

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.,

Petitioner

v.

**THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA
AND REGENXBIO INC.,**

Patent Owners

U.S. Patent No. 11,680,274

“Method of Increasing the Function of an AAV Vector”

IPR2024-00580

**DECLARATION OF DAVID V. SCHAFFER, PH.D.,
IN SUPPORT OF PETITION FOR *INTER PARTES* REVIEW OF
U.S. PATENT NO. 11,680,274**

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I, David V. Schaffer, Ph.D., hereby declare as follows.

1. I have been retained as an expert witness on behalf of Sarepta Therapeutics, Inc. (“Sarepta”) in connection with the above-captioned request for *inter partes* review (“IPR”). I am being compensated for my time in connection with this IPR at my standard consulting rate, which is \$1000 per hour.

2. I understand that Sarepta is petitioning for *inter partes* review of U.S. Patent No. 11,680,274 (“the ’274 patent”) (EX1001) and requests that the United States Patent and Trademark Office cancel claims 1, 3-6, and 8 of the ’274 patent as unpatentable. The following discussion and analysis provides my opinions as to why claims 1, 3-6, and 8 would have been obvious to a person of ordinary skill in the art (“POSA”).

I. BACKGROUND AND QUALIFICATIONS

3. I am the Hubbard Howe Jr. Distinguished Professor of Biochemical Engineering and Professor of Chemical and Biomolecular Engineering, Bioengineering, Molecular and Cell Biology at the University of California, Berkeley, where I also serve as the Director of the California Institute for Quantitative Biosciences (QB3), Director of the Bakar Labs, and Director of the Bakar BioEnginuity Hub.

4. My research group is also part of the Helen Wills Neuroscience Institute at UC Berkeley.

5. I graduated from Stanford University with a B.S. degree in Chemical Engineering in 1993.

6. Afterward, I attended the Massachusetts Institute of Technology and earned my Ph.D., also in Chemical Engineering, in 1998 with advisor Professor Doug Lauffenburger, while minoring in Molecular and Cell Biology.

7. I conducted a postdoctoral fellowship in the laboratory of Fred Gage at the Salk Institute for Biological Studies in La Jolla, CA, before moving to UC Berkeley in 1999.

8. At Berkeley, I apply engineering principles to enhance stem cell and gene therapy approaches for neuroregeneration. This work includes mechanistic investigation of stem cell control, as well as molecular evolution and engineering of viral gene delivery vehicles.

9. I have received an NSF Career Award, Office of Naval Research Young Investigator Award, Whitaker Foundation Young Investigator Award, and was named a Technology Review Top 100 Innovator.

10. I was also awarded the American Chemical Society BIOT Division Young Investigator Award in 2006, the Biomedical Engineering Society Rita Shaffer Young Investigator Award in 2000, the American Chemical Society Marvin Johnson Award in 2016, the American Institute of Chemical Engineers Andreas Acrivos Professional Progress Award in 2020, and was elected to the College of

Fellows of the American Institute of Medical and Biological Engineering in 2010 and the National Academy of Inventors in 2021.

11. I have authored over 240 publications in peer-reviewed journals, including Nature, the Proceedings of the National Academy of Sciences USA, Nature Biotechnology, Nature Neuroscience, Nature Communications, Nature Nanotechnology, Nature Chemistry, Journal of Virology, and Neuron.

12. I have been working and publishing in the gene therapy field since 1993. Over 67 of my publications are related to gene therapy using viral vectors. Of these, over 47 relate to adeno-associated virus (AAV) vectors.

13. I have authored eight book chapters, two of which are directed to gene therapy.

14. I have advised 58 Ph.D. students and 36 postdoctoral fellows, including 26 alumni who have become professors (at Caltech, Purdue, UCSF, UCSB, UC Davis, University of Illinois Urbana-Champaign, University of Pennsylvania, University of Wisconsin, Yale, and others), 35 in industry (*e.g.*, Abbott, Bayer, BioMarin, Genentech, Sanofi, as well as two CEOs and three CSOs of companies), and others in patent law and management consulting. I have published >240 peer-reviewed scientific manuscripts; have >30 issued patents with >60 more in prosecution (and 26 licensed to companies); have served on 7 journal editorial boards, 21 company scientific advisory boards, and 5 company boards of directors

(including two public companies, NASDAQ symbols FDMT and QURE); and have co-founded 8 companies (1 IPO, 1 acquired by Pfizer, 1 by Intellia, and 5 successfully growing). I have delivered >250 invited lectures, and technologies that I co-invented have entered into 10 human clinical trials.

15. EX1006 is a copy of my *curriculum vitae* setting forth my educational experience, employment history, publications, and awards.

16. I have not previously testified as an expert witness by trial or deposition.

II. MATERIALS CONSIDERED

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

- U.S. Patent No. 11,680,274 (“the ’274 patent”) (EX1001);
- U.S. Publication No. 2003/0138772 (“the ’772 Publication”) (EX1007);
- Xie *et al.*, “The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy,” *Proc. Nat’l Acad. Sci.* (2002) at 10405-10410 (“Xie”) (EX1008);
- Snowdy, “Nuclear targeting by adeno-associated virus capsid proteins and virions,” a dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the curriculum of Neurobiology (“Snowdy”) (2003) (EX1009); and
- Fabb *et al.*, “Adeno-associated virus vector gene transfer and sarcolemmal expression of a 144 kDa micro-dystrophin effectively the

dystrophin-associated protein complex and inhibits myofibre degeneration in nude/*mdx* mice,” *Human Molecular Genetics*, 2002, Vol. 11, No. 7, pp. 733-741 (“Fabb”) (EX1010).

Exhibit Number	Description
EX1001	U.S. Patent No. 11,680,274 (“the ’274 patent”)
EX1002	Prosecution history of U.S. Patent No. 11,680,274 (“the ’274 Prosecution History”)
EX1003	U.S. Provisional Patent Application No. 60/669,083, filed on April 7, 2005 (“the ’083 Provisional”)
EX1004	U.S. Provisional Patent Application No. 60/733,497, filed on November 4, 2005 (“the ’497 Provisional”)
EX1005	Declaration of Dr. David V. Schaffer under 37 C.F.R. § 1.68 (“Schaffer Decl.”)
EX1006	<i>Curriculum vitae</i> of Dr. David V. Schaffer (“Schaffer CV”)
EX1007	U.S. Patent Application Publication 2003/0138772 (“the ’772 Publication”)
EX1008	Xie <i>et al.</i> , “The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy,” <i>Proc. Nat’l Acad. Sci.</i> , 2002, pp. 10405-10410 (“Xie”)
EX1009	Snowdy, “Nuclear Targeting by adeno-associated virus capsid proteins and virions,” A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the curriculum of Neurobiology (2003) (“Snowdy”)
EX1010	Fabb <i>et al.</i> , “Adeno-associated virus vector gene transfer and sarcolemmal expression of a 144 kDa micro-dystrophin effectively the dystrophin-associated protein complex and inhibits myofibre degeneration in nude/ <i>mdx</i> mice,” <i>Human Molecular Genetics</i> , 2002, Vol. 11, No. 7, pp. 733-741 (“Fabb”)
EX1011	Opening Expert Report of Paola Leone (Public Excerpts) (“Leone Opening Report”)

Exhibit Number	Description
EX1012	Rebuttal Expert Report of Paola Leone (Public Excerpts) (“Leone Rebuttal Report”)
EX1013	Rebuttal Expert Report of Michael Metzker (Excerpts) (“Metzker Rebuttal Report”)
EX1014	U.S. Patent No. 10,526,617 (“the ’617 patent”)
EX1015	Declaration of Thomas Hyatt under 37 C.F.R. § 1.68 (“Hyatt Decl.”)
EX1016	Declaration of Christopher DuBose under 37 C.F.R. § 1.68 (“DuBose Decl.”)
EX1017	Jans and Jans, “Negative charge at the casein kinase II site flanking the nuclear localization signal of the SV40 large T-antigen is mechanistically important for enhanced nuclear import,” <i>Oncogene</i> (1994), Vol. 9, No. 10, pp. 2961-2968 (“Jans & Jans”)
EX1018	Sequence Alignment of AAVrh.46 (SEQ ID NO: 4) from the ’274 Patent and AAVrh.10 (SEQ ID NO: 81) from the ’772 Publication (“AAVrh.10/AAVrh.46 Alignment”)
EX1019	Sequence Alignment of AAVrh.10 (SEQ ID NO: 81) and AAV8 (SEQ ID NO: 95) Capsid Protein Sequences from the ’772 Publication (“AAVrh.10/AAV8 Alignment”)
EX1020	Sequence Alignment of AAV2, AAVrh.10, and AAV8 Capsid Protein Sequences (“AAV2/AAVrh.10/AAV8 Alignment”)
EX1021	NetPhos/AAVrh.10 Phosphorylation Sites (“NetPhos-AAVrh.10”)
EX1022	Sequence Alignment of AAVrh.10 (SEQ ID NO: 81) and AAV8 (SEQ ID NO: 95) Capsid Protein Sequences from the ’772 Publication Showing Differences In Phosphorylation Sites (“AAVrh.10/AAV8 Phosphorylation Sites”)
EX1023	Table Showing Xie Surface Amino Acids (“Xie Surface Table”)

Exhibit Number	Description
EX1026	12/20/23 Regenzbio's Initial Claim Chart Relating Accused Product to the Asserted Claims of U.S. Patent 11,680,274, Regenzbio Inc., <i>et al.</i> v. Sarepta Therapeutics, Inc. <i>et al.</i> , C.A. No. 23-667-RGA (D. Del.) ("Initial Claim Chart")
EX1029	Kwon and Schaffer, "Designer Gene Delivery Vectors: Molecular Engineering and Evolution of Adeno-Associated Viral Vectors for Enhanced Gene Transfer," <i>Pharm. Research.</i> , 2008, Vol. 25, No. 3, pp. 489-99 ("Kwon")
EX1030	Kotterman and Schaffer, "Engineering adeno-associated viruses for clinical gene therapy," <i>Nature Reviews, Genetics</i> , 2014, Vol. 15, pp. 445-451 ("Kotterman")
EX1031	Grimm and Kay, "From Virus Evolution to Vector Revolution: Use of Naturally Occurring Serotypes of Adeno-associated Virus (AAV) as Novel Vectors for Human Gene Therapy," <i>Curr. Gene Therapy</i> , 2003, Vol. 3, pp. 281-304 ("Grimm")
EX1032	Wu <i>et al.</i> , "Mutational Analysis of the Adeno-Associated Virus Type 2 (AAV2) Capsid Gene and Construction of AAV2 Vectors with Altered Tropism," <i>J. Virol.</i> , 2000, Vol. 74, No. 18, pp. 8635-8647 ("Wu")
EX1033	Blom <i>et al.</i> , "Sequence and Structure-based Prediction of Eukaryotic Protein Phosphorylation Sites," <i>J. Mol. Biol.</i> , 1999, Vol. 294, pp. 1351-1362 ("Blom")
EX1034	Ivanov <i>et al.</i> , "Phosphorylation of the Potyvirus Capsid Protein by Protein Kinase CK2 and Its Relevance for Virus Infection," <i>The Plant Cell</i> , 2003, Vol. 15, pp. 2124-2139 ("Ivanov")
EX1035	Chen <i>et al.</i> , "Signal-induced site specific phosphorylation targets IκBα to the ubiquitin-proteasome pathway," <i>Genes & Dev.</i> , 1995, Vol. 9, pp. 1586-1597 ("Chen")
EX1036	Lange <i>et al.</i> , "Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome," <i>Proc. Nat'l Acad. Sci.</i> , 2000, Vol. 97, pp. 1032-1037 ("Lange")

Exhibit Number	Description
EX1037	Patrick <i>et al.</i> , “p35, the Neuronal-specific Activator of Cyclin-dependent Kinase 5 (Cdk5) Is Degraded by the Ubiquitin-Proteasome Pathway,” <i>J. Biol. Chem.</i> , 1998, Vol. 273, pp. 24057-24064 (“Patrick”)
EX1038	Penrose and McBride, “Proteasome-Mediated Degradation of the Papillomavirus E2-TA Protein Is Regulated by Phosphorylation and Can Modulate Viral Genome Copy Number,” <i>J. Virol.</i> , 2000, Vol. 74, No. 13, pp. 6031-6038 (“Penrose”)
EX1039	Whiteside <i>et al.</i> , “N- and C-Terminal Sequences Control Degradation of MAD3/I κ B α in Response to Inducers of NF- κ B Activity,” <i>Mol. and Cell. Biol.</i> , 1995, Vol. 15, pp. 5339-5345 (“Whiteside”)
EX1040	Alberts, MOLECULAR BIOLOGY OF THE CELL, 3rd Ed., Chapters 2 (excerpt), 3, and 6 (“Alberts I”)
EX1041	https://www.genome.gov/about-genomics/fact-sheets/Deoxyribonucleic-Acid-Fact-Sheet (“DNA Fact Sheet”)
EX1042	https://www.genome.gov/about-genomics/educational-resources/fact-sheets/human-genomic-variation (“Genomic Variation”)
EX1043	https://www.genome.gov/genetics-glossary/Central-Dogma (“Central Dogma”)
EX1044	https://www.genome.gov/genetics-glossary/Genetic-Code (“Genetic Code”)
EX1045	https://www.genome.gov/about-genomics/fact-sheets/Polymerase-Chain-Reaction-Fact-Sheet (“PCR Fact Sheet”)
EX1046	https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/what-gene-therapy (“Gene Therapy”)
EX1047	https://patienteducation.asgct.org/gene-therapy-101/vectors-101 (“Vectors 101”)

Exhibit Number	Description
EX1048	Mandal <i>et al.</i> , “Optical Backbone-Sidechain Charge Transfer Transitions in Proteins Sensitive to Secondary Structure and Modifications,” <i>Faraday Discussions</i> , 2018, Vol. 207, pp. 115-135 (“Mandal”)
EX1049	Ponnazhagan <i>et al.</i> , “Adeno-associated Virus for Cancer Gene Therapy,” <i>Cancer Research.</i> , 2001, Vol. 61, pp. 6313-6321 (“Ponnazhagan”)
EX1050	Chiorini <i>et al.</i> , “Cloning and Characterization of Adeno-Associated Virus Type 5,” <i>J. Virol.</i> , 1999, Vol. 73, No. 2, pp. 1309-1319 (“Chiorini”)
EX1051	Mori <i>et al.</i> , “Two novel adeno-associated viruses form cynomolgus monkey: pseudotyping characterization of capsid protein,” <i>Virology</i> , 2004, Vol. 330, pp. 375-383 (“Mori”)
EX1052	Hauck and Xiao, “Characterization of Tissue Tropism Determinants of Adeno-Associated Virus Type 1,” <i>J. Virol.</i> , 2003, Vol. 77 pp. 2768-2774 (“Hauck”)
EX1053	Matsushita <i>et al.</i> , Adeno-associated virus vectors can be efficiently produced without helper virus,” <i>Gene Therapy</i> , 1998, Vol. 5, pp. 938-945 (“Matsushita”)
EX1054	Kim <i>et al.</i> , “Substrate Specificities and Identification of Putative Substrates of ATM Kinase Family Members,” <i>J. Biol. Chem.</i> , 1999, Vol. 274, pp. 37538–37543 (“Kim”)
EX1056	Alberts, MOLECULAR BIOLOGY OF THE CELL, 3rd Ed., Chapters 7 and 8 (“Alberts II”)

III. LEGAL STANDARDS – OBVIOUSNESS

18. In this section, I describe my understanding of certain legal standards relating to the issue of obviousness that I have been asked to consider for claims 1, 3-6, and 8 of the '274 patent. These legal standards have been explained to me in

connection with the preparation of this Declaration. I have applied these standards in my analysis, as described in the sections below.

19. I understand that a claim is obvious when the differences between the claim and the prior art are such that the claim as a whole would have been obvious to a POSA at the relevant time. It is my understanding that four factors are applied in determining whether a claim is unpatentable as obvious under 35 U.S.C. § 103: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at issue; (3) the level of ordinary skill in the art; and (4) objective evidence indicating obviousness or non-obviousness – also referred to as “secondary considerations” – if present.

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

21. I understand that a claim can be obvious over a single reference in combination with the knowledge of a POSA, or based on the teachings in a combination of references. For obviousness based on a combination of prior art references, I understand that a POSA must have a motivation to combine the

references. I understand that the prior art references themselves may provide a suggestion, motivation, or reason to combine. I further understand that a motivation to combine two or more prior art references need not be express, but may be based upon common sense or the knowledge available to a POSA.

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

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

IV. SUMMARY OF INVALIDITY GROUNDS

24. The table below summarizes the invalidity grounds for claims 1, 3-6, and 8 of the '274 patent that I address in this declaration.

Ground	Claims	Description
1	1, 3-6	Obvious in view of the '772 Publication
2	1, 3-6	Obvious in view of the '772 Publication and Xie

Ground	Claims	Description
3	1, 3-6	Obvious in view of the '772 Publication and Snowdy
4	8	Obvious in view of the '772 Publication and Fabb
5	8	Obvious in view of the '772 Publication, Xie, and Fabb
6	8	Obvious in view of the '772 Publication, Snowdy, and Fabb

25. I note that Xie, Snowdy, and Fabb were not considered by the Patent Office during prosecution. EX1001 (“References Cited”); EX1002. Although the ’772 Publication is listed in the “References Cited” section of the ’274 patent, the Patent Office did not discuss the ’772 Publication during prosecution of the challenged claims. EX1002. The ’772 Publication, Xie, Snowdy, and Fabb were all published and/or accessible to the public before April 7, 2004, one year before the earliest priority date listed on the face of the ’274 patent. *See* Section VIII.A-D.

V. TECHNICAL BACKGROUND AND STATE OF THE PRIOR ART

A. DNA, RNA, Proteins, and the Genetic Code

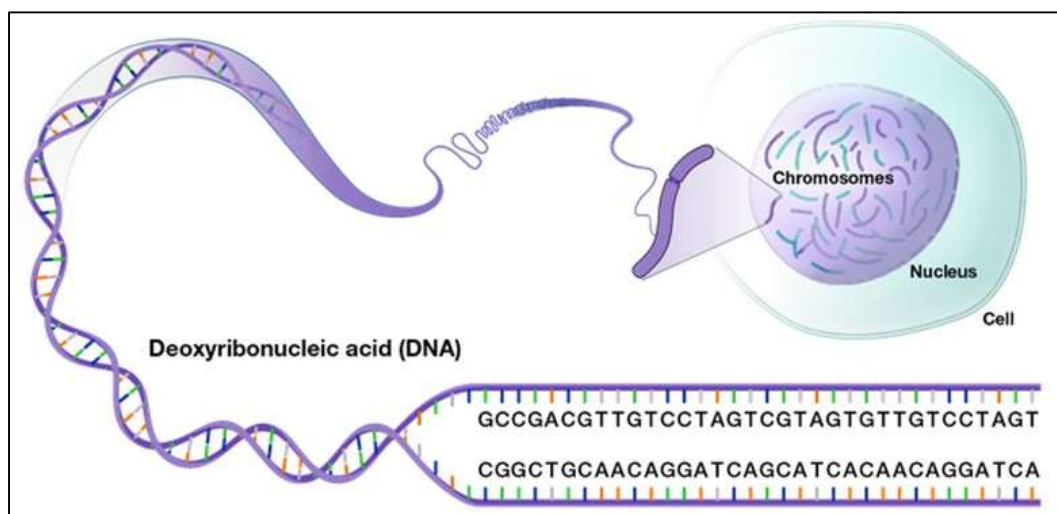
1. Genes and DNA

26. Deoxyribonucleic acid (DNA) is a molecule that contains the biological instructions for each organism. *See* Alberts, MOLECULAR BIOLOGY OF THE CELL, 3rd Ed., pp. 15-19 (EX1040); <https://www.genome.gov/about-genomics/fact-sheets/Deoxyribonucleic-Acid-Fact-Sheet> (EX1041), p. 1. For certain organisms, including human beings, DNA is located in a structure in cells known as the

“nucleus.” EX1040 (Alberts I), p. 22, Fig. 3-15; EX1041 (DNA Fact Sheet), p. 1.

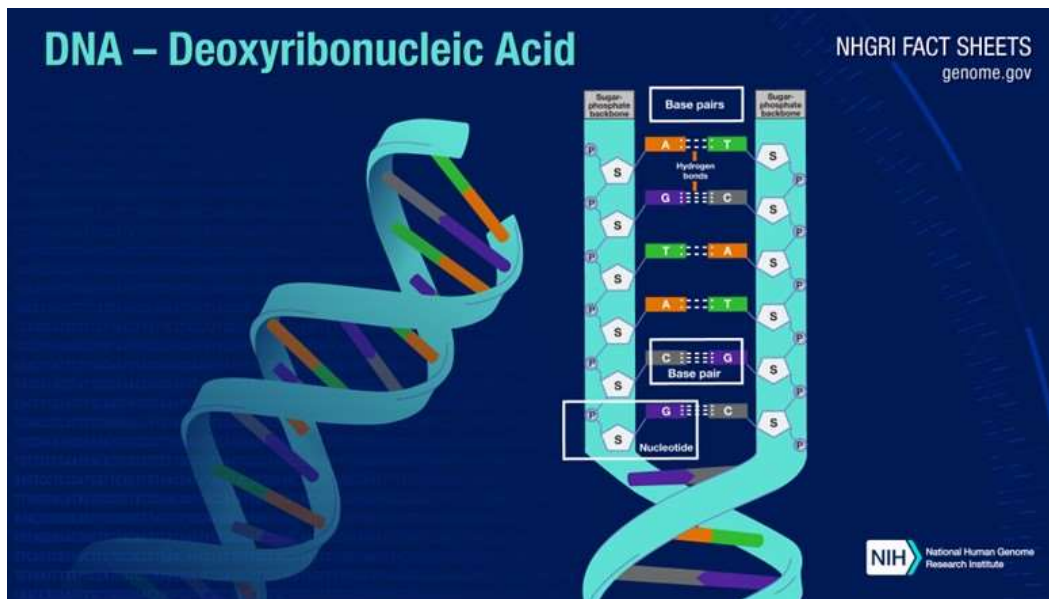
The instructions in DNA molecules are passed from adult organisms to their offspring during reproduction. EX1040 (Alberts I), pp. 16-19; EX1041 (DNA Fact Sheet), p. 1.

27. In human beings, DNA is packaged into “chromosomes” in the nucleus as shown in the figure below:



<https://www.genome.gov/about-genomics/educational-resources/fact-sheets/human-genomic-variation>. EX1042 (Genomic Variation Fact Sheet), p. 2.

28. The structure of a DNA molecule – which resembles a twisted ladder – is known as a “double helix.” EX1040 (Alberts I), pp. 15-19. This structure allows information to be copied and passed along from generation to generation:



EX1041 (DNA Fact Sheet), p. 1; EX1040 (Alberts I), pp. 15-19.

