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

PETITION FOR *INTER PARTES* REVIEW

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EX1074	Po and Senozan, "The Henderson-Hasselbalch Equation: Its History and Limitations," *Journal of Chemical Education* 78.11 (2001): 1499 ("Po")
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LIST OF CHALLENGED CLAIMS

Claim	Element
1 [pre]	1. 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
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.

Claim	Element
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.

Sarepta Therapeutics, Inc. ("Sarepta" or "Petitioner") respectfully requests *inter partes* review of claims 1-4, 6, 7, and 11 (the "challenged claims") of U.S. Patent No. 7,704,721 ("the '721 patent") (EX1001). The '721 patent is assigned to Genzyme Corporation.

Petitioner is concurrently filing a petition requesting *inter partes* review of various claims of a related patent, U.S. Patent No. 9,051,542 ("the '542 patent). The '542 patent is a continuation of U.S. Patent Application No. 11/141,996, which issued as the '721 patent.

I. INTRODUCTION

Adeno-associated virus (AAV) has been studied for decades as a useful tool to deliver therapeutic genes to patients to treat diseases such as Duchenne Muscular Dystrophy, cystic fibrosis, and various diseases of the eye. For both preclinical and clinical applications, researchers have sought to develop efficient methods to purify high titer, recombinant AAV (rAAV) preparations at a large scale.

Decades before the earliest priority date for the '721 patent, aggregation of viral particles was known to decrease viral infectivity. Aggregation was also known to be dependent on the concentration of viral particles in a preparation. A number of factors were also known to inhibit viral particle aggregation, including high ionic strength, multivalent ions, non-ionic surfactants, and, for AAV in particular, pH values around 7.5.

The challenged claims are directed to a method of preventing aggregation in a purified preparation of rAAV virions. The challenged claims recite a straightforward set of steps for the method, and properties of the resulting preparation, such as pH, ionic strength, the presence of multivalent ions, and particle concentration, all of which were well known to affect aggregation in the prior art for years before the earliest possible priority date for the '721 patent.

Specifically, the claimed method comprises purifying rAAV particles by creating a lysate, using ultracentrifugation or chromatography, and adding salts of multivalent ions (citrate, phosphate, sulfate, or magnesium) to produce a preparation with an ionic strength greater than 200 mM, at a concentration between 1 x 10^{13} vg/ml and 6.4 x 10^{13} vg/ml, in a buffer within the pH range of 7.5 and 8.0. The challenged claims also contain various additional limitations, such as an average particle radius for the rAAV particles of less than about 20 nm as measured by dynamic light scattering (claim 6), or a recovery of at least 90% following filtration of the composition through a 0.22 μm filter (claim 7).

Challenged claims 1-4, 6, 7, and 11 are obvious over two different combinations of prior art references: (1) Auricchio and Konz, and (2) Potter and Konz.

Auricchio and Konz. Auricchio is directed towards an efficient and cost-effective method to purify high physical and infectious rAAV formulations.

Auricchio is also directed towards inhibiting aggregation and producing an rAAV formulation that will be stable over time. To this end, Auricchio teaches the avoidance of CsCl gradients during purification. Auricchio further teaches the elution of purified rAAV in a high salt buffer with multivalent ions.

While Auricchio is directed towards an efficient purification method that would be suitable for large scale rAAV preparation, the only analytical 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. This method is time and labor intensive and is not compatible with large scale purification.

A POSA would therefore have been motivated to combine Auricchio with a reference that disclosed more efficient and cost-effective analytical techniques to assay for the presence of aggregates.

Konz is such a reference. Konz is directed to efficient purification methods suitable for large scale rAAV purification and production. Konz discloses analytical techniques including dynamic light scattering (DLS) and filtration through a 0.22 μm filter to evaluate aggregation in the purified preparations. Konz also discloses formulation buffers similar to the elution buffer in Auricchio, with an ionic strength greater than 200 mM, containing multivalent ions, with a pH within the claimed pH

range of 7.5 to 8.0. Konz further discloses the addition of non-ionic surfactants to reduce the possibility of aggregation even further.

The challenged claims are therefore obvious over the combination of Auricchio and Konz.

Potter and Konz. Potter is directed towards production of a high physical titer rAAV stock for use as a reference standard stock for preclinical studies. Potter discloses that the rAAV stock produced by their method was intended to be distributed to various laboratories for use in preclinical research, indicating that it was stable during storage and distribution. Potter discloses an efficient method for large scale rAAV purification, involving three column chromatography steps. Using this method, Potter produced a stock formulation of purified rAAV particles at a concentration of about 1.12 x 10^{13} vg/ml to 1.46 x. 10^{13} vg/ml, in a buffer that a POSA would have understood to have a pH of about 7.4 or 7.5 to 8.0, which contained multivalent ions, and had an ionic strength greater than 200 mM. Potter discloses electron microscopy studies showing that the rAAV stock formulation did not exhibit any aggregation.

A POSA would have sought to replace Potter's electron microscopy with more efficient and cost-effective analytical techniques to assay for the presence of aggregates. A POSA would therefore have been motivated to combine Potter with

Konz. The challenged claims are therefore obvious over the combination of Potter and Konz.

II. MANDATORY NOTICES

A. Real Parties-in-Interest (37 C.F.R. §42.8(b)(1))

Petitioner identifies Sarepta Therapeutics, Inc., Sarepta Therapeutics Three, LLC, and Catalent, Inc. as real parties-in-interest.

B. Related Matters (37 C.F.R. §42.8(b)(2))

Petitioner identifies the following related matters. The '721 patent is being asserted in currently-pending litigation: *Genzyme Corp. v. Sarepta Therapeutics, Inc.*, C.A. No. 24-cv-00882-RGA (D. Del.), D.I. 81. EX1011.

A related patent, the '542 patent, is also asserted in both this litigation and a prior litigation to which Petitioner was not a party, *Genzyme Corp. v. Novartis Gene Therapies, Inc.*, C.A. No. 21-1736 (RGA) (D. Del.) (D.I. 17). EX1012. The question of the validity of the '542 patent, however, was never presented to a jury, because the parties entered into a Joint Stipulation and Order of Dismissal With Prejudice on February 14, 2024, terminating the litigation. EX1013.

C. Related Patent Office Proceedings

Claims 1, 2, 5, and 6 of the '542 patent were the subject of two petitions for *inter partes* review, IPR2023-00608 and IPR2023-00609, brought by Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation. EX1014; EX1015.

Patent Owner statutorily disclaimed claims 1 and 2 during these prior proceedings. EX1019. The Patent Trial and Appeal Board (PTAB) denied institution of both IPRs. EX1017; EX1018.

Auricchio and Konz were not cited or discussed during the prior proceedings on the related '542 patent. Potter was discussed as a background reference by Novartis, but not addressed by the Board in either decision denying institution. EX1014, 20, 22, 63; EX1015, 22, 24; EX1017; EX1018. Notably, as discussed in detail below (Section IV.B.3), Patent Owner and its expert, Dr. Martyn Davies, materially mischaracterized Potter and the state of the art as of 2004 in responding to the 608 Petition (they did not address Potter in responding to the 609 Petition).

D. Lead and Back-up Counsel and Service Information

Petitioner provides the following counsel and service information. Pursuant to 37 C.F.R. §42.10(b), a Power of Attorney accompanies this Petition.

