

**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,

Patent Owner

---

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

“Method of Increasing the Function of an AAV Vector”

---

IPR2024-00580

**REPLY DECLARATION OF DAVID V. SCHAFFER, PH.D.**

## **TABLE OF CONTENTS**

|                                                                                                                        | <b><u>Page</u></b> |
|------------------------------------------------------------------------------------------------------------------------|--------------------|
| I. MATERIALS CONSIDERED .....                                                                                          | 1                  |
| II. LEGAL STANDARDS – OBVIOUSNESS .....                                                                                | 2                  |
| III. DR. SAMULSKI’S OPINIONS ARE NOT RESPONSIVE TO MY<br>OPINIONS OR RELEVANT TO THE ISSUES IN THIS<br>PROCEEDING..... | 2                  |
| A. The Meaning of “Tropism” .....                                                                                      | 3                  |
| B. The State of the Art in 2005 .....                                                                                  | 3                  |
| C. Snowdy’s Teachings .....                                                                                            | 6                  |
| IV. DR. SAMULSKI’S UNUSUAL DEFINITIONS OF “TROPISM”.....                                                               | 10                 |
| A. The ’772 Publication Contradicts Dr. Samulski’s Definitions of<br>“Tropism” .....                                   | 16                 |
| B. Dr. Samulski Mischaracterizes My Publications .....                                                                 | 21                 |
| C. Dr. Samulski’s Patents and Applications Differ from His<br>Definitions of “Tropism” .....                           | 24                 |
| D. Dr. Samulski’s Research Publications Differ from His<br>Definitions of “Tropism” .....                              | 27                 |
| E. My Declaration Does Not Support Dr. Samulski’s Unusual<br>Definitions of Tropism.....                               | 32                 |
| V. THE STATE OF THE ART IN 2005 REGARDING SINGLE<br>AMINO ACID MODIFICATIONS TO CHANGE TROPISM.....                    | 34                 |
| A. Snowdy Contradicts Dr. Samulski’s Argument .....                                                                    | 36                 |
| B. The ’772 Publication Contradicts Dr. Samulski’s Argument .....                                                      | 37                 |
| C. Dr. Samulski’s ’126 Provisional Application Differs from His<br>Argument.....                                       | 41                 |
| 1. Single Amino Acid Substitutions to Alter Tropism.....                                                               | 42                 |

|     |                                                                                                           |    |
|-----|-----------------------------------------------------------------------------------------------------------|----|
| (a) | Sequence Alignments .....                                                                                 | 46 |
| (b) | Crystal Structure Analysis .....                                                                          | 47 |
| (c) | Identification of a Single Amino Acid Responsible<br>for Enhanced Transduction.....                       | 52 |
| 2.  | Single Amino Acid Changes to Alter Tropism by<br>Altering Phosphorylation .....                           | 56 |
| D.  | Dr. Samulski's '809 Patent Contradicts His Argument .....                                                 | 68 |
| 1.  | Single Amino Acid Changes to Alter Tropism by<br>Altering Phosphorylation .....                           | 69 |
| 2.  | Single Amino Acid Changes That Expand the Tropism of<br>an rAAV Vector .....                              | 71 |
| E.  | Dr. Samulski's Scientific Publication (Wu 2006) Contradicts<br>His Argument .....                         | 75 |
| F.  | A 2003 Publication from Another Research Group Contradicts<br>Dr. Samulski's Argument.....                | 80 |
| G.  | My 2005 Published Patent Application Contradicts Dr.<br>Samulski's Argument.....                          | 86 |
| H.  | My Publications Do Not Support Dr. Samulski's Argument<br>Regarding Single Amino Acid Substitutions ..... | 87 |
| 1.  | Jang .....                                                                                                | 87 |
| 2.  | Bartel.....                                                                                               | 88 |
| VI. | DR. SAMULSKI INCORRECTLY DESCRIBES AND LIMITS<br>SNOWDY'S TEACHINGS .....                                 | 91 |
| A.  | Snowdy's Teachings Are Not Limited to Nuclear Localization<br>of AAV .....                                | 92 |
| B.  | Snowdy's Teachings Are Not Limited to Substitution of<br>Alanine for Serine .....                         | 96 |

|       |                                                                                                                                              |     |
|-------|----------------------------------------------------------------------------------------------------------------------------------------------|-----|
| C.    | The Fact That Some Mutations Disclosed in Snowdy Had No Effect Does Not Diminish Snowdy’s Teachings .....                                    | 101 |
| VII.  | DOSAGE AND OFF-TARGET LOCALIZATION .....                                                                                                     | 104 |
| A.    | Tissue-Specific Promoters .....                                                                                                              | 104 |
| B.    | Route of Administration.....                                                                                                                 | 104 |
| C.    | Dr. Samulski Conflates Biodistribution and Infectivity.....                                                                                  | 106 |
| D.    | My Publications Are Wholly Consistent with My Opinions in This Proceeding and Do Not Support Dr. Samulski’s Arguments .....                  | 111 |
| 1.    | Kotterman .....                                                                                                                              | 111 |
| 2.    | Schaffer 2014.....                                                                                                                           | 112 |
| VIII. | MOTIVATION TO MODIFY AAVrh.10 BASED ON AAV8.....                                                                                             | 114 |
| A.    | A POSA Would Not Have Chosen AAV8 Instead of Modifying AAVrh.10.....                                                                         | 118 |
| B.    | A POSA Would Have Arrived at the S665N Substitution.....                                                                                     | 121 |
| 1.    | A POSA Would Have Used a Rational Design Approach Rather Than Making Large Numbers of Mutations.....                                         | 121 |
| 2.    | The Fact That Asparagine and Serine Both Have Uncharged Polar Side Chains Would Not Have Dissuaded a POSA From Making the Substitution ..... | 125 |
| C.    | A POSA Would Not Have Used Methods of Modifying Tropism Other Than Single Amino Acid Substitutions .....                                     | 127 |
| D.    | A POSA Would Have Been Motivated to Maintain the Superior Tropism of AAVrh.10 Across All Three Tissues .....                                 | 128 |
| E.    | I Did Not Express a “New” Opinion at My Deposition Regarding Preserving Tropism of AAVrh.10 in Muscle .....                                  | 134 |

|     |                                                                                                                                                         |     |
|-----|---------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| F.  | I Discussed Muscle Tropism in the Section of My Declaration Addressing Ground 1 of the Petition, and I Did Not “Admit” Otherwise at My Deposition ..... | 137 |
| IX. | DR. SAMULSKI INCORRECTLY DESCRIBES MY ARGUMENTS ABOUT CLAIM 8 .....                                                                                     | 139 |
| A.  | I Discussed Targeted Delivery to Muscle in My Declaration in the Sections Addressing Grounds 4-6 .....                                                  | 139 |
| B.  | Dystrophin Is Expressed Throughout the Body .....                                                                                                       | 141 |
| C.  | A POSA Would Not Have Started with AAV7 .....                                                                                                           | 143 |
| X.  | DR. SAMULSKI DOES NOT CORRECTLY CHARACTERIZE JANS & JANS .....                                                                                          | 149 |
| A.  | CKII Binding.....                                                                                                                                       | 151 |
| B.  | Phosphorylation of Serines Adjacent to the CKII Site.....                                                                                               | 157 |
| XI. | SECONDARY CONSIDERATIONS .....                                                                                                                          | 165 |

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

1. I previously submitted a Declaration in this matter (EX1005), which provides my background and qualifications in Section I and a copy of my CV (EX1006). I have been asked to provide this Reply Declaration to respond to specific issues raised in a declaration from Dr. Jude Samulski (EX2027) and in the Patent's Owners' Response.

## **I. MATERIALS CONSIDERED**

2. In formulating my opinions, I considered my original Declaration, the Patent Owners' Preliminary Response ("POPR"), the Institution Decision, the Patent Owners' Response ("POR"), Dr. Samulski's Declaration, and all of the materials cited therein, along with the additional materials cited in this Reply Declaration, set out in the table below:

| <b>Exhibit Number</b> | <b>Description</b>                                                                                                                                                                                                                |
|-----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| EX1058                | Haberman <i>et al.</i> , "Therapeutic Liabilities of <i>in vivo</i> Viral Vector Tropism: Adeno-Associated Virus Vectors, NMDAR1 Antisense, and Focal Seizure Sensitivity," <i>Molecular Therapy</i> (2002): 495-500 ("Haberman") |
| EX1059                | U.S. Provisional Patent Application 60/636,126 ("the '126 provisional")                                                                                                                                                           |
| EX1060                | Wu <i>et al.</i> , "Single Amino Acid Changes Can Influence Titer, Heparin Binding, and Tissue Tropism in Different Adeno-Associated Virus Serotypes," <i>Journal of Virology</i> (2006), pp. 11393-97 ("Wu 2006")                |
| EX1061                | U.S. Patent No. 7,892,809 ("the '809 patent")                                                                                                                                                                                     |
| EX1062                | Bowles <i>et al.</i> "Phase 1 Gene Therapy for Duchenne Muscular Dystrophy Using a Translational Optimized AAV Vector," <i>Molecular Therapy</i> (2012): 443-455 ("Bowles")                                                       |

| <b>Exhibit Number</b> | <b>Description</b>                                                                                                                                                                                                              |
|-----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| EX1063                | March 6, 2025 Samulski Deposition Transcript (“Samulski Dep. Tr.”)                                                                                                                                                              |
| EX1064                | Nov. 1, 2024 Schaffer Deposition Transcript, with errata (“Schaffer Dep. Tr.”)                                                                                                                                                  |
| EX1065                | U.S. Patent No. 9,012,224 (“the ’224 patent”)                                                                                                                                                                                   |
| EX1066                | U.S. Patent Application Publication 2005/0053922 (“the ’922 publication”)                                                                                                                                                       |
| EX1067                | PCT/US2005/045552 (“the ’552 PCT”)                                                                                                                                                                                              |
| EX1068                | Kern <i>et al.</i> , “Identification of a Heparin-Binding Motif on Adeno-Associated Virus Type 2 Capsids,” <i>Journal of Virology</i> (2003): 11072-11081 (“Kern”)                                                              |
| EX1069                | U.S. Provisional Patent Application 60/484,111 (“the ’111 provisional”)                                                                                                                                                         |
| EX1070                | Maersch <i>et al.</i> “Optimization of stealth adeno-associated virus vectors by randomization of immunogenic epitopes,” <i>Virology</i> (2010): 167-175 (“Maersch”)                                                            |
| EX1071                | Wang <i>et al.</i> , “Comparative Study of Liver Gene Transfer with AAV Vectors Based on Natural and Engineered AAV Capsids,” <i>Molecular Therapy</i> (2015): 1877-1887 (“Wang”)                                               |
| EX1073                | Lu <i>et al.</i> , “506. Transductional and Genome Biodistribution of Novel AAV Serotype Vectors after Systemic Delivery and Their Liver Specific Dose Response Profiles in Mice,” <i>Molecular Therapy</i> (2005): S196 (“Lu”) |

## **II. LEGAL STANDARDS – OBVIOUSNESS**

3. I incorporate by reference the legal standards relevant to my opinions in Section III of my original Declaration (EX1005).

## **III. DR. SAMULSKI’S OPINIONS ARE NOT RESPONSIVE TO MY OPINIONS OR RELEVANT TO THE ISSUES IN THIS PROCEEDING**

4. Dr. Samulski’s deposition testimony demonstrated that his opinions are not responsive to my opinions or relevant to the issues in this proceeding.