29. Sometimes mistakes are made during the copying process. Those mistakes can lead to mutations in the DNA sequence. EX1040 (Alberts I), pp. 19-20.

30. In the DNA double helix, the sides of the ladder run in opposite directions, shown in the diagram above by the small white pentagons, which represent the sugar portions of the DNA bases. On one side, the pentagons point “up,” while on the other side, they point “down.”

31. The ladder contains rungs, where each rung is made up of a pair of nucleotide bases. There are four different nucleotide bases: Adenine (A), Thymine (T), Guanine (G), or Cytosine (C). Base A always pairs with base T, and base C always pairs with base G. Bases A and T are said to be “complementary,” and bases C and T are similarly said to be “complementary.” EX1040 (Alberts I), p. 18. As a

result, if the sequence of the bases on one strand of a DNA double helix is known, the sequence of the bases on the other strand can easily be determined, because each base on the second strand will be complementary to the corresponding base on the first strand. *See* EX1040 (Alberts I), pp. 15-19.

32. Sequences of bases in DNA contain coded instructions to make a protein – large biomolecules that carry out most biological functions of the cell. *Id.*, p. 21; EX1041 (DNA Fact Sheet), p. 2. The sequences that encode proteins are known as “genes,” which can vary greatly in size. In the human genome, genes can range from about 1,000 bases, to 1 million bases. EX1041 (DNA Fact Sheet), p. 2.

33. The protein coding sequences make up only about one percent of the DNA in cells. *Id.*, p. 2. Other sequences of DNA are involved in regulating the way in which genes are “expressed,” meaning (as I discuss further below) they are used by the cell to create RNA molecules (in a process known as “transcription”), which can then be used by the cell to make proteins (in a process known as “translation”). *Id.*; EX1040 (Alberts I), p. 22, Fig. 3-15; p. 58, Fig. 6-1. Often, particular genes are expressed only in particular cell types, and only at particular times during the development of an organism. EX1040 (Alberts I), pp. 62, 79.

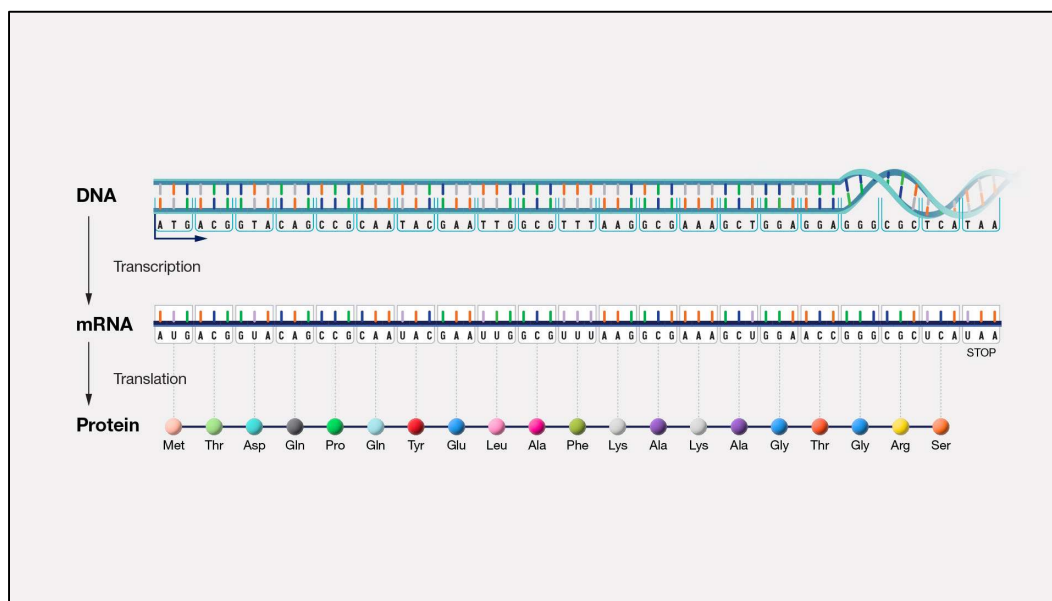
2. RNA, Proteins, and the Genetic Code

34. Cells “transcribe” RNA from DNA in the nucleus. *Id.*, p. 22, Fig. 3-15, pp. 58-60; EX1041 (DNA Fact Sheet), p. 2. A type of RNA known as “messenger

RNA” travels from the nucleus to the cytoplasm, where the cell “translates” the RNA message into protein molecules. EX1040 (Alberts I), pp. 21-22, Fig. 3-15. In this way, the DNA instructions in the nucleus direct the manufacture of proteins in the cytoplasm. *Id.*, p. 22. The protein-making factories in the cytoplasm are known as “ribosomes.” *Id.*, pp. 24-25.

35. There are about 20 different naturally occurring amino acids, which can be joined together in many different orders to form a wide variety of proteins. *Id.*, pp. 4-5, 23; EX1041 (DNA Fact Sheet), p. 2.

36. The diagram below shows this sequence of events – in which DNA is “transcribed” into mRNA, which, in turn, is “translated” into protein:

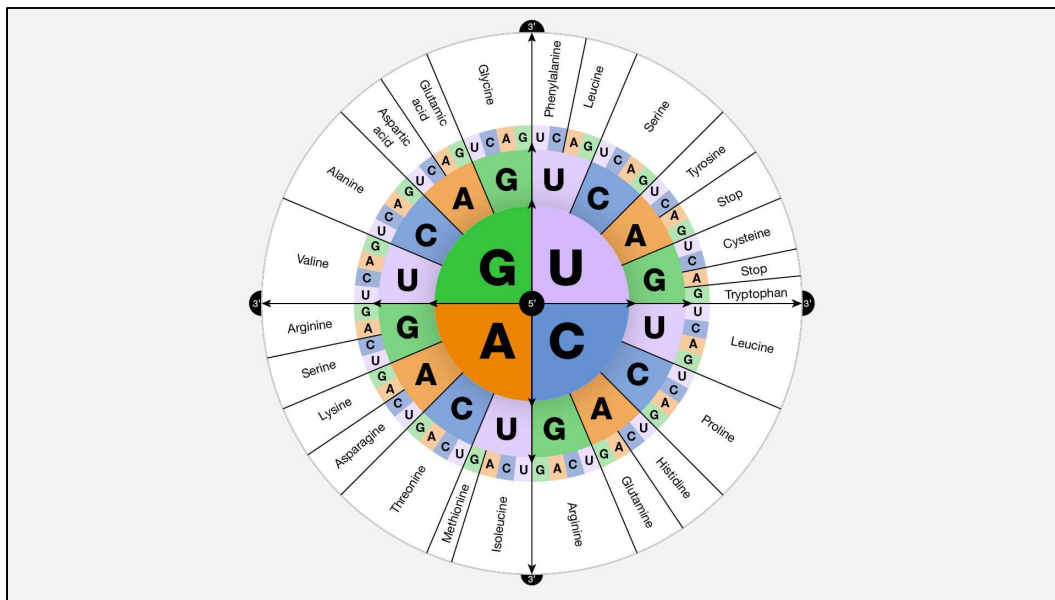


<https://www.genome.gov/genetics-glossary/Central-Dogma> (EX1043).

37. The graphic above depicts a theory in biology known as the “central dogma,” which states that genetic information flows only in one direction, from

DNA, to RNA, to protein, and cannot flow in reverse, out of protein and back to nucleic acid. EX1043 (Central Dogma).

38. The term “genetic code” refers to the instructions contained in a gene that tell a cell how to make a specific protein. <https://www.genome.gov/genetics-glossary/Genetic-Code> (EX1044). The genetic code is constructed from the four nucleotide bases of DNA, adenine (A), cytosine (C), guanine (G) and thymine (T). These four nucleotide bases are used to spell out three-letter “codons” that specify which amino acid is needed at each position within a protein, as shown in the figure below:



EX1044 (Genetic Code); *see also* EX1040 (Alberts I), pp. 4-5, 23, 65-67.

39. Note that in the diagram, the base “U” or “uracil” replaces the base “T”. In mRNA, there are “U” bases, which are chemically modified versions of the “T”

bases that are a component of DNA, but not RNA. EX1040 (Alberts I), pp. 21, 65-67.

40. As the diagram above also shows, the genetic code has certain redundancies – for example, multiple different codons code for the same amino acid. Some amino acids such as arginine are coded for by six codons (CGU, CGC, CGA, CGG, AGG, AGA), while others, such as glutamic acid are represented by only two codons (GAA, GAG). This redundancy is referred to as the “degeneracy” of the genetic code. *Id.*, pp. 23, 65-67.

3. Complementary DNA (“cDNA”)

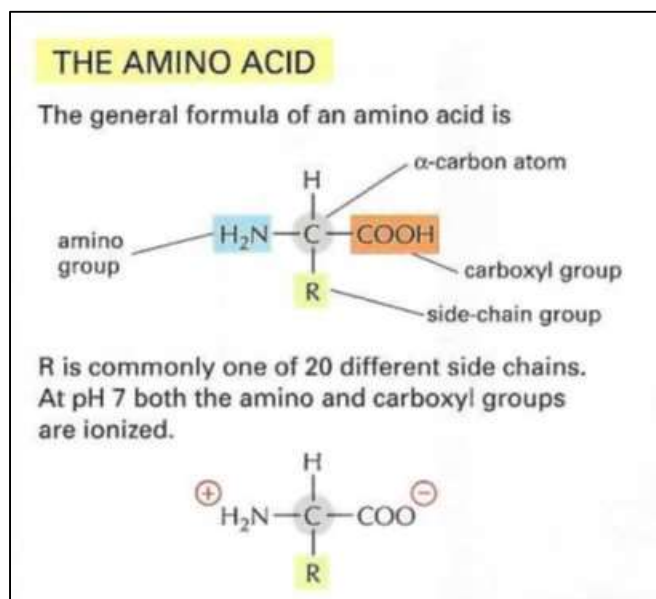
41. Genes in eukaryotes are made up of coding regions (“exons”) and non-coding regions (“introns”). EX1040 (Alberts I), pp. 22-23. A messenger RNA is the product of a cellular process known as “splicing,” in which a primary RNA transcript made directly from the genomic DNA is processed to remove the introns and join the exons together. *Id.*, pp. 22-23. The product of this splicing process is the mRNA that is translated on the ribosome into protein. *Id.*, pp. 22-23; Alberts II (EX1056), pp. 87-89, Fig. 8-56.

42. It is possible to use an enzyme, “reverse transcriptase,” to reverse transcribe an mRNA molecule back into DNA. This process does not occur naturally in eukaryotic cells, but instead, the enzyme used to reverse transcribe mRNA is from a type of virus. EX1040 (Alberts I), pp. 119-20; EX1056 (Alberts II), pp. 21-24.

The DNA made through this artificial process is known as “complementary DNA” or “cDNA.” EX1056 (Alberts II), pp. 21-24. cDNA contains only the coding portions of a gene – and is therefore shorter than the full length gene containing both coding and non-coding regions. *Id.*

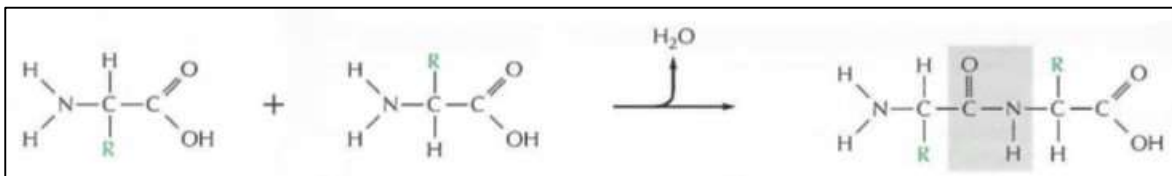
B. Protein Structure and Function

43. Proteins are made up of amino acids, linked together in a chain along a peptide backbone. Amino acids have the following general structure – a central carbon atom surrounded by four different groups – a hydrogen atom, a carboxyl group (COOH), an amino group (NH₂), and a side chain (R):



EX1040 (Alberts I), pp. 4-5. The 20 different side chains (R) distinguish the amino acids from each other. *Id.*

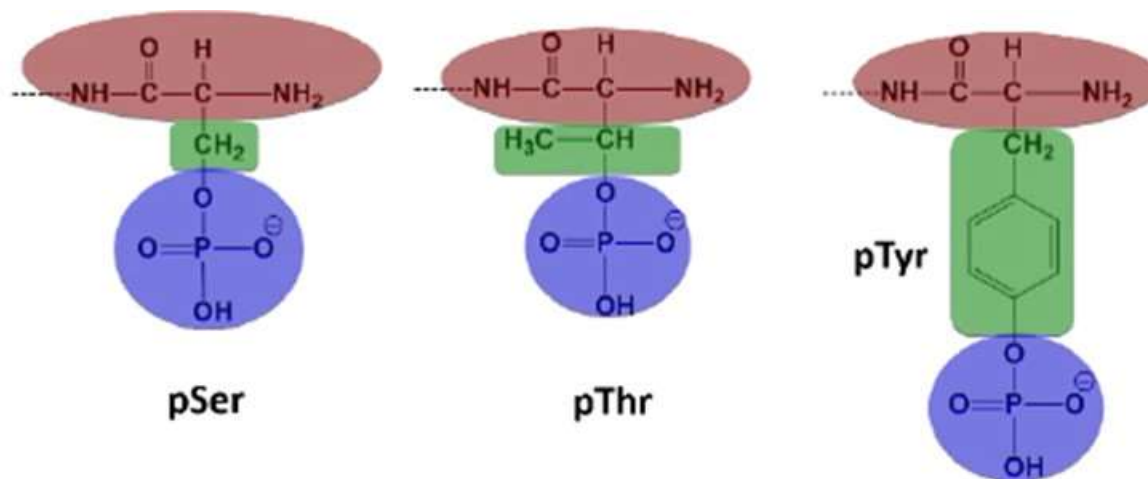
44. When the carboxyl group of one amino acid reacts with the amino group of another amino acid, a link in the chain (called a “peptide bond”) is formed:



Id., p. 4.

1. Phosphorylation

45. Certain amino acids have, as part of their R groups, an OH group that can be phosphorylated. The amino acids that are most commonly phosphorylated are serine, threonine, and tyrosine. *See, e.g., Blom et al., “Sequence and Structure-based Prediction of Eukaryotic Protein Phosphorylation Sites,” J. Mol. Biol.*, 1999, Vol. 294, pp. 1351-1362 (EX1033), Abstract. The figure below shows phosphorylated serine, threonine, and tyrosine amino acids:



EX1048 (Mandal) 4, Fig. 1.

46. Phosphorylation plays an important role in the regulation and function of proteins, including, for example, the proteins that make up viral capsids. EX1033 (Blom), p. 1; Ivanov *et al.*, “Phosphorylation of the Potyvirus Capsid Protein by Protein Kinase CK2 and Its Relevance for Virus Infection,” *The Plant Cell*, 2003, Vol. 15, pp. 2124–2139 (EX1034), Abstract; Penrose and McBride, “Proteasome-Mediated Degradation of the Papillomavirus E2-TA Protein Is Regulated by Phosphorylation and Can Modulate Viral Genome Copy Number,” *J. Virol.*, 2000, Vol. 74, No. 13, pp. 6031-6038 (EX1038), Abstract.

47. Phosphorylation of a variety of different proteins, often at serine residues, has been known for many years to regulate degradation of these proteins in cells by a pathway known as the ubiquitin pathway. *See, e.g.*, Chen *et al.*, “Signal-induced site specific phosphorylation targets I κ B α to the ubiquitin-proteasome pathway,” *Genes & Dev.*, 1995, Vol. 9, pp. 1586-1597 (EX1035), Abstract; Lange *et al.*, “Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome,” *Proc. Nat’l Acad. Sci.*, 2000, Vol. 97, pp. 1032-1037 (EX1036), Abstract; Patrick *et al.*, “p35, the Neuronal-specific Activator of Cyclin-dependent Kinase 5 (Cdk5) Is Degraded by the Ubiquitin-Proteasome Pathway,” *J. Biol. Chem.*, 1998, Vol. 273, pp. 24057-24064 (EX1037), Abstract; Penrose and McBride (EX1038), Abstract; Whiteside *et al.*, “N- and C-Terminal Sequences Control Degradation of

MAD3/I κ B α in Response to Inducers of NF- κ B Activity,” *Mol. and Cell. Biol.*, 1995, Vol. 15, pp. 5339-5345 (EX1039), Abstract.

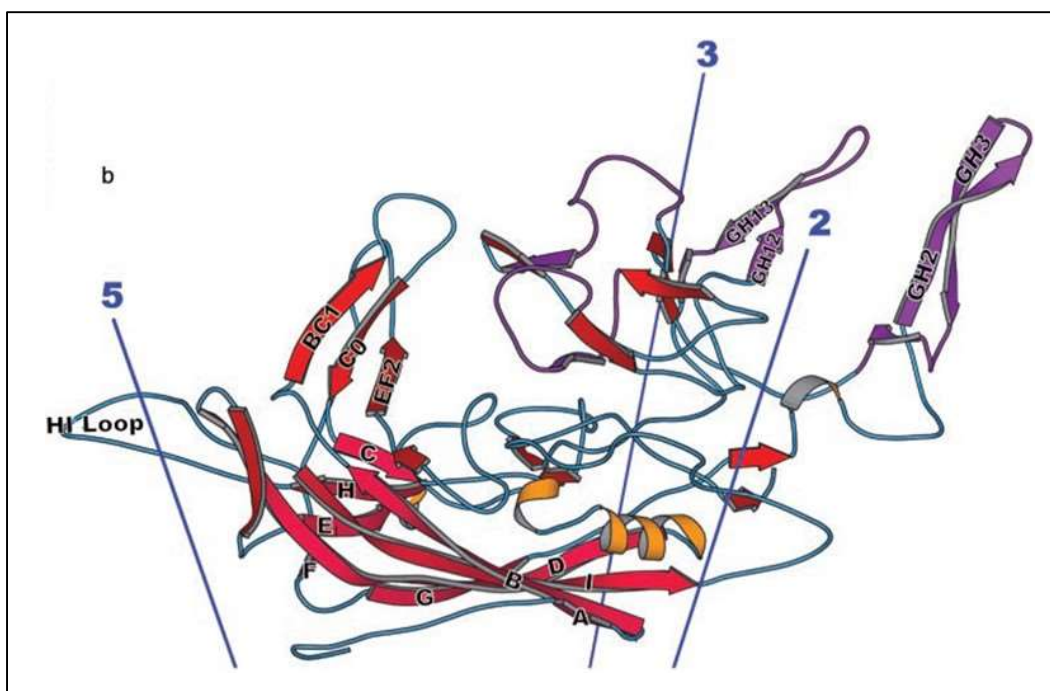
48. Researchers observed that mutation of these phosphorylation sites to non-phosphorylatable amino acids could reduce the rate of degradation or prevent degradation of these proteins altogether. *See, e.g.*, EX1036 (Lange), Abstract; EX1038 (Penrose and McBride), Abstract.

2. Three Dimensional Protein Structure

49. Proteins are not merely strings of amino acids; they also have three dimensional structures that are necessary for them to carry out the work of the cell. EX1040 (Alberts I), pp. 30-32. Although the sequence of amino acids in a protein determines the protein’s structure, the structure cannot be accurately predicted from knowing the sequence alone. *Id.*, p. 30.

50. The folded conformation (*i.e.*, the three-dimensional structure) of proteins can be determined through a technique known as X-ray crystallography, which is carried out on crystals of the protein. *Id.*, p. 30; EX1056 (Alberts II), p. 32.

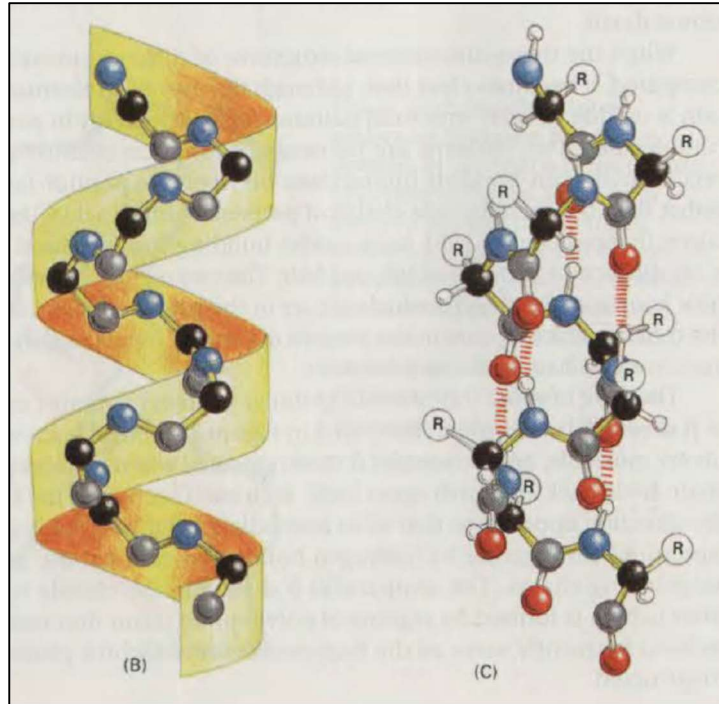
51. There are structural patterns that recur in three dimensional protein structures – two of the most common are known as an “ α [alpha] helix” and a “ β [beta] sheet.” EX1040 (Alberts I), pp. 30-32. These structures are shown in the diagram of the AAV capsid protein below (which I discuss later on):



EX1008 (Xie), p. 3, Fig. 1.

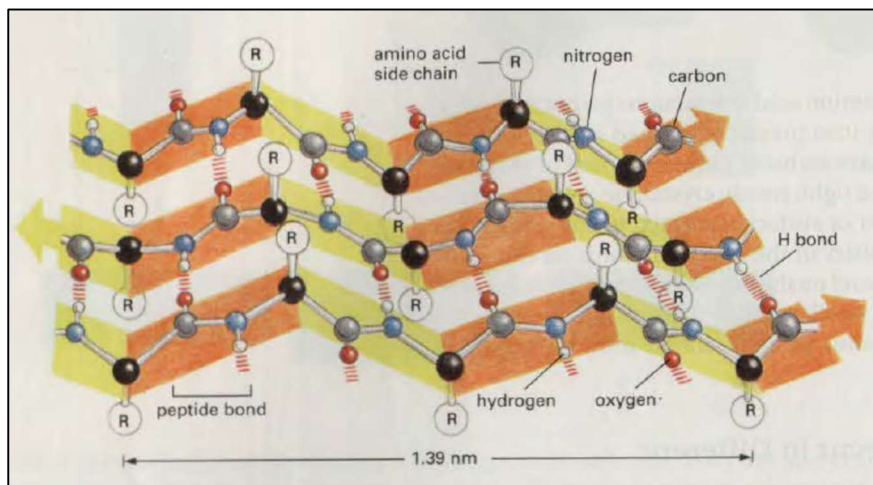
52. In the diagram above, the red and purple flat arrows are beta “strands” that make up beta “sheets” – collections of beta strands that run parallel (arrows all pointing in the same direction) or anti-parallel (arrows pointing in opposite directions, in an alternating fashion). EX1008 (Xie), p. 3, Fig. 1, Fig. 1 legend; EX1040 (Alberts I), pp. 30-32. The orange coil is an alpha helix. EX1008 (Xie), p. 3, Fig. 1; EX1040 (Alberts I), pp. 30-32. The grey strings connecting these structures are loops that do not fold into either beta sheets or alpha helices, and tend to have less well defined structures. EX1040 (Alberts I), pp. 35-36.