Lead Counsel	Back-Up Counsel
Robert Wilson (Reg. No. 45,227) Quinn Emanuel Urquhart & Sullivan, LLP 295 Fifth Avenue, 9th Floor New York, New York 10016 robertwilson@quinnemanuel.com (212) 849-7000	Anne Toker (Reg. No. 53,692) Quinn Emanuel Urquhart & Sullivan, LLP 295 Fifth Avenue, 9th Floor New York, New York 10016 annetoker@quinnemanuel.com (212) 849-7000

Lead Counsel	Back-Up Counsel
	James Glass (Reg. No. 46,729) Quinn Emanuel Urquhart & Sullivan, LLP 295 Fifth Avenue, 9th Floor New York, New York 10016 jimglass@quinnemanuel.com (212) 849-7000

III. REQUIREMENTS FOR IPR

A. Payment of Fees

The undersigned authorizes the Office to charge the fee required for this Petition for *inter partes* review to Deposit Account No. 50-5708.

B. Grounds for Standing

Petitioner certifies that the ’721 patent is available for IPR and Petitioner is not barred or estopped from requesting IPR on the grounds identified herein. Petitioner further certifies that the prohibitions of 35 U.S.C. §§315 (a)-(b) are inapplicable.

C. Statement of Relief Requested

Petitioner respectfully requests review and cancellation of claims 1-4, 6, 7, and 11 of the ’721 patent. The challenged claims should be found unpatentable on the following grounds:

Prior Art References
Auricchio (EX1007); published in 2001; prior art under pre-AIA §102(b).
Konz (EX1008), published November 27, 2003; prior art under at least pre-AIA §102(e).
Potter (EX1009); published in 2002; prior art under pre-AIA §102(b).

Ground	Claims	Description
1	1, 2, 3, 4, 6, 7, 11	Obvious in view of Auricchio and Konz
2	1, 2, 3, 4, 6, 7, 11	Obvious in view of Potter and Konz

Auricchio, Konz, and Potter were not considered by the Patent Office during prosecution. EX1001 ("References Cited"); EX1002.

IV. BACKGROUND

A. Overview of the Technology

Since the 1990s, researchers have been working to develop methods to produce high titer, pure, large scale preparations of rAAV for use in gene therapy. EX1029; EX1005, ¶¶26-34. 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. EX1009, 14-17; EX1005, ¶34.

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. EX1023, 5; EX1005, ¶¶35-90. It was known that empty AAV capsids have a tendency to aggregate during dialysis. EX1023, 6; EX1005, ¶35. 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. EX1043; EX1005, ¶35.

Researchers were actively working on developing methods to inhibit rAAV aggregation during purification of high titer rAAV preparations. EX1043. 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. EX1030; EX1031; EX1032; EX1041, 6; EX1005, ¶36. The Floyd studies showed that dilution of viral particle preparations, which reduces ionic strength, can result in aggregation. EX1030, Abstract, 2; EX1005, ¶¶37-64.

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, Abstract, 4-6, 8, 10; EX1005, ¶82. 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, 5-6, 8, 10; EX1005, ¶82. The unencapsulated DNA bound to empty capsids was shown to be as susceptible to digestion by nucleases as DNA free in solution. EX1051, 6; EX1005, ¶82. Empty polyoma particles similarly were known to bind to viral DNA *in vitro*. EX1051, 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"); EX1005, ¶82.

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. EX1042; EX1052; EX1054; EX1005, ¶¶83-84. A POSA at the relevant time would have further 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. EX1042; EX1052; EX1054; EX1005, ¶¶83-84.

B. THE '721 PATENT

The '721 patent is titled "Compositions and Methods to Prevent AAV Vector Aggregation." EX1001. The patent names John Fraser Wright and Guang Qu as inventors. *Id*. The '721 patent issued on April 27, 2010. *Id*.

The '721 patent is assigned to Genzyme Corporation. *Id*.

1. The Claims

The challenged claims of the ’721 patent are directed to a method of preventing aggregation in a purified preparation of rAAV virions. *Id.*, 14:9-29, 33-40, 49-51. The challenged claims recite certain properties of the claimed method, namely, that it comprises creating a lysate, purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, and adding citrate, phosphate, sulfate, or magnesium multivalent ions to produce a preparation with an ionic strength greater than 200 mM, with an rAAV concentration between 1 x 10^{13} vg/ml and 6.4 x 10^{13} vg/ml, and a pH between 7.5 and 8.0.

Dependent claim 6 requires the rAAV particles in the composition to have an average particle radius less than about 20 nm as measured by DLS. Dependent claim 7 recites that recovery of the purified rAAV particles is at least about 90% following filtration through a 0.22 μm filter.

2. The Specification

The specification of the ’721 patent discusses the effect of different buffers and methods of purification on aggregation of AAV2-FIX particles. *Id*., Figs. 1B, 2, 44:11-29, 6:62-9:4, 10:18-11:49; EX1005, ¶¶96-124. “AAV2-FIX” vectors are AAV2 serotype viral vectors containing a human coagulation factor IX (“FIX”) transgene. EX1001, 10:55-56; EX1005, ¶96. AAV2 is the only serotype tested in the ’721 patent. EX1005, ¶96. The specification discusses “dilution stress”

experiments in which rAAV2 aggregation was measured after dilution in various buffers containing various different ions and excipients. EX1001, Figs. 1B, 2, 4:11-29, 6:5-7:45; 10:17-11:50; EX1005, ¶¶98-105. The specification discusses various methods to detect viral particle aggregation, including ultrafiltration and diafiltration, and dynamic light scattering. EX1001, 11:55-13:3.

The specification also discusses the effect of storage at 4°C, and of freeze-thaw cycles on the stability and infectivity 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, 7:65-10:15, Table 3, 11:65-12:2, 13:9-50; EX1005, ¶¶97, 122-24.

3. The Prosecution History

(a) Prosecution of the ’721 Patent

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, 83-90. The Examiner also issued a 35 U.S.C. § 103(a) rejection over several of these prior art references. EX1002, 90-95. In response, the applicant amended the claims. EX1002, 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, 104-14.

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

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

The Examiner then issued another 35 USC § 103(a) rejection, after which the applicant amended the claims again. EX1002, 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, 297.

The Examiner then proposed an Examiner's Amendment, which was agreed to by the applicant. EX1002, 318-22. The Examiner's Amendment added limitations including 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, 320-21. After the Examiner's Amendment was entered, the case was allowed and issued.

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

(b) Prior IPR Petitions Challenging the Related '542 Patent

As discussed above (Section II.C), a different petitioner (Novartis), previously brought IPR petitions challenging claims 1, 2, 5, and 6 of a related patent, the '542 patent. EX1014; EX1015. The PTAB denied institution of both IPRs. EX1017; EX1018.

Auricchio and Konz[1] were not before the Board in these proceedings. EX1014; EX1015.

(i) Potter

The Potter reference was cited only as a background reference by Novartis, and was materially mischaracterized by Patent Owner in its POPR and Patent Owner's expert, Martyn Davies, in his declaration. EX1014, 20, 22, 63; EX1015, 22, 24; EX1016; EX1060, ¶¶121, 123-24, 151-52; EX1005, ¶¶25, 131-32, 164-65,

[1] For the avoidance of confusion, a *different* publication by Konz is cited in Patent Owner's preliminary response in IPR2023-00608. EX1016, 10.

260-68. Potter was never addressed by the Board in the decisions denying institution of the Novartis petitions. EX1017; EX1018.