**A. The Meaning of “Tropism”**

5. First, Dr. Samulski offers two different and highly unusual definitions of the term “tropism.” EX2027 (Samulski Dec.), ¶¶ 38, 73. As I set out below, both of these unusual definitions differ from the ’772 Publication and Dr. Samulski’s own patented and published work from the relevant time.

6. Dr. Samulski’s testimony at his deposition showed that his definitions of tropism, and his opinions based on his definitions of tropism, are not responsive to the opinions I set out in my original Declaration, or relevant to the issues in this proceeding. Dr. Samulski testified that his definition of tropism was not informed at all by the way the term is used in the ’772 Publication, with which he disagrees. *See, e.g.*, EX1063 (Samulski Dep. Tr.). 43:5-12, 44:17-20, 44:22-45:4, 45:6. My opinions, however, use the term “tropism” as it is used in the ’772 Publication, which I believe to be the way a POSA and others in the field would have understood the term at the relevant time.

**B. The State of the Art in 2005**

7. Second, Dr. Samulski’s deposition testimony demonstrated that his opinions on the state of the art as of 2005 are similarly aside from the point. Dr. Samulski argues in his declaration that people in the art at the time, and even for many years afterwards, would not have considered making single amino acid

modifications to the AAV capsid to alter tropism. *See, e.g.*, EX2027 (Samulski Dec.), ¶¶ 28, 32.

8. At his deposition, Dr. Samulski testified that his opinions are directed to the question whether a POSA as of 2005 could have looked at the linear sequence of an AAV capsid protein and, without any other information, picked out a single amino acid to mutate, confident that such a mutation would affect tropism. *See, e.g.*, EX1063 (Samulski Dep. Tr.), 81:2-20.

9. But that is not responsive to any opinion that I set out in my original Declaration. My opinion is that a POSA at the relevant time, given the guidance of the '772 Publication and Snowdy, would have been motivated to create a single amino acid mutation in the AAVrh.10 capsid to try to confer some of the favorable liver tropism of AAV8 onto AAVrh.10. It is also my opinion that a POSA at the time, to create such a single amino acid mutation in the AAV capsid, would have aligned the capsid sequences of AAVrh.10 and AAV8, identified positions where AAVrh.10 has a phosphorylatable residue and where AAV8 has a nonphosphorylatable residue, and substituted in the non-phosphorylatable residue from AAV8 to replace the phosphorylatable residue in AAVrh.10.

10. Dr. Samulski acknowledged at his deposition that I have not argued that a POSA would have been able to look at, for example, the 738 amino acids in the AAVrh.10 capsid and select a single amino acid to mutate without any other

information, confident that the change would favorably alter a particular property of the virus. EX1063 (Samulski Dep. Tr.), 85:17-22, 24-25.

11. Instead, Dr. Samulski acknowledged that my opinions in fact are based on the ability of a POSA, in light of the teachings of the '772 Publication and Snowdy, to select several amino acids in the AAV capsid to mutate to see if any altered the liver tropism of the AAV vector. EX1063 (Samulski Dep. Tr.). 85:1-22, 24-25; 86:1-4, 6.

12. Importantly, Dr. Samulski also testified that “one approach” a POSA could have taken at the relevant time was to select several amino acids to test to see if any of them affected functions such as tropism or transduction. EX1063 (Samulski Dep. Tr.), 83:18-23. That is the basis of my obviousness opinions as set out in my original Declaration. *See, e.g.*, EX1005 (Schaffer Dec.), ¶¶ 277-80.

13. He also appears to have disregarded or to have dismissed the work of others in the field at the time investigating single amino acid mutations to change tropism and other characteristics of the virus, including the '772 Publication, a 2003 research publication (Kern), and my own 2005 patent application publication, all of which I discuss below. *See infra*, Sections V.B, V.F, and V.G.

14. Dr. Samulski also did not discuss in his declaration his own work at the time, disclosed in a 2004 patent filing and a 2006 research publication, using single amino acid substitutions in the AAV capsid to alter tropism. *See* Sections V.C, D,

and *E infra*. At his deposition, he attempted to walk back his broad generalizations about the state of the art as of 2005 by testifying that when he said no one was working on single amino acid substitutions to change tropism, he meant no one other than himself and his research group. EX1063 (Samulski Dep. Tr.), 143:13-18.

15. Therefore, like his opinions on the definition of the word “tropism,” Dr. Samulski’s arguments about the state of the art in 2005, and specifically the teachings in the art about making single amino acid mutations to change tropism, are not on point, and do not address my opinions in this proceeding.

### **C. Snowdy’s Teachings**

16. Third, Dr. Samulski’s deposition testimony demonstrates that he did not give Snowdy’s teachings appropriate weight in formulating his opinions in this proceeding.

17. Dr. Samulski argues in his declaration 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.” EX2027 (Samulski Dec.) ¶ 72. By his own admission, Dr. Samulski accorded “little . . . weight” to Snowdy’s teachings because the portion I have relied on for my opinions was “never peer-reviewed or otherwise published.” EX2027 (Samulski Dec.) ¶ 72.

18. Given that Dr. Samulski nowhere challenges the validity of the results set out in Snowdy, and in fact signed off on the dissertation as Snowdy's advisor and a member of his thesis committee, it is my opinion that these results are entitled to the same weight as published, peer-reviewed results. I believe that a POSA at the time would similarly have understood them to be worthy of the same weight as published, peer-reviewed results.

19. It is particularly surprising to me that Dr. Samulski attempts to diminish the import of the work included in his own student's dissertation. Dr. Samulski, after all, was Snowdy's thesis advisor, helped design his experiments, supervised his work and his thesis, and was a member of the committee that awarded Snowdy his Ph.D. Dr. Samulski even testified that as an older student and former Navy Seal, Snowdy was more mature and needed less supervision than younger graduate students, fresh out of college. EX1063 (Samulski Dep. Tr.), 16:3-8, 16:23-25, 17:1-6, 17:11-25.

20. It is my understanding that as a matter of patent law, it does not matter whether a prior art reference was "peer-reviewed" or published in a scientific journal. For example, patents and patent publications are not "peer reviewed" by the academic community or published in scientific journals, yet they are valid prior art references nonetheless. *See also* EX1063 (Samulski Dep. Tr.), 25:1-2.

21. Moreover, Dr. Samulski testified that he was Snowdy’s advisor on this dissertation, the work was carried out in his lab, and he supervised, approved, and signed the dissertation, indicating that he believed the work to be sufficiently valuable to support the award of a Ph.D. degree, as did the rest of the faculty members who reviewed and signed-off on the dissertation. EX1009 (Snowdy), p. 1; EX1063 (Samulski Dep. Tr.), 16:3-17:6, 18:10-12, 19:10-13, 19:20-20:14.

22. Dr. Samulski characterized Snowdy’s dissertation at his deposition as merely “acceptable,” not “exceptional.” EX1063 (Samulski Dep. Tr.), 18:23-19:1-3-7. And yet, in a 2004 patent filing, Dr. Samulski disclosed and claimed making single amino acid substitutions in AAV2 to confer the enhanced muscle transduction of AAV1 onto AAV2 – where the rational design approach he employed involved intentionally making single amino acid substitutions to replace a phosphorylatable threonine in AAV2 with the corresponding, non-phosphorylatable asparagine in AAV1. *See infra*, Section V.C.

23. According to Dr. Samulski, this work involved “extraordinary” skill and was wholly uninformed by Snowdy’s results from his own laboratory, which he

nonetheless testified had been available over a year prior to his subsequent patent filing.<sup>1</sup> EX1063 (Samulski Dep. Tr.), 142:24-143:18, 194:6-22, 25, 195:1-196:10.

24. Dr. Samulski's testimony demonstrates that he did not give proper weight in his analysis to the teachings of Snowdy as they would have been understood by a POSA at the relevant time.

---

<sup>1</sup> Dr. Samulski at his deposition did not offer a scientific reason that his '126 provisional could not have been informed by Snowdy's results. Instead, he testified that he did not believe the '126 provisional had been influenced by Snowdy's work because they had not named Snowdy as an inventor. EX1063 (Samulski Dep. Tr.), 195:2-10, 13-25, 196:1-10. He further testified that he believed he had arrived independently at the idea to substitute in non-phosphorylatable amino acids to replace phosphorylatable amino acids to enhance transduction based on protein chemistry, "the chemistry of a charged amino [acid] versus an uncharged amino acid." EX1063 (Samulski Dep. Tr.), 195:2-10, 13-25, 196:1-10. Of course, the chemistry of charged and uncharged amino acids does not explain the specific choice in the '126 provisional to focus on the ability of particular residues to be phosphorylated.

#### IV. DR. SAMULSKI'S UNUSUAL DEFINITIONS OF "TROPISM"

25. Dr. Samulski's unusual definitions of "tropism" appear to be offered to support his argument that a POSA would not have combined the teachings of Snowdy with those of the '772 Publication. *See, e.g.*, EX2027 (Samulski Dec.), ¶ 73. Specifically, Dr. Samulski states that Snowdy's teachings are directed to nuclear localization, which "relates to *infectivity*, and is unrelated to the tissue-specific *tropism* driving Sarepta's motivation to combine." *Id.* (emphases added).

26. I note preliminarily that the '772 Publication touts the superior gene transfer capability and *transduction* of AAVrh.10 across three tissues of AAVrh.10. EX1007, [0257]. And Snowdy teaches that removing a putative phosphorylation site in the AAV capsid can greatly improve *transduction* of target tissues. EX1005, ¶¶ 95-96, 187-89, 272-76. Therefore, as I set out in my original Declaration, a POSA would also be motivated to combine these references to improve *transduction* of AAVrh.10. *See, e.g.*, EX1005, ¶ 276.

27. Dr. Samulski testified that the argument from his declaration that nuclear localization relates to *infectivity* rather than *tropism* is premised on his own definition of tropism that he has adopted in this case, regardless of how the term is used in the art. EX1063 (Samulski Dep. Tr.), 51:7-52:10, 52:12.

28. For example, as I discuss further below (Section IV.D), Dr. Samulski admitted that he used the term "tropism" differently in his own scientific articles,

such as Haberman, than in his declaration. EX1063 (Samulski Dep. Tr.) 39:6-9; EX1058 (Haberman).

29. Dr. Samulski further testified that there are different meanings for the word “tropism,” and he was not trying to cover all the meanings of tropism in his declaration. EX1063 (Samulski Dep. Tr.), 38:21-39:1, 39:3-5, 40:22-25, 41:2-9. According to Dr. Samulski, the word “tropism” is used differently by different scientists focusing on different areas of research in the field of gene therapy. EX1063 (Samulski Dep. Tr.), 40:22-25, 41:2-9.

30. Taken together, Dr. Samulski offers multiple definitions of tropism in his declaration and deposition testimony that are inconsistent with each other, and also inconsistent with how the term is generally used in the art, including in the ’772 Publication and his own patents and scientific publications.

31. In ¶ 38 of his declaration, Dr. Samulski offers the following unusual definition of the term “tropism”: “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.” EX2027 (Samulski Declaration), ¶ 38 (emphasis in original).

32. In this part of his declaration, Dr. Samulski distinguishes between “tropism” and “infectivity”: “‘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.” EX2027 (Samulski Declaration), ¶ 38 (emphasis in original).