53. These diagrams are ways of representing the arrangement of amino acids in the protein, in three dimensions. Each alpha helix is made up of amino acids in a spiral structure, as shown in the diagram below:



Id., p. 31.

54. Each beta sheet is similarly made up of amino acids, as shown in the diagram below:



Id.

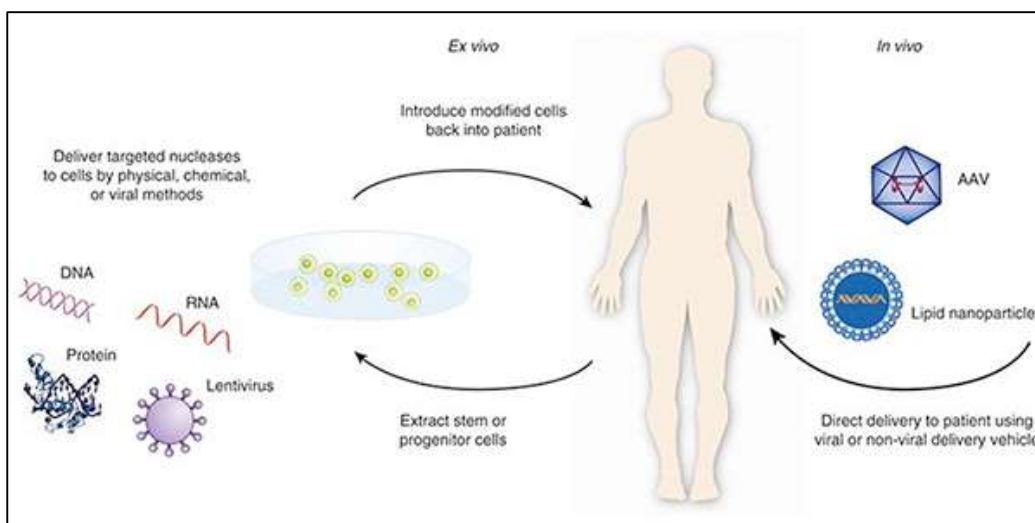
C. Gene Therapy

55. The term “gene therapy” refers to a technique that modifies a person’s genes to treat or cure disease. <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/what-gene-therapy> (EX1046).

56. “Gene therapy” approaches include adding a gene to supply a good copy of a protein that is present in the patient only in a functionally deficient form, or to provide a protein with therapeutic effects. *Id.* Gene therapy also includes trying to fix a mutation in a gene or to reduce or remove the activity of a gene that is mutated and has gained an undesirable activity. *Id.*

57. Tools known as “vectors” are means of adding exogenous DNA into a cell. *Id.*; <https://patienteducation.asgct.org/gene-therapy-101/vectors-101> (EX1047).

58. Many different types of viruses have been studied as potential vectors for gene therapy, including adeno-associated viruses (AAV), adenoviruses, retroviruses, and lentiviruses. EX1047 (Vectors 101). In the figure below, AAV is shown on the right hand side as one type of gene therapy vector:



EX1046 (Gene Therapy).

59. Viruses are generally made up of two components: (1) a protein coat or “capsid” that forms the outer shell of the virus, and (2) a viral genome, contained within the capsid, made up of either DNA or RNA, that encodes the instructions necessary for the virus to make copies of itself. EX1040 (Alberts I), pp. 111-114. When a virus infects a cell, it releases its DNA or RNA and most often takes over the cell’s copying machinery, which is used to make multiple copies of the viral DNA or RNA. *Id.*, pp. 115-17. The instructions in the viral DNA or RNA also direct the infected cell to produce capsid proteins and other proteins required to package the viral DNA or RNA into capsids to form new viral particles. *Id.* The new copies of the virus are then released from the cell and are available to infect other cells. EX1040 (Alberts I), p. 111.

60. A viral vector can be thought of as a vehicle that is used to deliver a specific DNA sequence to a person’s cells. EX1047 (Vectors 101), p. 1. The viral

capsid is used to package the therapeutic gene of interest for delivery to the patient's cells. *Id.* Most of the virus's genome inside the capsid is replaced with the therapeutic gene. *See* EX1031 (Grimm), pp. 6-7, Fig. 2, Fig. 2 legend. Short viral regulatory sequences from the viral genome are retained, because they are required to package the therapeutic gene inside the capsid. Ponnazhagan *et al.*, "Adeno-associated Virus for Cancer Gene Therapy," *Cancer Research.*, 2001, Vol. 61, pp. 6313-6321 (EX1049), pp.1-3, Fig. 1, Fig. 1 legend, Fig. 2, Fig. 2 legend; EX1031 (Grimm), pp. 6-7, Fig. 2 Fig. 2, Fig. 2 legend.

D. Adeno-Associated Virus (AAV)

61. The ability to deliver genes with high efficiency and specificity is important for gene therapy. Kwon and Schaffer, "Designer Gene Delivery Vectors: Molecular Engineering and Evolution of Adeno-Associated Viral Vectors for Enhanced Gene Transfer," *Pharm. Research.*, 2008, Vol. 25, No. 3, pp. 489-499 (EX1029), p. 1; Kotterman and Schaffer, "Engineering adeno-associated viruses for clinical gene therapy," *Nature Reviews, Genetics*, Vol. 15, pp. 445-451 (EX1030), p. 5.

62. Vectors based on adeno-associated virus (AAV) have several desirable features as gene delivery vectors. EX1029 (Kwon), p. 1.

63. First, AAV is non-pathogenic (up to 90% of the human population has been exposed to AAV with no significant issues). *Id.*

64. Second, all viral genes can be deleted from recombinant AAV (rAAV) vectors (discussed further below), thereby yielding an efficient transgene delivery vehicle. *Id.*

65. Third, AAV can efficiently infect both dividing and non-dividing cells in a variety of tissues, *e.g.*, muscle, liver, brain, lung, retina, heart, and pancreas. *Id.*

66. Fourth, although AAV serotype 2 (AAV2) is the best characterized AAV variant, there are a number of other available AAV variants with differences in cellular tropism – *i.e.*, the ability to target and infect particular types of cells. *Id.*

1. The AAV Genome

67. AAVs have a single-stranded DNA genome, about 4.7 kb long. *Id.*; EX1030 (Kotterman), p. 2, Fig. 1, Fig. 1 legend. The AAV genome contains two open reading frames (ORF) flanked by inverted terminal repeat elements (“ITRs”) (52–54). Sequences of DNA that are transcribed into mRNA for translation into protein are characterized by the absence of frequent stop codons – they are sometimes referred to as “open reading frames,” or “ORFs.” EX1040 (Alberts I), pp. 23-24, 69; EX1056 (Alberts II), pp. 9-12, Fig. 7-9; EX1030 (Kotterman), p. 2, Fig. 1 legend.

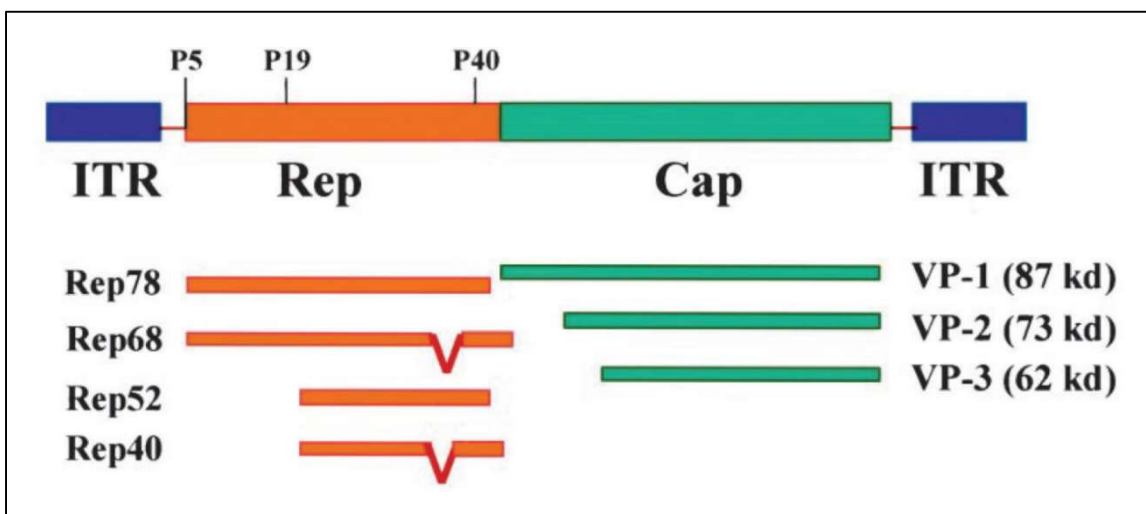
68. ITRs are DNA sequences at the ends of the viral genome that are required for various functions during the viral lifecycle. EX1029 (Kwon), p. 1; EX1030 (Kotterman), p. 2, Fig. 1, Fig. 1 legend.

69. These ITRs are necessary for viral genome integration, replication, and packaging into the capsid shell. EX1029 (Kwon), p. 1.

70. The first ORF (the AAV *rep* gene) encodes four Rep proteins that are involved in replication of the viral genome. EX1029 (Kwon), p. 1; EX1030 (Kotterman), p. 2, Fig. 1, Fig. 1 legend.

71. The *second* ORF (the AAV *cap* gene) encodes three structural proteins (vp1, vp2, and vp3) that are translated from one mRNA via alternative splicing and translation initiation at different start codons, and that make up the viral capsid. EX1029 (Kwon), pp. 1-2; EX1030 (Kotterman), p. 2, Fig. 1, Fig. 1 legend.

72. The genomic organization of AAV is illustrated below:

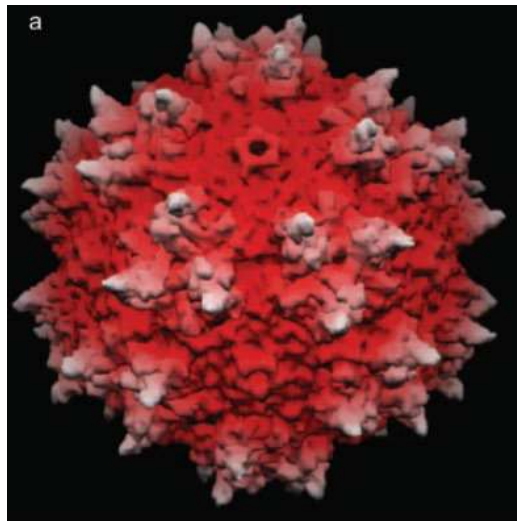


EX1049 (Ponnazhagan), p. 2, Fig. 1, Fig. 1 legend.

73. As the figure shows, the vp1, vp2, and vp3 proteins encoded by the *cap* gene differ in only their N-terminal sequences and length – the vp2 and vp3 proteins are subsets of the vp1 sequence, missing different amounts of N-terminal vp1

sequence. The protein derived from the unspliced mRNA transcript is the vp1 protein (which has an approximate weight of 87 kDa); vp1 is the biggest of the three capsid proteins. The smaller proteins, resulting from translation initiation at different start codons, are the vp2 protein (with an approximate mass of 73 kDa) and vp3 protein (with an approximate mass of 62 kDa). EX1029 (Kwon), pp. 1-2; EX1049 (Ponnazhagan), p. 2, Fig. 1, Fig. 1 legend.

74. Sixty total copies of the vp1, vp2, and vp3 proteins assemble into a capsid having an icosahedral structure in a 1:1:10 (vp1:vp2:vp3) molar ratio as illustrated below, where the vp1, vp2, and vp3 proteins combine to hold the structure together in its capsid form. EX1030 (Kotterman), p. 2, Fig. 1, Fig. 1 legend; EX1031 (Grimm), p. 3.



EX1008 (Xie), 4, Fig. 3.

75. The viral genome loads into the capsid. EX1008 (Xie), p. 1; EX1029 (Kwon), p. 2.

2. The AAV Capsid

76. The capsid is the portion of an AAV virus, and therefore of an AAV gene therapy vector, that is “seen” by the patient’s immune system. EX1031 (Grimm), Abstract; EX1008 (Xie), p. 3.

77. Moreover, specific amino acids in the AAV capsid proteins point outwards in the capsid structure, and therefore face outwards towards the patient’s immune system, while others do not. EX1008 (Xie), pp. 3-4, Fig. 2, Fig. 2 legend, Fig. 4, Fig. 4 legend. These outward facing amino acids in the AAV capsid were understood to be likely responsible for the differences in tropism and serology observed among different AAV variants. *See* EX1031 (Grimm), p. 3; Wu *et al.*, “Mutational Analysis of the Adeno-Associated Virus Type 2 (AAV2) Capsid Gene and Construction of AAV2 Vectors with Altered Tropism,” *J. Virol.*, 2000, Vol. 74, No. 18, pp. 8635-8647 (EX1032), Abstract, *passim*, Table 1, Table 1 legend (focusing on mutagenesis of regions of the AAV2 capsid believed to be on the surface); EX1008 (Xie), p. 3 (stating that loops in the crystal structure of the capsid protein “constitute surface features of the virus that govern interactions with antibodies and cellular receptors”).

78. These properties are exploited in creating AAV gene therapy vectors, in which *rep* and *cap* are excised from between the ITRs, a gene of interest is inserted

in their place, and the *rep* and *cap* genes are supplied externally as helper genes to package the therapeutic transgene inside the capsid. EX1029 (Kwon), p. 2.

79. Certain amino acids of the vp1, vp2, and vp3 capsid proteins have been found to be essential for capsid formation based on mutagenesis studies. EX1032 (Wu), at Abstract.

3. AAV Infection

80. AAV infection begins with viral binding to the cell surface, followed by viral uptake, intracellular trafficking, translocation to the nucleus, virion uncoating, synthesis of double stranded DNA, and viral gene expression. EX1029 (Kwon), p. 2.

81. Although these steps collectively influence AAV tropism, viral binding to the cell surface is particularly important and has thus been a primary target for engineering to alter AAV tropism. *Id.*

82. AAVs are prevalent in primate populations. As of April 2005, researchers had isolated a number of AAV serotypes from human and nonhuman primate tissues. *See, e.g.,* Mori *et al.*, “Two novel adeno-associated viruses from cynomolgus monkey: pseudotyping characterization of capsid protein,” *Virology* 330 (2004) 375-383 (EX1051), pp. 1-2.

83. Researchers named certain variants with a number following “AAV” – *i.e.*, AAV1 through AAV11. *Id.* Other variants are identified by a two letter

abbreviation, such as “rh”, followed by a number. The first two letters reflect the original source of the naturally occurring variant – *i.e.*, “rh” for Rhesus macaque. EX1007 (’772 Publication), at [0046], Table 1 (pp. 112-13).

E. Recombinant AAV (rAAV) Vectors for Gene Therapy

84. By the priority date, AAVs were well understood to be useful vectors for gene therapy. EX1031 (Grimm), Abstract. In the early 1980s, researchers created the first recombinant adeno-associated virus (rAAV) by modifying a wild-type AAV genome. EX1029 (Kwon), p. 2. This work showed that rAAVs can operate as vectors and deliver genes of interest into mammalian cells.

85. Before the priority date, researchers were working to improve the tropism (ability to target particular cell types) and transduction efficiency (efficiency of delivering a therapeutic gene and expressing it in targeted cells) of known AAV variants. *Id.*, pp. 1-5. In particular, people of skill in the art understood that there was a need to improve transduction of liver cells (for the treatment of hemophilia, glycogen storage diseases, and various metabolic disorders), lung cells (for the treatment of diseases such as cystic fibrosis), and muscle cells (for the treatment of muscular dystrophies), and to circumvent the problem of neutralizing antibodies in the patient for clinical applications. EX1031 (Grimm), pp. 12-17.

86. More than 50 naturally occurring AAV variants had been isolated by the priority date. EX1029 (Kwon), p. 2; EX1051 (Mori), Abstract, p. 2; Chiorini *et*

al., “Cloning and Characterization of Adeno-Associated Virus Type 5,” *Journal of Virology*, 2004, Vol. 330, pp. 375-383 (EX1050), Abstract. Sequence alignments for the vp1 capsid proteins of the known AAV variants had been published, and conserved regions and variable regions had been identified. EX1050 (Chiorini), Abstract (“Alignment of the *cap* open reading frame with that of the other AAV serotypes identifies both conserved and variable regions which could affect tissue tropism and particle stability”). AAV vectors had been widely tested in animal studies and evaluated in human clinical trials. EX1031 (Grimm), Abstract.

87. People of skill in the art recognized that one limitation of AAV as a gene therapy vector is that there is about a 5 kb limit to the size of the foreign gene that an AAV vector can encapsidate and deliver. EX1031 (Grimm), p. 6 (“Since the capsids of AAV types 1 to 6 are similar in diameter (this is likely also true for AAV-7 and -8), the size of the foreign DNA to be encapsidated, including the ITRs, must not exceed the approximate 5 kb packaging limit of AAV”).

88. As a result of the 5kb size limitation for AAV vectors, researchers designed smaller but still functional versions of large therapeutic genes, including “mini-” or “micro-” versions, for use with AAV vectors. EX1010 (Fabb), Abstract.

89. Researchers also sought to modify and improve the function of the naturally occurring variants for gene therapy applications. For example, a 2003 review discusses some of the limitations of AAV2 as a gene therapy vector:

. . . there is concern that two restrictions of AAV-2 vectors might limit their clinical use in humans. ***First, these vectors are rather inefficient at transducing some cells of therapeutic interest, such as liver and muscle cells. Second, gene transfer might be hampered by neutralizing anti-AAV-2 antibodies, which are highly prevalent in the human population.*** In efforts to overcome both limitations, an increasing number of researchers are now focusing on the seven other naturally occurring serotypes of AAV (AAV-1 and AAV-3 to -8), which are structurally and functionally different from AAV-2. To this end, several strategies have been devised to cross-package an AAV-2 vector genome into the capsids of the other AAV serotypes, resulting in a new generation of “pseudotyped” AAV vectors. In vitro and in vivo, these novel vectors were shown to have a host range different from AAV-2, and to escape the anti-AAV-2 immune response, thus underscoring the great potential of this approach.

EX1031 (Grimm), Abstract (emphasis added).

90. This review specifically calls out the need to improve transduction of liver and muscle cells, and to identify or obtain additional serotypes to circumvent the problem of neutralizing antibodies in the patient.

91. Researchers tended to focus on the capsid proteins, as determinants both of tropism and of the potential for neutralization by the patient’s own antibodies. Hauck and Xiao, “Characterization of Tissue Tropism Determinants of Adeno-Associated Virus Type 1,” *J. Virol.*, 2003, Vol. 77, pp. 2768-2774 (EX1052), Abstract; EX1032 (Wu), Abstract. Studies ranged from the investigation of single amino acid mutations in naturally occurring capsid proteins, to the creation of chimeras – artificial proteins made up of capsid protein segments from different variants. EX1052 (Hauck), Abstract; EX1032 (Wu), Abstract.

92. Efforts to improve the tropism of AAV gene therapy vectors included mixing and matching amino acids from different AAV serotypes to confer the properties of one serotype on the other, with much success. EX1052 (Hauck), Abstract.

93. Capsid engineering strategies (including changes as minor as single amino acid modifications) yielded novel rAAVs with altered properties. EX1032 (Wu), at Abstract, pp. 5-6, Table 1.

94. Efforts to improve transduction efficiency and other properties of AAV viral vectors also included altering phosphorylation sites on the capsid proteins, by substituting amino acids that can be phosphorylated (*e.g.*, serine) with those that cannot (*e.g.*, alanine or asparagine). EX1009 (Snowdy), pp. 4-5, 125-30; *see also* Jans and Jans, “Negative charge at the casein kinase II site flanking the nuclear localization signal of the SV40 large T-antigen is mechanistically important for enhanced nuclear import,” *Oncogene*, 1994, Vol. 9, No. 10, pp. 2961-2968 (EX1017), p. 3 (substituting asparagine (N) for aspartic acid (D) to remove a phosphorylation site in SV40 large T-antigen).

95. For example, Snowdy reports that an S181A mutation – *i.e.*, mutating serine (S) 181 in the AAV2 capsid to alanine (A), and thereby eliminating this potential phosphorylation site – improved the transduction efficiency of the virus. EX1009 (Snowdy), pp. 5, 128.

96. By comparison, to simulate constitutive phosphorylation (the state of being continuously phosphorylated), Snowdy mutated serine (S) 181 to aspartic acid (D). EX1009 (Snowdy), pp. 5, 128; EX1017 (Jans & Jans), pp. 2-3. Snowdy reported that when serine (S) 181 in AAV2 was mutated to aspartic acid (D) – thereby simulating a phosphorylated amino acid at this location – transduction of the AAV2 virus was substantially reduced. EX1009 (Snowdy), pp. 5, 128.

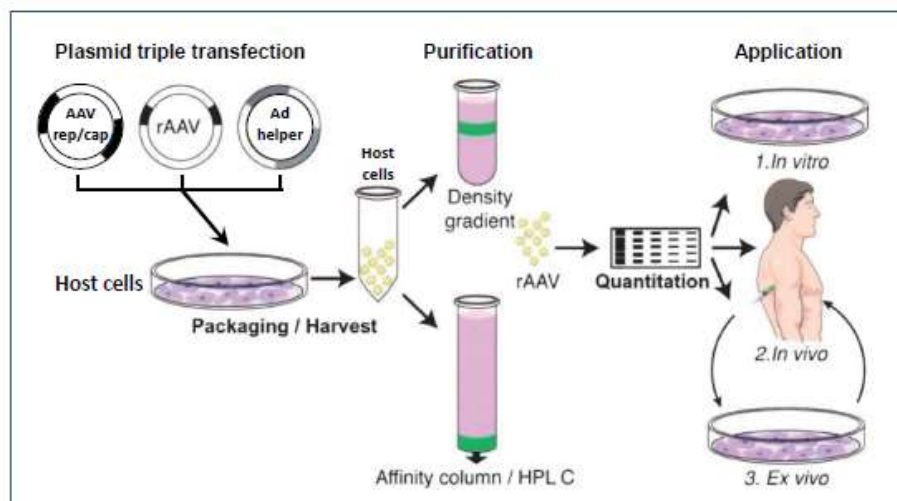
F. Production of rAAV in Host Cells

97. Recombinant AAV vectors are typically made using cells grown in culture. In a method referred to as the “triple transfection” method, three genetic elements are introduced into the cultured host cells: (i) an engineered DNA gene flanked by two inverted terminal repeats (ITRs) capable of encapsidation within an AAV capsid, (ii) AAV *rep* and *cap* genes, and (iii) genes that provide helper functions necessary for AAV viral replication. *See, e.g.*, EX1031 (Grimm), p. 7, Fig. 1, Fig. 1 legend; Matsushita *et al.*, Adeno-associated virus vectors can be efficiently produced without helper virus,” *Gene Therapy*, 1998, Vol. 5, pp. 938-945 (EX1053), at Abstract.

