

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Title: METHOD OF INCREASING THE FUNCTION OF AN AAV VECTOR

DECLARATION OF DR. RICHARD JUDE SAMULSKI

I currently hold the opinions expressed in this declaration. However, my analysis may continue, and I may acquire additional information and/or attain supplemental insights that may result in added observations.

I hereby declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true. I further declare 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.

Dated: 12/5/2024

By: _____

Richard Jude Samulski, Ph.D.

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I, RICHARD JUDE SAMULSKI, PH.D., hereby declare the following:

I. QUALIFICATIONS AND BACKGROUND INFORMATION

1. My education and experience are described more fully in my *curriculum vitae* (EX2028). For ease of reference, I have highlighted certain information below.

2. Since 1993, I have been a professor in the Department of Pharmacology at the University of North Carolina. For over twenty years, I was the Director of the Gene Therapy Center at the University of North Carolina School of Medicine. My research interest is in gene therapy and specifically, adeno-associated viruses (AAV) and their treatment. Currently, I am the co-founder and Chief Scientific Officer of M34, Inc., which develops gene therapies for treating neurological diseases.

3. In 1976, I received my Bachelors of Science (B.S.) in Microbiology from Clemson University in Clemson, South Carolina. In 1982, I received my Ph.D. in Molecular Biology from the University of Florida in Gainesville, Florida under the supervision of Nicholas Muzyczka. As a graduate student, I was the first to demonstrate that AAV could be used as a viral vector and my work culminated in the first U.S. patent involving non-AAV genes inserted into AAV.

4. After graduate school I went on to continue studying AAV, and have now studied AAV for over forty years. In 1982, I began my postdoctoral research

at The State University of New York (SUNY) and Princeton University. There, I began studying AAV with Dr. Thomas Shenk, the James A. Elkins Professor of Life Sciences, Emeritus in the Department of Molecular Biology at Princeton University. From 1986 to 1993, I was a faculty member in the Department of Biology at the University of Pittsburgh. In 1993, I was recruited to be the Director of the University of North Carolina at Chapel Hill's School of Medicine's Gene Therapy Center, a position I held until 2016.

5. In 1993, I also co-founded an AAV-based gene therapy company called Merlin. Research at Merlin first demonstrated AAV-mediated long-term gene transduction in muscle tissue. This breakthrough led to the merger of Merlin with Somatix Corp., which then merged with Cell Genesys Inc.

6. From 1994 until 1997 I served as a member of the Recombinant DNA Advisory Committee, which assists FDA with gene therapy clinical trial approvals in the United States. Since then I have also frequently served as a gene therapy consultant to FDA.

7. In 2001, I co-founded Asklepios BioPharmaceutical Inc. ("AskBio"), now a subsidiary of Bayer Pharmaceuticals, which has developed AAV therapeutics for over twenty years. At AskBio we developed a cell line for generating AAV therapeutics. It was the first to use human embryonic kidney (HEK) cells in serum-free suspension media to develop and produce AAV vectors

at scale. It currently yields among the highest in the industry. At that time we were one of the few in the field of AAV gene therapy that were focused on the importance of manufacturing. In 2020, AskBio was acquired by Bayer for \$4 billion, and it continues to operate today as an independent scientific entity with nearly 1,000 employees worldwide.

8. I also created two subsidiaries of AskBio, Bamboo Therapeutics, which focused on Duchenne muscular dystrophy, and Chatham Therapeutics, which focused on genetic solutions for hemophilia. I was the Vice President at Bamboo Inc. until it was acquired by Pfizer in 2016. Upon Bamboo's acquisition, I worked with Pfizer for 16 months to ensure the successful transition of Bamboo's therapeutic pipeline. Chatham was similarly acquired by Baxter (Takeda). In addition to this work, throughout my career I have helped advance other AAV therapeutics into human clinical trials including for giant axonal neuropathy, Canavan disease, Parkinson's disease, Duchenne muscular dystrophy, Pompe disease, and congestive heart failure.

9. In 2018, I helped found the Columbus Children's Foundation, a non-profit organization that helps provide treatment for children with rare genetic diseases. My other public service work includes speaking at high schools, museums, hospitals, to U.S. state senators, and to the North Carolina General Assembly on gene therapy. I am also a member of other non-profit organizations

including Phoenix Nest, Rain Bio, the Muscular Dystrophy Association, the Progeria Research Foundation, and others.

10. In 2019, I joined the Foundation for National Institutes of Health as a member of the Accelerating Medicines Partnership Bespoke Gene Therapy Consortium (AMP Bespoke Gene Therapy), which brings together members of private, public, and non-profit sectors to develop rare genetic diseases that are not the focus of commercial development.

11. For over four decades, I have taught numerous graduate level courses on biotechnology and gene therapy including at the University of Pittsburgh from 1986 to 1993 and at the University of North Carolina since 1993.

12. I have received significant research support (over \$82 million in funding) for my work in AAV including from the National Institute of Health (NIH), the National Institute of Allergy and Infectious Diseases (NIAID), the Cystic Fibrosis Foundation, the Muscular Dystrophy Association, and the North Carolina Biotechnology Center, among many others throughout my career.

13. Throughout my career I have received a variety of awards including the Life Sciences Award Winner (2024), Columbus Hero Award (2022), European Society of Gene & Cell Therapy Founder's Award (2022), and a Top 12 Pioneer Award for Seminal Work in Gene and Cell Therapy (2013). In 2008 I was

honored by the American Society of Genetics and Cell Therapy (ASGCT) with its inaugural Outstanding Achievement Award.

14. Other awards include being named in the Top 100 Most Inspiring People in the Life Sciences Industry, PharmaVoice, (2020), Society of Chinese Bioscientists in America (SCBA) Award (2013), Senator Paul B. Wellstone Muscular Dystrophy Cooperative Research Center Award (2008), and Researcher/Innovator Healthcare Hero Award, Triangle Business Journal (2005). I was also the first non-MD to be placed on the University of Florida's "Wall of Fame" in 2016. I was also honored to receive an audience with Pope Francis in 2015 for my work in treating rare genetic disorders, including Canavan disease.

15. I am an author or co-author on over 340 peer-reviewed publications, 45 book chapters, and I have edited numerous textbooks and journals. I understand that in 2022, it was reported that I had published 151 papers relating specifically to AAV in my career, more than any other scientist besides Dr. James Wilson, one of the inventors of the '274 patent. My publications include articles published in *Gene Therapy*, *Molecular Therapy: The Journal of the American Society of Gene Therapy*, *Human Gene Therapy*, *Journal of Virology*, *Nature Reviews: Genetics*, *Nature Protocols*, *Nature Medicine*, *Nature Biotechnology*, *The New England Journal of Medicine*, and *Science Translational Medicine*. I have been on the editorial board of many journals as well, including *Human Gene Therapy*, *PLOS*

Pathogens, Stem Cells, World Journal of Biological Chemistry, Molecular Therapy, Journal of Pharmaceutical Sciences, Virus Research, The Journal of Gene Medicine, and Journal of Virology, among others. I understand my publications have been cited over 17,000 times. *See* EX2042, 7.

16. I currently hold over 90 patents directed to gene therapy and AAV.

17. I have been invited to speak on my work in gene therapy and AAV by numerous national/international associations and universities including The Ohio State University, Sorbonne University, American Society of Cell & Gene Therapy, Children's Research Institute, GlaxoSmithKline LLC, the Nature Biotechnology Conference, Duke University, the Cystic Fibrosis Foundation, and the Muscular Dystrophy Association.

18. I am a member of many professional organizations and societies including the NIH, Recombinant DNA Advisory Committee, the American Association for the Advancement of Science, Society of Virology, the American Society of Gene Therapy, UNC Lineberger Comprehensive Cancer Center, National Gene Vector Laboratories Panel, the American Society of Hematology, and the Gene Therapy for Rare Diseases Consortium Foundation.