Patent Owner addressed Potter in the 608 POPR (but did not discuss Potter in the 609 POPR). EX1016, 47-48, 68-69. Patent Owner and Dr. Davies materially mischaracterized Potter in several different respects.

First, Patent Owner and Dr. Davies mischaracterized the concentration of the disclosed formulations in Potter. EX1005, ¶263, 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***." EX1016, 47-48(emphasis added); EX1060, ¶121; EX1005, ¶¶164, 263.

However, as 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 claims of the '721 patent. EX1009, 9-10, 12, Table II; EX1005, ¶¶165, 264. 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, 47; EX1060, ¶121; EX1005, ¶264.

Patent Owner also incorrectly characterized the analytical method, electron microscopy, that Potter used to assess aggregation of the purified AAV preparations.

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, 68-69; EX1005, ¶265.

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." EX1005, ¶266. 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. EX1036, Abstract; EX1005, ¶¶71, 266. It is simply wrong to say, as Patent Owner did in the prior IPR proceeding, that electron microscopy "cannot accurately detect" AAV aggregates. EX1005, ¶266.

A POSA would generally have understood "visual methods" to mean methods such as visual inspection, or even light microscopy, rather than electron microscopy. EX1005, ¶267. Notably, 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, light microscopy. EX1005, ¶267. The '721 patent repeatedly discloses assessing aggregation of rAAV preparations by visual inspection using light microscopy. EX1001, 1:62-2:8, 8:50-56, 9:50-53; EX1005, ¶268. Given these disclosures in the '721 patent, it is therefore

particularly surprising that the Patent Owner disparaged "visual inspection" techniques in the 608 POPR. EX1005, ¶268.

(ii) Evans

In addition, the Evans reference, which was a principal reference in IPR2023-00608, is discussed in this Petition solely as incorporated by reference into Konz. EX1014, 11; EX1015, 13. Here, Petitioner relies on Konz's disclosure, as incorporated via Evans, of formulation buffers that meet the ionic strength, multivalent ion, non-ionic surfactant, citrate, and pH limitation recited by the challenged claims.

The express teachings of Evans that Konz points to, and that Petitioner relies on here, are materially different from the disclosures of Evans that were contested and that the Board addressed in IPR2023-00608. EX1017, 17-21 (addressing issues of whether particle radius and product recovery were inherently taught by the prior art combination that included Evans, discussing Evans's disclosure of physical titer, and also addressing whether Evans taught ionic strength as a "results-effective variable for rAAV aggregation").

Here, Petitioner relies on the express disclosures of Auricchio and Potter regarding high physical titer, along with their disclosures of high ionic strength buffers containing multivalent ions in the claimed pH range, in combination with corresponding disclosures in Konz. Petitioner further relies on Konz's express

disclosures of particle radius measurement by DLS and product recovery measurement by sterile filtration through a 0.22 μm filter.

And given that neither Auricchio nor Konz was cited in the prior IPRs, Petitioner's arguments here (and limited reliance on Evans) are therefore materially different from the issues in the prior IPRs, including those involving Novartis's reliance on Evans as a principal reference.

Moreover, Petitioner here relies on background, state of the art references (that were also not before the Patent Office during prosecution or the PTAB during the prior IPR proceedings) to demonstrate that a POSA at the relevant date would have been well aware that ionic strength was a "results-effective variable" for viral particle aggregation. EX1030, EX1031, EX1032; EX1005, ¶¶37-64.

4. Priority Date

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; EX1003; EX1004. The '721 patent issued from U.S. Patent Application No. 11/141,996, filed on June 1, 2005. EX1001.

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, because the '997 provisional does not sufficiently describe or enable the full scope of the challenged claims. EX1005, ¶¶135-58. First, the challenged claims encompass

purification methods that produce empty capsids in addition to full capsids. EX1005, ¶¶136-38. The '997 provisional, however, does not sufficiently describe or enable compositions of viral particles that include empty capsids. EX1005, ¶¶135, 138, 140-53. Second, the '997 provisional does not sufficiently describe or enable compositions of viral particles at the pH range of 7.5-8.0. EX1005, ¶¶139-40, 154-58.

The '997 provisional does not disclose any composition containing empty capsids that does not have "significant aggregation," other than purified preparations that have been treated with a nuclease. EX1005, ¶144. In fact, the only data relating to a composition containing empty capsids in the '997 provisional are the dilution stress data shown in Appendix D (the "HS" and "HS + DNAse" formulations). EX1003, 13; EX1005, ¶¶144-53. And there are no data in this figure that show inhibition of aggregation in the HS preparation, which contains purified, empty capsids without a nuclease. EX1003, 13; EX1005, ¶¶144-49. These data therefore do not provide written description support for, or enable, the full scope of challenged claims 1, 4, 6, and 7. EX1005, ¶¶136-38, 144, 153.

Second, the '997 provisional must describe and enable compositions at the claimed pH range of 7.5 to 8.0. EX1005, ¶139. 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. EX1003, 11-13, Appendices B, C,

and D; EX1005, ¶¶154-56. These data therefore do not provide any support for the pH range of 7.5-8.0 recited in the challenged claims. EX1005, ¶¶157-58.

Therefore, the challenged claims are not entitled to the June 1, 2004 priority date, and the earliest possible priority date is December 22, 2004. EX1005, ¶140.

V. LEVEL OF ORDINARY SKILL IN THE ART

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. EX1005, ¶¶159-63. 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. EX1005, ¶¶159-63.

VI. OVERVIEW OF THE PRIOR ART

A. Auricchio

Auricchio was published in 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 discloses a "simple and scalable" method for purifying rAAV. EX1007, Overview Summary; EX1005, ¶167. 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, 1; EX1005, ¶¶167-68. 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, 1, Abstract; EX1005, ¶167.

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, Overview Summary, 1, 4; EX1005, ¶¶183. For example, the Overview and 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, 1 (Overview Summary); EX1005, ¶183.

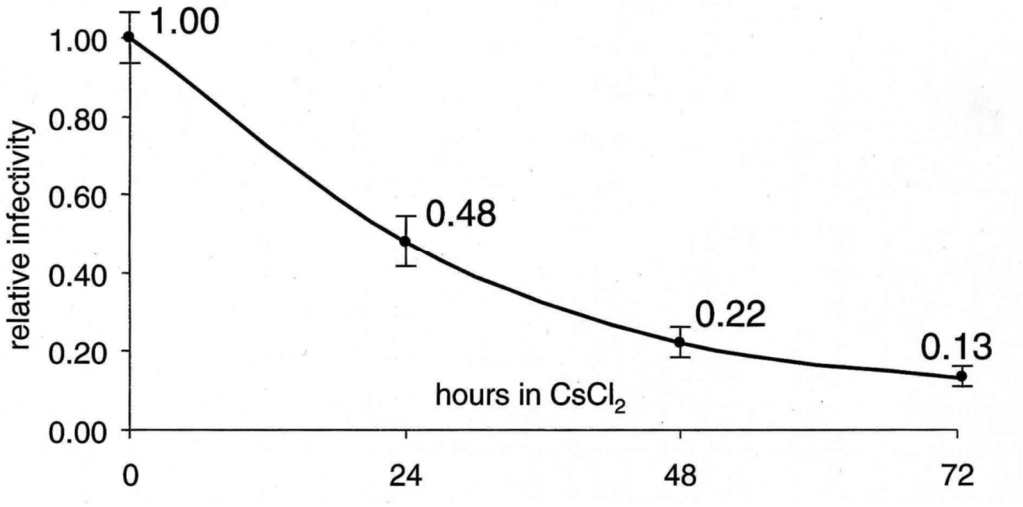
A POSA would have understood from these disclosures that Auricchio's method included the steps up through elution and concentration in high salt, and that Auricchio's method produced rAAV2 with high physical and infectious titer. EX1007, 2 (describing the last steps of the method as "On binding of the virus to the

matrix and after two washes, the vector is eluted by a change in buffer and concentrated"); EX1005, ¶184. 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. EX1005, ¶184.