98. Typically, researchers clone these genetic elements into three separate expression plasmids, which are then introduced into the production host cells by a method known as triple transient transfection. EX1031 (Grimm), p. 7, Fig. 2, Fig. 2 legend. Once all three genetic elements are cloned into the host cells, they are grown

in culture. EX1049 (Ponnazhagan), p. 2, Fig. 2 and Fig. 2 legend. During culturing, the host cells replicate the engineered DNA and produce capsid proteins (which assemble and package the engineered DNA genome). EX1049 (Ponnazhagan), p. 2, Fig. 2 and Fig. 2 legend; EX1031 (Grimm), p. 7, Fig. 2, Fig. 2 legend. Ultimately, researchers harvest the rAAVs containing the gene of interest from the host cell culture. EX1049 (Ponnazhagan), p. 2, Fig. 2 and Fig. 2 legend.

99. The Figure below illustrates the overall “triple transfection” process of generating rAAVs.



Modified from EX1049 (Ponnazhagan), Fig. 2.

VI. THE '274 PATENT

100. The '274 patent is titled “Method of Increasing the Function of an AAV Vector.” EX1001 ('274 patent), 1. The patent names Luk Vandenberghe, Guangping Gao, and James Wilson as inventors. *Id.* The '274 patent issued on June 20, 2023. *Id.*

101. The '274 patent is assigned to The Trustees of the University of Pennsylvania. *Id.* I understand that the '274 patent is exclusively licensed to REGENXBIO Inc. I use the term “Patent Owners” to refer collectively to The Trustees of the University of Pennsylvania and REGENXBIO Inc.

A. The Claims

102. The challenged claims of the '274 patent are directed to recombinant AAVs having vp1 capsid proteins with a particular amino acid sequence – specifically, the naturally occurring vp1 capsid protein sequence for AAVrh.46 or a sequence at least 95% identical to AAVrh.46, and where the amino acid at position 665 is asparagine (N). *Id.*, 193:1-15.

103. I have reproduced the challenged claims in the table below:

Claim	Element
1 [pre]	A recombinant adeno-associated virus (AAV) comprising
1[a]	an AAV capsid and
1[b]	a minigene having AAV inverted terminal repeats and a heterologous gene operably linked to regulatory sequences which direct expression of the heterologous gene in a host cell,
1[c]	wherein the AAV capsid comprises AAV vp1 proteins, AAV vp2 proteins, and AAV vp3 proteins,
1[d]	wherein the AAV vp1 proteins have i) the sequence of amino acids 1 to 738 of SEQ ID NO: 4 (AAVrh46), or ii) an amino acid sequence at least 95% identical to the full length of amino acids 1 to 738 of SEQ ID NO: 4

Claim	Element
1[e]	wherein the amino acid residue corresponding to position 665 in SEQ ID NO: 4 is N when aligned along the full length of amino acids 1 to 738 of SEQ ID NO: 4.
3	The recombinant AAV according to claim 1, wherein the AAV vp1 capsid proteins have an amino acid sequence which is at least 97% identical to the full length of amino acids 1 to 738 of SEQ ID NO: 4, wherein the amino acid residue corresponding to position 665 is N when aligned along the full length of amino acids 1 to 738 of SEQ ID NO: 4.
4	The recombinant AAV according to claim 1, wherein the AAV inverted terminal repeats are from a different AAV than the AAV supplying the capsid proteins.
5	A composition comprising the recombinant AAV according to claim 1 and a physiologically compatible carrier.
6	The recombinant AAV according to claim 1, wherein the heterologous gene encodes an ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, arginase, fumarylacetate hydrolase, carbamoyl phosphate synthetase I, phenylalanine hydroxylase, alpha-1 antitrypsin, glucose-6-phosphatase, porphobilinogen deaminase, cystathione beta-synthase, branched chain ketoacid decarboxylase, isovaleryl-coA dehydrogenase, propionyl-CoA carboxylase, methylmalonyl-CoA mutase, glutaryl-CoA dehydrogenase (GCDH), betaglucosidase, pyruvate carboxylate, hepatic phosphorylase, phosphorylase kinase, β -glucuronidase (GUSB), glycine decarboxylase, a low density lipoprotein (LDL) receptor, high density lipoprotein (HDL) receptor, very low density lipoprotein (VLDL) receptor, scavenger receptor, glucocorticoid receptor, estrogen receptor, Vitamin D receptor, nuclear receptor, cystic fibrosis transmembrane regulator (CFTR) sequence, Factor IX or variants thereof, Factor VIII or variants thereof, a dystrophin gene product, or an immunoglobulin.
8	The recombinant AAV according to claim 6, wherein the dystrophin gene product is a mini-dystrophin or micro-dystrophin.

104. As the table shows, claim 1 is an independent claim. *Id.*, 193:1-15. Claims 3-6 and 8 are dependent claims, which recite additional elements that, as I discuss further below, were well-known, routine, and conventional in the art as of the earliest priority date for the '274 patent. *Id.*, 193:19-194:23.

B. The Specification

105. The specification of the '274 patent discusses the prior identification of naturally occurring AAV variants: “Recently, investigators have described a large number of AAVs of different sequences [G. Gao, et al., Proc Natl Acad Sci USA, 100(10):6081-6086 (May 13, 2003); US-2003-0138772-A1 (Jul. 24, 2003)] and characterized these AAVs into different serotypes and clades [G. Gao, et al., J. Virol., 78(12):6381-6388 (June 2004); International Patent Publication No. WO 2005/033321]. It has been reported that different AAVs exhibit different transfection efficiencies, and also exhibit tropism for different cells or tissues.” *Id.*, 1:51-59.

106. The specification discloses the capsid protein sequences for certain naturally occurring AAV variants isolated from Rhesus macaques – designated rh.20, rh.32/33, rh.39, rh.46, rh.73, and rh.74. *Id.*, 2:37-58, Figs. 4, 5, 6; Sequence Listing, cols. 33-192. The specification also discloses alignments of the amino acid sequences for the vp1 capsid proteins of these naturally occurring AAV variants. *Id.*, 2:37-58, Figs. 5, 6.

107. The '274 patent contains no examples, experimental data, or other disclosure indicating that the asparagine (N) at position 665 of AAVrh.46 has any unexpected biological significance or confers any favorable properties on AAVrh.46 for use as a gene therapy vector or otherwise. In fact, the '274 patent contains no discussion of position 665 in AAVrh.46 at all, or any discussion of why it may be important to preserve asparagine (N) at that position in the capsid protein of an rAAV vector. The first identification of asparagine (N) at position 665 is in a set of amended claims filed during prosecution. EX1002, pp. 122-26 (June 12, 2019 Preliminary Amendment).

C. The Prosecution History

108. During prosecution, the Examiner issued two 35 U.S.C. §103 rejections based on prior art references that describe AAV capsid sequences. *Id.*, pp. 265-82. Patent Owners overcame the first rejection on the ground that the cited reference was disqualified under pre-AIA 35 U.S.C. §103(c). *Id.*, p. 341. In response to the second rejection, Patent Owners cancelled two pending claims. *Id.*, p. 342. The Examiner allowed the remaining claims, but Patent Owners filed two subsequent Requests for Continued Examination. *Id.*, pp. 425-29, 503-507. The '274 patent issued on June 20, 2023.

109. The Examiner did not evaluate the challenged claims in light of the primary prior art at issue here – the '772 Publication, Xie, Snowdy, and Fabb.

D. Priority Date

110. The '274 patent claims priority to two provisional applications, 60/669,083 (“the '083 application”) filed April 7, 2005, and 60/733,497 (“the '497 application”), filed November 4, 2005. EX1001 ('274 patent); EX1003 ('083 application); EX1004 ('497 application). The earliest non-provisional application listed on the face of the '274 patent is PCT/US2006/013375, filed on April 7, 2006. EX1001.

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

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

112. I understand that the earliest priority applications listed on the face of the '274 patent are U.S. Provisional Patent Application Nos. 60/669,083, filed on April 7, 2005 (EX1003), and 60/733,497, filed on November 4, 2005 (EX1004). I also understand that the earliest non-provisional application listed on the face of the '274 patent is PCT/US2006/013375, filed on April 7, 2006. EX1001.

113. I have carried out my analysis of obviousness from the perspective of a POSA as of April 2005.

114. I have been asked to consider the level of education and experience of a POSA for the '274 patent. In evaluating the qualifications of a POSA, I have

considered the following factors: (i) the types of problems encountered in the art, (ii) prior art solutions to those problems, (iii) the rapidity with which innovations are made, (iv) the sophistication of the technology, and (v) the educational level of active workers in the field. I have also relied on my experience working with and supervising others in the field of gene therapy.

115. In my opinion, a POSA in the technical field of the '274 patent would have had at least a Ph.D. in biochemistry, molecular biology, or a related field and between one and four years of post-doctoral experience in the field of gene therapy. Alternatively, a POSA would have had at least a Master's or Bachelor's Degree in biochemistry, molecular biology, or a related field, with a corresponding number of additional years of experience in the field of gene therapy.

VIII. THE PRIOR ART

A. The '772 Publication

116. The '772 Publication is titled "Method of detecting and/or identifying adeno-associated virus (AAV) sequences and isolating novel sequences identified thereby." EX1007 ('772 Publication). The '772 Publication lists Guangping Gao, James Wilson, and Mauricio Alvira as named inventors. *Id.*

117. The '772 Publication was published on July 24, 2003 – more than a year before the filing date of the earliest provisional application for the '274 patent, on April 7, 2005. *Id.*

118. The '772 Publication discloses nucleic acid sequences encoding the capsid proteins for a group of naturally occurring AAV variants that were identified in tissue samples taken from non-human primates. *Id.*, [0046]. The disclosed AAV variants are listed in Table 1. *Id.*, pp. 112-13. Figure 1 is an alignment of the nucleic acid sequences encoding the capsid proteins of the disclosed AAV variants. *Id.*, pp. 2-97.

119. The '772 Publication also discloses the amino acid sequences of the capsid proteins for these AAV variants. *Id.*, Fig. 2. Figure 2 is an alignment of the amino acid sequences for the disclosed AAV capsid proteins. *Id.*, 98-103.

120. The '772 Publication teaches that the disclosed sequences may be used to make rAAV vectors for gene therapy applications. *Id.*, [0075], [0086]-[0095].

1. The '772 Publication Identifies AAVrh.10 as a Particularly Preferred Embodiment

121. The '772 Publication identifies AAVrh.10 as a particularly preferred embodiment of the disclosed, naturally occurring AAVs. *See id.*, [0253]-[0257]. AAVrh.10 is sometimes referred to in the '772 Publication as the “44-2” or “44.2” variant. *Id.*, Table 1 (pp. 112-13), [0089], [0142].

122. The '772 Publication discloses the results of various experiments in the Examples. *Id.*, pp. 125-38. The data and discussion in the Examples highlight the unique properties of the newly identified AAVrh.10 variant as a potential rAAV vector, particularly in the transduction of lung cells. Specifically, Example 9

discloses two transduction experiments, in each of three different tissues: lung, liver, and muscle. *Id.*, p. 133. Example 9 states: “The data from both these experiments confirmed the superb tropism of clone 44.2 [AAVrh.10] in lung-directed gene transfer.” *Id.*, [0256].

123. The '772 Publication compares AAVrh.10 to another variant, AAV8, which showed superior performance in liver, and which has an asparagine (N) at position 665. Example 9 states: “Interestingly, performance of clone 44.2 in liver and muscle directed gene transfer was also outstanding, close to that of the best liver transducer, AAV8 and the best muscle transducer AAV1, suggesting that this novel AAV has some intriguing biological significance.” *Id.*, [0257]; *see also* [0253].

124. Examples 3 and 4 disclose experiments involving “chimeric packaging constructs,” in which AAV2 *rep* gene sequences were fused with *cap* sequences from the newly identified variants. *Id.*, [0213]-[0228].

125. The resulting plasmid constructs were added to cells along with plasmid constructs containing the AAV2 ITR sequences fused with other genes replacing the AAV2 genome (in this case a reporter green fluorescent protein (GFP) sequence), and also with plasmid constructs containing helper adenovirus sequences. *Id.*, [0214]. The '772 Publication identifies this process as a form of “triple transfection.” *Id.*

126. The experiments in this example are referred to as “pseudotyping” – meaning the creation of a viral particle where the viral capsid coat is not from the same variant as the genome packaged inside. For example, constructs created by combining AAV2 *rep* and ITR sequences with sequences from AAV7 are “pseudotyped” constructs described in the ’772 Publication as “AAV2/7.” *Id.*

127. Example 5 describes similar pseudotyping experiments, except using capsid proteins from AAV1, AAV5, and AAV8, rather than AAV7. *Id.*, [0229]-[0232]. Various different reporter genes were used to measure the yields of vector for the different serotypes. *Id.*, [0232], Table 4. Example 5 discloses that “[t]he yields of vector were high for each serotype with no consistent differences between serotypes.” *Id.*

128. In Example 6, serologic analysis of the pseudotyped vectors was performed. *Id.*, [0233]-[0236]. Serologic assays are carried out to determine whether antibodies against one serotype will react against another serotype, neutralizing the other serotype’s ability to produce an infection. Example 6 describes experiments in which mice were injected with vectors of different serotypes, and the resulting serum was then used to test whether it could inhibit infection of cells in culture by a reporter virus (containing a GFP construct) of each serotype. *Id.*, [0234].

129. In general, the more closely related the serotypes, the more likely cross-neutralization will be to occur between them. For example, a particular serotype would be expected to cross-neutralize itself in these assays.

130. Example 6 discloses that “[a]ntisera to AAV1, AAV2, AAV5, and AAV8 neutralized transduction of the serotype to which the antiserum was generated (AAV5 and AAV8 to a lesser extent than AAV1 and AAV2) but not to the other serotypes (i.e., there was no evidence of cross neutralization, suggesting that AAV8 is a truly unique serotype).” *Id.*, [0235] and Table 5.

131. Notably, the fact that AAV8 is called out in Example 6 as a “truly unique” serotype would inform a POSA at the relevant time that AAV8, like AAVrh.10, had properties that would make it a promising candidate for use in gene therapy. People of ordinary skill in the art at the relevant time would have understood that neutralization of a gene therapy vector by antibodies in the host, *i.e.*, the patient undergoing the gene therapy, is undesirable because it renders the gene therapy vector ineffective. *See, e.g., id.*, [0024] (“the vectors of the invention containing the AAV capsid proteins of the invention are particularly well-suited for use in applications in which the neutralizing antibodies diminish the effectiveness of other AAV serotype based vectors, as well as other viral vectors”).

132. Example 6 also discloses that human sera from 52 normal subjects were screened for neutralization of selected serotypes. *Id.*, [0235]. Once again, a person

of ordinary skill would understand the '772 Publication to be pointing to the advantages of AAV8 in this serological study: “No serum sample was found to neutralize AAV2/7 and AAV2/8 while AAV2/2 and AAV2/1 vectors were neutralized in 20% and 10% of sera, respectively.” *Id.*, [0236].

133. Similarly, Example 6 discloses: “A fraction of human pooled IgG representing a collection of 60,000 individual samples did not neutralize AAV2/7 and AAV2/8, whereas AAV2/2 and AAV2/1 vectors were neutralized at titers of serum equal to 1/1280 and 1/640, respectively.” *Id.* These results suggest that human patients generally may not have pre-existing immunity to AAV2/7 and AAV2/8, again, making AAV8 a favorable candidate for gene therapy.

134. Example 7 discloses experiments in which recombinant AAV2 genomes containing various reporter constructs to drive tissue-specific expression of the reporter genes were packaged with capsids from different serotypes. *Id.*, [0237]-[0251]. In these experiments, the performance of vectors with capsids from the new serotypes were evaluated in mouse models of muscle- and liver-directed gene transfer and compared to vectors with capsids from the known serotypes AAV1, AAV2, and AAV5. *Id.*, [0241].

135. Example 7 discloses that AAV2/8 produced the highest level of transgene expression in mouse liver. *Id.*, [0245], Table 6. Specifically, AAV2/8 produced about nine times as much expression of the transgene in liver as the next

best construct, AAV2/7, and over 100 times as much expression in liver as the most poorly expressed construct, AAV2. *Id.*, Table 6. I have reproduced Table 6 below:

TABLE 6		
Expression of β -unit of Rhesus Monkey Chorionic Gonadotropin (rhCG)		
Vector	Muscle	Liver
AAV2/1	4.5 $\pm$ 2.1	1.6 $\pm$ 1.0
AAV2	0.5 $\pm$ 0.1	0.7 $\pm$ 0.3
AAV2/5	ND*	4.8 $\pm$ 0.8
AAV2/7	14.2 $\pm$ 2.4	8.2 $\pm$ 4.3
AAV2/8	4.0 $\pm$ 0.7	76.0 $\pm$ 22.8

*Not determined in this experiment.

Id., p. 132.

136. Additional experiments showed that the number of transduced liver cells corresponded to the levels of transgene expression observed. EX1007 (the '772 Publication), [0245]. The amount of vector DNA found in liver also correlated with the levels of transgene expression. *Id.*, [0246], Table 7. I have reproduced Table 7 below:

TABLE 7	
Real Time PCR Analysis for Abundance of AAV Vectors in nu/nu Mouse Liver Following Injection of 1×10^{11} Genome Copies of Vector.	
AAV vectors/Dose	Genome Copies per Cell
AAV2/1AlbA1AT	0.6 $\pm$ 0.36
AAV2AlbA1AT	0.003 $\pm$ 0.001
AAV2/5AlbA1AT	0.83 $\pm$ 0.64
AAV2/7AlbA1AT	2.2 $\pm$ 1.7
AAV2/8AlbA1AT	18 $\pm$ 11

Id., p. 132.

137. Example 7 touts the advantages of AAV8 for transduction in liver: “These studies indicate that AAV8 is the most efficient vector for liver-directed gene transfer due to increased numbers of transduced hepatocytes.” *Id.*, [0246].

138. The end of Example 7 sums up the advantages of AAV8 as a liver transducer:

[0249] Vectors based on AAV7 and AAV8 appear to be immunologically distinct (i.e., they are not neutralized by antibodies generated against other serotypes). Furthermore, sera from humans do not neutralize transduction by AAV7 and AAV8 vectors, *which is a substantial advantage over the human derived AAVs currently under the development for which a significant proportion of the human population has pre-existing immunity that is neutralizing* [Chirmule, N., et al., (1999) *Gene Ther* 6, 1574-83].

[0250] The tropism of each new vector is favorable for in vivo applications. . . . *Importantly, AAV2/8 provides a substantial advantage over the other serotypes in terms of efficiency of gene transfer to liver that until now has been relatively disappointing in terms of the numbers of hepatocytes stably transduced.* AAV2/8 consistently achieved a 10 to 100-fold improvement in gene transfer efficiency as compared to the other vectors. . . . *This improved efficiency will be quite useful in the development of liver-directed gene transfer where the number of transduced cells is critical, such as in urea cycle disorders and familial hypercholesterolemia.*

Id., [0249]-[0250] (emphases added).

139. Example 9 discloses additional transduction experiments in three different tissues, lung, liver, and muscle. *Id.*, [0252]-[0258]. These experiments

showed that another AAV capsid clone, 44.2, was “found to be a very potent gene transfer vehicle in all three tissues with a big lead in the lung tissue particularly.” *Id.*, [0253]. Clone 44.2 is AAVrh.10, according to Table 1. *See id.*, Table 1 (pp. 112-13).

140. Table 8 shows the data from day 14 of the study – meaning 14 days after delivery of the various constructs to mice:

TABLE 8			
Vector	Target Tissue		
	Lung	Liver	Muscle
AAV2/1	ND	ND	45 ± 11
AAV2/5	0.6 ± 0.2	ND	ND
AAV2/8	ND	84 ± 30	ND
AAV2/rh.2 (43.1)	14 ± 7	25 ± 7.4	35 ± 14
AAV2/rh.10 (44.2)	23 ± 6	53 ± 19	46 ± 11
AAV2/rh.13 (42.2)	3.5 ± 2	2 ± 0.8	3.5 ± 1.7
AAV2/rh.21 (42.10)	3.1 ± 2	2 ± 1.4	4.3 ± 2

Id., p. 133.

141. Table 8 shows that AAVrh.10 produced 1.6 times as much reporter expression in lung as the next best construct (AAV2/rh.2), and nearly 40 times as much reporter expression in lung as AAV2/5. Table 8 also shows that AAVrh.10 performed second best in liver, producing about 60% of the reporter expression of the best construct in liver, AAV2/8. The table also shows that AAVrh.10 performed well in muscle, producing about the same amount of reporter expression as the other best construct in muscle, AAV2/1.

142. Additional experiments were conducted to “confirm the superior tropism of AAV 44.2 in lung tissue.” *Id.*, [0254]. AAV2 with a minigene construct for lung expression was pseudotyped (combined) with capsids from other serotypes. *Id.*, [0254]-[0255]. These pseudotyped AAVs were administered to immune deficient mice, and then to immune competent mice, and the lung tissue was evaluated for gene transfer of the various pseudotyped AAVs. *Id.*, [0254]-[0255], Tables 9 and 10.