19. In writing this Declaration, I have considered the following: my own knowledge and experience, including my work experience in the fields of gene therapy and AAV; my experience in teaching those subjects; and my experience in

working with others involved in those fields. In addition, I have analyzed the following publications and materials:

- U.S. Patent No. 11,680,274 B2 (Exhibit 1001)
- Declaration of Dr. David Schaffer (Exhibit 1005)
- U.S. Patent App. No. 2003/0138772A1 (Exhibit 1007)
- 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”) (Exhibit 1009)
- 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 (“Jans & Jans”) (Exhibit 1017)
- All other materials cited in my declaration.

II. GENE THERAPY OVERVIEW

20. Gene therapy represents a revolutionary approach for treating genetic conditions by modifying the genetic structure of cells. EX1046. At a high level, this technique involves either introducing, altering, or replacing genes in a patient’s cells to correct a defective or missing gene responsible for a disease, or to

introduce a new gene that can produce a therapeutic product, like an antibody.

EX1046. There are various strategies for delivering these therapeutic genes into the body. One method uses viral vectors—modified viruses designed to carry the gene into target cells. EX2001, 2. Alternatively, non-viral delivery methods, such as direct injection of DNA or RNA, have also been explored. EX2001, 6. While non-viral vectors are simpler and generally considered safer, they often have lower efficiency in getting the gene into cells or ensuring it works effectively. EX2001, 6. On the other hand, viral vectors tend to result in stronger gene expression, potentially offering more powerful therapeutic outcomes. EX2001, 2. Research into viral vectors aims to optimize their safety and accuracy, ensuring they can deliver the right gene to the right location without causing harmful side effects.

A. Early Gene Therapy and Discovery of AAVs

21. Gene therapy emerged as a promising treatment for diseases in the 1990s, but early attempts saw only limited success. Initially, non-viral methods, such as injecting “naked” DNA directly into the body, were explored but proved to have minimal clinical impact. EX2002, 1. In an effort to improve outcomes, researchers shifted their focus to viral vectors, which could deliver therapeutic proteins more effectively. EX2002, 1. Early candidates included recombinant adenoviruses, lentiviruses, and herpesviruses. EX2002, 1. However, by the late 1990s, these viral vectors showed a range of safety issues, including inflammation

and serious side effects, which hindered the development of reliable, clinically safe gene therapies. EX2002, 2. Despite these setbacks, researchers in the field, including myself, remained optimistic and we continued our search for viral vectors that would be both safe and effective for gene delivery. EX2001, 2.

22. Adeno-associated virus (AAV), first discovered in 1965 as a contaminant in adenovirus preparations, presented itself as a potential solution. EX2003, 1. Unlike its adenoviral counterparts, AAVs were known to be non-pathogenic, meaning they did not cause disease on their own, making them a safer option for gene therapy. For decades, only six AAV types—AAV1 through AAV6—were known. EX2003, 1.

23. However, in late 2002, the lab of Dr. James Wilson, a physician and researcher at the University of Pennsylvania, identified new AAVs in primate tissue that had never been associated with either adenovirus or AAV infections. EX2003, 1, 7; EX2004, 2; EX2005, 1-2 (distinguishing this type of infection from “productive infection”). Remarkably, these AAVs persisted in the host cells without causing symptoms or adverse effects, suggesting they could be used safely in gene therapy. EX2001, 5. The work in Dr. Wilson’s lab led to the discovery of over 100 new AAV sequences in primate tissues, all sharing phylogenetic similarities with the original six. EX2003, 2. His research helped pave the way for

further exploration, and many of these newly identified serotypes were detailed in the '274 patent.

B. AAVs as Viral Vectors and Vector Modification

24. By 2005, when the application leading to the '274 patent was filed, AAV-based gene therapy was still an emerging field. *See, e.g.*, EX2006, 1. (“AAV virologists and gene therapists alike have just begun to scratch the surface in terms of the utility of this small virus in a clinical setting.”). The first AAV-based therapy, Glybera, wouldn’t be approved in Europe until 2012, and it took another five years before Luxturna became the first AAV-based gene therapy approved in the United States. EX2007, 3-4.

25. In 2005, the understanding of AAV biology was still in its early stages. Research was primarily focused on the six AAV serotypes that had been identified (AAV1-6). EX2003, 1. It was known that AAV’s outer protein shell, or “capsid,” was composed of three proteins—vp1, vp2, and vp3—encoded by the AAV cap gene. EX2008, 1. These proteins were expressed as “splice variants,” with vp1 being the largest and containing the full amino acid sequence, while vp2 is translated from the same mRNA but utilizing a different start codon, and vp3 was expressed as shorter portions of the vp1 sequence. EX2009, 1. In gene therapy, the AAV capsid serves as the delivery vehicle, housing the genetic material such as a transgene or minigene. We use the term “serotype” to refer to specific AAV

capsids, like AAV1, AAVrh10, or AAVrh46. Different serotypes exhibit varying efficiencies in delivering genetic material to specific cells or tissues, a phenomenon known as “tropism,” that I discuss in more detail below. *See, e.g.*, EX2009, 1; EX2002, 2; EX2007, 2; EX1032, 1; EX1052, 1.

26. Early AAV research focused on AAV2, one of the first identified serotypes, and in 2005 scientists were still working to map the functional domains of the capsid proteins (vp1, vp2, and vp3) through genetic analysis. *See* EX1032, 1. The atomic structure of AAV2, published in 2002, provided key insights into how the virus’s structure related to its function, fueling further research into its potential for gene delivery. *See* EX1008, 1, 5.

27. At the time, AAV research for gene therapy was largely limited to basic science—we were figuring out how these vectors worked, and determining which portions of the vectors were important for specific AAV functions. By and large, mutagenesis and genetic engineering were not yet being used for the goal of enhancing a specific AAV function for clinical benefit. Instead, much of the research focused on understanding how variations in the AAV capsid structure influenced its functionality. For example, studies like the one by Hauck that Petitioner relies upon, (EX1052), explored how different regions of the AAV capsid affected its ability to infect muscle tissue. EX1052, 1-2 (“In the present study, a series of AAV capsid mutants were generated to identify the major regions

affecting AAV transduction efficiency in muscle.”). But these studies were not aimed at engineering AAVs for specific therapeutic purposes; instead, they sought to better understand the relationship between capsid structure and tropism. *See, e.g.,* EX1052, 6 (“[W]e draw the conclusion that amino acids 350 to 423 are important in determining AAV1 tropism.”).

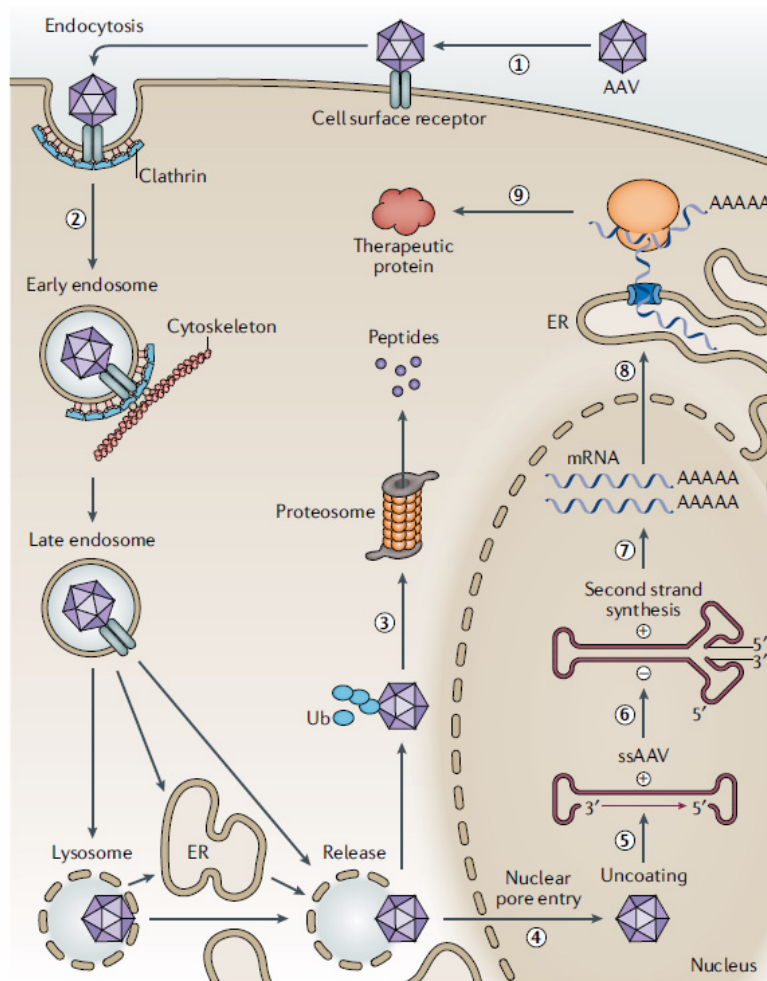
28. The kind of modifications that I understand are being proposed by Petitioner here—targeting a single amino acid in the AAV capsid to allegedly improve or change its functionality, such as its tissue targeting ability—had not been reported in the literature by 2005. It was not until years later that high-throughput mutagenesis studies began to explore this kind of detailed engineering. *See, e.g.,* EX2010, 1 (reporting in 2019 that “recent high-throughput AAV mutagenesis studies have focused on limited capsid regions” and that their study generated “all single-codon insertions and deletions”). By 2005, AAV-based vectors for gene therapy were still in the very early stages of exploration.