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, 1, Abstract; EX1005, ¶¶168-69. The last step of Auricchio's method is concentration after elution using a high salt phosphate buffer. EX1007, 2; EX1005, ¶¶170.

Auricchio teaches that purified rAAV2 is not stable in CsCl. EX1007, 2-3, 6, Fig. 1; EX1005, ¶171. 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, 2-3, Fig. 1; EX1005, ¶171.

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, 2-3, Fig. 1, reproduced below:



EX1007, Fig. 1; EX1005, ¶172.

In contrast, Auricchio's SSCP method, which was 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, 2-3; EX1005, ¶173. 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, 2; EX1005, ¶173.

After some additional steps, 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, 2; EX1005, ¶¶174-76. The crude lysate was applied to the column. EX1007, 2; EX1005, ¶177. 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, 2; EX1005, ¶177.

The rAAV was eluted with 15 ml of PBS, pH 7.4, plus 0.4 M NaCl. EX1007, 2; EX1005, ¶178. The eluate was concentrated to about 1 ml with a Millipore Biomax-100K NMWL filter device (UFV2BHK40) by centrifugation.[2] EX1007, 2; EX1005, ¶178.

Auricchio further discloses that in two independent runs of this method, they obtained physical titers of 1.4 x 10^{13} genome copies, and 1.1 x 10^{13} genome copies. EX1007, 4, Table 2; EX1005, ¶180. 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, 2; EX1005, ¶180.

Given that the rAAV2 was eluted in 15 mls of PBS, pH 7.4, plus 0.4 M NaCl,[3] and then concentrated to 1 ml of this buffer, the concentration of these two runs was 1.4 x 10^{13} vg/ml, and 1.1 x 10^{13} vg/ml. EX1007, 2; EX1005, ¶181.

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[2] The NaCl concentration was adjusted to physiological levels by refilling the filter device with PBS pH 7.4. EX1007, 2; EX1005, ¶1005, 179.

[3] A POSA would have understood that the concentration of each component of 1X PBS is: $Na_2HPO_4 \cdot H_2O$ (8.9 mM); NaCl (138 mM); KCl (2.7 mM); KH_2PO_4 (1.5 mM). EX1065, 250-51; EX1005, ¶¶306-310. A POSA would have further

Auricchio touts the high titers obtained: "In these experiments, up to 1.4 x 10^{13} particles of AAV2 could be purified and concentrated using 8 ml of agarose-heparin suspension." EX1007, 4; EX1005, ¶¶182, 184.

Auricchio describes *in vitro* and *in vivo* experiments that showed the high infectivity of the concentrated rAAV2 preparation produced by their method. EX1007, 2, 4-5; EX1005, ¶¶185-201.

B. Konz

Konz, titled, "Methods of Adenovirus Purification," is an international publication of a PCT application, filed in English and designating the United States. EX1008. 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. Therefore, to the extent that the challenged claims are not entitled to the '997 provisional date, at least for the reasons set out above (Section IV.B.4), Konz is 102(b) prior art against the challenged claims.

Should the Patent Office determine that the challenged claims are entitled to the priority date of the '997 provisional, then Konz is 102(e) prior art against the

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understood that the ionic strength of Auricchio's elution buffer, PBS plus 0.4 M NaCl, is more than 550 mM. EX1005, ¶¶306-15

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.

Konz is directed towards efficient methods of purification of recombinant viral particles, including rAAV, in light of a "need for large scale manufacture and purification of clinical-grade virus," for applications including gene therapy. EX1008, 1:25-27, 2:23-26, 5:1-3, 5:28-30, 15:33-16:2; 14:24-29; EX1005, ¶¶202-19.

Konz discloses high concentration viral particle preparations. EX1008, 6:23-25, 7:30-32, 12:21-23, 30:11-12, 34:18-21; EX1005, ¶213. Konz discloses that their invention is an improvement over the prior art "industry norm," which involved low column loadings ("$<1 \times 10^{12}$ vp/ml resin"). EX1008, 24:12-13; EX1005, ¶213.

Konz further discloses that "an appropriate formulation buffer (e.g., see PCT publication WO 01/66137) can be used to maximize product stability." EX1008, 22:15-16, 25:20-22; EX1005, ¶¶210-11, 214, 216. In turn, WO 01/66137 (Evans), discloses high ionic strength buffers in a pH range somewhat higher than the pH of Auricchio's buffer, including a buffer at pH 8.0, containing a multivalent ion and a non-ionic surfactant, with an ionic strength above 200 mM. EX1020, 11:31-12:4; EX1005, ¶304.

Konz also discloses the addition of non-ionic surfactants to "inhibit aggregation in anion exchange and throughout the process." EX1008, 23:17-24:9; EX1005, ¶212.

Konz discloses the use of dynamic light scattering (DLS) to determine yields and mean particle sizes, to assess aggregation. EX1008, 30:13-30, 48:12-14; EX1005, ¶218. Konz found the mean particle size of an adenoviral preparation by DLS to be "123 nm, consistent with theoretical expectations," indicating that the particles were monomers and not aggregates. EX1008, 30:19-20,48:4-14; EX1005, ¶¶218-19.

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; EX1005, ¶¶218-19.

Konz also discloses sterile filtration through a 0.22 μm filter as a means of assaying the extent of particle aggregation. EX1008, 23:1-5, Table 1, 25:29-30, 30:13-30, 36:24-28; EX1005, ¶217. Konz discloses high yields from sterile filtration, including yields above 90%. EX1008, 36:24-27, 37:1-6, Table 2; 48:1-21, Table 12; EX1005, ¶217.

C. Potter

Potter, "Streamlined Large-Scale Production of Recombinant Adeno-Associated Virus (rAAV) Vectors," 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; EX1001.

Potter is directed towards large scale production of rAAV vectors to develop a National Reference Standard (NRS). EX1009, 1-2; EX1005, ¶¶221-268. Potter explains that there was a need for an NRS 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, 2; EX1005, ¶224.

Potter explains that their goal was to generate the NRS stock, aliquot it into a large number of individual user vials, validate its utility as a reference standard among a handful of rAAV laboratories, and then transfer it to an appropriate distribution service for wider distribution. EX1009, 2; EX1005, ¶224.

Potter describes the generation of the NRS with the newly developed protocol. EX1009, 2; EX1005, ¶¶225-26. Potter used the AAV2 serotype for the capsids. EX1009, 1 (citing EX1026, which, in turn, cites EX1066 (describing protocol for purification of rAAV2)); EX1005, ¶225.

Potter 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, 5-7; EX1005, ¶¶227-32. After the third and final column chromatography purification step, the sample was eluted with PBS (phosphate-buffered saline) containing 0.5 M NaCl. EX1009, 5-7; EX1005, ¶¶233-35.