143. The results are shown in Tables 9 and 10, which I have reproduced below:

Vector	Total GC in 50 μ l vector	μ g of A1AT/ml with 50 μ l vector	μ g of A1AT/ml with 1×10^{11} vector	Relative Gene transfer as compared to rh.10 (clone 44.2)
2/1	3×10^{12}	2.6 ± 0.5	0.09 ± 0.02	2.2
2/2	5.5×10^{11}	<0.03	<0.005	<0.1
2/5	3.6×10^{12}	0.65 ± 0.16	0.02 ± 0.004	0.5
2/7	4.2×10^{12}	1 ± 0.53	0.02 ± 0.01	0.5
2/8	7.5×10^{11}	0.9 ± 0.7	0.12 ± 0.09	2.9
2/ch.5 (A.3.1)	9×10^{12}	1 ± 0.7	0.01 ± 0.008	0.24
2/rh.8 (43.25)	4.6×10^{12}	26 ± 21	0.56 ± 0.46	13.7
2/rh.10 (44.2)	2.8×10^{12}	115 ± 38	4.1 ± 1.4	100
2/rh.13 (42.2)	6×10^{12}	7.3 ± 0.8	0.12 ± 0.01	2.9
2/rh.21 (42.10)	2.4×10^{12}	9 ± 0.9	0.38 ± 0.04	9.3
2/rh.22 (42.11)	2.6×10^{12}	6 ± 0.4	0.23 ± 0.02	5.6
2/rh.24 (42.13)	1.1×10^{11}	0.4 ± 0.3	0.4 ± 0.3	1

TABLE 10		
AAV Vector	μg of A1AT/ml with 1×10^{11} vector	Relative Gene transfer as compared to rh.10 (clone 44.2)
2/1	0.076 ± 0.031	2.6
2/2	0.1 ± 0.09	3.4
2/5	$0.0840.033$	2.9
2/7	0.33 ± 0.01	11
2/8	1.92 ± 1.3	2.9
2/ch.5 (A.3.1)	0.048 ± 0.004	1.6
2/rh.8 (43.25)	1.7 ± 0.7	58
2/rh.10 (44.2)	2.93 ± 1.7	100
2/rh.13 (42.2)	0.45 ± 0.15	15
2/rh.21 (42.10)	0.86 ± 0.32	29
2/rh.22 (42.11)	0.38 ± 0.18	13
2/rh.24 (42.13)	0.3 ± 0.19	10

Id., p. 133.

144. I note that the experiments set out in Table 10 involved the administration of a standardized amount of vector (1×10^{11} copies), while Table 9 does not appear to involve a standardized amount (50 ul).

145. Table 9 shows that, in the first set of experiments, AAVrh.10 performed 200 times as well in lung as some of the other variants (2/5 and 2/7), and 7 times better than the next best variant (2/rh.8). AAVrh.10 performed nearly 35 times as well in lung as AAV8. *Id.*, Table 9, [254]-[257].

146. Similarly, Table 10 shows that, in the second set of experiments, AAVrh.10 performed about 35 times as well in lung as some of the other variants (2/1 and 2/5), and nearly twice as well as the next best variant (2/rh.8). Again, in these experiments, AAVrh.10 performed about 35 times as well in lung as AAV8. *Id.*, Table 9, [254]-[257].

147. Example 9 discloses additional serological testing, with the data shown in Tables 11a, 11b, 12, 13a, and 13b. These experiments were carried out by creating pseudotyped vectors (*e.g.*, AAV2/44.2 [AAVrh.10]) that were used to immunize rabbits, and then the serum from the immunized rabbits was mixed with various vectors (10^9 genome copies of each) that contained GFP reporter constructs. The amount of infectivity in cells in culture was assayed by measuring the resulting GFP fluorescence in the cells. *Id.*, [0258]. I have reproduced Table 11a below:

TABLE 11a				
Cross-NAB assay in 8431 cells and adenovirus (Adv) coinfection				
Infection in 8431 cells (coinfecting with Adv) with:				
Serum from rabbit immunized with:	10^9 GC rh.13 AAV2/42.2	10^9 GC rh.21 AAV2/42.10	10^9 GC rh.22 AAV2/42.11	10^{10} GC rh.24 AAV2/42.13
AAV2/1	1/20	1/20	1/20	No NAB
AAV2/2	1/640	1/1280	1/5120	No NAB
AAV2/5	No NAB	1/40	1/160	No NAB
AAV2/7	1/81920	1/81920	1/40960	1/640
AAV2/8	1/640	1/640	1/320	1/5120
Ch.5 AAV2/A3 rh.8	1/20	1/160	1/640	1/640
AAV2/43.25 rh.10	1/20	1/20	1/20	1/320
AAV2/44.2 rh.13	No NAB	No NAB	No NAB	1/5120
AAV2/42.2 rh.21	1/5120	1/5120	1/5120	No NAB
AAV2/42.10 rh.22	1/5120	1/10240	1/5120	1/20
AAV2/42.11 rh.24	1/20480	1/20480	1/40960	No NAB
AAV2/42.13	No NAB	1/20	1/20	1/5120

Id., p. 134.

148. A POSA would have understood that the fractions shown in Table 11a (e.g., 1/20, 1/640, 1/1280 etc.) represent dilutions of antisera raised against the various vectors (listed vertically on the left side of the table) required to neutralize some percentage of the infectivity (say 50%) of the 10^9 or 10^{10} genome copies of the other viral vectors carrying GFP reporter constructs (listed horizontally across the top of the table).¹ For example, it took a 1/320 dilution of AAV2/8 to neutralize AAV2/42.11, while a much higher dilution factor of AAV2/7 (1/40960) was sufficient to neutralize the same percentage of AAV2/42.11. In other words, over 125 times as much antiserum from AAV2/8 immunized rabbits as from AAV2/7 immunized rabbits was required to effect the same neutralization in cells in culture.

149. Table 11a similarly indicates that no neutralization was observed with AAV2/rh.10 of any of the vectors other than AAV2/42.13 (the table indicates “No NAB” meaning “no neutralizing antibody”).

150. A POSA would have understood that lack of cross neutralization across different serotypes is desirable for an AAV gene therapy vector.

¹ A POSA at the time would have understood that antibodies raised against a particular capsid construct (say AAV2/42.11) would powerfully neutralize viral vectors made from that same construct, so that only a very small amount of the antibodies would be needed.

151. Table 11b shows cross neutralization of the pseudotyped vectors against different vectors from those in Table 11a. The results in Table 11b are more mixed. For example, in these experiments, AAV2/44.2 [AAVrh.10] did not appear to neutralize itself to the extent that would be expected (a 1/5120 dilution was required), and there does not appear to be a large amount of cross neutralization by AAVrh.10 (AAV2/44.2). AAV8 appears to have cross neutralized AAV2/44.2 [AAVrh.10] (1/81920 dilution), but did not cross neutralize AAV2/3, where AAV3 was a previously identified serotype. I have reproduced Table 11b below:

TABLE 11b					
Cross-NAB assay in 8431 cells and Adv coinfection					
Infection in 8431 cells (coinfecting with Adv) with:					
Serum from rabbit immunized with:	10 ⁹ GC rh.12	10 ¹⁰ GC ch.5	10 ¹⁰ GC rh.8	10 ⁹ GC rh.10	10 ⁹ GC rh.20
	AAV2/42.1B	AAV2/A3	AAV2/43.25	AAV2/44.2	AAV2/42.8.2
AAV2/1	No NAB	1/20480	No NAB	1/80	ND
AAV2/2	1/20	No NAB	No NAB	No NAB	ND
AAV2/5	No NAB	1/320	No NAB	No NAB	ND
AAV2/7	1/2560	1/640	1/160	1/81920	ND
AAV2/8	1/10240	1/2560	1/2560	1/81920	ND
ch.5 AAV2/A3	1/1280	1/10240	ND	1/5120	1/320
rh.8 AAV2/43.25	1/1280	ND	1/20400	1/5120	1/2560
rh.10 AAV2/44.2	1/5120	ND	ND	1/5120	1/5120
rh.13 AAV2/42.2	1/20	ND	ND	No NAB	1/320
rh.21 AAV2/42.10	1/20	ND	ND	1/40	1/80
rh.22 AAV2/42.11	No NAB	ND	ND	ND	No NAB
rh.24 AAV2/42.13	1/5120	ND	ND	ND	1/2560

Id., p. 134.

152. Table 12 shows self-neutralization experiments, confirming the ability of serum raised against each vector to neutralize that vector, as would be expected:

[0260]

TABLE 12

	Titer of rabbit sera		Titer after
	Vector	Titer d21	Boosting
ch.5	AAV2/A3	1/10,240	1/40,960
rh.8	AAV2/43.25	1/20,400	1/163,840
rh.10	AAV2/44.2	1/10,240	1/527,680
rh.13	AAV2/42.2	1/5,120	1/20,960
rh.21	AAV2/42.10	1/20,400	1/81,920
rh.22	AAV2/42.11	1/40,960	ND
rh.24	AAV2/42.13	1/5,120	ND

Id., p. 134.

153. Tables 13a and 13b provide a control for the data presented in the previous tables. In Tables 13a and 13b, each of the constructs listed horizontally across the top of the table was added to cells in culture, in the absence of any serum, and the amount of GFP fluorescence was read out to determine the amount of inhibition of infection of cells in culture that occurred. *Id.*, pp. 134-35. AAV2/44.2 [AAVrh.10] and AAV2/8 showed similar infectivity. EX1007 (the '772 Publication), at Tables 13a and 13b. I have reproduced Tables 13a and 13b below:

TABLE 13a						
<u>Infection in 8431 cells (coinfecting with Adv) with GFP</u>						
	10 ⁹ GC/well AAV2/1	10 ⁹ GC/well AAV2/2	10 ⁹ GC/well AAV2/5	10 ⁹ GC/well AAV2/7	10 ⁹ GC/well AAV2/8	10 ⁹ GC/well ch.5 AAV2/A3
# GFU/field	128	>200	95	56	13	1
	83	>200	65	54	11	1

TABLE 13b							
<u>Infection in 8431 cells (coinfecting with Adv) with GFP</u>							
	10 ⁹ GC/well rh.8 AAV2/43.25	10 ⁹ GC/well rh.10 AAV2/44.2	10 ⁹ GC/well rh.13 AAV2/42.2	10 ⁹ GC/well rh.21 AAV2/42.10	10 ⁹ GC/well rh.22 AAV2/42.11	10 ⁹ GC/well rh.24 AAV2/42.13	10 ⁹ GC/well rh.12 AAV2/42.1B
# GFU/field	3	13	54	62	10	3	18
	2	12	71	60	14	2	20
			48	47	16	3	12

Id., pp. 134-35.

154. I note that there are two examples titled “Example 10,” *id.* [0263]-[0284], and [0285]-[0303], but no Example titled “Example 8.” The first Example 10 discloses experiments involving a mouse model of familial hypercholesterolemia, where two different pseudotyped vectors, AAV2/7 and AAV2/8, carrying an LDL minigene were introduced into the mouse, and the amount of rescue of the condition by the vectors was observed. *Id.*, [0263]-[0284].

155. The first Example 10 discloses that both pseudotyped vectors rescued some of the deficiencies of the mouse model of hypercholesterolemia:

[0284] All of the vectors pseudotyped in an AAV2/8 or AAV2/7 lowered total cholesterol, LDL and triglycerides

as compared to the control. These test vectors also corrected phenotype of hypercholesterolemia in a dose-dependent manner. A reduction in plaque area for the AAV2/8 and AAV2/7 mice was observed in treated mice at the first test (2 months), and the effect was observed to persist over the length of the experiment (6 months).

Id., [0284].

156. The second Example 10 sets out experiments involving a mouse hemophilia B model. *Id.*, [0285]-[0303]. In these experiments, pseudotyped vectors carrying a Factor IX minigene were assayed for their ability to rescue hemophilia B in the mouse. The second Example 10 discloses that AAV2/8 and AAV2/7 produced the best expression in the liver as compared with other serotypes (AAV1, AAV2, AAV5), consistent with the previous tropism experiments that I discussed above and that showed favorable tropism for AAV8 in liver tissue. *Id.*, [0292]; *see also* Table 8, p. 133.

157. The second Example 10 further discloses that about 70-80% of hepatocytes stained positive for Factor IX in hepatocytes from liver tissue harvested from the mice injected with the AAV2/8.cFIX vector. *Id.*, [0297].

158. Finally, the second Example 10 states that the ultimate results regarding correction of hemophilia B in the mice had not yet been obtained. *Id.*, [0303].

2. The '772 Publication Teaches the Modification of Naturally Occurring Sequences to Create Artificial AAV Vectors

159. The '772 Publication teaches the creation of “artificial” AAV vectors through modification of the AAV capsid proteins encoded by the naturally occurring

sequences disclosed in Figures 1 and 2. Specifically, the '772 Publication teaches that artificial AAV vectors may be made by combining AAV capsid sequences from one or more different AAV variants. *Id.*, [0074], [0075]. The '772 Publication explains:

In addition to including the nucleic acid sequences provided in the figures and Sequence Listing, the present invention includes . . . artificial AAV serotypes generated using these sequences and/or unique fragments thereof.

As used herein, artificial AAV serotypes include, without limitation, AAV with a non-naturally occurring capsid protein. Such an artificial capsid may be generated by any suitable technique, using a novel AAV sequence of the invention (e.g., a fragment of a vp1 capsid protein) in combination with heterologous sequences which may be obtained from another AAV serotype (known or novel), non-contiguous portions of the same AAV serotype, from a non-AAV viral source, or from a non-viral source. An artificial AAV serotype may be, without limitation, a chimeric AAV capsid, a recombinant AAV capsid, or a “humanized” AAV capsid.

Id.

160. As an example, the '772 Publication discloses that a “novel NHP [non-human primate] clone was made by splicing capsids fragments of two chimp adenoviruses [*sic*] into an AAV2 rep construct. This new clone, A3.1, is also termed Ch.5 [SEQ ID NO: 20] [*sic*].” *Id.*, [0067].²

² I note that the description of this clone as a chimera of *adenovirus* capsid fragments appears to be an error – and should state instead “*adeno-associated*” virus capsid fragments. Moreover the description of this clone as SEQ ID NO: 20 also

161. The '772 Publication also discusses sequences with varying degrees of homology to the naturally occurring sequences: “Further included in this invention are nucleic acid sequences which are greater than 85%, preferably at least about 90%, more preferably at least about 95%, and most preferably at least about 98 to 99% identical or homologous to the sequences of the invention, including FIG. 1 and the Sequence Listing [SEQ ID NOS: 1, 9-59, and 117-120].” *Id.*, [0069].

B. Xie

162. Xie, “The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy,” discloses the three-dimensional crystal structure of the vp3 protein of AAV2. EX1008 (Xie), Abstract, p. 2. Xie was published in 2002, more than one year before the earliest priority date for the '274 patent, April 7, 2005. EX1008 (Xie).

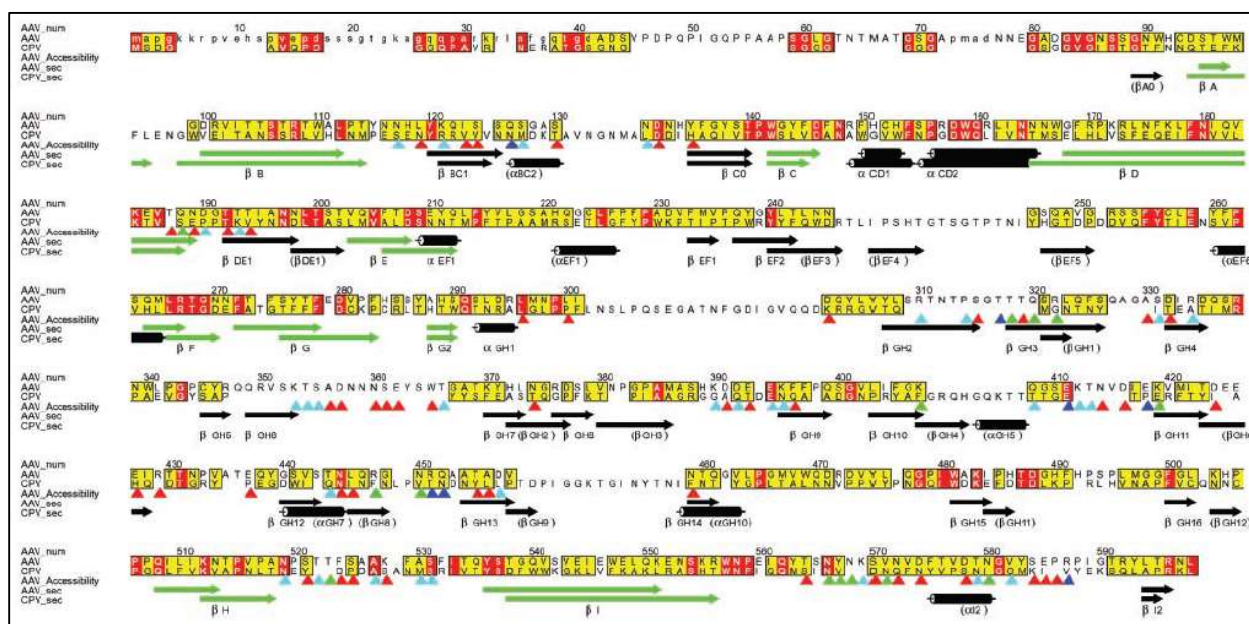
163. Xie discusses the importance of amino acids on the surface of the AAV capsid for determining characteristics of the virus such as tropism, transduction

appears to be an error. This clone is described as A3.1, which matches the description of SEQ ID NO: 120, not SEQ ID NO: 20. *Id.*, pp. 170, 331 The description of SEQ ID NO: 120 states: “adeno-associated virus serotype, clone A3.1 vp1.” *Id.*, at p. 331.

efficiency, and immunogenicity. *Id.*, p. 3 (discussing “the surface features of the virus that govern interactions with antibodies and cellular receptors”).

164. Xie discloses that the surface of the AAV2 viral capsid is composed of 60 triangular facets made-up of vp1, vp2, and vp3 capsid proteins. *Id.*, p. 4, Fig. 4, Fig. 4 legend.

165. Figure 2 of Xie, reproduced below, shows a sequence alignment in which the triangles indicate the AAV2 vp3 amino acids that are on the surface, according to the crystal structure:



Id., p. 3, Fig. 2, Fig. 2 legend.

C. Snowdy

166. Snowdy is a doctoral thesis published in 2003. EX1009 (Snowdy), p. 1. Snowdy states that the advisor for the thesis was Dr. R. Jude Samulski, a

recognized figure in the field of gene therapy and the use of AAV vectors for gene therapy applications. *Id.*

1. Snowdy Was Publicly Accessible by at Least February 2004

167. Snowdy is a printed publication that was publicly accessible by at least February 2004 – more than one year before the earliest priority date for the '274 patent on April 7, 2005.

168. A “Record” of the Snowdy thesis was first published to the ProQuest Dissertations and Theses database on December 15, 2003. 2024-01-18 Declaration of Thomas Hyatt (EX1015), ¶¶3, 4, 7, 8. This Record included information about the dissertation – including a complete copy of the abstract, index record, and citation information (title, author name, degree granting institution, and degree date). *Id.*, ¶3.

169. The full text of Snowdy was also available before the priority date of the '274 patent for purchase from ProQuest as of December 15, 2003. *Id.*, ¶¶8, 9.

170. By December 15, 2003, ProQuest Records were part of the ProQuest Abstract Database, which was available in the ProQuest® Digital Dissertations and Theses database (“PQDT”). *Id.*, ¶4. PDQT is accessible via the world wide web to authenticated subscribers. *Id.* The Abstract Database allows for text searching of the Records, including the title, author, index terms, granting institution, and the abstract. *Id.*

171. Therefore, as of December 15, 2003, the abstract, title, author, granting institution, and degree date of Snowdy were available digitally on PQDT, as part of the Record. *Id.*, ¶¶10-11.

172. As of December 15, 2023, PDQT could be searched electronically for the Record for Snowdy, so that, for example, Snowdy could be found in PDQT by searching for words and phrases from the title such as “adeno-associated virus,” “capsid proteins,” and “nuclear targeting.” *Id.*, ¶12.

173. At the time of Snowdy’s publication on December 15, 2003, the Record for Snowdy was made available directly from ProQuest in electronic format and online via third party vendors, including Dialog, as part of the Abstract Database which was made available via such vendors. *Id.*, ¶14.

174. A POSA in 2003 was familiar with the ProQuest database and would have been able to find the thesis readily in a number of ways, including keyword search and author search.

175. As an alternative to the full copy of Snowdy available for purchase from ProQuest as of December 15, 2003, the full Snowdy thesis was on the shelf of the Wilson Library at the University of North Carolina (UNC) no later than February 2004. *See* 2023-12-21 Declaration of Christopher DuBose (EX1016). ¶¶8-10, 12. Thus, a POSA, researcher, or other member of the public who had located the entry for the Snowdy thesis in the ProQuest index in December 2003 would have been

able to locate the full copy of the thesis in the Wilson Library by no later than February 2004.

176. EX1009 is a true and correct copy of the original Snowdy thesis in the Wilson Library. *Id.*, ¶¶5-7. I have compared EX1009 to the copy of Snowdy attached to the Hyatt Declaration, and found them to be identical. EX1009 (Snowdy); EX1015 (Hyatt Decl.), pp. 5-162. According to the Hyatt Declaration, the copy of Snowdy attached to the declaration is a true and correct copy of the full Dissertation as originally published and kept by ProQuest. EX1015 (Hyatt Decl.), ¶ 15. Therefore, both EX1009 and the copy of Snowdy attached to the Hyatt Declaration are true and correct copies of the original Snowdy thesis in the Wilson Library.

2. Snowdy Teaches the Removal of Phosphorylation Sites in AAV Capsid Proteins to Improve Transduction Efficiency

177. Snowdy discloses methods of improving AAV as a gene therapy tool. EX1009 (Snowdy), pp. 4-5. In particular, Snowdy examined the effect of modifying possible phosphorylation targets (serines and threonines) in the AAV2 capsid on transduction efficiency. *Id.* Snowdy found that substituting one of these phosphorylation targets with the non-phosphorylatable amino acid alanine (A) increased transduction efficiency, while substituting that same phosphorylation target with aspartic acid (D), which mimics a constitutively phosphorylated state, decreased transduction efficiency. *Id.*

178. I note that there is a typographical error in the Introduction and Background section of Snowdy. *Id.*, pp. 28-29. Snowdy states: “Studies establishing the importance of phosphorylation sites in regulating nearby NLSs [nuclear localization signals] depend on the finding that, at least in the context of regulating the efficiency of NLSs, a phosphorylated serine or threonine can be mimicked by substitution of the residue with *asparagine*, which functions as a constitutively phosphorylated residue, and a constitutively dephosphorylated residue can be mimicked by alanine (Jans & Jans 1994).” *Id.* (emphasis added).

179. A POSA at the time would immediately understand that the term “asparagine” is a typographical error and that the correct term should be “aspartic acid.” A POSA at the time would have been well aware that asparagine is a nonphosphorylatable amino acid and that aspartic acid was commonly used to mimic a phosphorylated state. EX1017 (Jans & Jans), pp. 5-6 (“Asp [aspartic acid] closely resembles phosphoserine/phosphothreonine with regard to size and charge characteristics, and has been shown to simulate phosphorylated Ser/Thr residues in functional terms in a number of studies.”).