C. AAV Vector Biology

29. For an AAV vector to deliver its genetic payload to the nucleus of a cell, a number of steps must occur. Delivery begins when the AAV vector is administered to a patient, generally through intravenous (IV) injection into the patient’s bloodstream. Depending on the target tissue, however, administration can also occur via direct injection to the tissue. For example, direct injection can be

used to more effectively administer a gene therapy to the retina, brain tissue, or skeletal muscle. Regardless of route of administration, the AAV vector must end up in proximity to the tissue type that it is targeting for delivery to succeed, i.e., have tropism to that tissue.

30. Arriving at the correct cell or tissue type is only the first step in delivery, however. An overview of the pathway that AAV vector takes after it is administered to a patient is shown below in an image from a recent review I co-authored:



EX2029, 4. Specifically, after administration, to gain entry into a cell, the AAV vector interacts with the receptors on the cell surface (step 1, above). Specifically, the vp3 region of the AAV capsid protein will interact with the cell surface receptors. Once the capsid protein finds a valid binding site, the AAV vector will enter the cell through cell-mediated endocytosis, meaning the cell forms an endosome, or lipid compartment, around the AAV vector (step 2). After the AAV is released from the endosome, it will either be marked for degradation (step 3) or trafficked intracellularly into the nucleus through a nuclear pore, often referred to as nuclear translocation (step 4). Once inside the nucleus, the AAV is uncoated and the AAV genome is released (step 5). The genome is transcribed into mRNA (steps 6-7), exported from the nucleus (step 8), and the resulting therapeutic protein is translated and expressed by ribosomes in the rough endoplasmic reticulum (step 9). EX2029, 4.

D. General Principles of AAV Vector Selection

31. At a high level, when selecting an AAV vector for use in gene therapy, in 2005 a skilled artisan would have had two primary concerns—specificity and efficiency. Specificity, meaning that a skilled artisan would have wanted an AAV vector to be preferentially taken up by a certain cell or tissue type, to maximize expression in the cell or tissue type of interest. Efficiency, meaning

that a skilled artisan would have wanted an AAV vector to deliver the gene of interest to the cell nucleus and have the gene be subsequently expressed and produced.

32. To meet these goals, a skilled artisan would generally have begun by selecting an AAV vector that was known at the time to target the cell type or tissue of interest, i.e., a vector that has known preferential tropism to that cell type or tissue. For example, if target disease was a disease of the liver, a skilled artisan would have selected an AAV vector that was the best for delivery to the liver. Or if the disease at issue was a disease that targeted muscle, a skilled artisan would have instead selected an AAV vector that had the best delivery to muscle tissue. In other words, in 2005, the general approach would have been to simply select a known AAV vector that could target the tissue type that the skilled artisan wanted to target, and test that vector *in vitro* and *in vivo* to see if it could deliver the gene of interest. In 2005, we were only beginning to gain an understanding of tropism and how to improve tropism to a particular tissue. Altering tropism remains a challenge, even today, and researchers in the field are still actively studying new potential mechanisms for altering tropism. *See, e.g.*, EX2035; EX2024, 6; EX2026, 8. Notably, as I will discuss more below, while some techniques had been shown by 2005 to improve or change tropism, none involved a single amino acid substitution. Nor am I aware today of any example where tropism is

significantly increased or changed to a materially different tissue type because of a single amino acid substitution.

33. In selecting an AAV vector, in my opinion it would also have been important to a skilled artisan (both in 2005 and today) generally to limit off-target expression of the gene of interest. The reason for this is two-fold. At the outset, as a matter of common sense, if a portion of my AAV therapy is going to tissues or parts of the body beyond the intended target, a higher dosage of AAV will likely need to be administered to reach an effective level in the target tissue.

34. Administering a higher dosage of AAV to a patient may have significant repercussions. First, it means manufacturing more AAV. AAVs, like other biological therapeutics, are expensive to manufacture and difficult to manufacture in bulk while maintaining quality. EX2036, 1. Scaling up from benchtop to clinic remains one of the greatest challenges in developing an AAV product. EX2036, 2. Requiring increased AAV dosages could lead to the AAV gene therapy being potentially cost prohibitive to produce, particularly if the disease being treated is a rare, and few patients are available to take (and therefore pay for) the gene therapy product. Thus, in 2005, a skilled artisan would generally seek to minimize a patient's dosage while maximizing expression in the relevant target tissue.

35. Second, separate from increasing manufacturing costs, increasing the amount of AAV that is injected into a patient can significantly increase the risk of invoking an immune response in that patient. Immune responses to AAV gene therapies can be life-threatening, and unfortunately a handful of deaths have been attributed to high dose AAV therapeutics. Therefore, critical steps are taken throughout treatment to reduce their likelihood. For example, prior to being treated with AAV, a patient's blood is tested for pre-existing antibodies against the specific AAV serotype being used in the gene therapy. If the patient is found to have high preexisting anti-AAV antibodies, not only may the AAV gene therapy have reduced efficiency, but it is also likely that the patient will have an immune response if the AAV gene therapy product is administered. Immune responses can range from transient liver toxicity to death. *See, e.g.*, EX2037; EX2038; EX2039. Therefore, seropositivity (testing positive for high levels of antibodies to an AAV) generally contraindicates a patient from receiving that particular gene therapy. In addition to antibodies stemming from preexisting innate immunity, increasing the amount of AAV administered is known today (and was known in 2005) to increase the likelihood that the patient will generate antibodies during treatment with gene therapy, i.e. have an adaptive immune response. EX2037, 3-4. In my career, I have seen many clinical stage gene therapy products ultimately fail at the clinical trial stage because of harmful immune responses such as liver toxicity and

thrombotic microangiopathy, a rare but serious disease that affects a patient's small blood vessels. *See, e.g.*, EX2037; EX2039.

36. For these reasons, I strongly disagree with Dr. Schaffer's statements that it's not "necessarily problematic" or "not too much of a concern" if the AAV is delivered to off-target tissues. EX2030 (Schaffer Tr.), 55:10-19, 56:1-21. Increasing manufacturing costs and potentially putting patients at a higher risk of adverse effects are certainly concerns that a skilled artisan would have had in 2005, and would have sought to minimize through careful selection of the AAV vector.

37. As an aside, I also take issue with the positions Dr. Schaffer took at his deposition that the "entire delivery process is a determinant of tropism," and that "increasing infectivity" is the "same as altering tropism." EX2030 (Schaffer Tr.), 80:13-21, 234:11-13; *see also* EX2030 (Schaffer Tr.), 312:5-11 (stating prior art is "talking about AAVs' ability to infect a target cell, and that's an inherent part of tropism"); EX2030 (Schaffer Tr.), 76:3-21 ("Tropism is a complicated word that is really the sum, I think, or the result of all of those steps that the virus has to traverse to be able to make it from the outside of the cell to the inside of the nucleus.")). In my opinion, such a definition for tropism overgeneralizes the term to cover all aspects of gene delivery, and that is not how the term tropism is traditionally understood or used by those of us who are working in this field, both today and back in 2005.