Physical particle titers were determined by both a dot-blot assay (DBA) and a real-time polymerase chain reaction (PCR) assay (RTPA). EX1009, 7-17, Table II; EX1005, ¶¶236, 236-39. Notably, Potter states that the DBA and RTPA are based on "quantification of packaged genomes, rather than on the assay of assembled particles." EX1009, 17; EX1005, ¶237. Therefore, removal of empty capsids would have no effect on titers determined via these analytical methods. EX1009, 17; EX1005, ¶237.

Potter's physical titers therefore provide "vector genomes/ml" ("vg/ml") concentrations, despite the fact that they are referred to in Table II as "particles/ml." EX1010, 9, Table II; EX1005, ¶237. As Potter explains, and as a POSA would have understood, the meaning of "particles/ml" in Potter's Table II is "packaged genomes/ml," which is the same as "vg/ml." EX1067, 3, Table 2 (listing the "Unit Determination" for both the dot-blot assay and quantitative PCR as "Viral genome-containing particles/ml (vg/ml)"); EX1005, ¶237.

The purified rAAV was also analyzed using electron microscopy. EX1009, 16-17, Fig. 5A; EX1005, ¶¶251-53. Potter examined multiple grids in carrying out

the electron microscopy. EX1009, 16-17, Fig. 5; EX1005, ¶254. A POSA would have understood that because a sample was placed on multiple grids, particles from each sample were visualized across multiple grids. EX1005, ¶254. Therefore, a POSA would have understood that the electron micrographs in Figure 5 of Potter were representative of particles on multiple grids. EX1009, 16-17, Fig. 5; EX1005, ¶254. There is no evidence of aggregation in these micrographs. EX1005, ¶255.

VII. CLAIM CONSTRUCTION

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, 14:9-29, 33-40, 49-51.

Challenged claims 1-4, 6, 7, and 11 should be analyzed according to their plain and ordinary meaning. EX1005, ¶270.

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 Corp. v. Novartis Gene Therapies, Inc.*, C.A. No. 21-1736 (RGA) (D. Del.), D.I. 268. EX1061. The following terms of the '542 patent that are the same as terms in the '721 patent were construed (shaded terms were agreed upon, the others were disputed between the parties and construed by the Court):

Claim Term	Claim(s)	District Court's Construction
"filtration . . . through a 0.22	7	passing a liquid through a 0.22

Claim Term	Claim(s)	District Court's Construction
µm filter"		µm filter to remove materials
"ionic strength"	1, 2, 3, 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, 2, 3, 4, 6, 7, 11	an ionic species having a charge valency greater than one (whether positive or negative)
"recombinant adeno-associated virus (AAV) vector particles" / "AAV vector particles" / "recombinant virus particles"	1, 2, 3, 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, 2, 3, 4, 6, 7, 11	having been subjected to a purification procedure

EX1063, 12; EX1062, 18-26; EX1061, 7.

The arguments presented here do not change if the District Court's constructions above are applied to the challenged claims of the '721 patent, rather than the plain and ordinary meaning. EX1005, ¶273.

VIII. DETAILED EXPLANATION OF GROUNDS

A. Ground 1: Claims 1-4, 6, 7, and 11 Are Obvious Over Auricchio and Konz

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. Auricchio touts the simplicity and reproducibility of its method, which involves the use of a single column. EX1007, 1. Auricchio also touts the high infectious titer, in addition to high physical titer, of the viral preparations produced using its method. EX1007, 1 (Overview and Summary), 4-6; EX1005, ¶¶274-75.

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, 3, 6, Fig. 1; EX1005, ¶276.

Like Auricchio, Konz is directed to fulfilling a need for large scale manufacture and purification of clinical grade virus. EX1008, 1:25-27; EX1005, ¶278.

Like Auricchio, Konz focuses on stability, which will help large scale production and distribution of rAAV preparations. *See* EX1008, 22:15-16 (stating, "an appropriate formulation buffer (e.g., see PCT publication WO 01/66137) can be used to maximize product stability."); EX1005, ¶280.

Konz, and Evans as incorporated into Konz, teach 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. EX1041, 6 (finding that purified AAV

particles aggregated at pH 7.2 and below, but that no aggregates were observed at pH 7.5); EX1008, 30:13-20 (disclosing a formulation buffer with pH 8.0), 42:12-17 (same); EX1020, 11:31-12:4 (disclosing a high salt formulation buffer with pH 8.0), 14:15-28 (disclosing a high salt formulation buffer, pH 8.0, with a free radical inhibitor such as sodium citrate); EX1005, ¶281.

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, 24:1-9, 25:29-30, 48:11-21, Table 12; EX1005, ¶282.

Notably, 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, 2-4, Fig. 1; EX1005, ¶277.

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. EX1005, ¶283.

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. EX1005, ¶284. 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. EX1068, 2 (discussing removal of residual solvents); EX1005, ¶284.

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. EX1005, ¶285. 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. EX1005, ¶285.

1. Claim 1

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

Auricchio discloses a method of producing high physical and high infectious titer rAAV2. EX1007, 2-5; EX1005, ¶286.

Auricchio is directed to preventing aggregation of purified rAAV. 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, 3, 6, Fig. 1; EX1005, ¶288.

Auricchio also discloses that its method produced good yields, high physical titer, and high infectious titer rAAV2 *in vitro* and *in vivo*, results that would indicate to a POSA that the SSCP purified preparation did not contain large numbers of aggregates of viral particles. EX1007, 3-6; EX1005, ¶¶289-90.

Konz is expressly directed towards inhibition of viral particle aggregation during purification. EX1005, ¶291. For example, Konz teaches the addition of non-ionic surfactants to inhibit viral particle aggregation throughout the purification. EX1008, 23:17-24:12; EX1005, ¶291.

Konz also discloses choosing formulation buffers to maximize product stability. EX1008, 22:15-16; EX1005, ¶292. Konz, refers to a PCT publication (Evans), which it incorporates by reference. EX1008, 22:15-16 ("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.").

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); EX1005, ¶293.

Auricchio, in combination with Konz, thus meets this limitation of claim 1. EX1005, ¶294.

(b) "providing a lysate comprising rAAV virions;"

Auricchio discloses creating a lysate as one of the earliest steps in the SSCP purification. EX1007, 2; EX1005, ¶295.

Konz similarly discloses creating a lysate as one of the earliest steps in the purification. EX1008, 27:23-25, 27:25-28:4; EX1005, ¶296. Given the express teachings in Konz, including by reference to Evans, 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. EX1008, 14:24-29; EX1020, 3:12-14; EX1005, ¶296.

Auricchio and Konz thus both meet this limitation of claim 1. EX1005, ¶297.

(c) "purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, wherein said virions are purified; and"

Auricchio's SSCP method for purifying rAAV virions includes directly applying the lysate to a preequilibrated heparin column. EX1007, 2; EX1005, ¶298. A POSA would have understood that a heparin column is a form of chromatography. EX1042, Abstract; EX1005, ¶298.

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. EX1007, 4, Fig. 2; EX1005, ¶299.

Konz expressly discloses the use of column chromatography to purify rAAV. EX1008, 14:20-34; EX1005, ¶300.

Auricchio and Konz thus both meet this limitation of claim 1. EX1005, ¶301

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

Auricchio discloses eluting the purified rAAV from the heparin column with a phosphate buffer (PBS). EX1007, 2; EX1005, ¶302.

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. EX1008, 22:15-17, 24:27-25:22; EX1005, ¶303.

Evans, in turn, discloses formulation buffers that include multivalent ions, in particular, magnesium ions. EX1020, 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); *see also* 9:6-9, 36:25-27; EX1005, ¶304. The presence of $MgCl_2$ in this buffer meets this limitation of the claims. EX1005, ¶304.