33. In ¶ 73 of his Declaration, by contrast, Dr. Samulski refers to a figure from a 2020 review he authored: “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.” EX2027 (Samulski Declaration), ¶ 73; EX2029 (Li), p. 4, Fig. 1.

34. In ¶ 30, Dr. Samulski reproduces Figure 1 from his 2020 review, which I have reproduced below, along with the caption, which, I note, does not mention the term “tropism”:

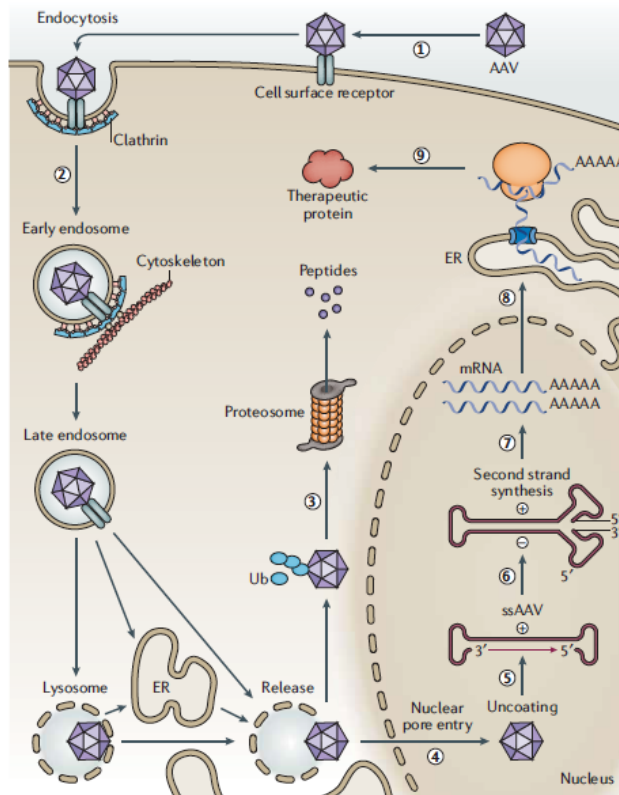


Fig. 1 | AAV vector transduction pathway. Adeno-associated virus (AAV) vector virions bind to receptors and co-receptors on the surface of target cells (step 1) and are taken into endosomes within these cells through endocytosis (step 2). Following their release from endosomes, AAV virions are either ubiquitinated and targeted for proteasome-mediated degradation (step 3) or intracellularly trafficked to the nucleus (step 4). Once in the nucleus, AAV virions are uncoated and the AAV genome is released (step 5). The AAV single-stranded DNA genome then is converted into double-stranded DNA (step 6), followed by transcription (step 7) and the nuclear export of mRNA (step 8) for translation and expression of the therapeutic transgene (step 9). Engineering AAV vectors to affect any step of their transduction pathway impacts their transduction efficiency. ER, endoplasmic reticulum; ssAAV, single-stranded AAV; Ub, ubiquitin.

EX2029, p. 4, Figure 1.

35. According to ¶ 73 of his declaration, “tropism” includes only steps 1 and 2 in the figure above, namely, binding to cell surface receptors and being taken into endosomes through endocytosis. EX2027 (Samulski Declaration), ¶ 73.

36. Notably, however, this second definition differs from Dr. Samulski’s first definition, from ¶ 38, which defines tropism to exclude “intake” into the cell: “‘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.” EX2027 (Samulski Declaration), ¶ 38 (first emphasis in original, second emphasis added).

37. Therefore, the ¶ 38 definition of tropism excludes “intake of the AAV vector into the cell,” while his ¶ 73 definition includes intake as part of tropism. At his deposition, Dr. Samulski also contradicted ¶ 73 of his declaration, testifying that, while his tropism definition includes being “taken up” by the cell, that part of tropism is not actually shown in the figure he cites in ¶ 30 in support of that version of his definition. EX1063 (Samulski Dep. Tr.), 31:1-2, 4-7.

38. At his deposition, Dr. Samulski attempted to reconcile these two definitions, testifying that he views endocytosis as different from “intake,” and that endocytosis is properly viewed as an event that happens *before* the AAV reaches the cell. EX1063 (Samulski Dep. Tr.), 32:20-25, 33:3-13. In this way, Dr. Samulski added an unusual definition of “intake” to his unusual definition of “tropism.”

39. In any event, Dr. Samulski testified that neither of these definitions of “tropism” includes steps that occur inside the cell – most notably, gene expression. EX1063 (Samulski Dep. Tr.), 33:14-17.

40. Finally, Dr. Samulski offered yet another definition of tropism at his deposition, that “cell culture cannot define things related to tropism.” EX1063

(Samulski Dep. Tr.), 55:19-21, 23-24. Instead, he testified that cells in culture can only be used to study “infectivity.” EX1063 (Samulski Dep. Tr.), 57:9-13, 15-21. However, one of Dr. Samulski’s publications from 2006 (Wu 2006) that I discuss below (Section IV.D), references experiments using cells in culture to provide insights about the tropism of different AAV variants. EX1060 (Wu), p. 4 (“It is also noteworthy to mention that preincubation of AAV6 vectors with heparin does not block AAV6 transduction in HepG2 cells in vitro (data not shown). These results suggest that the heparin binding ability and liver tropism of AAV6 might constitute two unrelated phenotypic characteristics caused by the same lysine 531 residue”).

41. In my opinion, people of ordinary skill in the art at the relevant time, and even today, would not understand the term “tropism” to have any of these meanings. Instead, in my opinion, people of ordinary skill in the art would have understood “tropism” to have the meaning that I set out in my Declaration and at my deposition, namely, “the ability to target and infect particular types of cells.” EX1005 (Schaffer Dec.), ¶ 66.

42. As I discuss below, Dr. Samulski’s multiple definitions and variations on those definitions, are contradicted by the ’772 Publication, other references published in the 2005-2006 timeframe, as well as Dr. Samulski’s own patents and scientific publications.

**A. The '772 Publication Contradicts Dr. Samulski's Definitions of "Tropism"**

43. Dr. Samulski's definition of "tropism" is not consistent with the use of the term in the '772 Publication. I note that at his deposition, Dr. Samulski agreed that his definition of "tropism" is different from the use of the term in the '772 Publication, and that he did not modify his definition in any way to account for the usage of the term in the '772 Publication. EX1063 (Samulski Dep. Tr.), 43:5-12, 44:17-20, 44:22-45:4, 6. Dr. Samulski testified that "they [the '772 Publication] are using tropism in another manner." EX1063 (Samulski Dep. Tr.), 48:2-3, 43:5-12, 44:17-20, 44:22-45:4, 6.

44. Dr. Samulski further testified, "I would not have defined my definition by their [the '772 Publication] definition," although he would have considered whether the '772 Publication was consistent or inconsistent with his definition. EX1063 (Samulski Dep. Tr.), 44:17-20, 44:22-45:4, 6.

45. He further testified that after considering the '772 Publication's definition, he would nonetheless have offered his own definition. EX1063 (Samulski Dep. Tr.), 44:22-45:4, 6.

46. Dr. Samulski testified that he was not bound by the way the term "tropism" is used in the '772 Publication. EX1063 (Samulski Dep. Tr.), 43:5-12.

47. Importantly, the '772 Publication expressly differs from Dr. Samulski's definitions of tropism and demonstrates that a POSA would be motivated to combine the teachings of the '772 Publication with those of Snowdy.

48. Example 9 of the '772 Publication discloses a set of experiments titled, "Tissue Tropism Studies." EX1007 ('772 Publication), [0252]-[0262]. I discussed these experiments in detail in my original declaration. *See, e.g.*, EX1005 (Schaffer Dec.), ¶¶ 122-23, 139-146.

49. Example 9 describes these experiments as "*gene transfer studies* targeting 3 different tissues, liver, lung and muscle *to screen for tissue tropism* of a particular AAV construct." EX1007 ('772 Publication), [0253] (emphases added).

50. Dr. Samulski testified that these studies used gene expression to evaluate tropism. EX1063 (Samulski Dep. Tr.), 46:24-47:1. He further testified that the '772 Publication uses the term "tropism" in a different way from the way he uses it in his declaration. EX1063 (Samulski Dep. Tr.), 47:22-48:6, 50:10-18 (analogizing "tropism" to opening the door to a room – the cell – without entering the room), 50:20-25, 51:2-6 (testifying that both the '772 Publication and Haberman use the term "tropism" differently from the definitions in his declaration).

51. The experiments in Example 9 of the '772 Publication involved infecting lung, liver, and muscle tissues with different AAV serotypes carrying "an

easily detectable and quantifiable reporter gene,” human  $\alpha 1$  anti-trypsin gene (A1AT). EX1007 (’772 Publication), [0253].

52. After infection of the three tissues, the serum concentration of the protein (in  $\mu\text{g}$  A1AT/ml serum) produced by the reporter gene was measured in each infected animal. EX1007 (’772 Publication), [0253], Table 8. The results are shown in Table 8:

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

EX1007 (’772 Publication), [0253], Table 8.

53. This study therefore measured *reporter gene expression* in various different tissues to evaluate *tropism*.

54. A POSA would have understood that gene expression is an AAV function that takes place in the nucleus of infected cells, rather than outside the cell, at the cell surface, or in an endosome. A POSA would have further understood that the experiments in Example 9 were not designed to measure activities outside the cell, or binding to cell surface receptors, or uptake into endosomes. Instead, they evaluated gene expression as a measure of “tropism.”

55. The '772 Publication discloses that in these experiments, “a novel AAV capsid clone, 44.2, was found to be a very potent gene transfer vehicle in all 3 tissues with a big lead in the lung tissue particularly.” EX1007 ('772 Publication), [0253]. The '772 Publication describes these experiments as demonstrating the “superior tropism of AAV44.2 in lung tissue,” which was then confirmed in other experiments that also measured reporter protein expression. EX1007 ('772 Publication), [0254]-[0256].

56. I note that “expression of the gene of interest” is a function that Dr. Samulski expressly excludes from both his ¶ 38 and his ¶ 73 definitions of “tropism.” EX2027 (Samulski Dec.), ¶¶ 38, 73. EX1063 (Samulski Dep. Tr.), 47:22-24.

57. Dr. Samulski’s definitions of the term tropism therefore cannot be reconciled with the meaning of the term as it is used in the '772 Publication. His opinions, therefore, cannot speak to whether a POSA would have been motivated by the teachings of the '772 Publication about the superior tropism of AAVrh.10 across three separate tissues, in combination with Snowdy’s teachings, to make the S665N substitution to improve the tropism of AAVrh.10 in liver tissue.

58. As a result, to the extent that Dr. Samulski has based his opinions on his own, different definitions of tropism rather than on the definition of tropism used

in the '772 Publication, they are not responsive to my opinions or relevant to the question of obviousness of the '274 patent at issue here.

59. The '772 Publication also describes the experiments in Example 9 as demonstrating the ability of the various AAV serotypes to “transduce” liver, lung, and muscle tissues. EX1007 ('772 Publication), [0257].

60. As I set out in my original declaration, the '772 Publication touts the excellent transduction of AAVrh.10 across all three tissues: “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.” EX1007 ('772 Publication), [0257] (emphases added); EX1005 (Schaffer Dec.), ¶¶ 121-23, 207-208. Accordingly, the '772 Publication teaches that AAVrh.10 is an excellent “transducer” of lung, liver, and muscle, but is not quite as good as the best liver “transducer,” AAV8.