38. Rather, tropism and infectivity are better understood as separate functions. Tropism refers to the process of the AAV vector reaching a particular tissue or cell type; i.e. the steps that occur *before* the AAV reaches the cell or tissue. I note that elsewhere in Dr. Schaffer's deposition, he appears to agree with such a definition, stating that "'Tropism' refers to the collection of cells that a given AAV is capable of infecting." EX2030 (Schaffer Tr.), 54:5-10. I agree with this definition, as it is closer to how a skilled artisan would have used the word "tropism" in 2005. "Infectivity," on the other hand, generally refers to the steps that occur *after* the AAV vector reaches the cell or tissue of interest, i.e. intake of the AAV vector into the cell, translocation to the nucleus, and expression of the gene of interest. I also note that Dr. Schaffer's peer-reviewed publications appear to largely use the terms tropism and infectivity in the same way that I and the rest of the field at large generally do. EX1030, 6 (defining tropism as "The cell or tissue type that can be infected by a virus or a gene delivery vector"); EX2024, 2 ("envelope proteins are the primary determinants of the cell specificity or tropism of a retrovirus"); EX2025, 1 ("upon systemic administration, natural serotypes typically exhibit tropism for liver, or alternatively for multiple organs, which renders targeted delivery to other tissues difficult").

III. RESPONSE TO SAREPTA'S ARGUMENTS

39. Counsel for Patent Owners have asked me to rebut certain arguments and positions taken by Sarepta and Sarepta's expert, Dr. Schaffer. I address each argument in turn below.

A. Sarepta's Alleged Motivation to Modify AAVrh10 Based on AAV8 to Target Liver

1. A Skilled Artisan Targeting Liver in 2005 Would Have Chosen AAV8

40. Sarepta's initial premise—that a skilled artisan in 2005 would have looked at the AAV vectors disclosed in the '772 Publication with the goal of finding or creating a vector that targeted liver—would have led a skilled artisan at that time to begin her analysis with AAV8. Not only was AAV8 known by 2005 to have strong tropism to the liver, but the '772 Publication itself confirmed this superior tropism, including as compared to some of the new serotypes disclosed in the '772 Publication. *See, e.g.*, EX1007, ¶ 250 (describing how AAV8 “provides a substantial advantage over the other serotypes in terms of efficiency of gene transfer to liver”).

41. In the '772 Publication, the inventors tested known and new AAV capsids by generating chimeras that fused together the AAV2 rep gene (which controls replication) with various cap genes (which generate the AAV capsid proteins). *See* EX1007, ¶ 214. These chimeras were created to allow the inventors

to test and compare the tropism of the AAV capsid proteins, among other properties of the capsid, separately from the AAVs' ability to replicate. As the '772 Publication showed to a POSITA in 2005, AAV8 was expressed more strongly in liver than any other AAV tested. Table 6 compares the expression of known AAVs (AAV1, AAV2, AAV5, AAV7, and AAV8), and shows that AAV8 was expressed *nearly ten times* more strongly in liver than the next closest known tested AAV, AAV7:

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.

EX1007, ¶¶ 244-245. Moreover, the '772 Publication showed AAV8 had relatively low expression in muscle, which is desirable for reducing off-target expression if the goal was targeting liver.

42. Additionally, when the inventors compared AAV8's expression in the liver against new serotypes AAVrh2, AAVrh10, AAVrh13, and AAVrh21, they found that AAV8 remained superior:

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

EX1007, ¶ 253. Notably, AAV8 was shown to have *over fifty percent (50%) greater expression* in liver than AAVrh10. As I discuss more below, a skilled artisan in 2005 would not have expected a single amino acid substitution to significantly alter tropism in order to target to a different tissue. Thus, in my opinion, in 2005, a skilled artisan with the goal of targeting the liver would have selected the best known vector for delivery to liver at that time—AAV8.

43. In my opinion, a skilled artisan in 2005 would not have looked solely to modify a vector to try to improve tropism to a particular tissue. As discussed throughout this declaration, changing tropism is difficult to predict, and a skilled artisan would not have had a reasonable expectation that changes could be made to improve or alter tropism. A skilled artisan would also have been concerned that changes, even small ones, could negatively impact other properties of the capsid protein. Therefore, rather than seek to modify a vector, a skilled artisan in 2005 instead would have merely selected the best available vector for the tissue she

wanted to target. If that tissue was liver, a skilled artisan in 2005 would have chosen AAV8.

2. A Skilled Artisan Would Not Have Arrived at S665N Substitution

44. If we nevertheless accept Sarepta's premise that a skilled artisan would select AAVrh10 and modify it based on the sequence of AAV8 in order to improve tropism to liver, I disagree that a skilled artisan would have arrived at a substitution of an asparagine for a serine at position 665, to the exclusion of all other options. Instead, in my opinion, a skilled artisan would have made a large number of potential substitutions, insertions, deletions, and other modifications to try to test which sites might be important to different properties of the capsid protein. A skilled artisan undertaking this experimentation would not have had an expectation that any particular change, and certainly not a single amino acid change, would have improved AAVrh10's tropism to the liver.

45. As I discuss above, in 2005 we were still in the process of mapping out the various functional domains in different AAV vectors to figure out which portions of the AAV capsid were responsible for various AAV functions, including tropism. *See, e.g.,* EX1032; EX1052; EX2033; EX2034. To do that we generally used a technique called alanine scanning mutagenesis. *See* EX1032; EX2033; EX2034. Alanine scanning involves replacing a non-alanine amino acid residue

with alanine. Alanine is an amino acid that carries a methyl side chain, i.e. a neutral single carbon atom bonded to three hydrogen atoms. Alanine is the preferred residue for these types of mapping analyses because it generally does not alter the conformation of the protein, and it does not introduce electrical charge or polarity. Therefore, it allows a skilled artisan to identify which residues in an AAV capsid protein contribute to specific functions.

46. Essentially, when conducting alanine scanning, a skilled artisan would identify each residue of potential interest, replace one or more of them with an alanine in a separate mutant, and test all of them to see which residue caused either a loss or (less likely) a gain in a particular function. I sometimes refer to this as a “wreck and check” approach. *See, e.g.*, EX1032. Critically, until each mutant was checked, at that time in 2005 a skilled artisan would have had no way of predicting which specific residue contributed to any particular function of AAV, and they particularly would not have been able to predict which amino acids might impact tropism. While a skilled artisan would have been able to reasonably predict whether a capsid would *form*, based on known conserved and variable regions within the known AAVs, that is not the same as predicting whether a residue affected *tropism* specifically, whether any particular change would affect the tissue or cells that the AAV binds.