180. Moreover, a POSA at the time would have clearly understood from the Jans & Jans reference cited that the authors of this reference were using aspartic acid to mimic a phosphorylated state and in fact were using asparagine to obtain an unphosphorylated state. *Id.*, p. 3 (“The T-Ag(Asn-Asn-Gln^{113/115}) and T-ag(Gly-

Ala^{111/112}) fusion proteins contain T-ag amino acids 111-135, but possess non-functional CKII-phosphorylation sites, whereby the latter contains the nonphosphorylatable Ala-Gly residues in place of Ser^{111/112}, whilst T-Ag(Asn-Asn-Gln^{113/115}) retains Ser^{111/112} but the CKII recognition sequence Asp-Asp-Glu^{113/115} is mutated to Asn-Asn-Gln (Table 1”).

181. As a result, Snowdy, in citing Jans & Jans, teaches substituting asparagine for aspartic acid to remove the potential for phosphorylation.

182. A POSA would also have understood that substituting asparagine (N) for serine (S) would be a more conservative substitution than substituting alanine (A) for serine (S), because both serine (S) and asparagine (N) are polar, uncharged amino acids, while alanine (A) is nonpolar. EX1040 (Alberts I), p. 5.

183. Snowdy focused on the effect of phosphorylation on putative nuclear localization signals in the AAV2 capsid protein. EX1009 (Snowdy), pp. 4-5. Snowdy sought in particular to mutate phosphorylation sites near putative nuclear localization signals. *Id.*

184. Using a software program known as NetPhos,³ Snowdy identified three potential phosphorylation sites in the vicinity of a putative nuclear localization signal: serine 148, threonine 159, and serine 181. *Id.*

³ The version of NetPhos currently available, v.3.1 (<https://services.healthtech.dtu.dk/services/NetPhos-3.1/>), lists for each phosphorylatable position in a sequence a number of specific kinases, and provides the likelihood that the position is the target for one or more of them. From my own knowledge of how these programs work, I understand that the programs search for known kinase recognition sequences – meaning a specific series of amino acids flanking a phosphorylatable amino acid such as serine, threonine, or tyrosine – that is known to be a site recognized by particular kinases.

For AAVrh.10, specifically, NetPhos v.3.1 identifies two specific kinases as having a likely recognition sequence that includes the serine at position 665: ATM (ataxia telangiectasia mutated) kinase and DNAPK (DNA dependent protein kinase). NetPhos/AAVrh.10 Phosphorylation Sites (EX1021), p. 20. It is not clear from Snowdy whether this specific information was provided as part of v.2.0.

Nonetheless, programs such as NetPhos, as of 2003, were based on knowledge of kinase recognition sequences available in the scientific literature at the time. And the recognition sequence for at least one of the kinases predicted by NetPhos v.3.1

185. Snowdy mutated each of these three amino acids in the vicinity of the nuclear localization signal to either a nonphosphorylatable alanine (A), to simulate an unphosphorylated serine, or to an aspartic acid (D) to simulate a constitutively (*i.e.*, always) phosphorylated serine – resulting in six mutants altogether: S148A, S148D, T159A, T159D, S181A, and S181D. EX1009 (Snowdy), pp. 5, 125.

186. Snowdy found that all of the mutants were capable of forming virus at normal titers.⁴ The S148A, S148D, T159A, and T159D mutations had no effect, or

to phosphorylate the serine at position 665 of AAVrh.10, ATM kinase, was known as of 2003. Kim *et al.*, “Substrate Specificities and Identification of Putative Substrates of ATM Kinase Family Members,” *J. Biol. Chem.*, 1999, Vol. 274, pp. 37538–37543 (EX1054), p. 5. Therefore, NetPhos v.2.0 would have predicted S665 to be an actual phosphorylation site, even if it did not specifically identify the kinase most likely to phosphorylate at that site.

⁴ Snowdy infected cultured HeLa cells with 500 particles/cell of each of the six mutants to establish whether the potential phosphorylation sites were important to the virus’ ability to deliver its genome to the target cell during an infection. Infected cells were assayed for green fluorescence by fluorescent microscopy. AAV2-eGFP made from wild-type capsids was used as a positive control. EX1009 (Snowdy), p. 126.

little effect, on the virus' ability to infect the HeLa cells and to deliver the eGFP gene carried as their cargo. *Id.*, p. 126.

187. However, the S181A and S181D substitutions had large effects on virus infectivity. The same number of particles containing the S181A mutation infected far more cells than did the wild-type, and the same number of particles containing the S181D mutation infected almost no cells at all. *Id.*, pp. 5, 126-30.

188. Based on studies such as Jans & Jans, which found that removal of a phosphorylation site near a nuclear localization signal of SV40 significantly decreased import of a key viral protein (T antigen) into the nucleus of the infected cell, Snowdy expected the serine to alanine mutations to lower transduction efficiency, if the phosphorylation site was important for regulating the putative nuclear localization signal. *Id.*, pp. 128-29; EX1017 (Jans & Jans), Abstract. However, Snowdy's results for the S181A substitution, where the non-phosphorylatable alanine (A) was substituted in for the phosphorylatable serine (S), showed the opposite – *i.e.*, an **increased** level of infection compared to wild type. EX1009 (Snowdy), pp. 128-29.

189. These results suggested that the effect of mutating a phosphorylation site to a non-phosphorylatable amino acid in the AAV2 capsid had an effect on some characteristic of the virus other than nuclear localization, resulting in **greater** transduction efficiency. *Id.*, p. 129 (“Until we establish unequivocally that basic

region 3 of AAV2 is an NLS [nuclear localization signal], it is premature to assign the cause of the changes in transduction efficiency resulting from mutations to S181 as interfering with the function of an NLS”).

D. Fabb

190. Fabb *et al.*, “Adeno-associated virus vector gene transfer and sarcolemmal expression of a 144 kDa micro-dystrophin effectively restores the dystrophin-associated protein complex and inhibits myofibre degeneration in nude/*mdx* mice,” *Human Molecular Genetics*, 2002, Vol. 11, No. 7, pp. 733-741 (EX1010), discloses a “micro-dystrophin” used as part of an AAV vector for gene therapy. Abstract, pp. 2-3. Fabb was published in 2002, more than one year before the earliest priority date for the ’274 patent of April 7, 2005.

191. Fabb discloses that the full-length dystrophin gene is too large to be packaged into an AAV vector. *Id.*, Abstract, p. 2. Fabb also discloses the construction of a smaller version of the dystrophin cDNA known as a “micro-dystrophin.” *Id.*, Abstract, pp. 2-3. Fabb discloses that this micro-dystrophin, delivered in an AAV vector, restored dystrophin associated protein complexes and ameliorated dystrophic pathology at the cellular level in an animal model. *Id.*, Abstract, pp. 3-5.

IX. CLAIM CONSTRUCTION

192. Challenged claims 1, 3-6, and 8 of the '274 patent recite an AAV capsid protein having an amino acid sequence “at least 95% identical” or “at least 97% identical” to the full length of amino acids 1 to 738 of SEQ ID NO: 4. The '274 patent provides that for purposes of determining percent identity, “[a]lignments are performed using any of a variety of publicly or commercially available Multiple Sequence Alignment Programs.” EX1001 ('274 patent), at 5:31-45. The '274 patent identifies examples of available programs. *Id.*, at 5:45-6:8. However, the '274 patent does not specify a particular program or set of program parameters that should be used to align sequences and calculate percent identity for purposes of determining whether a particular test sequence falls within the scope of the claims.

193. I understand that in a currently-pending litigation in the District of Delaware, Patent Owners have asserted the '274 patent against Sarepta. I further understand that in their preliminary infringement contentions, served on December 20, 2023, Patent Owners used the Clustal Omega program (available at <https://www.ebi.ac.uk/jdispatcher/msa/clustalo>) at default settings to perform sequence alignments and calculate percent identity. EX1026 (Initial Claim chart), p. 3. For purposes of this Declaration, I have analyzed the percent identity elements as Patent Owners did in their preliminary infringement contentions, using the Clustal

program at default settings to generate amino acid sequence alignments and calculate percent identity values.

X. GROUND 1: CLAIMS 1 AND 3-6 ARE OBVIOUS OVER THE '772 PUBLICATION, AS CONFIRMED BY THE TESTIMONY OF PATENT OWNERS' EXPERT IN AN EARLIER LITIGATION

194. In my opinion, claims 1 and 3-6 of the '274 patent are obvious over the '772 Publication, as confirmed by the testimony of Patent Owners' expert in an earlier litigation.

A. Claim 1

1. "A recombinant adeno-associated virus (AAV)"

195. The '772 Publication discloses "[a] recombinant adeno-associated virus (AAV)." *See, e.g.*, EX1007, [0037]; [0023] ("The AAV sequences and fragments thereof are useful in production of rAAV . . ."); [0024] ("The rAAV vectors of the invention are particularly advantageous in rAAV re-administration and repeat gene therapy."), [0086] ("IV. Production of rAAV with Novel AAV Capsids"); [0092] ("The vectors described herein, *e.g.*, a plasmid, are useful for a variety of purposes, but are particularly well suited for use in production of a rAAV containing a capsid comprising AAV sequences or a fragment thereof. These vectors, including rAAV, their elements, construction, and uses are described in detail herein."); [0139] ("Thus, the invention further provides vectors generated using the nucleic acid and amino acid sequences of the novel AAV of the invention. Such vectors are useful for a variety of purposes, including for delivery of therapeutic molecules

Particularly desirable for delivery of therapeutic molecules are recombinant AAV containing capsids of the novel AAV of the invention.”); [0141] (“Using the techniques described herein, one of skill in the art may generate a rAAV having a capsid of a novel serotype of the invention, or a novel capsid containing one or more novel fragments of an AAV serotype identified by the method of the invention.”).

2. “An AAV capsid”

196. The ’772 Publication discloses recombinant AAVs comprising an AAV capsid. *See, e.g., id.*, [0086] (“IV. Production of rAAV with Novel AAV Capsids”); [0092] (“The vectors described herein, *e.g.*, a plasmid, are useful for a variety of purposes, but are particularly well suited for use in production of a rAAV containing a capsid comprising AAV sequences or a fragment thereof.”); [0139] (“Particularly desirable for delivery of therapeutic molecules are recombinant AAV containing capsids of the novel AAV of the invention.”); [0141] (“Using the techniques described herein, one of skill in the art may generate a rAAV having a capsid of a novel serotype of the invention, or a novel capsid containing one or more novel fragments of an AAV serotype identified by the method of the invention.”).

3. “A minigene having AAV inverted terminal repeats and a heterologous gene operably linked to regulatory sequences which direct expression of the heterologous gene in a host cell”

197. The ’772 Publication discloses minigenes, which are DNA constructs that include, for example, therapeutic genes that can be delivered by AAV vectors.

See, e.g., id., [0090] (“Optionally, the vectors of the invention further contain a minigene comprising a selected transgene which is flanked by AAV 5’ ITR and AAV 3’ ITR.”).

198. The version of the therapeutic gene included in the minigene is referred to as a “transgene” or a “heterologous gene,” because it is not endogenous to AAV. *Id.*, [0099] (“The transgene is a nucleic acid sequence, heterologous to the vector sequences flanking the transgene, which encodes a polypeptide, protein, or other product, of interest.”).

199. In addition to the transgene, the minigene includes regulatory sequences that determine how the transgene will be expressed in a cell. *Id.* (“The nucleic acid coding sequence is operatively linked to regulatory components in a manner which permits transgene transcription, translation, and/or expression in a host cell.”).

200. The minigene also includes sequences of DNA from the AAV genome known as “inverted terminal repeats” (“ITRs”), which I discussed above, that are required for viral functions such as replication and packaging. *Id.*, [0097] (“The minigene is composed of, at a minimum, a transgene and its regulatory sequences, and 5’ and 3’ AAV inverted terminal repeats (ITRs).”).

4. “The AAV capsid comprises AAV vp1 proteins, AAV vp2 proteins, and AAV vp3 proteins”

201. The '772 Publication discloses AAV capsids comprising AAV vp1 proteins, AAV vp2 proteins, and AAV vp3 proteins. *Id.*, [0080] (“The sequences of many of the capsid proteins of the invention are provided in an alignment in FIG. 2 and/or in the Sequence Listing, SEQ ID NO: 2 and 60 to 115, which is incorporated by reference herein. The AAV capsid is composed of three proteins, vp1, vp2 and vp3, which are alternative splice variants.”).

202. Based on the vp1 sequences and other disclosures in the '772 Publication, a POSA could readily determine the sequences of the corresponding vp2 and vp3 proteins for a given AAV variant. *Id.*, [0080] (“The full-length sequence provided in these figures is that of vp1. Based on the numbering of the AAV7 capsid [SEQ ID NO:2], the sequences of vp2 span amino acid 138-737 of AAV7 and the sequences of vp3 span amino acids 203-737 of AAV7. With this information, one of skill in the art can readily determine the location of the vp2 and vp3 proteins for the other novel serotypes of the invention.”)

5. “The AAV vp1 proteins have i) the sequence of amino acids 1 to 738 of SEQ ID NO: 4 (AAVrh46), or ii) an amino acid sequence at least 95% identical to the full length of amino acids 1 to 738 of SEQ ID NO: 4”

203. The '772 Publication also discloses the limitation, “wherein the AAV vp1 proteins have i) the sequence of amino acids 1 to 738 of SEQ ID NO: 4

(AAVrh46), or ii) an amino acid sequence at least 95% identical to the full length of amino acids 1 to 738 of SEQ ID NO: 4.”

204. The '772 Publication discloses sequences that are at least 95% identical to the full length of amino acids 1 to 738 of SEQ ID NO: 4. Specifically, the '772 Publication discloses the amino acid sequence of the AAVrh.10 vp1 capsid protein – SEQ ID NO: 81. *Id.*, pp. 264-66, Table 1 (SEQ ID NO: 81, also referred to as “rh.10” and “clone 44.2”) (pp. 112-113), Fig. 2 (pp. 98-103).

205. I have aligned SEQ ID NO: 81 (AAVrh.10) from the '772 Publication with SEQ ID NO: 4 (AAVrh.46) from the '274 patent using Clustal Omega at default settings. Sequence Alignment of AAVrh.46 (SEQ ID NO: 4) from the '274 Patent and AAVrh.10 (SEQ ID NO: 81) from the '772 Publication (EX1018), pp. 1-2. As shown in the alignment, Clustal reports that the percent identity for these two sequences is 97.29% – which is greater than the “at least 95% identical” threshold recited in claim 1. EX1018 (AAVrh.10/AAVrh.46 Alignment), pp. 1-2.

6. “The amino acid residue corresponding to position 665 in SEQ ID NO: 4 is N when aligned along the full length of amino acids 1 to 738 of SEQ ID NO: 4”

206. The '772 Publication also teaches the limitation “wherein the amino acid residue corresponding to position 665 in SEQ ID NO: 4 is N when aligned along the full length of amino acids 1 to 738 of SEQ ID NO: 4.”

(a) The '772 Publication Teaches the Substitution of Asparagine (N) for Serine (S) at Position 665 of AAVrh.10

207. The '772 Publication singles out AAVrh.10 as a particularly preferred embodiment, and touts “the superb tropism of clone 44.2 [AAVrh.10] in lung-directed gene transfer.” *Id.*, [0256]. The '772 Publication also identifies AAV8, which has an asparagine (N) at position 665, as providing “a substantial advantage over the other serotypes in terms of efficiency of gene transfer to liver.” *Id.*, [0250].

208. As I discussed above, the '772 Publication compares the performance of AAVrh.10 to AAV8: “Interestingly, performance of clone 44.2 [AAVrh.10] in liver and muscle directed gene transfer was also outstanding, close to that of the best liver transducer, AAV8 and the best muscle transducer AAV1, suggesting that this novel AAV has some intriguing biological significance.” *Id.*, [0257].

209. As I also discussed above, the '772 Publication teaches that it was particularly important to identify serotypes with good “efficiency of gene transfer to liver that until now has been relatively disappointing” *Id.*, [0250].

210. The '772 Publication thus teaches the superiority of AAVrh.10 overall and in lung in particular, the superiority of AAV8 in liver, and the importance of efficient gene transfer to the liver for a variety of gene therapy applications. Given these teachings and the express comparison between AAVrh.10 and AAV8 in the '772 Publication, a POSA would have considered substitutions between AAVrh.10

(best overall and best in lung) and AAV8 (best in liver) as a promising strategy for obtaining an artificial variant of AAVrh.10 with even more efficient gene transfer in liver.

211. Moreover, given the already superior performance of AAVrh.10 in lung, a POSA would not have sought to make sweeping substitutions throughout the AAVrh.10 sequence, such as changing multiple amino acids at once, for fear of damaging or destroying the desirable properties of AAVrh.10. Instead, a POSA would have taken a finer, more directed approach, modifying a single amino acid residue at a time. *See, e.g.*, EX1032 (Wu), pp. 5-6, Table 1.

212. A POSA would have understood from the alignment in Figure 2 of the '772 publication that AAVrh.10 and AAV8 differ at only 48 positions. EX1007 ('772 Publication), pp. 98-103, Fig. 2, [0071]. I have reproduced the alignment of AAVrh.10 and AAV8 using Clustal O at default settings, similar to the Clustal W program used to create the alignment in Figure 2. Sequence Alignment of AAVrh.10 (SEQ ID NO: 81) and AAV8 (SEQ ID NO: 95) capsid protein sequences from the '772 Publication (EX1019); EX1007 ('772 Publication), [0071].

213. One of the differences between AAV8 and AAVrh.10 is at position 665, where AAV8 has an asparagine (N), and AAVrh.10 has a serine (S). EX1019 (AAVrh.10/AAV8 Alignment). Thus, based on the teaching in Figure 2 and the experimental data regarding AAVrh.10 and AAV8 disclosed in the Examples, a

POSA would have been motivated to make this single amino acid change in AAVrh.10 – namely, substituting an N for the S at position 665. EX1007 ('772 Publication), [0074], [0075] (“An artificial AAV serotype may be, without limitation, a chimeric AAV capsid, a recombinant AAV capsid, or a ‘humanized’ AAV capsid.”).

**(b) Patent Owners’ Expert in an Earlier Litigation
Agrees that the ’772 Publication Teaches the
Substitution of Asparagine (N) for Serine (S) at
Position 665 of AAVrh.10**

214. It is my understanding that, in an earlier litigation against Sarepta, Patent Owners served expert reports that include opinions about how a POSA would understand the teachings of U.S. Patent No. 10,526,617 (“the ’617 patent”), which has the same specification and claims priority to the same applications as the ’772 Publication. EX1014 (’617 patent); Opening Expert Report of Paola Leone (Public Excerpts) (EX1011), 4, ¶10; Rebuttal Expert Report of Paola Leone (Public Excerpts) (EX1012), ¶1; Rebuttal Expert Report of Michael Metzker (Excerpts) (EX1013).

215. In its submissions in the earlier case, Patent Owners contend that a POSA would understand the alignment of AAV capsid protein sequences in Figure 2 to teach the creation of artificial sequences, where an amino acid in a naturally occurring sequence (*e.g.*, AAVrh.10) has been substituted with the amino acid

appearing in the corresponding position in the capsid sequence of a different variant in the alignment. EX1012 (Leone Rebuttal Report), ¶¶313, 316-19.

216. Plaintiffs' expert, Dr. Paola Leone, sets out certain guidelines for the creation of these artificial sequences. First, she explains that, from the alignment in Figure 2, a POSA could readily identify "conserved" and "non-conserved" regions of the aligned capsid protein sequences. *Id.*, ¶315. In her expert report, Dr. Leone includes an annotated version of a portion of Figure 2 where she colored the "conserved" regions in red. *Id.*, ¶316. Dr. Leone explains that, "[p]ersons of ordinary skill in the art would have understood that changes should be avoided in the red regions, and that changes should be concentrated in the regions that are not highlighted in red." *Id.*, ¶318.

217. Next, Dr. Leone explains that for those positions where substitutions may be made, Figure 2 guides a POSA as to which alternative amino acids to choose. According to Dr. Leone, a POSA would have understood to substitute an "alternate amino acid" at one of the non-conserved positions recruited from the corresponding position of one of the other AAV capsid protein sequences in the Figure 2 alignment. *Id.*, ¶398.

218. Dr. Leone concludes that the alignment in Figure 2 teaches a POSA what amino acid positions "can be changed," and for those positions that can be

changed, Figure 2 teaches a POSA “what those changes should be.” EX1012 (Leone Rebuttal Report), ¶¶310, 311.