61. As I also discussed in detail in my original declaration, Snowdy teaches that removing a putative phosphorylation site in the AAV capsid can greatly improve *transduction* of target tissues. EX1005 (Schaffer Dec.), ¶¶ 95-96, 187-89, 272-76.

62. Therefore, both references expressly address the ability of AAV to transduce cells, and Snowdy teaches one means of improving AAV transduction. *See, e.g.*, EX1009 (Snowdy), pp. 5, 112, 114.

63. Therefore, Dr. Samulski's argument that a POSA would not be motivated to combine the teachings of Snowdy with those of the '772 Publication based on his unusual definitions of tropism fails for this additional reason.

**B. Dr. Samulski Mischaracterizes My Publications**

64. Dr. Samulski states that my "peer-reviewed publications appear to largely use the terms tropism and infectivity in the same way that [he] and the rest of the field at large generally do." EX2027, ¶ 38. Dr. Samulski cites three of my publications in support of this statement, EX1030 (Kotterman), EX2024 (Jang), and EX2025 (Schaffer). Dr. Samulski incorrectly characterizes my publications. I discuss each in turn below.

65. Kotterman is a 2014 article I wrote with Melissa Kotterman titled, "Engineering adeno-associated viruses for clinical gene therapy." EX1030 (Kotterman). We provide the following definition of the term "Tropism" in a "Glossary" at the end of the article: "The cell or tissue type that can be infected by a virus or a gene delivery vector." EX1030 (Kotterman), p. 6. As a preliminary matter, this definition is completely consistent with the definition of "tropism" I provided in my Declaration and at my deposition ("the ability to target and infect particular types of cells"). And this definition is inconsistent with Dr. Samulski's definition, involving only the steps preceding contact of the virus with the cell – and not involving contact or entry into the cell.

66. The meaning of the term “tropism” is also clear from the context in which it is used in Kotterman. For example, we say, “[i]n another example of rational design, the incorporation of high-affinity ligands can confer binding to alternative cell surface receptors and thereby restrict or redirect viral tropism.” EX1030 (Kotterman), p. 3. Here, we discuss engineering high affinity ligands into an AAV capsid to change the ability of the capsid to bind to cell surface receptors to change tropism. Clearly, “tropism” is referring to binding and entry into the cell, and not just to the steps preceding contact with the cell.

67. Dr. Samulski also cites an article I wrote with colleagues in 2007, titled, “Library Selection and Directed Evolution Approaches to Engineering Targeted Viral Vectors.” EX2024 (Jang). Dr. Samulski quotes the statement from this article that “envelope proteins are the primary determinants of the cell specificity or tropism of a retrovirus.” EX2024 (Jang), p. 2. I note preliminarily that Dr. Samulski does not quote the entire sentence, but omits the first part, shown here in bold italics: “*Since they bind to a specific cell surface receptor*, envelope proteins are the primary determinants of the cell specificity or tropism of a retrovirus.” EX2024 (Jang), p. 2 (emphasis added).

68. Nothing in this statement contradicts the definition of tropism I provided in my Declaration and at my deposition – the statement is wholly consistent with the definition, in that it uses the term “tropism” to describe the cell types that a

virus is capable of infecting. The “envelope proteins” of a retrovirus are the viral proteins that, as Jang states, bind to a receptor on the cell surface as a first step in viral entry into the cell. EX2024 (Jang), p. 2.

69. In the section of Jang that discusses AAV, we similarly use the term “tropism” consistently with the definition in my Declaration and that I discussed at my deposition. For example, Jang discusses studies that “have attempted to enhance selective AAV vector gene delivery to specific cell types via altering binding affinity to receptors expressed on target cells,” describing such studies as “strategies to modify AAV tropism.” EX2024 (Jang), p. 6. Here again, the term “tropism” is used to describe a process of binding to receptors expressed on target cells so that the virus can gain entry to those cells.

70. Dr. Samulski also quotes the comment I published in 2014, titled, “AAV shuffles to the liver: commentary on Lisowski *et al*,” which I discussed above. EX2025 (Schaffer 2014). Dr. Samulski quotes the statement from this publication that “upon systemic administration, natural serotypes typically exhibit tropism for liver, or alternatively for multiple organs, which renders targeted delivery to other tissues difficult.” EX2025 (Schaffer 2014), p. 1. I am at a loss to understand how this statement could be read as inconsistent with the definition I gave in my Declaration and at deposition. This paper further states, “the recent hemophilia B trial harnessed the natural tropism of AAV8 for the liver.” EX2025

(Schaffer 2014), p. 1. Exactly as I did in my Declaration, I am describing here the natural ability of AAV8 to target and infect liver tissue, and describing that as “tropism.”

**C. Dr. Samulski’s Patents and Applications Differ from His Definitions of “Tropism”**

71. On December 15, 2004, Dr. Samulski filed Provisional Application No. 60/636,126 (“the ’126 provisional”), titled “Chimeric Vectors.” EX1059 (’126 provisional).

72. U.S. Patent No. 7,892,809, on which Dr. Samulski is listed as an inventor, claims priority to this provisional application. EX1061 (’809 patent). The ’809 patent also claims priority to PCT/US2005/045552, which was filed on December 15, 2005. EX1061 (’809 patent); EX1067 (’552 PCT). The ’809 patent issued on February 22, 2011, and it is my understanding that it expires in 2026.

73. U.S. Patent No. 9,012,224, on which Dr. Samulski is listed as an inventor, also claims priority to the ’126 provisional application. EX1065 (’224 patent). The ’224 patent issued from Application No. 12/983,547, which is a continuation of Application No. 11/793,430, which issued as the ’809 patent. It is my understanding that the ’224 patent expires in 2029.

74. The ’126 provisional sets out the following definition for the term “tropism”: “The term ‘tropism’ as used herein refers to *preferred entry of the virus*

*into the cell*, optionally and preferably *followed by expression (e.g., transcription and, optionally, translation) of sequences carried by the viral genome in the cell*, e.g., for a recombinant virus, *expression of the heterologous nucleotide sequence(s)*.” EX1059 (’126 provisional), 12:1-10 (emphases added).

75. According to this definition, “tropism” means, at the very least, “preferred entry of the virus into the cell,” rather than the steps that *precede* entry into, or even arrival at, the cell, as Dr. Samulski has argued in his declaration, at least with respect to one of his definitions of “tropism.” EX2027 (Samulski Dec.), ¶ 38.

76. Moreover, in his declaration, Dr. Samulski attempts to draw a distinction between “tropism” and “infectivity,” arguing that “tropism and infectivity are better understood as separate functions.” EX2027, ¶ 38. According to Dr. Samulski’s declaration, “infectivity” “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.” EX2027, ¶ 38.

77. Notably, the ’126 provisional does not use the term “infectivity” anywhere, and, in fact, defines “tropism” to include the features that Dr. Samulski separates out into “infectivity” in his declaration, such as expression of the viral genome, which a POSA would understand to take place after translocation to the nucleus. EX1059 (’126 provisional), 12:1-10; EX2027, ¶ 38.

78. Dr. Samulski confirmed at his deposition that the definition of “tropism” in the ’126 provisional is different from the definitions in his declaration. EX1063 (Samulski Dep. Tr.), 159:11-21, 162:11-16. Specifically, he testified that the definition of the term “tropism” in the ’126 provisional “is not referring to tropism as I described it, but it’s modifying the tropism, as I described it,” and that “it’s different than the one I’m declaring.” EX1063 (Samulski Dep. Tr.), 159:11-21, 162:11-16.

79. Both the ’809 patent and the ’224 patent include very similar definitions of “tropism” as the ’126 provisional. EX1061 (’809 patent), col. 8:60-67; EX1065 (’224 patent), col. 8:58-65. Both the ’809 patent and the ’224 patent define tropism (in pertinent part) as follows: “The term ‘tropism’ as used herein refers to *preferential entry of the virus into certain cell or tissue types or preferential interaction with the cell surface that facilitates entry into certain cell or tissue types*, optionally and preferably *followed by expression (e.g., transcription and, optionally, translation) of sequences carried by the viral genome in the cell*, e.g., for a recombinant virus, *expression of the heterologous nucleotide sequence(s)*”). EX1061 (’809 patent), col. 8:60-67; EX1065 (’224 patent), col. 8:58-65 (emphases added).

80. Dr. Samulski testified that this definition of tropism adds on to the definitions in his declaration that tropism can include expression of a transgene. EX1063 (Samulski Dep. Tr.), 242:24-243:4.

81. Both of these patents, like the '126 provisional, therefore, do not exclude gene expression from tropism. Dr. Samulski's definitions of tropism are therefore inconsistent with the '809 patent and the '224 patent.

**D. Dr. Samulski's Research Publications Differ from His Definitions of "Tropism"**

82. In 2002, Dr. Samulski was a co-author on a research paper titled, "Therapeutic Liabilities of *in Vivo* Viral Vector Tropism: Adeno-Associated Virus Vectors, NMDAR1 Antisense, and Focal Seizure Sensitivity." EX1058 (Haberman). I note that Stephen Snowdy is also an author on this publication. EX1058 (Haberman).

83. In the Introduction, Haberman states that preferential binding of neurons by AAV, combined with "promoter tropisms," results in differential patterns of transduction across various parts of the brain. EX1058 (Haberman), p. 1. The phrase "promoter tropisms" shows that Haberman is not using the term "tropism" according to any of the definitions Dr. Samulski offered in his declaration, but, instead is using the term to include functions inside the cell, such as gene expression regulated by different promoters.

84. Dr. Samulski testified that he used the term “tropism” in Haberman in a different way from the definitions he offered in his declaration. EX1063 (Samulski Dep. Tr.), 38:25-39:1, 39:3-9 (“There are other ways to define tropism other than by the virus specifically binding to step one and two”). In particular, Dr. Samulski testified “tropism” in Haberman was “based on the promoter expression.” EX1063 (Samulski Dep. Tr.), 38:25-39:1, 39:3-9. He further testified that he does his best to ensure that everything in his scientific publications is true and correct. EX1063 (Samulski Dep. Tr.), 34:18-21.

85. Haberman investigated whether delivery of antisense RNA directed against an NMDA receptor (NMDAR1) via an rAAV vector could affect the receptor function and seizure activity related to the receptor. EX1058 (Haberman), pp. 1-2.

86. Haberman infused rats with two different rAAV vectors, one containing NMDAR1 antisense cDNA under the control of the tTAK promoter, and the other containing NMDAR1 antisense cDNA under the control of the CMO promoter. EX1058 (Haberman), p. 3. They found that the tTAK construct caused the rats to become more sensitive to seizures, while the CMO construct caused the rats to become less sensitive to seizures. EX1058 (Haberman), p. 3.

87. The only difference between the two different constructs was the promoter in each construct controlling expression of the transgene. EX1063 (Samulski Dep. Tr.), 39:12-19.

88. Haberman hypothesized that the cause of these disparate results was differences in tropism between the two vectors, produced by the different promoters. EX1058 (Haberman), p. 3. Specifically, Haberman theorized that “the ***tropism*** of the two AAV vectors was such that in one case ***the transduction pattern*** favored primary inhibitory interneurons, whereas in the other case ***the transduction pattern*** favored primary output neurons.” EX1058 (Haberman), p. 3 (emphases added).