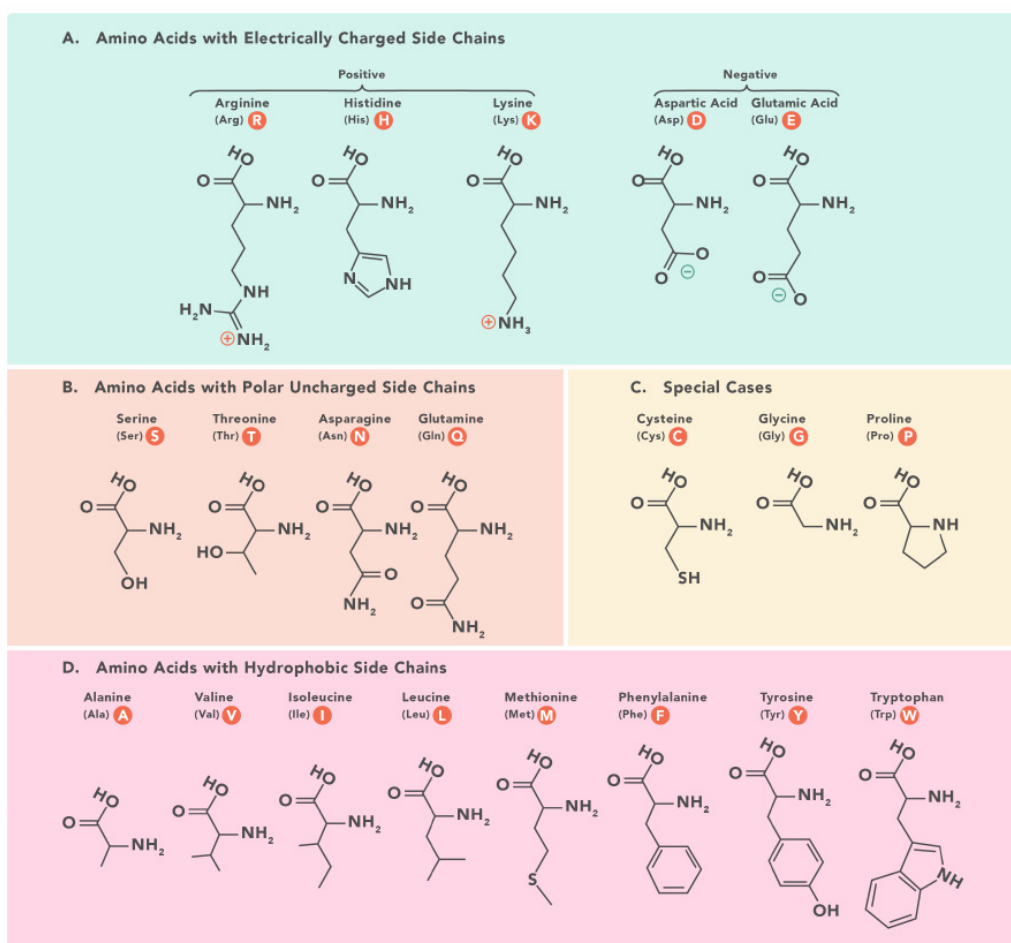
47. Here, at minimum, following Sarepta's logic, where there were 48 amino acid differences between AAVrh10 and AAV8, a skilled artisan would have made multiple different alanine substitutions depending on their position in the AAV capsid (i.e. conserved or non-conserved regions), and determined whether any affected tropism. In my opinion, a skilled artisan would have also tested mutants that had two, three, four, or more alanine substitutions, to determine whether particular regions, in addition to particular amino acids, might impact various functions. EX1032, 5-6, 9-10.

48. In my opinion, however, a skilled artisan would not have expected a single amino acid change to alter tropism in any meaningful way. I am unaware of any studies before 2005 where a single amino acid change was shown to alter AAV tropism. I note that neither Sarepta nor Sarepta's expert has cited to any such study either.

49. The Wu paper that Sarepta cites (Pet., 27), in my opinion, stands for the opposite proposition. For example, Wu makes a number of mutations, including several single alanine wreck and check substitutions (mutant numbers 29, 31, 45, and 48). EX1032, 5. Two of those mutants (mut29 and mut45) were shown to have no impact on infectivity. EX1032, 5 (showing mutants 29 and 45 are Class 1); EX1032, 4 ("Class 1 contained mutants that have an infectious titer similar to the wt titer."). The other two mutants (mut31 and mut48) formed a

capsid but were found to have reduced or abolished infectivity. EX1032, 5 (showing mut31 was class 4c, non-infectious with an “empty particle” and mut48 was class 2a); EX1032, 4 (“Class 2 contained partially defective mutants with infectious titers 2 to 3 logs lower than the wt titer”), (“This meant that mut31 formed intact virus particles that were empty.”). Nothing in Wu suggests that any of these four amino acid substitutions could potentially change the tropism of the AAV. Nor am I aware of any other paper suggesting that.

50. Additionally, I would expect Sarepta’s proposed substitution—substituting an asparagine in place of a serine—to have little to no effect on tropism, or of any other function, because it is a conservative substitution. A conservative substitution is a substitution that does not alter the electrical charge or polarity of the side chain of the amino acid. A chart of the various amino acids categorized by the nature of their side chains is shown below:



EX2031, 5; *see also* EX1040 (Alberts I), 4-5.

51. Both serine and asparagine have polar uncharged side chains. Therefore a substitution between these two amino acids would be considered conservative. I note that Dr. Schaffer agrees with this understanding. *See* EX1005, ¶ 182 (“A POSA would also have understood that substituting asparagine (N) for serine (S) would be a more conservative substitution than substitution alanine (A) for serine (S), because (S) and asparagine (N) are polar, uncharged amino acids, while alanine (A) is nonpolar”). Therefore, to the extent that a skilled

artisan would have made that change, in my opinion a skilled artisan would expect little to no functional difference to result from such a substitution. A skilled artisan would certainly not expect that such a substitution would impact tropism.

3. A Skilled Artisan Would Have Used Other Known Methods of Altering Tropism

52. In 2005, if a skilled artisan wanted to improve tropism to a particular tissue, and did not want to use a known AAV, approaches other than single amino acid substitution were available. For example, Sarepta's Wu reference teaches additional modifications including, rather than substituting a single amino acid, instead inserting larger fragments of DNA into the AAV2 vector to try to change tropism. *See, e.g.*, EX1032, 9 ("To determine whether we could change the tropism of rAAV by inserting a novel receptor ligand into the capsid, we constructed two mutant plasmids that contained a serpin receptor ligand."). Wu inserted a five-amino acid fragment (specifically a serpin ligand) into vp2 of AAV2 and, separately, a ten-amino acid fragment into vp3 of AAV2 (specifically a serpin receptor ligand). EX1032, 9. The insertion of both serpin ligands significantly increased the virus's ability to target IB3 cells, a "lung epithelial cell line believed to express the serpin receptor." EX1032, 9. According to Wu, these results suggested "that the tropism of the mutant rAAVs had been changed." EX1032, 9; *see also* EX1032, 12 ("[we] have demonstrated that insertion of a

foreign receptor ligand at some of these positions can change the tropism of the virus”).

53. Wu’s teaching is also entirely consistent with the disclosures in the ’772 Publication, the reference I understand Sarepta bases all of its arguments around in its Petition. At the outset, I note that the ’772 Publication is silent as to using single amino acid substitutions to alter tissue tropism. Instead, the ’772 Publication discusses modifying AAV vectors by combining fragments of AAV sequences with portions of other AAVs to generate what it refers to as an “artificial capsid.” EX1007, ¶ 75 (discussing combining “a fragment of a vp1 capsid protein” with “heterologous sequences which may be obtained from another AAV serotype”); *see also* EX1007, ¶¶ 83, 118, 141. The ’772 Publication explains such modifications “may be to increase expression, yield, and/or to improve purification in the selected expression systems, or for another desired purpose (e.g., to change tropism or alter neutralizing antibody epitopes.” EX1007, ¶ 0091. Additionally, the ’772 Publication discloses that “fragments may be readily utilized in a variety of vector systems and host cells” and “may be used alone, in combination with other AAV sequences or elements, or in combination with elements from other AAV or non-AAV viral sequences.” EX1007, ¶ 17.

B. Sarepta's Alleged Motivation to Modify AAVrh10 to "Preserve" Tropism to Muscle

54. I understand that at his deposition, when asked about what would motivate a skilled artisan to modify AAVrh10 based on AAV8, Dr. Schaffer expressed opinions not found in his expert declaration, stating that, in addition to wanting to improve tropism to liver, in his opinion a skilled artisan would also seek to preserve or maintain AAVrh10's tropism to muscle and lung. I disagree with Dr. Schaffer. For the reasons I discussed above, a skilled artisan seeking to improve or focus tropism to one tissue of interest would generally seek to *minimize* tropism to off-target tissues that would not benefit from receiving the gene of interest. Put another way, there would generally be no benefit to increasing delivery of the gene of interest to the off-target tissues.

55. To the extent Dr. Schaffer is suggesting that some gene of interest exists that would be desirable to target to liver, muscle, and lung at the same time, his declaration is silent as to any such gene. Indeed, in my opinion, a skilled artisan targeting any other tissue location besides liver would seek to *avoid* targeting to the liver, because the liver is often the default destination for gene therapies, making it more difficult to target non-liver tissues. *See, e.g.,* EX1030, 3.