219. Dr. Leone provides an example in her report of how a POSA would have been guided by Figure 2. *Id.*, ¶319. She examines positions 609 and 610, which she has not highlighted in red, and which she therefore says are positions where a POSA would understand that substitutions may be made. *Id.*

220. The page from Dr. Leone’s annotated version of Figure 2 showing positions 609 and 610 in Figure 2 is reproduced below. Positions 609 and 610 are highlighted in the blue box, and the AAVrh.10 (clone 44_2) capsid protein sequence is indicated with a blue arrow:

	610	620	630	640	650	660
C1\VP1	NATTAPITGNVTAMGVLPQNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
C2\VP1	NATTAPITGNVTAMGVLPQNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
C5\VP182	NATTAPITGNVTAMGVLPQNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
AAV4\VP1	SNSNLETVDRITALDAVPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
AAV1	SSSTDPATGDRHMGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
AAV6\VP1	SSSTDPATGDRHMGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
A3_3	SQNTTASVGSVDSQGLPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
A3_7	SQNTTASVGSVDSQGLPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
A3_4	SQNTTASVGSVDSQGLPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
A3_5	SQNTTASVGSVDSQGLPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
AAV2	EGNQAAZADNTQGVLPQNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
AAV3	SSNTAPTITSTNHCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
13.3b\VP1	AANTAAQIQVWNNQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
AAV7	AANTAAQIQVWNNQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
223_4	AASTAAQIQVWNNQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
223_5	AASTAAQIQVWNNQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
223_10	AASTAAQIQVWNNQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
223_2	AASTAAQIQVWNNQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
223_7	AASTAAQIQVWNNQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
223_6	AASTAAQIQVWNNQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
44_1	QQNAAPIVGAINSCGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
44_5	QQNAAPIVGAINSCGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
44_2	QQNAAPIVGAINSCGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
29.3\VP1	QQNAAPIVGAINSCGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
29.5\VP1	QQNAAPIVGAINSCGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
42_15	QQNAAPIVGAINSCGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
42_8	QQNAAPIVGAINSCGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
42_13	QQNAAPIVGAINSCGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
42_3A	QQNAAPIVGAINSCGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
42_4	QQNAAPIVGAINSCGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
42_5A	QQNAAPIVGAINSCGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
42_1B	QQNAAPIVGAINSCGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
42_5B	QQNAAPIVGAINSCGALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
43_1	QTNGAPIVGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
43_12	QTNGAPIVGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
43_5	QTNGAPIVGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
AAV8	QONTAPQIGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
43_21	AANTQAQIGLHNQGVIPQNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
43_25	AANTQAQIGLHNQGVIPQNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
43_23	AANTQAQIGLHNQGVIPQNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
43_20	AANTQAQIGLHNQGVIPQNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
AAV_9	AANTQAQIGLHNQGVIPQNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
24_1	ESTAGPQIGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
42.2REAL	SSTAGPQIGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
7.2\VP1	SSTAGPQIGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
27.3\VP1	SSTAGPQIGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
16.3\VP1	SSTAGPQIGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
42_10	SSTAGPQIGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
42_3B	SSTAGPQIGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
42_11	SSTAGPQIGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
F1\VP1	SSTAGPQIGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
F5\VP183	SSTAGPQIGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
F3\VP1	SSTAGPQIGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
42_6B	SSTAGPQIGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					
42_12	SSTAGPQIGTNSQCALPGNVVQNRDIYYQGPINAKIPHTDGNTHPSPLMGGFGLNHPFF					

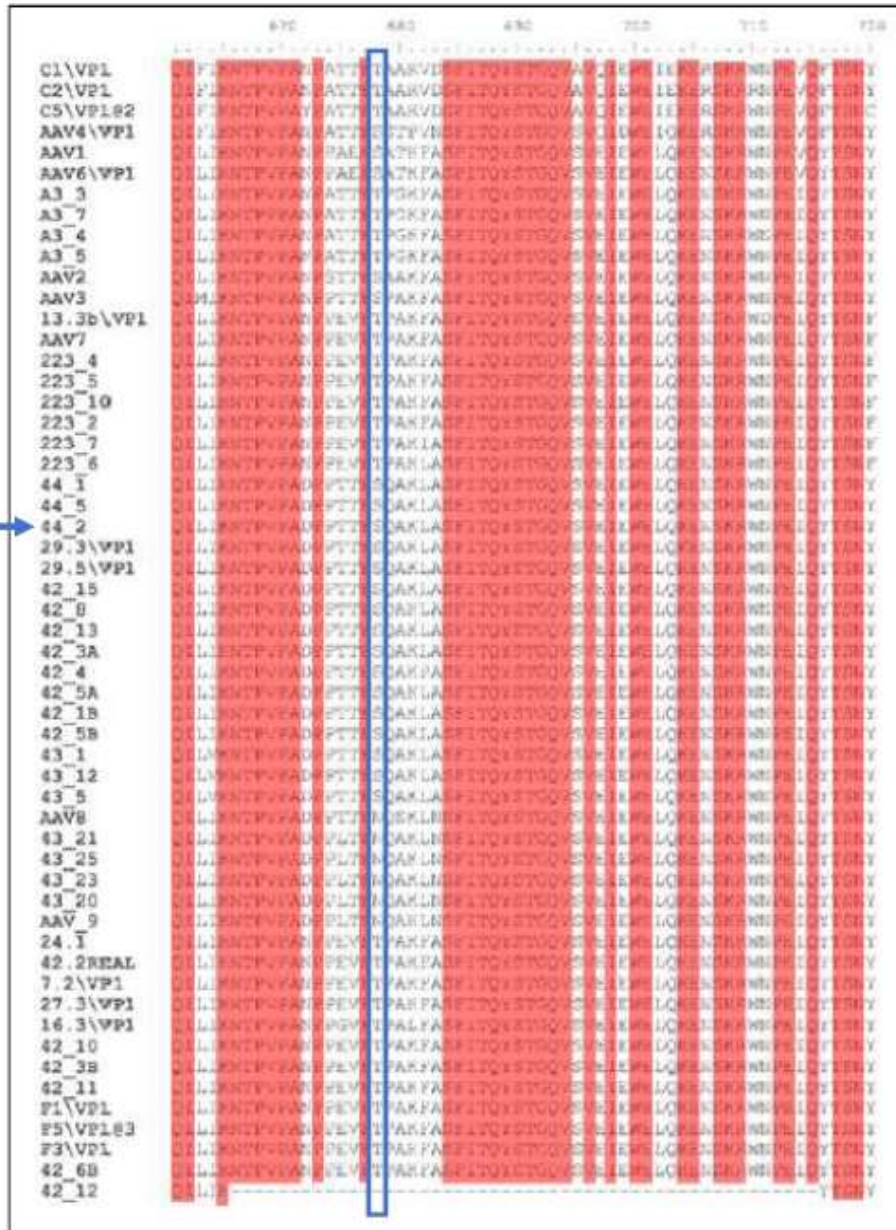
Id., 33 (blue arrow and blue box added).

221. As Dr. Leone explains, the AAVrh.10 sequence (identified as variant “44_2”) has a “G” (glycine) at position 609 and “A” (alanine) at position 610. *Id.*, ¶319. Other sequences in Figure 2 have D (aspartic acid), A (alanine), or Q

(glutamine) at position 609; and N (asparagine), R (arginine), D (aspartic acid), S (serine), T (threonine), V (valine), or L (leucine) at position 610. *Id.*

222. Dr. Leone contends that a POSA would have reasonably expected that changing the G or A in positions 609 and 610 in sequence 44_2 (AAVrh.10) to one of the amino acids present present at the corresponding position in other sequences in Figure 2 would result in a capsid protein that may be used to form an AAV capsid. *Id.*

223. According to Dr. Leone's opinions in the earlier case, one of the substitutions that Figure 2 teaches would be the substitution of asparagine (N) for the serine (S) at position 665 of AAVrh.10. Position 665 in AAVrh.10 corresponds to position 678 in the alignment in Figure 2. The page from Dr. Leone's annotated Figure 2 that includes position 678 is reproduced below. Once again, the relevant position (here position 678) is highlighted in the blue box, and AAVrh.10 (clone 44_2) is indicated by a blue arrow:



Id., 34 (blue arrow and blue box added).

224. As shown in the annotated figure, position 678 is in a non-red, non-conserved region, and therefore is a position at which, according to Dr. Leone, a POSA would understand that substitutions could be made. At this position, AAVrh.10 (variant “44_2”) has an S (serine) amino acid, while other sequences in

the figure have a T (threonine) or N (asparagine) amino acid. Thus, according to Dr. Leone, a POSA would have understood Figure 2 to teach the creation of artificial variants of AAVrh.10, in which T or N has been substituted for the S at position 665 in AAVrh.10.

225. In her report, Dr. Leone also explains that “persons of ordinary skill in the art would have understood from the specification that *the fewer amino or nucleic acids that were changed*, the more likely the sequences would encode proteins that, if expressed, may be used to form an AAV capsid” *Id.*, ¶334 (emphasis added).

226. Applying Dr. Leone’s analysis, the ’772 Publication teaches the creation of artificial variants of AAVrh.10, containing single amino acid substitutions of amino acids at the corresponding positions of other sequences disclosed in Figure 2 – including at position 665, and including the substitution of N for S at that position – resulting in the sequence recited in claim 1.

**(c) A POSA Would Have Had a Reasonable Expectation
of Success in Making the Claimed Combination**

227. A POSA would have had a reasonable expectation of success in creating artificial variants of AAVrh.10 with single amino acid substitutions based on amino acids at corresponding positions of the other sequences disclosed in Figure 2. The creation of these artificial sequences would have required no more than routine experimentation, along with the use of various analytical techniques that were well within the skill of a POSA at the time.

228. First, a POSA would have analyzed the alignment of preferred embodiments AAVrh.10 and AAV8 disclosed in Figure 2 of the '772 Publication. Analyzing such an alignment to determine that the two sequences differed at 48 positions would have been routine for a POSA. The '772 Publication states that it was known in the art how to create and analyze such sequence alignments. EX1007 ('772 publication), [0018].

229. Then, making 48 variants of AAVrh.10, each containing a single substitution, and using those sequences to make rAAV vectors, was well within the skill of a POSA at the time, and required no more than routine experimentation. The '772 Publication states that making mutants of this type was routine experimentation. *Id.*, [0072] (describing site directed mutagenesis as a routine technique).

230. Testing 48 variants of AAVrh.10, each containing a single amino acid substitution, was also well within the skill of a POSA at the time, and required no more than routine experimentation. *Id.*, [0142]. The '772 Publication states that assays for determining the suitability of various AAV vector constructs in different tissues would have been well known to a POSA. *Id.*

231. The opinions of Patent Owners' expert, Dr. Leone, in the earlier litigation confirm my opinion that a POSA would have had a reasonable expectation of success in constructing and testing these 48 variants of AAVrh.10. As Dr. Leone

explained, a 2000 study which “tested 93 different sequences” is “an excellent example of conducting routine experimentation” EX1012 (Leone Rebuttal Report), ¶415 (citing Wu (EX1032)).

232. Moreover, a POSA would have had a reasonable expectation of success that a variant of AAVrh.10 with a serine (S) to asparagine (N) substitution at position 665 would form a stable AAV capsid capable of packaging a minigene. According to Patent Owners’ expert, Dr. Leone, a POSA would have known with a “reasonable degree of probability” that modified AAVrh.10 capsid sequences having only a small number of mutations – such as a single substitution according to the alignment in Figure 2 – would result in proteins that “may be used to form an AAV capsid.” *See id.*, ¶¶323, 334, 406.

233. Therefore, because a POSA would have been motivated to create a variant of AAVrh.10 containing an asparagine (N) instead of a serine (S) at position 665, and because that person would have had a reasonable expectation of success in making that variant, it is my opinion that claim 1 is obvious over the ’772 Publication.

B. Claim 3: “The recombinant AAV according to claim 1, wherein the AAV vp1 capsid proteins have an amino acid sequence which is at least 97% identical to the full length of amino acids 1 to 738 of SEQ ID NO: 4, wherein the amino acid residue corresponding to position 665 is N when aligned along the full length of amino acids 1 to 738 of SEQ ID NO: 4”

234. As I discussed above with respect to claim 1, the alignment of the AAVrh.10 capsid protein from the '772 Publication (SEQ ID NO: 81) with the AAVrh.46 capsid protein from the '274 patent (SEQ ID NO: 4) yields a percent identity of 97.29% – which meets the “at least 97% identical” threshold recited in claim 3. EX1018, pp. 1-2 (AAVrh.10/AAVrh.46 Alignment). As I discussed above for claim 1, a POSA would have been motivated to create this construct and would have had a reasonable expectation of success in creating it.

235. Therefore, in my opinion, dependent claim 3 of the '274 patent is obvious over the '772 Publication, as confirmed by the opinions of Patent Owners' expert in the earlier litigation.

C. Claim 4: “The recombinant AAV according to claim 1, wherein the AAV inverted terminal repeats are from a different AAV than the AAV supplying the capsid proteins”

236. The '772 Publication teaches the recombinant AAV of claim 1 with inverted terminal repeats from a different AAV than the AAV supplying the capsid proteins. In particular, the '772 Publication discloses a triple transfection method for creating recombinant AAVs where the AAV ITRs are from a different AAV than the AAV supplying the capsid protein. Using this method, a plasmid carrying the

cap gene (coding for the AAV capsid proteins) of one of the AAV variants – such as AAVrh.10 – is co-transfected with another plasmid containing the ITRs from a different AAV variant – such as AAV2: “Chimeric packaging constructs are generated by fusing AAV2 rep with cap sequences of novel AAV serotypes. These chimeric packaging constructs are used, initially, for pseudo typing recombinant AAV genomes carrying AAV2 ITRs by triple transfection in 293 cell using AdS helper plasmid.” EX1007 (’772 Publication), [0214].

237. A POSA would have been motivated to create, and would have had a reasonable expectation of success in creating, an rAAV where the AAV ITRs are from a different AAV than the AAV supplying the capsid protein to evaluate the transduction efficiency of different AAV variants: “These pseudotyped vectors are used to evaluate performance in transduction-based serological studies and evaluate gene transfer efficiency of novel AAV serotypes in different animal models including NHP and rodents, before intact and infectious viruses of these novel serotypes are isolated.” *Id.*

238. Here, the ’772 Publication discloses creating recombinant AAVs through triple transfection. Using this method, a plasmid carrying the *cap* gene (coding for the AAV capsid proteins) of one of the variants, such as AAVrh.10, is co-transfected with another plasmid containing the ITRs from a different AAV variant – here AAV2.

239. Therefore, in my opinion, dependent claim 4 of the '274 patent is obvious over the '772 Publication, as confirmed by the opinions of Patent Owners' expert in the earlier litigation.

D. Claim 5: “A composition comprising the recombinant AAV according to claim 1 and a physiologically compatible carrier”

240. The '772 Publication teaches a composition comprising the recombinant AAV according to claim 1 and a physiologically compatible carrier. *Id.*, [0148] (“The above-described recombinant vectors may be delivered to host cells according to published methods. The rAAV, preferably suspended in a physiologically compatible carrier, may be administered to a human or non-human mammalian patient. Suitable carriers may be readily selected by one of skill in the art in view of the indication for which the transfer virus is directed. For example, one suitable carrier includes saline, which may be formulated with a variety of buffering solutions (*e.g.*, phosphate buffered saline). Other exemplary carriers include sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, peanut oil, sesame oil, and water. The selection of the carrier is not a limitation of the present invention.”).

241. As the '772 Publication teaches, for example in the passage quoted above, a POSA would have been motivated to create a composition with the rAAV and with a physiological carrier to deliver the rAAV to host cells in a patient, and

could have done so according to methods that were known in the art. Thus, a POSA would have had a reasonable expectation of success in creating this composition.

242. Therefore, in my opinion, dependent claim 5 is obvious over the '772 Publication, as confirmed by the opinions of Patent Owners' expert in the earlier litigation.

E. Claim 6: “The recombinant AAV according to claim 1, wherein the heterologous gene encodes an ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, arginase, fumarylacetate hydrolase, carbamoyl phosphate synthetase I, phenylalanine hydroxylase, alpha-1 antitrypsin, glucose-6-phosphatase, porphobilinogen deaminase, cystathione beta-synthase, branched chain ketoacid decarboxylase, isovaleryl-coA dehydrogenase, propionyl-CoA carboxylase, methylmalonyl-CoA mutase, glutaryl-CoA dehydrogenase (GCDH), betaglucosidase, pyruvate carboxylate, hepatic phosphorylase, phosphorylase kinase, β -glucuronidase (GUSB), glycine decarboxylase, a low density lipoprotein (LDL) receptor, high density lipoprotein (HDL) receptor, very low density lipoprotein (VLDL) receptor, scavenger receptor, glucocorticoid receptor, estrogen receptor, Vitamin D receptor, nuclear receptor, cystic fibrosis transmembrane regulator (CFTR) sequence, Factor IX or variants thereof, Factor VIII or variants thereof, a dystrophin gene product, or an immunoglobulin”

243. The '772 Publication teaches the recombinant AAV according to claim 1 wherein the heterologous gene encodes any of the proteins in the list recited in dependent claim 6. Specifically, the '772 Publication identifies the following proteins listed in claim 6 as possible heterologous genes in the recombinant AAVs disclosed in the '772 Publication:

Other useful gene products include, carbamoyl synthetase 1, ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, arginase, fumarylacetate hydrolase, phenylalanine hydroxylase, alpha-1 antitrypsin, glucose-6-phosphatase, porphobilinogen deaminase, factor VIII, factor IX, cystathione beta-synthase, branched chain ketoacid decarboxylase, . . . isovaleryl-coA dehydrogenase, propionyl CoA carboxylase, methyl malonyl CoA mutase, glutaryl CoA dehydrogenase, . . . betaglucosidase, pyruvate carboxylase, hepatic phosphorylase, phosphorylase kinase, glycine decarboxylase, H-protein, T-protein, a cystic fibrosis transmembrane regulator (CFTR) sequence, and a dystrophin cDNA sequence.

Id., [0155], [0157].

244. As the '772 Publication teaches, a POSA would have been motivated to create rAAVs with these transgenes to treat various diseases where the transgenes could supply a missing or defective gene product.

[0102] However, desirably, the transgene is a non-marker sequence encoding a product which is useful in biology and medicine, such as proteins, peptides, RNA, enzymes, or catalytic RNAs. Desirable RNA molecules include tRNA, dsRNA, ribosomal RNA, catalytic RNAs, and antisense RNAs. One example of a useful RNA sequence is a sequence which extinguishes expression of a targeted nucleic acid sequence in the treated animal.

[0103] The transgene may be used to correct or ameliorate gene deficiencies, which may include deficiencies in which normal genes are expressed at less than normal levels or deficiencies in which the functional gene product is not expressed. A preferred type of transgene sequence encodes a therapeutic protein or polypeptide which is expressed in a host cell.

Id., [0102], [0103]

245. The '772 Publication also teaches that a POSA could have selected these transgenes and created rAAVs with these transgenes using techniques that were known in the art (along with the teachings of the '772 Publication). *Id.*, [0104] (“Suitable transgenes may be readily selected by one of skill in the art. The selection of the transgene is not considered to be a limitation of this invention.”); [0113] (“The combination of the transgene, promoter/enhancer, and 5' and 3' ITRs is referred to as a ‘minigene’ for ease of reference herein. Provided with the teachings of this invention, the design of such a minigene can be made by resort to conventional techniques.”).

246. Thus, a POSA would have had a reasonable expectation of success in creating rAAVs with the transgenes recited in claim 6.

247. Therefore, in my opinion, dependent claim 6 is obvious over the '772 Publication, as confirmed by the opinions of Patent Owners’ expert in the earlier litigation.

F. Secondary Considerations Do Not Change the Conclusion of Obviousness

248. For evidence of “secondary considerations” to be informative of obviousness, I understand that there must be a “nexus” or link between the alleged secondary consideration and the subject matter recited in the Asserted Claims. I am not aware of any secondary considerations of non-obviousness with the required nexus to the claims of the '274 patent. For example, I am not aware of any

commercial success attributable to a recombinant AAV vector having an AAVrh.46 capsid protein sequence with an asparagine (N) at position 665.⁵ Similarly, I am not aware of any licenses directed specifically to the '274 patent or the subject matter recited in challenged claims 1 and 3-6.

249. Finally, I am not aware of any unexpected results having a nexus to the claimed subject matter. The '274 patent does not disclose unexpected properties of an rAAV vector with capsid proteins having the amino acid sequence of AAVrh.46. To the extent the asparagine (N) at position 665 in the AAVrh.46 sequence eliminates a phosphorylation site found in other naturally occurring AAV capsid sequences, the potential benefits were not unexpected to a POSA. Instead, as I discussed above, the effects of phosphorylation on the biological properties of rAAV vectors, such as transduction efficiency, had been studied before the '274 patent and disclosed in prior art references such as Snowdy.

⁵ If Patent Owners attempt to rely on the commercial success of Sarepta's gene therapy treatment for Duchenne muscular dystrophy – ElevidysTM – there is no nexus to the challenged claims of the '274 patent. I understand that ElevidysTM uses the vp1 capsid protein from a different, naturally occurring AAV variant – AAVrh.74. Patent Owners cannot show a nexus between the commercial success of ElevidysTM and the AAVrh.46 sequence recited in the challenged claims.

250. To the extent that an asparagine (N) at position 665 of AAVrh.46 confers any favorable properties on AAVrh.46 for use as a gene therapy vector or otherwise, the '274 patent contains no such discussion. In fact, the '274 patent contains no discussion of position 665 in AAVrh.46 at all, other than the recitation in the claims.

251. To the extent Patent Owners attempt to raise secondary considerations that have only a marginal nexus, if any, to claims 1 and 3-6 of the '274 patent, such evidence of secondary considerations should not outweigh the compelling evidence of obviousness, discussed above. Thus, secondary considerations do not alter my opinion that claims 1 and 3-6 of the '274 patent are obvious over the '772 Publication, as confirmed by Patent Owners' expert in the earlier litigation.

XI. GROUND 2: CLAIMS 1 AND 3-6 ARE OBVIOUS OVER THE '772 PUBLICATION IN COMBINATION WITH XIE

252. As I discussed above, a POSA would have been motivated, based on the disclosures of the '772 Publication, to combine the preferred embodiment, AAVrh.10, with elements of another preferred embodiment, AAV8, to improve the beneficial properties of AAVrh.10 as a gene therapy vector. Such a combination would have led a POSA to create 48 artificial variants of AAVrh.10, each containing a single amino acid substitution with the amino acid at the corresponding position of AAV8. Combining the '772 Publication with Xie would have further directed a POSA to 18 of these 48 artificial variants.

253. Specifically, Xie discloses that 18 of the 48 amino acids that differ between AAVrh.10 and AAV8 lie on the surface of the capsid, and teaches that amino acids on the surface of the capsid govern characteristics of AAV such as tropism, transduction efficiency, and immunogenicity.

A. A POSA Would Have Been Motivated to Combine the '772 Publication with Xie to Obtain an AAVrh.10 Sequence with an Asparagine (N) Substitution at Position 665

254. As I discussed above for Ground 1, the '772 Publication discloses elements 1[pre], 1[a], 1[b], 1[c], and 1[d] in independent claim 1 of the '274 patent.

1. Applying the Teachings of Xie, a POSA Would Have Identified 18 Amino Acids on the Surface of the AAV Capsid as the Most Promising Substitution Sites in AAVrh.10

255. In considering which mutations to make in the AAVrh.10 capsid to confer some of the favorable properties of AAV8 on AAVrh.10, a POSA would have routinely consulted available crystal structures of AAV. In fact, another of Patent Owners' experts in the earlier litigation, Dr. Michael Metzker, stated that, in addition to looking at the one-dimensional sequences aligned in Figure 2 of the '772 Publication to determine where to make substitutions in AAVrh.10, "one of ordinary skill in the art would have consulted known crystal structures of related sequences to guide where to introduce mutations to a sequence." EX1013 (Metzker Rebuttal Report), ¶306.

256. A POSA would therefore have consulted the 2002 crystal structure of the AAV2 vp3 capsid protein published by Xie in 2002. EX1008 (Xie). As I discussed above, Xie teaches that the amino acids that lie on the surface of the capsid “govern interactions with antibodies and cellular receptors.” *Id.*, p. 3. A POSA at the time would have understood this disclosure to mean that the amino acids on the surface of the capsid govern the tropism, transduction efficiency, and immunogenicity of the AAV virus.

257. Therefore, a POSA, motivated by the '772 Publication to create 48 variants of AAVrh.10, each containing a single substitution with the amino acid at the corresponding position of AAV8, would have further focused on those variants of the 48 that involved amino acids that lie on the surface of the capsid according to Xie. *Id.*, pp. 3-4, Fig. 2, Fig. 2 legend, Fig. 4, Fig. 4 legend.