89. Haberman goes on to explain that an examination of the transduction patterns of the two different constructs, infused in a 1:1 mixture, showed that their hypothesis about tropism was correct. EX1058 (Haberman), pp. 3-4, Fig. 5. More than 60% of the transduced cells expressed one or the other of the constructs, but not both. EX1058 (Haberman), pp. 3-4, Fig. 5. Haberman concludes that “[t]his ***transduction pattern*** illustrates the likely difference in *in vivo tropism* between the two similar AAV vectors.” EX1058 (Haberman), p. 4, Fig. 5 (emphases added).

90. Haberman therefore again uses the term “tropism” to describe transduction patterns, *i.e.*, expression patterns within cells, of different constructs.

91. Haberman uses the term several times in this way, in the same context. For example, “these studies vividly illustrate how ***a modest change in the promoter element can change the in vivo tropism*** to such a degree as to produce opposite physiological effects.” EX1058 (Haberman), p. 4 (emphasis added). Haberman further states, “the studies also showed that ***modest changes in the promoter***

*element can alter the tropism* in a manner that produced dramatically divergent effects.” EX1058 (Haberman), p. 4 (emphasis added).

92. Haberman describes the experiments discussed above where a 1:1 mixture of the two constructs was infused as follows: “Further substantiation of this *altered tropism* was obtained using a mixture of two AAV vectors. It is clear that AAV-CMV-LacZ and AAV-tTAK-GFP vectors *transduce different neuronal populations*, albeit with some overlap between the two populations (Fig. 5). . . . Thus, *the transduction patterns reflect a difference in tropism*, not random variation. . . . The *most likely source* of this *divergent tropism* is the *promoters* of the two vectors.” EX1058 (Haberman), pp. 4-5 (emphases added).

93. Haberman states that further experiments would be needed “to *determine the elements that contribute to tropism* and *where they have their effects during the transduction process*.” EX1058 (Haberman), p. 5. Haberman thus repeatedly uses the term “tropism” to refer to transduction, *i.e.*, a process involving entry into different cell types by rAAV.

94. Based on these results, Haberman cautions that in a case where the same cell needs to be infused with two different constructs, one containing a transgene to be expressed, and another containing an activator of the transgene, “*differential cellular tropism* [of the two constructs] could result in *significantly diminished gene expression*.” EX1058 (Haberman), p. 499 5 (emphases added).

95. Haberman therefore uses the term “tropism” to describe differences in “transduction patterns,” *i.e.*, differences in observed expression of the AAV vector transgene in different cell types, between the two AAV vectors. Clearly, according to Haberman, transgene expression in different cell types is a key element of tropism.

96. Haberman therefore contradicts Dr. Samulski’s definition of tropism for purposes of this proceeding, which involves only processes *before* the AAV vector reaches the cell. Haberman, furthermore, is completely consistent with my definition of tropism, which encompasses a series of steps from cell surface binding to gene expression.

97. Another of Dr. Samulski’s publications that I discuss in detail below (Section V.E) also contradicts the definitions of tropism set out in his declaration. EX1060 (Wu 2006). Dr. Samulski testified that he used the word “tropism” in this paper to refer to a process “dictated by the promoter [of the transgene], not by the capsid.” EX1063 (Samulski Dep. Tr.), 215:17-25. Thus, as Dr. Samulski concedes, Wu 2006 relates “tropism” to the expression of the gene sequences carried by the AAV vector. Wu 2006 does not limit the term “tropism” to steps occurring outside the cell or to the initial steps of binding and uptake of the AAV virus, as Dr. Samulski contends the term should now be defined for purposes of this proceeding.

**E. My Declaration Does Not Support Dr. Samulski's Unusual Definitions of Tropism**

98. The POR states incorrectly that my Declaration was inconsistent with my deposition testimony regarding the meaning of the term “tropism,” and that my Declaration supports Dr. Samulski's opinions. POR, pp. 52-53.

99. The opinions I set out in my Declaration regarding the definition of “tropism” are completely consistent with my deposition testimony. In my Declaration, I defined tropism as “the ability to target and infect particular types of cells.” EX1005 (Schaffer Dec.), ¶ 66.

100. I also briefly defined “tropism” and “transduction efficiency” in a single sentence in my Declaration as follows: “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.” EX1005 (Schaffer Dec.), ¶ 85.

101. The POR misinterprets this statement. The fact that “tropism” and “transduction efficiency” are two different terms does not mean that they describe completely separate phenomena. As I intended to convey through the parentheticals, “tropism” is a broader term that encompasses “transduction efficiency.” For example, an rAAV vector might have “tropism” across several different tissues, but might have different “transduction efficiencies” for each tissue.

102. This paragraph in my Declaration, furthermore, cites a 2014 review of mine. EX1029 (Kwon). In this publication, I stated, “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*. Although *these steps collectively determine AAV tropism*, viral binding to the cell surface is particularly important and has thus been a primary target for engineering to alter AAV tropism.” EX1029 (Kwon), p. 2 (internal citations omitted) (emphases added).

103. Therefore, in this review, which I referenced to support my definition of tropism in my Declaration, I provided essentially the same definition of tropism that I gave at my deposition, namely, that “tropism” is the collection of steps from viral binding to the cell surface all the way through viral gene expression. *See, e.g.*, EX1064 (Schaffer Dep. Tr.), 54:8-10; 76:3-8 (“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”); 312:9-11, 17-19 (explaining that the “ability to infect a target cell” is an “inherent part” and an “important aspect” of tropism).

## **V. THE STATE OF THE ART IN 2005 REGARDING SINGLE AMINO ACID MODIFICATIONS TO CHANGE TROPISM**

104. Dr. Samulski responds to my opinions by arguing that people of skill in the art at the time would not have attempted to alter tropism of rAAV vectors by making single amino acid modifications to the capsid. *See, e.g.*, EX2027 (Samulski Dec.), ¶¶ 28, 32. In particular, Dr. Samulski argues that, as of the relevant date, single amino acid substitutions had not been found to improve or change tropism: “while some techniques had been shown by 2005 to improve or change tropism, none involved a single amino acid substitution.” EX2027 (Samulski Dec.), ¶ 32.

105. Dr. Samulski states, “By 2005, AAV-based vectors for gene therapy were still in the very early stages of exploration.” EX2027 (Samulski Dec.), ¶ 28. Dr. Samulski points to a study by Hauck, published in 2003, as an example of the state of the art as of 2005, which he characterizes as a domain-swapping approach. EX2027 (Samulski Dec.), ¶ 27; EX1052 (Hauck). Moreover, Dr. Samulski characterizes Hauck as “not aimed at engineering AAVs for specific therapeutic purposes.”<sup>2</sup> EX2027 (Samulski Dec.), ¶ 27.

---

<sup>2</sup> Notably, at his deposition, Dr. Samulski tried to revise his statements about Hauck representing the state of the art as of 2005, testifying that Hauck’s approach was merely “one common practice.” EX1063 (Samulski Dep. Tr.), 130:5-7, 9-11.

106. Dr. Samulski goes even further in his declaration, claiming that he is not aware even today of single amino acid mutations that could increase tropism “significantly” or change tropism to a “materially” different tissue type: “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.” EX2027 (Samulski Dec.), ¶ 32.

107. He further argues that “The kind of modifications 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.” EX2027 (Samulski Dec.), ¶ 28. Dr. Samulski cites a 2019 paper in support of this argument, suggesting that it took more than a decade for the field to begin exploring single amino acid changes in AAV capsids to improve function or change tropism. EX2027 (Samulski Dec.), ¶ 28.

108. However, contrary to these statements, Dr. Samulski admitted at his deposition that his own lab had employed a strategy as of 2004 that involved using single amino acids to change tropism, an approach that he distinguished from the domain-swapping approach of Hauck. EX1063 (Samulski Dep.), 124:25-125:20, 125:22-126:1, 3-5, 147:22-148:2.

109. As I discuss below, Dr. Samulski's description of the state of the art as of 2005 is not correct. Dr. Samulski's opinions are not only different from his own patent filings, patents, and research publications, but are also contradicted by the disclosures in Snowdy, the '772 Publication, and the published work of others in the field, including one of my own patent application publications.

**A. Snowdy Contradicts Dr. Samulski's Argument**

110. As a preliminary matter, the Snowdy reference, one of the principal references at issue in this proceeding, discloses a single amino acid substitution that improves tropism. I note that Dr. Samulski was Snowdy's advisor on this dissertation, and signed the dissertation. EX1009 (Snowdy), p. 1; EX1063 (Samulski Dep. Tr.), 16:3-17:6, 18:10-12, 19:10-13, 19:20-20:14.

111. Specifically, as I discussed in my original Declaration, Snowdy substituted an alanine for a serine at position 181 of AAV2 (mutant S181A) and found that "the result was a virus that transduces target cells with greater efficiency than did the wild-type virus." EX1009 (Snowdy), p. 128; *see also* pp. 126-30; EX1005 (Schaffer Dec.), ¶ 187; EX1063 (Samulski Dep. Tr.) 87:6-9, 11-16 (testifying that Snowdy found that he enhanced transduction efficiency with a single amino acid change in AAV2).

112. In contrast, when Snowdy substituted an aspartic acid for this same serine (mutant S181D), Snowdy found that the ability of the rAAV to infect target

cells was essentially eliminated. EX1009 (Snowdy), p. 126; EX1005 (Schaffer Dec.), ¶ 187. Snowdy further disclosed, “The same number of particles containing 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.” EX1009 (Snowdy), p. 126.

113. Snowdy therefore discloses two examples of a single amino acid substitution (S181A and S181D) in an rAAV capsid that significantly altered the efficiency of transduction of target cells, which, as I discussed above, alters tropism.

114. Dr. Samulski testified that he and Snowdy were using HeLa cells as a model *in vitro* system to investigate steps that are involved in the infectious pathway of AAV2, assuming that the results might be informative of the ability of AAV to infect a cell in tissues *in vivo*. EX1063 (Samulski Dep. Tr.), 92:5-21, 92:23-93:10.

#### **B. The ’772 Publication Contradicts Dr. Samulski’s Argument**

115. Dr. Samulski states inaccurately that “the ’772 Publication is silent as to using single amino acid substitutions to alter tissue tropism.” EX2027 (Samulski Dec.), ¶ 53.

116. However, contrary to Dr. Samulski’s statement, the ’772 Publication, dated July 24, 2003, discloses a single amino acid substitution in AAV that could alter properties of the virus including tropism. Nevertheless, Dr. Samulski testified that he did not consider the disclosures regarding single amino acid substitutions in

the '772 Publication as part of his opinion because they represent “a hypothesis I don’t believe in.” EX1063 (Samulski Dep. Tr.), 62:23-63:1, 63:3-23, 63:25-64:5, 64:7-10, 65:9-10, 12-18, 20-21.

117. Specifically, the '772 Publication provides:

It is notable that AAV7 has an unusual GTG start codon. With the exception of a few house-keeping genes, such a start codon has not previously been reported in DNA viruses. The start codons for vp1, vp2 and vp3 for other AAV serotypes have been believed to be such that they permit the cellular mechanism of the host cell in which they reside to produce vp1, vp2 and vp3 in a ratio of 10%:10%:80%, respectively, in order to permit efficient assembly of the virion. However, the AAV7 virion has been found to assemble efficiently even with this rare GTG start codon. ***Thus, the inventors anticipate this it is desirable to alter the start codon of the vp3 of other AAV serotypes to contain this rare GTG start codon, in order to*** improve packaging efficiency, to alter the virion structure and/or to ***alter location of epitopes*** (e.g., neutralizing antibody epitopes) ***of other AAV serotypes***. The start codons may be altered using conventional techniques including, e.g., site directed mutagenesis. Thus, the present invention encompasses altered AAV virions of any selected serotype, composed of a vp3, and/or optionally, vp1 and/or vp2 having start codons altered to GTG.