56. Because I can think of no reason why a skilled artisan in 2005 would have wanted to simultaneously target liver, muscle, and lung together, then in my

opinion, what Dr. Schaffer must be identifying are two separate motivations, or goals. These two separate goals—improving tropism to liver versus maintaining tropism in muscle and lung—would be addressed by a skilled artisan in completely different ways. I discuss in Section III(A) above the steps a skilled artisan would take if her goal was to *improve* tropism to liver.

57. If, a skilled artisan's goal was simply to *maintain* existing tropism in AAVrh10, however, such as to muscle and/or lung, then a skilled artisan would have had two options. First, a skilled artisan could simply have left the vector as is and unmodified, therefore ensuring tropism was maintained. Or, on the other hand, a skilled artisan would have understood that she had an exceedingly large number of potential changes that could reasonably be made to the vector without impacting the existing tropism.

58. Indeed, following the framework that Dr. Schaffer sets forth in paragraphs 214-226 of his declaration, based on Figure 2 in the '772 Publication, a skilled artisan would have known that amino acids within the non-conserved regions could have been changed to any of the other amino acids found at that residue among the other known AAVs. EX1005, ¶¶ 214-226; EX2030 (Schaffer Tr.), 192:4-197:18. I understand that there are roughly 390 non-conserved residues across the AAVrh10 vp1 sequence, and that at some of these residues could see up to 7 different amino acids across the known AAVs in Figure 2. Assuming an

average of 2 different amino acid possibilities at each location, that would be 780 possible single amino acid substitutions. A skilled artisan would have known she could modify multiple amino acid residues, increasing the possibilities exponentially, well into thousands or even millions of possibilities. As I discuss above, a skilled artisan would have also considered inserting entire fragments from other AAV capsid proteins or other unrelated proteins, increasing the number of potential substitutions even further. EX1032; *see also* EX1052.

59. In my opinion, at bottom, a skilled artisan would have understood that any number of these residues could be modified within the scope of Figure 2 and be highly unlikely to affect the existing tropism of AAVrh10 to muscle and lung. I further believe that a skilled artisan would have been extremely unlikely to pluck a single serine to asparagine substitution at position 665 out of thin air. There would have been no reason for a skilled artisan to select that particular substitution—a substitution that Sarepta only arrives at based on improving tropism to *liver* using AAV8—if the skilled artisan’s only goal was to maintain *muscle* and *lung* tropism. Instead, a skilled artisan would have made one of the thousands or millions of possible substitutions at one or more locations throughout the capsid protein, and tested whether those substitutions impacted tropism and a variety of other properties.

C. How a Skilled Artisan Would Have Targeted Dystrophin to Muscle Tissue

60. I understand that one of the claims of the '274 patent, claim 8, requires delivery of a “dystrophin gene product” that is either a “mini-dystrophin or micro-dystrophin.” EX1001, cl. 8. Dystrophin is a protein that is predominantly expressed in muscle, and a skilled artisan creating a gene therapy to deliver dystrophin would seek to target the muscles. *See* EX1010, 3 (“Expression of micro-dystrophin Δ 3788 in the skeletal muscle of nude/mdx mice transduced with rAAV”); EX1010, 4 (discussing analysis of muscle sections); EX1010, 5 (“In order to utilize an AAV vector system to provide gene transfer based on complementation of dystrophin deficiency in muscle tissues in vivo, we have reduced the cDNA sequence of this gene down from 14 to ~3.8 kb to produce a microdystrophin cDNA.”).

61. I have read Dr. Schaffer’s declaration and the Petition, and I did not see any discussion of delivery to muscle specifically. I also saw that at his deposition, Dr. Schaffer would not agree that dystrophin is largely expressed in muscle. EX2030 (Schaffer Tr.), 359:8-360:10. I do not agree. Dystrophin is a protein well-known to be expressed primarily in muscle, as even the Fabb reference that Dr. Schaffer relies upon explains. EX1010, 1 (“Duchenne *muscular* dystrophy is a severe life-threatening X-linked recessive disorder, caused by

mutations in the dystrophin gene”; describing how previous vector options were “less than ideal for gene transfer to *skeletal muscle*”); at 6 (“AAV vectors remain one of the most attractive systems for *muscle* gene transfer, and the studies described here further confirm the feasibility of using highly engineered *micro-dystrophin* AAV vectors as an approach towards gene therapy of DMD”); *see also* EX2040, 4-5 (“The cellular regulation of dystrophin expression appears to be a key aspect of the plasticity of muscle ... The dystrophin protein is also large (427 kDa) and requires a specialized intracellular structural niche within myofibers [i.e., muscle fibers] throughout the body (recalling that myofibers account for more cell volume in the body than any other cell type).”); EX2041 (“The dystrophin complex stabilizes the plasma membrane of striated muscle cells.”). Therefore, it is my opinion that a skilled artisan seeking to deliver a mini- or micro-dystrophin, as required by claim 8, would seek to target muscle tissue.

62. A skilled artisan desiring to target delivery of a gene therapy to muscle in 2005 would take an entirely different path than those I describe above, where the goal was either to improve tropism to the liver or to simply maintain the muscle/lung-specific tropism of AAVrh10. Assuming a skilled artisan were to begin, as Dr. Schaffer does, with the '772 Publication, a skilled artisan would have data from multiple experiments related to muscle delivery available to them.

63. Of the AAV vectors known before the '772 Publication, AAV1 was thought to be the best muscle transducer. EX1007, ¶ 243 (explaining that AAV1 “is the most efficient in skeletal muscle of the previously described serotypes”). In Example 7 in the '772 Publication, the inventors evaluated “[t]he performance of vectors base[d] on the new serotypes ... in murine models of muscle and liver-directed gene transfer and compared to vectors based on the known serotypes AAV1, AAV2 and AAV5.” EX1007, ¶ 241. Based on those experiments, the inventors generated the data found in Table 6 of the '772 Publication:

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.

EX1007, ¶ 244. These data show that the performance of AAV7 in muscle was approximately three times better than the previously known best muscle transducer, AAV1. I note that AAVrh10 was not tested in this experiment.

64. As I discuss above, in Example 9 of the '772 Publication, AAVrh10 and several other vectors were examined in gene transfer studies to the lung, liver,

and muscle. EX1007, ¶ 253. In those experiments, AAVrh10 was shown to transduce muscle at approximately the same rate as AAV1:

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

EX1007, ¶ 253. The inventors concluded that AAVrh10 had a “big lead in the lung tissue particularly” based on this experiment, and went on to conduct several additional experiments to “confirm the superior tropism of AAV 44.2 in lung tissue.” EX1007, ¶¶ 254-255.

65. Based on these experiments, a skilled artisan seeking to target delivery of a gene to muscle would have started her work with AAV7. The experiments in the '772 Publication show, collectively, that AAV7 has a significant lead in the muscle, transducing muscle three times better than AAV1, while AAVrh10 showed approximately the same performance in muscle as AAV1. The '772 Publication directs a skilled artisan to use AAV7 for muscle-directed delivery, explaining that “vectors based on AAV7 capsids of the invention are particularly well suited for use in muscle; whereas vectors based on rh.10 (44-2)

capsids of the invention are particularly well suited for use in lung.” EX1007, ¶ 142. The ’772 Publication also explains that “[t]he lack of pre-existing immunity to AAV7 and the favorable tropism of the vectors for muscle indicates that AAV7 is suitable for use as a vector in human gene therapy and other in vivo applications.” EX1007, ¶ 251.

66. To the extent Dr. Schaffer points to sections of the ’772 Publication indicating AAVrh10’s tropism to muscle now, after ignoring delivery to muscle in his prior declaration, I do not agree that a skilled artisan would have chosen AAVrh10 over AAV7 for muscle delivery. The data above shows the superiority of AAV7 over AAVrh10 for muscle directed-delivery. Based on those data, a skilled artisan seeking to develop a gene therapy vehicle for muscle-directed delivery would have started with AAV7.