Auricchio and Konz thus both meet this limitation of claim 1. EX1005, ¶305.

(e) "to produce a preparation of virions with an ionic strength of at least 200 mM"

The elution buffer disclosed in Auricchio contains PBS plus 0.4 M NaCl. EX1007, 2; EX1005, ¶306. A POSA would have understood that this buffer has an ionic strength of more than 550 mM, which meets this limitation of claim 1. EX1005, ¶¶306-15; EX1065, 250-51.

Konz incorporates Evans by reference, and Evans, in turn discloses formulation buffers that meet this limitation of the claims. EX1020, 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 (with a free radical inhibitor such as sodium citrate); *see also* 36:21-22 (claim 4) ("the salt is sodium chloride from about 25 mM to about 250 mM"), 41:14-15 (claim 37) (same); EX1005, ¶316.

A POSA would have understood that 250 mM NaCl would have an ionic strength of at least 250 mM, which is greater than the 200 mM limitation of the claims. EX1005, ¶317. Therefore, this buffer meets this limitation of the claims. EX1005, ¶317.

Auricchio and Konz thus both meet this limitation of claim 1. EX1005, ¶318.

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

Auricchio discloses purified rAAV particles at concentrations exceeding 1 x 10^{13} vg/ml and less than 6.4 x 10^{13} vg/ml. EX1007, 2, 4, Table 2. EX1005, ¶319.

Specifically, Auricchio discloses numbers of genome copies obtained from various runs of the SSCP method. EX1007, 4, Table 2. EX1005, ¶320. The genome copy numbers were obtained using real time PCR. EX1007, 2; EX1005, ¶321.

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. EX1007, 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"); EX1005, ¶320.

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. EX1007, 2; EX1005, ¶322. 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.[4] EX1005, ¶322.

Auricchio therefore meets this limitation of claim 1. EX1005, ¶323.

(g) "and wherein the pH of the purified preparation of rAAV virions is between 7.5 and 8.0."

Auricchio discloses eluting purified rAAV from the heparin column with a buffer at pH 7.4. EX1007, 2; EX1005, ¶325.

As discussed above, a POSA would have been motivated to combine Auricchio with Konz to improve the stability of the purified preparation and reduce

[4] The notation "vg/ml" in the '721 claims would be understood by a POSA to mean "vector genomes / ml." EX1001, 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 . . ."); EX1005, ¶322. 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. EX1005, ¶322. Empty capsids will not contain a "vector genome" and will not be counted in a concentration measured as vg/ml. EX1005, ¶322.

aggregation even further. EX1005, ¶326. Konz teaches that higher pH buffers improve the stability of the viral particles. EX1008, 26:10-172; EX1005, ¶329. 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, 6 (finding that purified AAV particles aggregated at pH 7.2 and below, but that no aggregates were observed at pH 7.5); EX1005, ¶329..

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. EX1005, ¶330. 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. EX1005, ¶330.

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 (via 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, 11:31-12:4, 14:15-28 (with a free radical inhibitor such as sodium citrate); EX1005, ¶¶327-28, 331.

Therefore, the combination of Auricchio and Konz discloses this limitation of claim 1. EX1005, ¶¶324, 332.

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

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, 22:4-6; EX1005, ¶333. In particular, Konz discloses that nuclease treatment can be applied during steps "downstream" of anion exchange chromatography. EX1008, 6:5-7; EX1005, ¶333. Konz further discloses that nuclease treatment leads to lower contaminating DNA levels in the final purified product. EX1008, 21:14-16; EX1005, ¶333.

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. EX1005, ¶¶334-35.

Konz therefore meets the additional limitation of dependent claim 2. EX1005, ¶336.

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

A POSA would have understood that the commonly used nuclease Benzonase was an endonuclease from *Serratia marcescens*. EX1053, 2; EX1005, ¶337. Konz expressly discloses the use of Benzonase. EX1008, 22:2-3; EX1005, ¶337. 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, 11; EX1005, ¶337.

Konz therefore meets the additional limitation of dependent claim 3. EX1005, ¶338.

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

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, 3:31-35, 13:8-34, 38:19-21; EX1005, ¶339. 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, 11:31-12:4, 14:15-28 (disclosing the addition of citrate); EX1005, ¶339.

Konz, by reference to Evans, therefore meets the additional limitation of dependent claim 4. EX1005, ¶340.

5. 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."

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. EX1005, ¶342.

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. EX1005, ¶343.

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. EX1008, 48:4-15. EX1005, ¶344. 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. EX1005, ¶344. 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, 14:24-29; EX1005, ¶344.

Auricchio, in combination with Konz, therefore meets the additional limitation of dependent claim 6. EX1005, ¶¶341-45

6. 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."

The combination of Auricchio and Konz discloses the additional limitation of dependent claim 7. EX1005, ¶¶346-50.

Again, as 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"); EX1005, ¶347.

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. EX1008, 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); EX1005, ¶348.

Example 2 of Konz discloses a protocol involving diafiltering the purified formulation, exchanging the virus into the formulation buffer using five diafiltration volumes. EX1008, 36:21-28; EX1005, ¶349. After diafiltration, the product was sterile filtered with a 0.22 μm filter. EX1008, 36:21-28; EX1005, ¶349. Notably, as shown in Table 2, the yield from the sterile filtration step was 94%. EX1008, 37:1-6, Table 2; EX1005, ¶349.

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. EX1005, ¶350. Specifically, Example 5 states that sterile filtration was carried out with a Millipore Millipak-20 filter (100 cm^2)." EX1008, 42:18-19; EX1005, ¶350. A Millipore Millipak-20 filter is a 0.22 μm filter. EX1008, 25:29-30; EX1069, 3; EX1005, ¶350. The yield from the sterile filtration step in Example 5 was 98%. EX1008, 43:1-5, Table 6; EX1005, ¶350.

In Example 9, Konz states that the "process specifics used are similar to those described in Example 5 . . . ." EX1008, 48:4-5; EX1005, ¶351. The yield from the sterile filtration step in Example 9 was 98%. EX1008, 48:16-21, Table 12; EX1005, ¶351.

Therefore, Auricchio, in combination with Konz, meets the additional limitation of dependent claim 7. EX1005, ¶352.

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

Konz discloses diafiltering purified rAAV virions into the final formulation buffer. EX1008, 22:13-16; EX1005, ¶353. 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. EX1005, ¶353.

Konz therefore meets the additional limitation of dependent claim 11. EX1005, ¶354.

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

A POSA would have had a reasonable expectation of success in combining Auricchio with Konz to arrive at the claimed purification method. EX1005, ¶355. 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. EX1005, ¶355.

Auricchio's purification method produced stable, high physical and infectious titer rAAV in a high ionic strength buffer containing multivalent ions. EX1005,

¶356. 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. EX1005, ¶356.

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. EX1005, ¶357. 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. EX1005, ¶357.

Moreover, a POSA would have had a reasonable chance of success that this method of purifying rAAV would prevent aggregation. EX1005, ¶358. 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, a POSA would have sought to maintain these characteristics of the preparation in combining them with Konz. EX1005, ¶358.

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 ($MgCl_2$), at a pH somewhat higher than Auricchio's (pH 8.0, for example), containing a non-ionic surfactant, in accordance with Konz's teachings. EX1005, ¶359. 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 after sterile filtration with a 0.22 μm filter, 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. EX1005, ¶359.