EX1007 ('772 Publication), [0072] (emphases added); EX1005 (Schaffer Dec.), ¶ 229.

118. Here, the '772 Publication discloses a single amino acid substitution in AAV, namely, replacing ATG, the codon for the amino acid methionine, with GTG, the codon for the amino acid valine. *See, e.g.,* EX1044 (Genetic Code); EX1063 (Samulski Dep. Tr.), 60:23-61:6. Mutating the GTG codon to ATG would have produced a single amino acid mutation, replacing methionine with valine, in the vp1

protein and vp2 protein. *See, e.g.*, EX1007 ('772 Publication), p. 99 (Fig. 2B), p. 140 (SEQ ID NO: 2); EX1063 (Samulski Dep. Tr.), 60:23-61:6, 61:14-19, 61:21-62:11.

119. Dr. Samulski testified that a POSA would have understood that the mutated codon (ATG to GTG, and therefore methionine to valine) would have been present in the vp1 and vp2 proteins in the mutant capsid.<sup>3</sup> EX1063 (Samulski Dep. Tr.), 60:23-61:6, 61:14-19, 61:21-62:11.

120. Moreover, the '772 Publication teaches that altering the common ATG start codon to the uncommon GTG start codon could “alter location of epitopes (*e.g.*, neutralizing antibody epitopes) of other AAV serotypes.” EX1007 ('772 Publication), [0072].

121. Dr. Samulski agreed that the '772 Publication discloses in [0072] that a single amino acid change in the AAV capsid could affect the location of epitopes

---

<sup>3</sup> Dr. Samulski also testified that the mutation of the ATG at the start of vp3 to GTG in serotypes other than AAV7 might result in failure to express vp3 and failure to assemble a capsid. EX1063 (Samulski Dep. Tr.), 62:12-13, 62:16-22. In my opinion, such an outcome is unlikely, given that AAV7 (as the '772 Publication teaches), naturally includes the GTG start codon for vp3, and both expresses vp3 and “assembles efficiently” into viral particles. EX1007 ('772 Publication), [0072].

on the surface of the capsid. EX1063 (Samulski Dep. Tr.), 67:4-17, 67:19-68:17, 19. Dr. Samulski testified that, even if the position of the mutation of the methionine (ATG) to valine (GTG) was such that the valine (or original methionine) did not lie on the surface of the capsid, the mutation could nonetheless potentially alter the surface of the capsid. EX1063 (Samulski Dep. Tr.), 67:4-17, 67:19-68:17, 68:19.

122. Moreover, a POSA would have understood that altering the location of epitopes on an AAV capsid could alter the ability of the AAV to bind to host cells and transduce them, altering tropism. *See, e.g.*, EX1032 (Wu), p. 2 (“Girod et al. showed that insertion of the L14 epitope into the capsid coding region can expand the tropism of this virus to cells nonpermissive for AAV infection that bear the L14 receptor”) (internal citation omitted).

123. Dr. Samulski testified that even according to his definition of “tropism,” which encompasses only the ability of an AAV vector to bind and “open the door to the cell,” a single amino acid change that altered an epitope on the surface of the capsid could alter whether or not the virus could “open the door” and enter the cell – thus altering the tropism of the mutant AAV. EX1063 (Samulski Dep. Tr.), 50:9-16 (testifying that in his view, if the cell is imagined as a room with a door, “tropism” refers to opening the door, but not entering the room), 68:20-23, 68:25-69:1.

124. In fact, the '772 Publication expressly teaches that one purpose of altering the common ATG start codon to the unusual GTG start codon would be to “change tropism”:

For example, it may be desirable to modify the coding regions of one or more of the AAV vp1, e.g., in one or more of the hypervariable regions (i.e., HPV1-12), or vp2, and/or vp3. In another example, *it may be desirable to alter the start codon of the vp3 protein to GTG. These 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 ('772 Publication), [0091] (emphases added).

125. The passage quoted above discloses that modifications such as the ATG to GTG mutation could be used to improve *in vitro* expression systems (“to increase expression, yield, and/or to improve purification in the selected expression systems) or to change tropism or alter neutralizing antibody epitopes *in vivo*).

126. Therefore, like Snowdy, the '772 Publication discloses single amino acid changes that can affect AAV tropism, contrary to Dr. Samulski's arguments.

**C. Dr. Samulski's '126 Provisional Application Differs from His Argument**

127. Snowdy is not the only example of single amino acid substitutions altering tropism around the relevant priority date of which Dr. Samulski should be aware based on his own personal experience. Dr. Samulski filed patents and published scientific articles around the relevant priority date (2005-2006) disclosing

single amino acid substitutions in AAV capsids that changed tropism.<sup>4</sup> See, e.g., EX1059 ('126 provisional); EX1061 ('809 patent); EX1060 (Wu 2006).

### 1. Single Amino Acid Substitutions to Alter Tropism

128. In the '126 provisional, the “Detailed Description of the Invention” states: “The inventors have discovered that in some cases *as little as a single amino acid* insertion or *substitution* from a first parvovirus (e.g., an AAV) into the capsid structure of another parvovirus (e.g., an AAV) to create a chimeric parvovirus *is sufficient to confer one or more of the desirable properties of the first parvovirus to the resulting chimeric parvovirus.*” EX1059 ('126 provisional), 8:23-27 (emphases added); EX1063 (Samulski Dep. Tr.), 109:5-110:10, 13-16, 18.

---

<sup>4</sup> As I discuss below, Dr. Samulski argued at his deposition that at least some of the work disclosed in the '126 provisional was the product of “extraordinary . . . skill.” EX1063 (Samulski Dep. Tr.), 142:24-143:18. I disagree, particularly in relation to the disclosures regarding the T450N and T716N substitutions that I also discuss below. But, in any event, the level of skill required for the disclosures of the '126 provisional does not address the fact that Dr. Samulski’s broad generalizations in his declaration about the state of the art as of 2005 are contradicted by his own work at the time.

129. The '126 provisional discusses the difference in ability to transduce different tissues among different AAV serotypes. EX1059 ('126 provisional), 14:28-15:12; 44:8-10. In particular, the '126 provisional calls out the superiority of AAV1 and AAV7 over AAV2 and AAV8 in transducing skeletal muscle. EX1059 ('126 provisional), 15:9-12; 44:8-15.

130. The '126 provisional notes that the amino acid sequences of AAV1 and AAV2 are 83% identical, and discusses Hauck, which involved exchanging large regions of AAV2 capsid with the corresponding amino acids of AAV1 and examined the ability of the resultant vectors to transduce skeletal muscle (Hauck et al., (2003) *J. Virology* 77:2768-2774). EX1059 ('126 provisional), 44:8-15.

131. As the '126 provisional states, Hauck (EX1052) identified two amino acids (I363V and E427D) that appeared to confer enhanced skeletal muscle transduction on AAV2, although not at the levels of AAV1. EX1052 (Hauck), p. 6; EX1059 ('126 provisional), 44:11-15.

132. The '126 provisional expressly distinguishes its approach of “‘selective’ amino acid change(s)” from previous work such as that of Hauck. EX1059 ('126 provisional), 16:27-33. The '126 provisional therefore contradicts Dr. Samulski’s statements, which I discussed above, that Hauck represented the state of the art as of 2005. EX2027 (Samulski Dec.), ¶ 27; EX1052 (Hauck).

133. Dr. Samulski also distinguished the approach in the '126 provisional from prior art alanine scanning. EX1063 (Samulski Dep. Tr.), 147:12-25.

134. Instead, the '126 provisional sets out a rational design scheme to make substitutions of amino acids in AAV2 with particular corresponding amino acids from AAV1, to try to confer on AAV2 the favorable muscle transduction properties of AAV1.<sup>5</sup> EX1059 ('126 provisional), 44:15-21 (“We wished to examine the phenomenon in a more detailed manner. Employing bioinformatic and structure-function analysis, we were able to identify key amino acids [*sic*] residues that account for enhanced AAV1 transduction to test in the context of majority AAV2 capsid. This approach would avoid the need to make all the different changes of

---

<sup>5</sup> At his deposition, Dr. Samulski referred to this as a “wreck and check” approach. EX1063 (Samulski Dep. Tr.), 139:6-13, 151:19-23 (testifying that “wreck and check” refers to looking at AAV1, seeing where the amino acid sequence differs from AAV2, and making mutations in AAV2 to make AAV2 look a little more like AAV1). I do not believe that others in the field as of 2005 or now would describe the rational design strategy of the '126 provisional that way. Dr. Samulski also testified that he considers any mutagenesis study where he does not know the outcome to be a “wreck and check” approach. EX1063 (Samulski Dep. Tr.), 141:11-18. Again, I do not believe others in the field use this term this way.

amino acids between the 2 serotypes and all of the different permutations”); EX1063 (Samulski Dep. Tr.) 107:24-108:4, 252:19-253:7 (testifying that the approach of the ’126 provisional was a rational design approach); EX1062 (Bowles), Abstract.

135. The ’126 provisional defines a “selective” amino acid change as one that results in the insertion and/or substitution and/or deletion of less than about 20, 18, 15, 12, 10, 9, 8, 7, 6, 5, 4 or 3 contiguous amino acids.” EX1059 (’126 provisional), 16:31-33. A “selective” amino acid change involves as few as a single point mutation. EX1059 (’126 provisional) 16:33-17:22 (“In particular embodiments, only two contiguous amino acids *or even point mutations (i.e., one amino acid)* are inserted and/or substituted into and/or deleted from the capsid subunits”) (emphasis added).

136. Therefore, Dr. Samulski’s ’126 provisional teaches single amino acid substitutions for the purpose of altering transduction of different cell types, and therefore altering tropism.

137. Notably, Dr. Samulski testified that once his results were published, it would have been “straightforward” for other researchers to understand and carry out the type of experiments his group had performed. EX1063 (Samulski Dep. Tr.), 283:14-23. This testimony supports my opinions that a POSA as of 2005 would have understood and been able to carry out the experiments, for example, that Snowdy disclosed in his dissertation, and that the ’772 Publication discloses.

**(a) Sequence Alignments**

138. To determine which substitutions to make in AAV2, the named inventors of the '126 provisional first carried out sequence alignments of AAV1 and AAV2, and analyzed them to determine positions at which AAV1 and AAV2 differed. EX1063 (Samulski Dep. Tr.), 111:21-112:10. They used this analysis to identify positions of interest for mutagenesis studies. EX1063 (Samulski Dep. Tr.), 111:21-112:10.

139. I note that this is essentially the same process that I explained in my Declaration that a POSA would have followed, based on the guidance in the '772 Publication, to make substitutions in AAVrh10 from AAV8, to confer some of the favorable liver transduction properties of AAV8 on AAVrh10. EX1005 (Schaffer Dec.), ¶¶ 272-80.

140. I explained that a POSA would have aligned AAVrh.10 with AAV8, identified the differences, and then carried out a rational design process to select which substitutions to make in AAVrh.10. EX1005 (Schaffer Dec.), ¶¶ 272-80. In particular, a POSA would have used the teachings of Snowdy about the effect of removing a phosphorylation site on transduction efficiency to determine which substitutions to make. EX1005 (Schaffer Dec.), ¶¶ 272-80.