67. I understand that Dr. Schaffer points to the statements in paragraph 257 of the ’772 Publication related to the performance of AAVrh10 in muscle. EX1007, ¶ 257 (“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.”). That statement alone is unlikely to have directed a skilled artisan seeking to develop a vector to improve delivery of genes to muscle by AAVrh10, given all of the statements and data in the ’772 Publication regarding the superior performance of AAV7 in muscle.

68. But even if a skilled artisan did choose AAVrh10 as a starting point for a vector for muscle-directed gene delivery and chose to modify it, she would not have chosen AAV8 as a modifier. Instead, she would have chosen to modify AAVrh10 with AAV7. To improve targeting muscle, she would have chosen AAV7. I have reviewed an alignment done at my direction comparing AAVrh10 with AAV7, AAV1, AAV2, AAV3, and AAV8 in both individual and multiple sequence alignments. EX2032. AAV7 and AAVrh10 are 88.6% identical, with 655 amino acids that are the same and 84 that are different. EX20XX, 9. Relevant to the claims, one of those differences is at position 665, where AAVrh10 has a serine and AAV7 has a threonine. EX2032.

69. Finally, as noted above, the Fabb paper cited by Petitioner and Dr. Schaffer discusses delivery to muscle. EX1010. Though it is not entirely clear which AAV vector Fabb used, it appears that they may have used AAV2. EX1010, 7 (noting that at least “flanking inverted terminal repeats (ITRs) of AAV serotype 2” were used). Fabb also explains that “recent observations have shown that AAV vectors based upon alternative serotypes, particularly type 1 but also types 3 and 5, can increase levels of protein production by 100-1000 fold.” EX1010, 6. As shown in the sequence alignments at EX2032, AAV1, AAV2, and AAV3 each have a serine at the position that corresponds to position 665 in

AAVrh10. EX2032, 2. While this alignment does not show AAV5, that capsid protein also has a serine at the equivalent of position 665. EX1001, Fig. 2E.

70. Thus, at bottom, a skilled artisan's motivation or goal is critical to understanding the steps that skilled artisan would have taken when selecting or modifying a gene therapy vector for targeted delivery. Three possible goals would have motivated a skilled artisan here: (i) improving delivery and expression in liver; (ii) maintaining existing tropism in muscle and lung; or (iii) improving delivery and expression in muscle. In my opinion, none of these three pathways would have led a skilled artisan to AAVrh10 with a serine to asparagine substitution at position 665.

D. Snowdy's Teachings on Nuclear Localization Signals are Irrelevant to Tissue Tropism

71. In Grounds 4 and 6, I understand that Sarepta relies on a section of a dissertation written by one of my former Ph.D. students, Stephen Snowdy, entitled "Nuclear targeting by adeno-associated virus capsid proteins and virions" (EX1009). Dr. Snowdy was a student in my lab at University of North Carolina Chapel Hill from ~1998-2003, and this work was performed under my supervision. I therefore have first-hand knowledge of the work described in Dr. Snowdy's dissertation, and can put this work in proper context. At a high level, Snowdy does not teach or discuss making mutations to change the tissue to which a particular

AAV serotype is targeted. Instead, Snowdy uses alanine and aspartic acid substitutions on or near putative nuclear localization signals to determine whether phosphorylation impacts nuclear localization of AAVs. The chapter of Snowdy that Sarepta cites also does not teach the modification of a serine to an asparagine. Further, Sarepta also ignores that, not only had asparagine substitutions been demonstrated to *negatively* affect functionality in Sarepta's other prior art references, but removing a phosphorylatable residue could also have contradictory effects.

1. Nuclear Localization Signals Would Not Have Been Expected to Affect Tissue Tropism

72. In my opinion, Sarepta's reliance on Chapter IV of the dissertation is misguided. As an initial matter, I note that the particular study described in Chapter IV was never peer-reviewed or otherwise published, and in my view a skilled artisan would have accorded little, if any, weight to the results.

73. Additionally, and perhaps more importantly, this study is unrelated to improving or changing tissue tropism, which is the sole motivation Sarepta identifies for combining Snowdy with the '772 Publication. *See* Pet., 49 ("A POSA, seeking to improve the transduction of AAVrh.10 in liver to approach that of AAV8, in view of Snowdy..."); *see also* EX1005, ¶ 277. Instead, Snowdy's study investigates his hypothesis that phosphorylation consensus sites could impact

nuclear localization signals—i.e., the ability of an AAV vector to be transported through a nuclear pore and into the nucleus for expression. EX1009, 123. In my view, nuclear localization relates to infectivity, and is unrelated to the tissue-specific tropism driving Sarepta’s motivation to combine. As I discuss in detail above in paragraph 30, tropism relates to the steps that occur that allow an AAV vector to reach and be taken up by the cell or tissue of interest—steps 1 and 2 in the Figure I describe above; the intracellular steps that follow affect infectivity. Snowdy’s hypothesis regarding the effect of phosphorylation was focused on step 4 in the Figure—nuclear pore entry. EX2029, 4. Increasing intracellular infectivity, and in particular, the transport of the virus across the nuclear pore, would not have been expected to increase extracellularly-determined tropism to a particular tissue.

74. The study in Chapter IV of Snowdy was designed to determine whether putative (potential) phosphorylation sites in the vicinity of a putative nuclear localization signal in AAV2 affected intracellular infectivity. Critically, Snowdy never showed that the putative nuclear localization signal was in fact a nuclear localization signal, or that the putative phosphorylation sites were in fact phosphorylated on AAV2. Just because an amino acid could potentially be phosphorylated does not in and of itself make it a phosphorylation site. Indeed, Snowdy found that mutating two of three potential phosphorylatable residues had

no impact, and never provided proof that any of the three were or were not phosphorylation sites. Therefore, any conclusions drawn are, in my opinion, too theoretical to be relied upon as determinative of the structure-function relationship of this particular site in AAV2. In any event, Snowdy's results and conclusion are completely irrelevant to tropism. There is no evidence that I am aware of, in Snowdy that suggests that removing a phosphorylation site at a nuclear localization signal would in any way change or improve tissue tropism.

75. I also note that Snowdy itself acknowledged the limitations of his study, cautioning against making a “premature” “assum[ption] that a given phosphorylation status will have the same effect on all NLSs, as there is *insufficient* evidence in the literature to support this assumption.” EX1009, 129 (“Until we establish unequivocally that basic region 3 of AAV2 is an NLS, 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”). Instead, Snowdy acknowledges this “issue will be better understood with future studies on other nuclear localization signals.” EX1009, 129. I agree with Snowdy on this point.

2. Snowdy Only Discloses Alanine and Aspartic Acid Substitutions

76. In the portion of Snowdy that Petitioner cites, Snowdy modifies a phosphorylatable residue, like a serine, to “either a nonphosphorylatable *alanine*, to simulate an unphosphorylated serine, or to an *aspartic acid* to simulate a constitutively [i.e. always] phosphorylated serine[.]” EX1009, 125. Neither substitution is to an asparagine. I note that this approach is consistent with the wreck and check alanine scanning approach that I describe above in Section III(A)(2). Snowdy goes on to find that one of the substitutions, the serine to alanine substitution at position 181 “infected far more cells than did the wild-type[.]” EX1009, 126.

77. While I see that Sarepta contends that “Snowdy also teaches the substitution of asparagine (N) as a nonphosphorylatable amino acid to remove a phosphorylation site,” (Pet., 48 (citing EX1009, 28-29; EX1005, ¶ 274)), Sarepta’s expert, Dr. Schaffer, (correctly, in my view) rebuts this. Earlier in his declaration, Dr. Schaffer explains that Snowdy’s reference to “asparagine” here is an unfortunate typo, because “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.’” EX1005, ¶¶ 178-179. Put differently, “asparagine” simply does not make sense in the context of the sentence, which discusses a substitution

“resulting in a *constitutively phosphorylated* residue,” meaning aspartic acid, not asparagine. EX1009, 29. Any skilled artisan would have understood this from the face of Snowdy. Therefore, none of the Snowdy’s disclosures that Sarepta relies upon involved modifying serine to *asparagine*. It follows, then, that they could not possibly teach the specific S665N modification required by the claims. If anything, a skilled artisan would understand Snowdy to teach an alanine substitution. *Id.*, 125. In my opinion, Sarepta provides no cogent explanation for why, if a skilled artisan were to modify a serine to a nonphosphorylatable amino acid, a skilled artisan would choose asparagine over alanine or any of the other possibilities.

