aggregation.

431. 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. Also, using nothing more than routine experimentation, after diafiltration, a POSA would have been able to sterile filter the preparation, determine the yield, and also apply DLS to determine whether aggregation was present.

432. 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 (about 8.0, given the teachings of Konz to use a higher pH to improve particle stability), 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 method that produced a concentrated rAAV stock where no aggregation was detected, and given Konz's data showing greater than 90% yields and no aggregation per assessment by DLS, a POSA would have had a reasonable expectation of success in achieving the claimed method – an rAAV purification method that produced a high titer, high ionic strength formulation in the claimed pH range containing a multivalent ion and a non-ionic surfactant without aggregation.

I. Secondary Considerations Do Not Change the Conclusion of Obviousness

433. 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 '721 patent. For example, I am not aware of any commercial success attributable to a purification method meeting the limitations of the challenged claims.[13] Similarly, I am not aware of any licenses directed specifically to the '721 patent or the subject matter recited in challenged claims 1-4, 6, 7, or 11.[14]

434. Finally, I am not aware of any unexpected results having a nexus to the claimed subject matter. The '721 patent does not disclose unexpected properties of

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[13] 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 '721 patent. There is no nexus between the commercial success of Elevidys® and the purification method recited in the challenged claims.

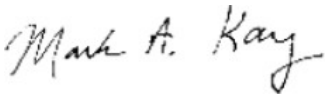
[14] If Patent Owner attempts to rely on any license to Novartis in the earlier case brought by Genzyme, I understand that any such license was executed in connection with the settlement of litigation and involved at least one other patent in addition to the '721 patent. Thus, there is no nexus between any Novartis license and the purification method recited in the challenged claims.

the claimed purification method. Effects of pH, multivalent ions, and ionic strength on viral particle aggregation had all been studied for decades before the '721 patent and disclosed in prior art references such as Floyd I, II, and III. And rAAV purification methods had been developed that produced high titer rAAV preparations where aggregation was not present before the '721 patent and disclosed in prior art references such as Potter. 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.

435. To the extent Patent Owner attempts to raise secondary considerations that have only a marginal nexus, if any, to claims 1-4, 6, 7, or 11 of the '721 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 1-4, 6, 7, and 11 of the '721 patent are obvious over the combination of Potter and Konz.

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.

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