141. Figure 1A of the '126 provisional shows that there were 83 amino acid differences between AAV1 and AAV2 in their alignment of these sequences.

EX1059 ('126 provisional), Figure 1A. They reduced this number to 36 mutagenesis candidates through analysis of the sequence alignments, and then to 12 based on additional analyses. EX1059 ('126 provisional), 5:15-29, Figures 1A, 1B; EX1063 (Samulski Dep. Tr.), 115:3-13. I note in addition that even making all 83 mutants would have been well within the skill of a POSA at the time. EX1005 (Schaffer Dec.), ¶ 231.

142. I note that AAVrh.10 and AAV8 are about 93.5% identical, and therefore more closely related than AAV1 and AAV2. EX1005 (Schaffer Dec.), ¶ 212; EX1019 (Sequence alignment); EX1063 (Samulski Dep. Tr.), 113:12-14, 16-17, 114:8-10. I note further that, as I set out in my declaration, the combination of the teachings of the '772 Publication with Snowdy would have reduced the number of mutagenesis candidates in AAVrh.10 to only four, fewer than the twelve that the inventors of the '126 provisional tested. EX1005 (Schaffer Dec.), ¶¶ 277-80.

### **(b) Crystal Structure Analysis**

143. The '126 provisional discloses that they relied on Xie's crystal structure data for selecting some mutagenesis sites. EX1059, 15:17-19 ("This initial pool of candidate positions was narrowed considerably based on analysis of the known crystal structure of AAV2, *e.g.*, to identify amino acid positions that are located in regions that are likely to affect the biology of the virus"); EX1063 (Samulski Dep. Tr.) 117:7-22.

144. Dr. Samulski testified at his deposition that it would have taken “extraordinary . . . skill” to interpret Xie’s crystal structure in 2004, as they did in the ’126 provisional. EX1063 (Samulski Dep. Tr.), 142:24-143:18.

145. In my opinion, a POSA at the relevant time would have understood Xie’s teachings about which amino acids in the AAV2 capsid lie on the surface of the capsid, and the significance of loop structures on the surface of the capsid in determining functions such as interactions with cellular receptors. *See, e.g.*, EX1005 (Schaffer Dec.), ¶ 77; 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”).

146. To the extent that Dr. Samulski is suggesting that modeling was required for whatever analysis he chose to carry out in the ’126 publication, programs were available to a POSA at the time to carry out such modeling using nothing more than ordinary skill.

147. One such publicly available and broadly used program is “Swiss Model,” which Dr. Samulski used in a 2006 publication (Wu 2006) for modeling a region of the AAV1 and AAV6 capsid. *See, e.g.*, EX1060 (Wu 2006), p. 3 (disclosing that they used Swiss Model to create the modeling data in Figure 2, by

plugging in the coordinates from Xie's crystal structure into the Swiss Model program).<sup>6</sup>

148. In my original Declaration, I set out my opinion that a POSA at the time would have been motivated to combine the teachings of the '772 Publication with Xie's teachings regarding amino acids on the surface of the AAV2 capsid, to identify positions at which to make single amino acid substitutions in AAVrh.10, substituting in corresponding amino acids from AAV8. *See, e.g.,* EX1005 (Schaffer Dec.), ¶¶ 255-66.

149. The POR argues incorrectly that a POSA would not have been motivated to mutate S665 because it is "less accessible than at least twelve other residues" that differ between AAV8 and AAVrh.10. POR, pp. 44-46. In fact, Dr.

---

<sup>6</sup> I note that several of the authors of Wu 2006 are inventors on the '126 provisional, including Dr. Samulski, Josh Grieger, and Mavis McKenna. EX1060 (Wu 2006); EX1059 ('126 Provisional). I note further that Dr. Samulski testified that the crystal structure analysis in the '126 provisional was carried out by Dr. McKenna. *See, e.g.,* EX1063 (Samulski Dep. Tr.), 115:21-116:1 (referring to Dr. McKenna as "Mavis"). It is therefore reasonable to conclude that if Dr. McKenna used Swiss Model for the modeling carried out in Wu 2006, she similarly used it two years earlier for whatever modeling was carried out as part of the '126 provisional.

Samulski's publication, Wu 2006, contradicts the argument that a POSA seeking to alter tropism would not have been motivated to mutate "less accessible" residues on the surface of the AAV capsid. EX1060 (Wu 2006). As I discuss below (Section V.E), Wu 2006 found that a single amino acid residue, K531 in AAV6, was largely responsible for the superior tropism of AAV6 as compared to AAV1, which has a glutamic acid at position 531.

150. Wu 2006 modeled the location of K531 in AAV6 and E531 in AAV1, using Xie's AAV2 crystal structure to generate the model. EX1060 (Wu 2006), p. 3, Figure 2. Wu 2006 found that K531 in AAV6 was located in a patch of basic residues "at the base of each threefold spike of the AAV6 capsid surface model." EX1060 (Wu 2006), p. 3, Figure 2.

151. At his deposition, Dr. Samulski agreed that even residues at the base of peaks on the surface of the AAV capsid can affect binding to receptors on the surface of target cells and therefore influence tropism. EX1063 (Samulski Dep. Tr.), 231:21-25, 232:4-233:9.

152. Thus, a POSA would have understood Wu 2006 to teach that single amino acids at the base of peaks on the AAV capsid surface can be important determinants of tropism.

153. The POR also argues incorrectly that Xie does not stand for the proposition that individual amino acids "govern characteristics of AAV such as

tropism, transduction efficiency, and immunogenicity,”” because Xie refers to “loops” that lie on the surface. POR, p. 44.

154. The Xie crystal structure provides data about the locations of individual amino acids on the surface of the AAV2 capsid in three-dimensional space. *See, e.g.,* EX1008 (Xie), p. 4, Figure 4. The topographical map of one of the triangular facets of the capsid surface shown in Figure 4 of Xie color codes each individual amino acid separately to represent its distance from the center of the capsid. EX1008 (Xie), p. 4, Figure 4. Xie therefore teaches throughout the importance of individual amino acids to AAV functions such as receptor binding.

155. Xie also repeatedly discusses the locations and functions of individual amino acids. *See, e.g.,* EX1008 (Xie), p. 5 (discussing R448, R451, R347, R350, K353).

156. Moreover, Xie states that the crystal structure will enhance the ability to map the functions of AAV through rational design and targeted site-directed mutagenesis:

The structure also provides a framework for rational site-directed mutagenesis, through which the molecular mechanisms of viral-host interactions can be probed. A rational, structure-assisted approach should accelerate the progress in mapping functional sites and modulating function that has, to date, been attempted through scanning mutagenesis. This mapping, in turn, will provide a foundation for the

engineering of recombinant AAV for the more efficient delivery of gene therapies to specifically targeted cells.

EX1008 (Xie), p. 5 (citations omitted).

157. This paragraph cites Wu, which, as I have discussed, carried out alanine scanning, creating single amino acid mutants of AAV2, to create mutants with altered tropism. EX1032 (Wu), Abstract, pp. 5-6, Table 1; EX1005 (Schaffer Dec.), ¶¶ 93, 211. A POSA would therefore have understood Xie to teach using the crystal structure to carry out rational design to alter the tropism of AAV by making mutations in the capsid, including single amino acid mutations.

**(c) Identification of a Single Amino Acid Responsible for Enhanced Transduction**

158. In the '126 provisional, Dr. Samulski and his colleagues identified one variant (that they named "AAV2.5") that exhibited "much higher muscle transduction than AAV2." EX1059 ('126 provisional), 45:5-6. This variant had five amino acid changes from wild-type AAV2. EX1059 ('126 provisional), 45:6-7, Figure 11. They made additional variants to test which of the five changes were most directly responsible for the improved muscle transduction. EX1059 ('126 provisional), 45:10-11. They found that *a single amino acid* was "mostly responsible for enhanced AAV1 transduction." EX1059 ('126 provisional), 45:16-19; EX1063 (Samulski Dep. Tr.), 151:24-152:2, 152:4-8, 10. The single amino acid

was the insertion of a threonine at the 265 position in the AAV2 capsid sequence. EX1059 ('126 provisional), 45:5-6.

159. The legend to Figure 5 in the '126 provisional states, “The threonine insertion at position 265 is responsible for the majority of the *enhanced muscle tropism* of 2.5.” EX1059 ('126 provisional), 7:9-10 (emphasis added). The '126 provisional therefore expressly discloses a single amino acid change that enhanced muscle tropism of an AAV variant. See EX1063 (Samulski Dep. Tr.), 155:3-17, 19.

160. I have reproduced below the portion of Figure 9 from the '126 provisional showing the location of this insertion, in the alignment of AAV2 with a mutant with substitutions at positions 263 and 265 – I have highlighted the “T” insertion in the mutant:

|          |       |                |                                     |     |
|----------|-------|----------------|-------------------------------------|-----|
|          |       | 251            |                                     | 300 |
| 263, 265 | (251) | TYNHLYKQISSAST | GASNDNHYFGYSTPWGYFDNRFHCHFSPRDWQRL  |     |
| aav2 cap | (251) | TYNHLYKQISSQS  | -GASNDNHYFGYSTPWGYFDNRFHCHFSPRDWQRL |     |

EX1059 ('126 provisional), 8:8-10, 22:28-31, Figure 9 (highlight added).

161. Figure 7 of the '126 provisional and Figure 7 of the '809 patent show the alignment of AAV1 and AAV2 in this region. I have reproduced Figure 7 from

the '809 patent below, which indicates the relevant “T” at position 265 of AAV1 with an arrow (I have also highlighted this residue):

|                 |                                                       |                         |     |     |     |     |   |   |     |     |     |
|-----------------|-------------------------------------------------------|-------------------------|-----|-----|-----|-----|---|---|-----|-----|-----|
|                 | (220)                                                 | 220                     | 230 | 240 | 250 | 260 | ↓ | ↓ | 270 | 280 | 290 |
| AAV1 CAP (217)  | GADGVGNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSAS-     | TGASNDNHYFGYSTPWGYFDNRF |     |     |     |     |   |   |     |     |     |
| AAV2 CAP (217)  | GADGVGNSSGNWHCDSTWVGDRVITTSTRTWALPTYNNHLYKQISSQS--    | GASNDNHYFGYSTPWGYFDNRF  |     |     |     |     |   |   |     |     |     |
| AAV3A CAP (217) | GADGVGNSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISSQS--    | GASNDNHYFGYSTPWGYFDNRF  |     |     |     |     |   |   |     |     |     |
| AAV3B CAP (217) | GADGVGNSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISSQS--    | GASNDNHYFGYSTPWGYFDNRF  |     |     |     |     |   |   |     |     |     |
| AAV4 CAP (211)  | GADGVGNASGDWHCDSTWSEGHVTTSTRTWLPTYNHLYKRLGESL----     | QSNTYNGFSTPWGYFDNRF     |     |     |     |     |   |   |     |     |     |
| AAV5 CAP (207)  | GADGVGNASGDWHCDSTWVGDRVITKSTRTWLPSYNNHLYKREIKSGS-     | VDGSNANAYFGYSTPWGYFDNRF |     |     |     |     |   |   |     |     |     |
| AAV6 CAP (217)  | GADGVGNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSAS-     | TGASNDNHYFGYSTPWGYFDNRF |     |     |     |     |   |   |     |     |     |
| AAV7 CAP (218)  | GADGVGNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSET-     | AGSTNDNTYFGYSTPWGYFDNRF |     |     |     |     |   |   |     |     |     |
| AAV8 CAP (218)  | GADGVGSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGAT | NDNTYFGYSTPWGYFDNRF     |     |     |     |     |   |   |     |     |     |
| AAV9 CAP (217)  | GADGVGSSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGS  | SNDNAYFGYSTPWGYFDNRF    |     |     |     |     |   |   |     |     |     |

FIG. 7

EX1061 ('809 patent), p. 12, Figure 7 (highlight added); *see also* EX1059 ('126 provisional), p. 74, Figure 7.