9. Secondary Considerations Do Not Change the Conclusion of Obviousness

Petitioner is not aware of any secondary considerations of non-obviousness with the required nexus to the claims of the '721 patent. EX1005, ¶360. For example, Petitioner is not aware of any commercial success attributable to a method

meeting the limitations of the challenged claims.[5] EX1005, ¶360. Similarly, Petitioner is not aware of any licenses directed specifically to the '721 patent or the subject matter recited in challenged claims 1, 6, or 7.[6] EX1005, ¶360.

Finally, Petitioner is not aware of any unexpected results having a nexus to the claimed subject matter. EX1005, ¶361. The '721 patent does not disclose unexpected properties of the claimed method. EX1005, ¶361. 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

[5] 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 method recited in the challenged claims. EX1005, ¶360.

[6] If Patent Owner attempts to rely on any license to Novartis in the earlier case brought by Genzyme, 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 method recited in the challenged claims. EX1005, ¶360.

I, II, and III. EX1005, ¶361. And purification methods to produce high titer rAAV formulations that inhibited aggregation had been developed before the '721 patent and disclosed in prior art references such as Auricchio. EX1005, ¶361. 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. EX1005, ¶361.

To the extent Patent Owner attempts to raise secondary considerations that have only a marginal nexus, if any, to claims 1-4, 6, 7, and 11 of the '721 patent, such evidence of secondary considerations should not outweigh the compelling evidence of obviousness, discussed above. EX1005, ¶362. Thus, secondary considerations do not alter the conclusion that claims 1-4, 6, 7, and 11 of the '721 patent are obvious over the combination of Auricchio and Konz. EX1005, ¶362.

B. Ground 2: Claims 1-4, 6, 7, and 11 Are Obvious Over Potter and Konz

Claims 1-4, 6, 7, and 11 of the '721 patent are obvious over Potter and Konz. EX1005, ¶¶363-435.

A POSA would have been motivated to combine Potter with Konz because both Potter and Konz are directed towards efficient, cost-effective methods to produce pure, stable, high titer formulations of rAAV. EX1009, 2; EX1008, 1:25-27; EX1005, ¶¶364-65. Nonetheless, Potter includes the analytical technique of

electron microscopy, which is labor and time intensive and difficult to adapt to scale. EX1009, 16-17, Fig. 5; EX1005, ¶365.

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* EX1008, 24:1-9, 48:11-21, Table 12; EX1005, ¶366.

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. EX1005, ¶367. 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. EX1005, ¶367.

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). EX1005, ¶368.

Moreover, a POSA would have understood that the methods of Potter produced a high physical 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 pH 8.0. EX1005, ¶369. 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. EX1005, ¶¶368-69.

1. Claim 1

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

Potter discloses a method for purifying rAAV vector particles to create a "reference standard stock of rAAV with a precisely defined titer." EX1009, 2; EX1005, ¶370. 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, 2; EX1005, ¶370. 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. EX1005, ¶370. 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. EX1005, ¶370.

Potter, moreover, discloses electron microscopic analysis of the purified rAAV preparation. EX1009, 16-17, Fig. 5; EX1005, ¶371. The analysis shows no evidence of aggregation in the purified rAAV preparation. EX1005, ¶371. Potter is therefore directed towards a method of purifying rAAV that results in a stable preparation without aggregation. EX1005, ¶371.

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. EX1008, 23:17-19. EX1005, ¶372.

Konz also discloses formulation buffers that will maximize product stability. EX1008, 22:15-16; EX1005, ¶374. Konz refers to a PCT publication (Evans), which it incorporates by reference. EX1008, 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."); EX1005, ¶¶374-75.

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); EX1005, ¶373.

Potter, in combination with Konz, therefore meets this limitation of claim 1. EX1005, ¶376.

(b) "providing a lysate comprising rAAV virions;"

Potter discloses creating a lysate as one of the earliest steps in the rAAV purification. EX1009, 4-5; EX1005, ¶377.

Konz similarly discloses creating a lysate as one of the earliest steps in the purification. EX1008, 27:23-25, 27:25-28:4; EX1005, ¶378. 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. EX1008, 14:24-29; EX1005, ¶378.

Potter and Konz thus both meet this limitation of claim 1. EX1005, ¶379.

(c) "purifying rAAV virions from the lysate using ultracentrifugation and/or chromatography, wherein said virions are purified; and"

Potter discloses the use of column chromatography to purify rAAV virions from the lysate. EX1009, 5-7; EX1005, ¶380. 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. EX1009, 5-7; EX1005, ¶380.

Figure 1 of Potter shows the increasing purity of the rAAV preparation after each successive column chromatography step. EX1009, 6, Fig. 1; EX1005, ¶381.

Konz expressly discloses the use of column chromatography to purify rAAV. EX1008, 14:20-34; EX1005, ¶382.

Potter and Konz thus both meet this limitation of claim 1. EX1005, ¶383.

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

Potter discloses a phosphate buffer (PBS) used to elute purified rAAV from the heparin affinity chromatography column (the third column chromatography purification step). EX1009, 7 (stating that the "virus is eluted with PBS containing 0.5 M NaCl"); EX1005, ¶384. A POSA would have understood "PBS" to mean "phosphate buffered saline," and would further have understood PBS to involve

addition of phosphate salts. EX1065, 250-51; EX1005, ¶384. Potter therefore meets this limitation of claim 1. EX1005, ¶384.

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. EX1008, 22:15-17, 24:27-25:22; EX1005, ¶385.

Evans, in turn discloses formulation buffers that include multivalent ions, in particular, magnesium ions. EX1020, 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 (with a free radical inhibitor such as sodium citrate); *see also* 9:6-9, 36:25-27; EX1005, ¶386. The presence of $MgCl_2$ in this buffer meets this limitation of the claims. EX1005, ¶386.

Potter and Konz thus both meet this limitation of claim 1. EX1005, ¶387.

(e) "to produce a preparation of virions with an ionic strength of at least 200 mM"

The buffer disclosed in Potter contains 0.5 M NaCl. EX1009, 7; EX1005, ¶388. A POSA would have understood that the ionic strength of this buffer is greater than 200 mM. EX1005, ¶¶388-89.

The buffer disclosed in Potter therefore meets this limitation of the challenged claims. EX1005, ¶389.

Konz incorporates Evans by reference, and Evans, in turn discloses formulation buffers that meet this limitation of the claims. EX1020, 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 (with a free radical inhibitor such as sodium citrate); *see also* 36:21-22 (claim 4) ("the salt is sodium chloride from about 25 mM to about 250 mM"), 41:14-15 (claim 37) (same); EX1005, ¶390.

A POSA would have understood that a buffer containing 250 mM NaCl would have an ionic strength greater than 200 mM. EX1005, ¶391.

Potter and Konz thus both meet this limitation of claim 1. EX1005, ¶392.

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

Potter discloses purified, rAAV particles at concentrations exceeding 1 x 10^{13} vg/ml and less than 6.4 x 10^{13} vg/ml. EX1005, ¶393. Table II discloses titers of the rAAV reference standard obtained through Potter's purification process. EX1009, 9, Table II; EX1005, ¶393. 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, 9, Table II; EX1005, ¶393.

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. EX1005, ¶¶394-95. 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." EX1005, ¶395. 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." EX1005, ¶395.