78. Additionally, Sarepta and Dr. Schaffer ignore that, in addition to the serine at position 181 that they focus on, Snowdy tested two other amino acids at putative phosphorylation sites—a threonine at position 159 and a different serine at position 148. EX1009, 125-126. Notably, mutating these two amino acids “had no effect, or little effect on the virus’ ability to infect the HeLa cells and to deliver the

eGFP gene carried as their cargo.”¹ EX1009, 126. Therefore, even within Snowdy, mutating a serine residue had significantly different results. This experiment demonstrates that removing a phosphorylatable amino acid would not have been expected to have any impact on the function of the AAV, and certainly would not have been expected to impact tissue tropism.

3. Jans & Jans Shows That Asparagine Abrogates Functionality

79. I note that another prior art reference cited by both Sarepta and Snowdy, Jans & Jans, showed that an asparagine, rather than improving functionality, actually “abrogates” (i.e. destroys) functionality. EX1009, 125 (citing EX1017). Dr. Schaffer states that “Snowdy, in citing Jans & Jans, teaches substituting asparagine for aspartic acid to remove the potential for phosphorylation.” EX1005, ¶ 181. But in my opinion, that is not what Jans & Jans discloses at all.

¹ I note that immediately following this sentence there is another typographical error. To be consistent with the data presented in Figure 23, this sentence should read “However, the **S181A** and **S181D** had large effects on the infectivity of the virus.” EX1009, 126.

80. As an aside, I note that Jans & Jans is unrelated to AAV; rather, Jans & Jans was focused on nuclear localization sequences in cellular proteins generally. EX1017, 2. Moreover, what Jans & Jans discloses is that, in one particular non-AAV protein (SV40 large T-antigen), substituting asparagine for aspartic acid had a negative effect, “result[ing] in nuclear import rates less than 4% wild type.” EX1017, 2. In other words, Jans & Jans teaches that substituting in asparagine could have a dramatic negative impact on functionality, the opposite of what Sarepta contends a skilled artisan would have gleaned from Snowdy.

81. I note that Snowdy recognized this very finding. In a section of Snowdy that Sarepta does not cite, Snowdy relates the Jans & Jans finding, stating that “[i]t has been shown previously that mutation of basic residues to asparagines abrogates [i.e., destroys] the function of NLSs.” EX1009, 78; see EX1017, 2. After performing his own asparagine mutation, Snowdy similarly found that while, “[t]o our surprise” the modified AAV2 “made recombinant virus at wild-type levels,” (EX1009, 72-79), it nevertheless failed to successfully infect cells, (EX1009, 81, 108-109), rendering it useless as a vector for gene therapy. Therefore, Snowdy provides no reason for a skilled artisan to modify any residue to asparagine, let alone the serine at 665, and particularly where Snowdy’s other tested alternatives—i.e., the mutations Snowdy does teach—were known.

4. Jans & Jans Shows Amino Acid Substitutions Can Have Contradictory Effects

82. Notably, Jans & Jans also teaches that substituting serine (i.e., the native residue of AAVrh10 at position 665) in place of aspartic acid in the T-antigen protein actually “enhanced nuclear import”—the opposite effect of what Snowdy found, thus further rebutting the idea that a skilled artisan seeking to improve functionality would seek to change the serine at all. EX1017, 2.

83. Other portions of Jans & Jans further demonstrate why a skilled artisan would not rely on a specific substitution from one study to draw inferences about what would happen if that substitution was made in a different study in a different protein. In a separate experiment, Jans & Jans identifies a phosphorylation site that contains two serines at position 111 and position 112. EX1017, 4. In Figure 5, Jans & Jans shows that mutating the serine at position 111 had very little effect on phosphorylation, but mutating the serine at position 112 reduced phosphorylation by roughly 80%:

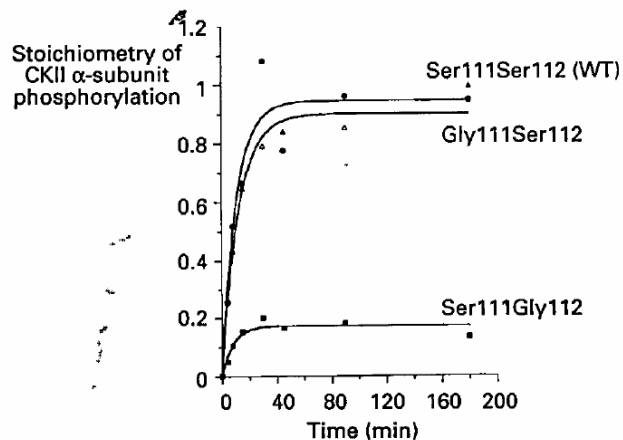


Figure 5 Kinetics of phosphorylation of T-ag fusion proteins by human casein kinase II α -subunit *in vitro*. Proteins were phosphorylated and the stoichiometry of phosphorylation quantitated as described in the legend to Figure 4. Results were fitted for the function $\text{Stoich.Phos.}(t) = \text{Max.Stoich.Phos.} \cdot (1 - e^{-kt})$, where t is time in min. The correlation coefficient (r) was 0.966, 0.992 and 0.954 respectively for T-ag(WT111–135), T-ag(Gly¹¹¹) and T-ag(Gly¹¹²). Phosphorylation of both T-ag(WT111–135) and T-ag(Gly¹¹¹) reached steady state at a stoichiometry of phosphorylation of about $1.0 \text{ mol Pi mol}^{-1}$ tetrameric fusion protein ($k = 94$ and 90×10^{-3} , respectively)

EX1017, 6.

84. Thus, not only would a skilled artisan not necessarily have known whether any amino acid was a phosphorylation site, they would not have been motivated to mutate a serine residue at any potential phosphorylation site, and they would have been unable to predict the effect of doing so. In my opinion, these contradictory effects highlight why relying on a specific substitution from one study to conclude generally that a skilled artisan would make a specific modification to a different AAV is tenuous and wholly speculative.

85. Thus, at bottom, in my opinion Sarepta's argument with regard to Snowdy fails because the results of mutating one specific serine in AAV2 has no

relevance whatsoever to mutating a different serine in a different AAV. Snowdy itself recognized this additional limitation regarding the applicability from one AAV to another AAV. See EX1009, 132 (noting that it was “unknown what receptor mediates infection by AAV1, AAV3, AAV7, and AAV8”). A skilled artisan simply would not have been motivated by the contradictory results in either Snowdy or Jans & Jans to substitute asparagine for the serine at position 665 in AAVrh10.

IV. LEGAL PRINCIPLES

A. Obviousness

86. I have been informed that a patent claim is invalid as “obvious” under 35 U.S.C. § 103 in light of one or more prior art references if it would have been obvious to a skilled artisan, taking into account (1) the scope and content of the prior art, (2) the differences between the prior art and the claims, (3) the level of ordinary skill in the art, and (4) any so called “secondary considerations” of non-obviousness, which include: (i) “long felt need” for the claimed invention, (ii) commercial success attributable to the claimed invention, (iii) unexpected results of the claimed invention, and (iv) “copying” of the claimed invention by others. For purposes of my analysis above and because I know of no indication from the patent owner or others to the contrary, I have applied a date of 2005, as the date of invention in my

obviousness analyses, although in many cases the same analysis would hold true even at a later time than 2005.

87. I have been informed that a claim can be obvious in light of a single prior art reference or multiple prior art references. To be obvious in light of a single prior art reference or multiple prior art references, there must be a reason to modify the single prior art reference, or combine two or more references, in order to achieve the claimed invention. This reason may come from a teaching, suggestion, or motivation to combine, or may come from the reference or references themselves, the knowledge or “common sense” of one skilled in the art, or from the nature of the problem to be solved, and may be explicit or implicit from the prior art as a whole. I have been informed that the combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results. I also understand it is improper to rely on hindsight in making the obviousness determination.