258. Xie discloses that 18 of the 48 amino acids that differ between AAVrh.10 and AAV8 lie on the surface of the capsid. *Id.*, 3-4, Fig. 2, Fig. 4, Fig. 4 legend; Table Showing Xie Surface Amino Acids (EX1023), 1-2. I have created a table of all the amino acids on the surface of the viral capsid according to Figures 2 and 4 of Xie. EX1023 (Xie Surface Table), pp. 1-2. In this table, I have highlighted

the 18 amino acids that differ between AAVrh.10 and AAV8 and lie on the surface of the capsid. *Id.*, pp. 1-2.⁶

⁶ I note that the numbering of the AAV2 amino acids in Xie is different from the numbering in the AAVrh.10/AAV8 alignment for two reasons. First, the AAV2 capsid protein has three fewer amino acids than the capsid proteins of AAVrh.10 and AAV8 (735 vs. 738). *See* Sequence Alignment of AAV2, AAVrh.10, and AAV8 Capsid Protein Sequences (EX1020). As a result, the positions 269, 496, 665, and 722 in AAVrh.10 and AAV8 correspond to positions 266, 493, 662, and 719 in AAV2. Second, Xie numbers the amino acids in the structure according to their position in vp2. EX1008 (Xie), p. 2 (“Although we follow the convention of VP2 numbering for parvoviral structures, it is the 533-residue VP3 that is visualized, along with the corresponding regions of VP1 and VP2 occupying equivalent positions in the capsid, and not the N termini unique to the minority proteins. All except 14 N-terminal residues of VP3 were clearly traceable, and no ambiguity occurred in the alignment of the chemical sequence to the atomic structure.”). Given that the start codon for vp2 is at amino acid 138 of vp1, to correlate the amino acid numbering in Xie to the amino acid numbering of vp1, it is necessary to subtract 137 from the vp1 numbering to obtain the Xie numbering – so that, for example, amino acid 1 of vp2 corresponds to amino acid 138 of vp1. As a result, it is necessary to

259. Therefore a POSA would have been motivated by the '772 publication in view of Xie to focus on mutating the 18 amino acids of AAVrh.10 that lie on the surface of the capsid out of the 48 total differences between AAVrh.10 and AAV8.

2. One of the 18 Most Promising Substitution Sites Is the Serine (S) at Position 665

260. Xie further discloses that the amino acid in AAV2 corresponding to the serine at position 665 of AAVrh.10 is also a serine that lies on the surface of the capsid. EX1008, 3-4, Fig. 2, Fig. 2 legend, Fig. 4, Fig. 4 legend. I have circled and marked with a blue arrow the serine that corresponds to position 665 of AAVrh.10 in Fig. 2 from Xie below:

subtract 137 from each AAV2 vp1 position to obtain the numbering in Xie Figures 2 and 4. EX1023 (Xie Surface Table) shows the numbering in AAV2, AAV8, AAVrh.10, and Xie Figures 2 and 4.

experimentation, along with the use of various analytical techniques that were well within the skill of a POSA at the time.

264. Moreover, it would have been well within the level of ordinary skill in the art at the time to have consulted the published crystal structure of the AAV2 capsid to determine which amino acids are positioned on the surface, and to have correlated those with the corresponding amino acids in AAVrh.10 and AAV8. EX1013 (Metzker Rebuttal Report), ¶306.

265. Additionally, Xie teaches that the surface amino acids in the AAV capsid tend to be in loop structures of the vp3 capsid protein, which are more flexible than, for example, the core, interior beta barrel of the vp3 protein, and therefore, likely more tolerant of single amino acid substitutions. EX1008, p. 3 (“These loops constitute the surface features of the virus that govern interactions with antibodies and cellular receptors.”).

266. For these reasons, a POSA would have had a reasonable expectation of success in combining the ’772 Publication with Xie to obtain an rAAV vector having the combination of elements recited in claim 1.

267. Thus, in my opinion, claim 1 of the ’274 patent is obvious over the combination of the ’772 Publication and Xie.

C. Claims 3-6 Are Obvious Over the '772 Publication and Xie

268. As I discussed above with respect to claim 1, a POSA would have been motivated to combine the '772 Publication with Xie. As I discussed above for Ground 1, claims 3-6, the '772 Publication teaches each of the additional elements of these claims. As I further discussed above for Ground 1 and for claim 1 of Ground 2, a POSA would have had a reasonable expectation of success in combining the '772 Publication with Xie to obtain an rAAV vector having the combination of elements recited in claims 3-6.

269. Thus, in my opinion, claims 3-6 of the '274 patent are obvious over the combination of the '772 Publication and Xie.

D. Secondary Considerations Do Not Change the Conclusion of Obviousness

270. For the reasons I discussed above for Ground 1, secondary considerations do not alter my opinion that claims 1 and 3-6 of the '274 patent are obvious over the combination of the '772 Publication and Xie.

XII. GROUND 3: CLAIMS 1 AND 3-6 ARE OBVIOUS OVER THE '772 PUBLICATION IN COMBINATION WITH SNOWDY

A. A POSA Would Have Been Motivated to Combine the '772 Publication with Snowdy to Obtain an AAVrh.10 Sequence with an Asparagine (N) Substitution at Position 665

271. As I discussed above for Ground 1, the '772 Publication discloses elements 1[pre], 1[a], 1[b], 1[c], and 1[d] in independent claim 1 of the '274 patent.

1. Snowdy Teaches That Mutating Phosphorylatable Amino Acids in the AAV Capsid to Non-Phosphorylatable Amino Acids Improved Transduction Efficiency

272. A POSA understood that, as I discussed above in Section V.B.1, certain amino acids were particularly interesting in viral capsids because they have the capability to be phosphorylated. Serine, threonine, and tyrosine are the amino acids most commonly phosphorylated. EX1033 (Blom), Abstract. It would have been well known to a POSA that phosphorylation of proteins was an important mechanism for regulation of their stability and degradation in cells, and was the subject of a great deal of research. EX1035 (Chen), Abstract; EX1036 (Lange), Abstract; EX1037 (Patrick), Abstract; EX1038 (Penrose), Abstract; EX1039 (Whiteside), Abstract. A POSA would also have understood that phosphorylation plays an important role in the regulation and function of viral proteins, in particular, viral capsid proteins. EX1034 (Ivanov), Abstract; EX1038 (Penrose), Abstract.

273. In particular, a POSA would have understood that mutation of phosphorylatable residues such as serine to non-phosphorylatable residues improved the stability and decreased the rate of degradation of various proteins. EX1035 (Chen), pp. 5-7; EX1036 (Lange), Abstract; EX1037 (Patrick), p. 7; EX1038 (Penrose), Abstract; EX1039 (Whiteside), p. 4.

274. Snowdy investigated the effects on the function of AAV of mutating phosphorylatable amino acids in AAV capsids to non-phosphorylatable amino acids,

with the goal of improving AAV as a gene therapy vector. EX1009 (Snowdy), pp. 4-5, 125-30 (reporting that an S181A mutation in the AAV2 capsid improved the transduction efficiency of the virus). Snowdy found that mutating a serine in the AAV2 capsid to a non-phosphorylatable alanine improved the transduction efficiency of AAV2. *Id.*, pp. 4-5, 125-30. Snowdy also teaches the substitution of asparagine (N) as a nonphosphorylatable amino acid to remove a phosphorylation site. *Id.*, pp. 28-29 (citing Jans & Jans (EX1017)).

275. Moreover, as I discussed above in Section VIII.C.2, Snowdy teaches that the increased transduction efficiency resulting from removing a capsid phosphorylation site could not be readily attributed to mechanisms relating to an NLS (nuclear localization signal). *Id.*, pp. 128-29. Therefore, Snowdy implicates a different mechanism and phosphorylation sites throughout the capsid, not merely those in the vicinity of an NLS. *Id.*

276. Therefore, a POSA at the time seeking to improve the transduction of AAVrh.10, would have been motivated to combine the '772 publication with Snowdy, given that Snowdy disclosed a method of improving the transduction of AAV vectors by removing phosphorylatable sites in the capsid proteins.

2. Applying the Teachings of Snowdy, a POSA Would Have Identified Four Phosphorylatable Amino Acids as the Most Promising Substitution Sites in AAVrh.10, One of Which Is the Serine (S) at Position 665

277. A POSA seeking to improve the transduction of AAVrh.10 in liver to approach that of AAV8, in view of Snowdy's teaching to substitute phosphorylatable amino acids with non-phosphorylatable amino acids, would have looked to see which positions in AAVrh.10 had phosphorylatable amino acids, where the corresponding position in AAV8 had a non-phosphorylatable amino acid.

278. Snowdy used a program, NetPhos – which would have been known to a POSA at the time – to determine which phosphorylatable amino acids in a protein are most likely to be the actual targets of kinases – enzymes that phosphorylate proteins. *See id.*, 125. As Snowdy states, it was known in the art that the context – meaning the amino acid sequence surrounding a phosphorylatable amino acid such as serine – could determine the probability that a given serine was phosphorylated by one or more kinases. *Id.*

279. When the AAVrh.10 sequence is run through NetPhos, the program identifies a number of different potential phosphorylation sites in AAVrh.10. EX1021 (NetPhos/ AAVrh.10 Phosphorylation Sites). However, there are only four such predicted phosphorylated positions in AAVrh.10 that correspond to non-phosphorylatable amino acids in AAV8 – S269A, S496G, S665N, and T722V – one

of which is S665N.^{7,8} EX1022 (Sequence Alignment of AAVrh.10 (SEQ ID NO: 81) and AAV8 (SEQ ID NO: 95) Capsid Protein Sequences from the '772

⁷ There is one additional serine in AAVrh.10, at position 559, which is identified by NetPhos as a phosphorylation site. EX1022 (AAVrh.10/AAV8 Phosphorylation Sites); EX1021 (NetPhos-AAVrh.10), p. 17. The corresponding amino acid at position 559 of AAV8, is an aspartic acid (D), which is technically a non-phosphorylatable amino acid. EX1022 (AAVrh.10/AAV8 Phosphorylation Sites). In my opinion, however, a POSA, in light of Snowdy, would not make this substitution (S559D), because Snowdy teaches expressly that substituting aspartic acid (D), for serine (S) almost completely eliminated the ability of AAV to infect HeLa cells. EX1009 (Snowdy), pp. 5, 126. Moreover, a POSA would have understood that aspartic acid (D), while technically “non-phosphorylatable,” was used to simulate a constitutively phosphorylated state. EX1009 (Snowdy), p. 125; EX1017 (Jans & Jans), p. 3.

⁸ Snowdy also raises the issue of whether any given phosphorylatable amino acid resides on the surface of the AAV capsid. EX1009 (Snowdy), pp. 135-36. As Xie discloses, three out of four of the positions where AAVrh.10 has a phosphorylatable amino acid and AAV8 has a nonphosphorylatable amino acid (S496G, S665N, and T722V) are on the surface of the AAV2 capsid, where they

Publication Showing Differences In Phosphorylation Sites); EX1021 (NetPhos/AAVrh.10 Phosphorylation Sites), pp. 7, 15, 20, 22-23.

280. As such, a POSA at the relevant time would have been motivated to construct all four of these mutant versions of AAVrh.10, and could have done so using only routine experimentation, per Dr. Leone's opinion in the earlier litigation, and as I discussed above for Ground 1. EX1012 (Leone Rebuttal Report), ¶415. One of these four mutations would be a substitution of asparagine (N) for serine (S) at position 665 of AAVrh.10. And, as I discussed above, Snowdy teaches that the increased transduction efficiency resulting from removing a capsid phosphorylation site could not be readily attributed to mechanisms relating to the NLS. Therefore, Snowdy implicates a different mechanism and phosphorylation sites throughout the capsid, not merely those in the vicinity of the NLS. EX1009 (Snowdy), pp. 128-30.

would be accessible to kinases. EX1008, pp. 3-4, Fig. 2, Fig. 2 legend, Fig 4, Fig. 4 legend; EX1023 (Xie Surface Table). Therefore, a POSA would have understood these three phosphorylatable positions in AAVrh.10 to be particularly interesting targets for mutagenesis to substitute with the corresponding non-phosphorylatable amino acid from AAV8.

281. As I also discussed above, Snowdy teaches the substitution of asparagine (N) as a nonphosphorylatable amino acid to remove a phosphorylation site. EX1009 (Snowdy), pp. 28-29 (citing Jans & Jans (EX1017)).

B. A POSA Would Have Had a Reasonable Expectation of Success in Combining the '772 Publication with Snowdy

282. For the reasons discussed above regarding Grounds 1 and 2, the combination of the '772 Publication with Snowdy to create substituted versions of AAVrh.10 would have required nothing more than routine experimentation, along with the use of various analytical techniques that were well within the skill of a POSA at the time. Therefore, a POSA would have had a reasonable expectation of success in making this combination.

283. It would have been routine for a POSA in view of Snowdy to have determined which of the 48 positions that differed between AAVrh.10 and AAV8 involved phosphorylatable amino acids in AAVrh.10, and then to have identified the subset of these positions at which the corresponding position in AAV8 had a non-phosphorylatable amino acid. EX1009 (Snowdy), p. 125; EX1033 (Blom), Abstract; EX1021 (NetPhos-AAVrh.10); EX1022 (AAVrh.10/AAV8 Phosphorylation Sites).

284. Given that the Snowdy reference is a Ph.D. thesis, Snowdy was likely at or below the level of a POSA at the time. Therefore, Snowdy's use of the NetPhos program to determine phosphorylation sites in an AAV capsid was also well within

the level of ordinary skill in the art at the time. EX1009 (Snowdy), p. 125; EX1033 (Blom), Abstract.

285. Making four variants of AAVrh.10, each containing a single substitution of a non-phosphorylatable amino acid for a phosphorylatable amino acid, and using those sequences to make rAAV vectors was well within the skill of a POSA at the time, and required no more than routine experimentation – as I discussed above for Ground 1. Indeed, Snowdy itself discloses this type of mutagenesis. EX1009 (Snowdy), pp. 125-126. And Snowdy found that “[a]ll of the mutants were capable of forming virus at normal titers,” confirming that a POSA would have had a reasonable expectation of success that the four AAVrh.10 capsid variants would similarly form rAAVs. *Id.*, p. 126.

286. For these reasons, a POSA would have had a reasonable expectation of success in combining the ’772 Publication with Snowdy to obtain an rAAV vector having the combination of elements recited in claim 1.

287. Thus, in my opinion, claim 1 of the ’274 patent is obvious over the combination of the ’772 Publication and Snowdy.

C. Claims 3-6 Are Obvious Over the ’772 Publication and Snowdy

288. As I discussed above for claim 1, a POSA would have been motivated to combine the ’772 Publication with Snowdy. As I discussed above for Ground 1, claims 3-6, the ’772 Publication teaches each of the additional elements of these

claims. As I further discussed above for Ground 1 and for claim 1 of Ground 3, a POSA would have had a reasonable expectation of success in combining the '772 Publication with Snowdy to obtain an rAAV vector having the combination of elements recited in each of claims 3-6. Thus, claims 3-6 of the '274 patent are obvious over the combination of the '772 Publication and Snowdy.

D. Secondary Considerations Do Not Change the Conclusion of Obviousness

289. For the reasons I discussed above for Ground 1, secondary considerations do not alter my opinion that claims 1 and 3-6 of the '274 patent are obvious over the combination of '772 Publication and Snowdy.

XIII. GROUND 4: CLAIM 8 IS OBVIOUS OVER THE '772 PUBLICATION, AS CONFIRMED BY THE OPINIONS OF PATENT OWNERS' EXPERT IN EARLIER LITIGATION, AND FABB

A. A POSA Would Have Been Motivated to Combine the '772 Publication with Fabb

290. Claim 8 recites: "The recombinant AAV according to claim 6, wherein the dystrophin gene product is a mini-dystrophin or micro-dystrophin."

291. The '772 Publication discloses "a dystrophin cDNA sequence." EX1007 ('772 Publication), [0157]. The '772 Publication also discloses the issue, well known to those of skill in the art at the time, that AAV vectors have a size limit on the transgenes that they can package and deliver. *Id.*, [0103], [0104]; EX1031 (Grimm), 6; EX1010 (Fabb), Abstract (reporting the use of a < 3.8kb micro-dystrophin cDNA in mice). The '772 Publication states:

In certain situations, a different transgene may be used to encode each subunit of a protein, or to encode different peptides or proteins. *This is desirable when the size of the DNA encoding the protein subunit is large, e.g., for an immunoglobulin, the platelet derived growth factor, or a dystrophin protein.*

Suitable transgenes may be readily selected by one of skill in the art.

EX1007 ('772 Publication), [0103], [0104] (emphasis added).

292. A POSA reading these disclosures would have understood that the large dystrophin gene would need to be reduced in size to be packaged in an AAV vector. The reference in the '772 Publication to a “dystrophin cDNA sequence” would also have indicated to a POSA that the '772 Publication teaches the use of a reduced size dystrophin made from cDNA rather than the longer, native DNA encoding the entire dystrophin gene.

293. A POSA at the time would have further understood that the full length cDNA of the dystrophin gene (14kb) is too large to be packaged into an AAV vector. EX1010 (Fabb), Abstract; EX1031 (Grimm), p. 6 (“Since the capsids of AAV types 1 to 6 are similar in diameter (this is likely also true for AAV-7 and -8), the size of the foreign DNA to be encapsidated, including the ITRs, must not exceed the approximate 5 kb packaging limit of AAV”). A POSA would therefore have been

motivated to create DNA constructs smaller in length than the full length dystrophin cDNA for use as part of an AAV vector.

294. Fabb teaches the construction of a smaller version of the dystrophin gene known as a “micro-dystrophin” for use with an AAV vector. EX1010 (Fabb), Abstract, pp. 2-3. Indeed, Fabb teaches that a < 3.8 kb micro-dystrophin delivered as part of an AAV gene therapy vector restored dystrophin associated protein complexes and inhibited degenerative dystrophic muscle pathology in an animal model. *Id.*, Abstract.

295. A POSA would therefore have been motivated to combine the ’772 publication with Fabb to create such a micro-dystrophin construct.

B. A POSA Would Have Had a Reasonable Expectation of Success in Combining the ’772 Publication with Fabb

296. A POSA would have needed only routine experimentation to create a micro-dystrophin that could fit within an AAV capsid. Given that working examples of such constructs were already known in the art, including the example disclosed in Fabb, a POSA could have used Fabb as a guide to create a micro-dystrophin that would fit within the claimed AAV capsid. *Id.*, pp. 2-3. A POSA would have needed only routine techniques to create such a micro-dystrophin, as taught by the ’772 publication, which states:

The preparation of a host cell according to this invention involves techniques such as *assembly of selected DNA sequences*. This assembly may be accomplished utilizing

conventional techniques. *Such techniques include cDNA and genomic cloning, which are well known* and are described in Sambrook et al., cited above, use of overlapping oligonucleotide sequences of the adenovirus and AAV genomes, combined with polymerase chain reaction, synthetic methods, and any other suitable methods which provide the desired nucleotide sequence.

EX1007 ('772 Publication), [0135] (emphases added). Given that a micro-dystrophin is constructed from a dystrophin cDNA, the cDNA techniques described in the '772 Publication as routine were the same techniques that would have been used by a POSA to create a micro-dystrophin. EX1010 (Fabb), pp. 2-3.

297. Therefore, in my opinion, claim 8 of the '274 patent is obvious over the combination of the '772 Publication with Fabb.

C. Secondary Considerations Do Not Change the Conclusion of Obviousness

298. For the reasons discussed above regarding Ground 1, secondary considerations do not alter my opinion that claim 8 of the '274 patent is obvious over the combination of the '772 Publication with Fabb.

XIV. GROUND 5: CLAIM 8 IS OBVIOUS OVER THE '772 PUBLICATION AND XIE, IN COMBINATION WITH FABB

A. A POSA Would Have Been Motivated to Combine the '772 Publication and Xie with Fabb

299. For the reasons discussed above regarding Ground 2, a POSA would have been motivated to combine the '772 Publication with Xie to improve the properties of AAVrh.10 as a gene therapy vector, by creating variants of AAVrh.10,

each containing a substitution of a single amino acid in the capsid protein from the preferred embodiment AAV8, where the substituted amino acids lie on the surface of the AAV capsid.

300. A POSA seeking to use such a variant of AAVrh.10 as a delivery vehicle for a dystrophin gene would have been motivated to combine the '772 Publication and Xie with Fabb, which teaches the construction of a smaller version of the dystrophin gene known as a “micro-dystrophin” for use with an AAV vector. EX1010 (Fabb), pp. 2-3.

301. A POSA would therefore have been motivated to combine the '772 publication and Xie with Fabb to create such a micro-dystrophin.

B. A POSA Would Have Had a Reasonable Expectation of Success in Combining the '772 Publication and Xie with Fabb

302. For the reasons discussed above with respect to Ground 4, a POSA would have needed only routine experimentation to create a micro-dystrophin that could fit within an AAV capsid.

303. Therefore, in my opinion, claim 8 of the '274 patent is obvious over the combination of Fabb with the '772 Publication and Xie.

C. Secondary Considerations Do Not Change the Conclusion of Obviousness

304. For the reasons discussed above with respect to Ground 1, secondary considerations do not alter my opinion that claim 8 of the '274 patent is obvious over the combination of Fabb with the '772 Publication and Xie.

XV. GROUND 6: CLAIM 8 IS OBVIOUS OVER THE '772 PUBLICATION AND SNOWDY, IN COMBINATION WITH FABB

A. A POSA Would Have Been Motivated to Combine the '772 Publication and Snowdy with Fabb

305. For the reasons discussed above regarding Ground 3, a POSA would have been motivated to combine the '772 Publication with Snowdy to improve the properties of AAVrh.10 as a gene therapy vector, by creating variants of AAVrh.10, each containing a substitution of a single amino acid in the capsid protein from the preferred embodiment AAV8, where non-phosphorylatable amino acids from AAV8 are substituted for phosphorylatable amino acids in AAVrh.10.

306. A POSA seeking to use such a variant of AAVrh.10 as a delivery vehicle for a dystrophin gene would have been motivated to combine the '772 Publication and Snowdy with Fabb, which teaches the construction of a smaller version of the dystrophin gene known as a “micro-dystrophin” for use with an AAV vector. EX1010 (Fabb), pp. 2-3.

307. A POSA would therefore have been motivated to combine the '772 Publication and Snowdy with Fabb to create the rAAV of claim 8.

B. A POSA Would Have Had a Reasonable Expectation of Success in Combining the '772 Publication and Snowdy with Fabb

308. For the reasons discussed above with respect to Ground 4, a POSA would have needed only routine experimentation to create a micro-dystrophin that could fit within an AAV capsid.

309. Therefore, in my opinion, claim 8 of the '274 patent is obvious over the combination of Fabb with the '772 Publication and Snowdy.

C. Secondary Considerations Do Not Change the Conclusion of Obviousness

310. For the reasons discussed above with respect to Ground 1, secondary considerations do not alter my opinion that claim 8 of the '274 patent is obvious over the combination of Fabb with the '772 Publication and Snowdy.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Respectfully submitted,

A handwritten signature in cursive script, appearing to read "David V. Schaffer", written in black ink.

David V. Schaffer

Date: February 21, 2024