As discussed above (Section VI.C), a POSA would have understood that Potter used two assays, the DBA and the RTPA, that both provide a "vg/ml" concentration. EX1005, ¶396. Both of these assays determine the number of AAV genomes present per ml of the preparation. EX1005, ¶396.

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." EX1009, 7-9, Table II; EX1005, ¶397. Potter found that the DBA and RTPA titers were very similar to one another, 1.12×10^{13} and 1.46×10^{13}, which provides confidence in both measurements. EX1009, 7-9, Table II; EX1005, ¶397. Table II is 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, 9, Table II; EX1005, ¶397.

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." EX1005, ¶398.

Potter thus meets this limitation of claim 1. EX1005, ¶399.

(g) "and wherein the pH of the purified preparation of rAAV virions is between 7.5 and 8.0."

The buffer used in Potter is PBS with 0.5 M NaCl. EX1009, 7 (stating that the "virus is eluted with PBS containing 0.5 M 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. EX1073; EX1065, 257, 294; EX1023, 2-3; EX1070, 9; EX1005, ¶400. Therefore, a POSA would have understood that Potter meets this limitation of the '721 patent claims. EX1005, ¶400.

As discussed above, Konz teaches that higher pH buffers improve the stability of the viral particles. EX1008, 26:10-17; EX1005, ¶401.

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. EX1005, ¶405. Specifically, a POSA would have selected a buffer with high NaCl concentration, divalent ions, in a similar pH range. EX1005, ¶405.

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 (via 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, 11:31-12:4; EX1005, ¶¶402-04.

Potter, in combination with Konz, therefore meets this limitation of claim 1. EX1005, ¶406.

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

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, 22:4-6; EX1005, ¶407. In particular, Konz discloses that nuclease treatment can be applied during steps "downstream" of anion exchange chromatography. EX1008, 5:13-20, 6:5-7; EX1005, ¶407. Konz further discloses that nuclease treatment leads to lower contaminating DNA levels in the final purified product. EX1008, 21:14-16; EX1005, ¶407.

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. EX1005, ¶¶408-09.

Konz therefore meets the additional limitation of dependent claim 2. EX1005, ¶410.

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

A POSA would have understood that the commonly used nuclease Benzonase was an endonuclease from *Serratia marcescens*. EX1053, 2; EX1005, ¶411. Konz expressly discloses the use of Benzonase. EX1008, 22:2-3; EX1005, ¶411. 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, 11; EX1005, ¶411.

Konz therefore meets the additional limitation of dependent claim 3. EX1005, ¶412.

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

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, 3:31-35, 13:8-34, 38:19-21; EX1005, ¶413. 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, 11:31-12:4, 14:15-28 (disclosing the addition of citrate); EX1005, ¶413.

Konz, by reference to Evans, therefore meets the additional limitation of dependent claim 4. EX1005, ¶414.

5. 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."

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. EX1008, 48:4-15; EX1005, ¶417. Konz discloses that the mean particle size by DLS analysis was as expected for individual particles that were not aggregated. EX1008, 48:4-15; EX1005, ¶417. Given that Konz states expressly that its teachings are applicable to rAAV, a POSA would have understood that these DLS results showing no aggregation of formulations prepared per Konz's methods would be applicable to rAAV. EX1008, 14:24-29; EX1005, ¶417.

Potter, in combination with Konz, therefore meets the additional limitation of dependent claim 6. EX1005, ¶¶415-16, 418.

6. 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."

Konz discloses using 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. EX1008, 30:13-30, 48:16-21, Table 12 (98% yield), 50:1-5, Table 14 (100% yield), 51:5-10, Table 16 (99% yield); EX1005, ¶¶421-25.

Therefore, Potter, in combination with Konz, meets the additional limitation of dependent claim 7. EX1005, ¶¶419-20, 426

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

Konz discloses diafiltering purified rAAV virions into the final formulation buffer. EX1008, 22:13-16; EX1005, ¶427. 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. EX1005, ¶427.

Konz therefore meets the additional limitation of dependent claim 11. EX1005, ¶428.

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

A POSA would have had a reasonable expectation of success in combining Potter with Konz to arrive at the claimed purification method. EX1005, ¶¶429-32. The techniques required to make the claimed combination, namely, diafiltration, sterile filtration, and the use of DLS, were well known to people of skill in the art at the time and would have required nothing more than routine experimentation. EX1005, ¶429.

Potter's methods produced high physical titer rAAV that did not aggregate in a high ionic strength buffer containing a multivalent ion. EX1005, ¶430. Konz teaches the addition of a non-ionic surfactant to high ionic strength buffers containing multivalent ions at a pH around 8.0 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. EX1005, ¶430.

Therefore, a POSA would have had a reasonable expectation of success in achieving the claimed combination. EX1005, ¶¶431-32.

9. Secondary Considerations Do Not Change the Conclusion of Obviousness

For the reasons set out regarding Ground 1, secondary considerations do not alter the conclusion that claims 1-4, 6, 7, and 11 of the '721 patent are obvious over the combination of Potter and Konz. EX1005, ¶¶433-35.

IX. CONCLUSION

Sarepta respectfully requests institution of IPR for claims 1-4, 6, 7, and 11 of the '721 patent based on the grounds specified in this Petition.

Respectfully submitted,

June 26, 2025

By: /Robert B. Wilson/

Robert B. Wilson (Reg. No. 45,227)
Anne S. Toker (Reg. No. 53,692)
James M. Glass (Reg. No. 46,729)
Quinn Emanuel Urquhart & Sullivan, LLP
295 Fifth Avenue, 9th Floor
New York, New York 10016
Tel.: 212-849-7000
Fax: 212-849-7100

Attorneys for Petitioner Sarepta Therapeutics, Inc.

WORD COUNT CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. §42.24(d), Petitioner hereby certifies, in accordance with and reliance on the word count provided by the word-processing system used to prepare this Petition, that the number of words in this paper is 13,127. Pursuant to 37 C.F.R. §42.24(d), this word count excludes the table of contents, table of authorities, mandatory notices under §42.8, certificate of service, certificate of word count, appendix of exhibits, and any claim listing.

Respectfully submitted,

June 26, 2025

By: /Robert B. Wilson/

Robert B. Wilson (Reg. No. 45,227)
Anne S. Toker (Reg. No. 53,692)
James M. Glass (Reg. No. 46,729)
Quinn Emanuel Urquhart & Sullivan, LLP
295 Fifth Avenue, 9th Floor
New York, New York 10016
Tel.: 212-849-7000
Fax: 212-849-7100

Attorneys for Petitioner Sarepta Therapeutics, Inc.

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §42.6 (e) and 37 C.F.R. §42.105, I hereby certify that on June 26, 2025, I caused the foregoing Petition for *Inter Partes* Review, Power of Attorney, and Exhibits 1001–[ ] to be served on Patent Owner by depositing them for shipment with Federal Express to the correspondence address of record listed on the Patent Center:

20855 - FOLEY & LARDNER LLP
3000 K STREET N.W.
SUITE 600
WASHINGTON, DC
UNITED STATES

Respectfully submitted,

June 26, 2025

By: /Robert B. Wilson/

Robert B. Wilson (Reg. No. 45,227)
Anne S. Toker (Reg. No. 53,692)
James M. Glass (Reg. No. 46,729)
Quinn Emanuel Urquhart & Sullivan, LLP
295 Fifth Avenue, 9th Floor
New York, New York 10016
Tel.: 212-849-7000
Fax: 212-849-7100

Attorneys for Petitioner Sarepta Therapeutics, Inc.