162. The alignment of AAV1 and AAV2 above shows that the insertion of the “T” at position 265 of AAV2 fills a gap in the sequence of AAV2 in a region where the two sequences are otherwise nearly identical. *See also* EX1059 ('126 provisional), 16:13-17 (“Likewise, when modifying AAV other than AAV2, the specific amino acid position(s) may be different than the position in AAV2. However, the corresponding amino acid position(s) will be readily apparent to those skilled in the art using well-known sequence alignment techniques (*see, e.g.*, Figure 7)”); EX1061 ('809 patent), col. 13:49-54.

163. Dr. Samulski testified that the threonine insertion in AAV2 was made because there is a threonine at the corresponding position of AAV1, and inserting

the threonine into AAV2 makes the two sequences align better at that position. EX1063 (Samulski Dep. Tr.), 151:24-152:2, 152:4-8, 10.

164. I note that the '126 provisional indicates that there is no meaningful distinction between a single amino acid *substitution* and a single amino acid *insertion* for the mutants they are disclosing: “A ‘corresponding’ modification can be an insertion and/or a substitution and/or a deletion. For example, a modification that is an insertion in AAV2 may be a substitution mutation in another AAV.” EX1059 ('126 provisional), 15:24-27, 16:10-13 (“Those skilled in the art will appreciate that for some AAV the corresponding modification will be an insertion and/or a substitution, depending on whether the corresponding amino acid positions are partially or completely present in the virus or, alternatively, are completely absent”); EX1061 ('809 patent), col. 13:45-49.

165. The '126 provisional further discloses that the five amino acid changes in the AAV2.5 variant “although small in number (0.6% total amino acids”) were “sufficient to significantly affect the immune profile for this virion when challenged with neutralizing antibodies specific for AAV2.” EX1059 ('126 provisional), 47:5-8. Specifically, the AAV2.5 variant was significantly less prone to neutralization by anti-AAV1 and anti-AAV2 antibodies, making the variant “suited when transducing individuals pre-exposed to AAV1, AAV2 or both” and “providing greater versatility in available vectors.” EX1059 ('126 provisional), 47:9-11.

## 2. Single Amino Acid Changes to Alter Tropism by Altering Phosphorylation

166. The '126 provisional also discloses making a single amino acid substitution in an AAV capsid that would be expected to affect phosphorylation. EX1059 ('126 provisional), 17:25-32; 21:20-22, 32-34.

167. I note that these changes to affect phosphorylation in the '126 provisional are not described as having anything to do with nuclear localization or nuclear localization signals. In fact, the '126 provisional does not mention the term “nuclear localization” or the term “NLS” anywhere. Instead, the '126 provisional teaches that these single amino acid changes to affect phosphorylation, like the other single amino acid substitutions taught by the '126 provisional, are intended to create a “chimeric virus vector [that] has enhanced transduction and/or an altered immunological profile as compared with the parent rAAV vector.” EX1059 ('126 provisional), 19:9-10.

168. The '126 provisional specifically discloses as an embodiment a single amino acid substitution mutant with a substitution of an amino acid that cannot be phosphorylated for an amino acid that can – so, removing a phosphorylation site. EX1059 ('126 provisional), 17:25-32; 21:20-22, 32-34.

169. The '126 provisional states:

In particular aspects of the embodiments described in the previous two paragraphs, the substitution is a *substitution of an amino acid that*

*cannot be phosphorylated for an amino acid that can be phosphorylated* (e.g., threonine). Alternatively, the substitution can be a substitution of a serine, tyrosine, glycine, alanine, proline, valine, leucine, isoleucine, asparagine or glutamine into the capsid subunit (e.g., substitution of the threonine at this position in AAV2). *One particular chimeric virus comprises an asparagine substituted at this position into the capsid subunits.*

EX1059 ('126 provisional), 21:20-27 (emphases added).

170. This paragraph refers to two previous paragraphs, which describe a series of AAV capsid mutants, with an amino acid substitution at the position corresponding to position 716 in AAV2. EX1059 ('126 provisional), 21:7-19 (“The invention also provides a chimeric virus vector comprising a chimeric AAV2 capsid comprising an amino acid substitution at amino acid position 716 in the AAV2 capsid subunits or at the corresponding position in an AAV2 capsid that has been modified to comprise non-AAV2 sequences”); EX1063 (Samulski Dep. Tr.), 164:23-165:20.<sup>7</sup>

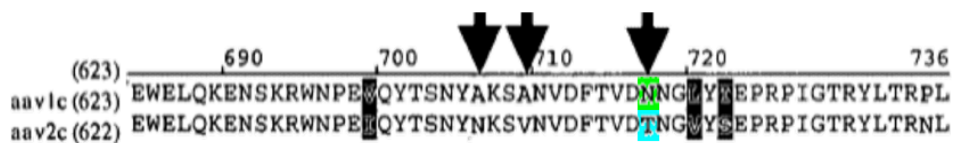
---

<sup>7</sup> I note that towards the end of his deposition, on redirect from counsel for Regeneron, Dr. Samulski initially mistakenly testified that threonine was being inserted rather than removed. EX1063 (Samulski Dep. Tr.), 288:9-15, 25, 289:1-14. On re-cross, however, he corrected this error, testifying that the threonine is being replaced by a non-phosphorylatable amino acid such as asparagine. EX1063 (Samulski Dep. Tr.), 289:20-290:15, 17.

171. Dr. Samulski testified that this substitution at position 716 of a non-phosphorylatable amino acid to replace a phosphorylatable amino acid was similar to the experiment in Snowdy at position 181, where a phosphorylatable amino acid was replaced with a non-phosphorylatable amino acid. EX1063 (Samulski Dep. Tr.), 165:21-24, 166:1.

172. Importantly, the '126 provisional calls out a “particular chimeric virus” that “comprises an *asparagine* substituted at this position [716] into the capsid subunits,” creating a T716N mutation. EX1059 ('126 provisional), 21:25-27 (emphasis added); EX1063 (Samulski Dep. Tr.), 168:11-15, 17-25, 169:1-3 (testifying that the '126 provisional singles asparagine out of ten possible amino acids mentioned).

173. I have reproduced below Figure 1B from the '126 provisional, showing an alignment of AAV1 and AAV2 in the region of amino acid 716. In the figure below, I have highlighted AAV2 T716 in blue, and the corresponding amino acid in AAV1 in green.



EX1059 ('126 patent), p. 65, Figure 1B (highlights added), 5:25-28 (legend for Figure 1B).

174. In the figure, the numbering above the line corresponds to the numbering for AAV1. The numbering for AAV2 is one less than the numbering for AAV1: the first amino acid on the left for AAV1 is 623, while the first amino acid on the left for AAV2 is 622. Therefore, AAV2 T716 in the figure is at position 717 according to the numbering above the line.

175. As can be seen from the figure, the corresponding amino acid at this position in AAV1 is “N” or asparagine, which I have highlighted in green. EX1063 (Samulski Dep. Tr.), 185:22-186:6.

176. Thus, the '126 provisional discloses substituting in asparagine, a nonphosphorylatable amino acid from AAV1, for the corresponding phosphorylatable amino acid, threonine, in AAV2 (T716N). EX1059 ('126 provisional), 21:20-22. In this way, the '126 provisional teaches removing a potential phosphorylation site in AAV2 by substituting in an asparagine, from the corresponding position of AAV1. EX1063 (Samulski Dep. Tr.), 186:7-15, 18-19.

177. And the '126 provisional teaches substituting a corresponding amino acid from one serotype into another to confer some of the properties of the first serotype on the second: “The inventors have identified regions within the AAV1/6/7 serotypes that confer desirable properties including, but not limited to, enhanced transduction ability (*e.g.*, enhanced transduction of skeletal muscle cells), higher expression of a transgene delivered by the recombinant genome and/or earlier onset

of transgene expression. These selected regions can be transferred or ‘engineered’ into other AAV capsids to confer the property(ies) of interest to the resulting chimeric particle.” EX1059 (’126 provisional), 14:28-33.

178. As I discussed above, the ’126 provisional expressly distinguishes the “selective amino acid change(s)” it teaches from “previous work with whole subunit or large domain swaps between AAV serotypes.” EX1059 (’126 provisional), 16:27-14:2. Specifically, a “selective amino acid change” can be a substitution of a single amino acid. EX1059 (’126 provisional), 16:27-17:2.

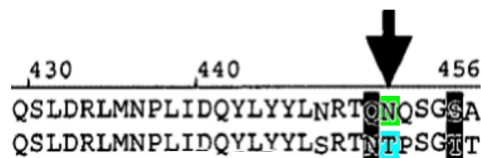
179. The following paragraph in the ’126 provisional discloses additional single amino acid mutants of AAV2 involving substitution of non-phosphorylatable asparagine in place of phosphorylatable threonine – in particular, a substitution of asparagine in for threonine at position 450 (T450N):

A further chimeric virus vector according to the invention is a chimeric virus vector comprising a chimeric AAV2 capsid comprising an amino acid substitution at any of amino acid positions 447, 448, 449, 450, 451, 452 or 453 (*e.g., at position 450*) in the AAV2 capsid subunits or at the corresponding position in an AAV2 capsid that has been modified to comprise non-AAV2 sequences. ***In particular embodiments, the substitution is a substitution of an amino acid that cannot be phosphorylated for an amino acid that can be phosphorylated (e.g., threonine).*** Alternatively, the substitution can be a substitution of a serine, tyrosine, glycine, alanine, proline, valine, leucine, isoleucine, asparagine or glutamine into the capsid subunit (*e.g., substitution of the threonine at position 450 in AAV2*). ***One particular chimeric***

*virus comprises an asparagine substituted at this position into the capsid subunits.*

EX1059 ('126 provisional), 21:28-22:5 (emphases added).

180. I have reproduced below the portion of Figure 1B from the '126 patent showing this region of the AAV2 capsid sequence, with the threonine at position 450 highlighted in blue (note that the numbering for AAV2 again is one number less than the numbering for AAV1, shown above the line, so that AAV2 T450 is shown at position 451 according to the numbering above the line<sup>8</sup>):



EX1059 ('126 patent), p. 65, Figure 1B (highlights added), 5:25-28 (legend for Figure 1B).

181. As can be seen from the figure above, the corresponding amino acid at this position in AAV1 is “N” or asparagine, which I have highlighted in green.

182. Again, the '126 provisional discloses substituting in asparagine, a nonphosphorylatable amino acid, from AAV1, for the corresponding

---

<sup>8</sup> Dr. Samulski agreed with this understanding of the numbering in Figure 1B.

EX1063 (Samulski Dep. Tr.), 181:25-183:9.

phosphorylatable amino acid, threonine, in AAV2 (T450N). EX1059 ('126 provisional), 21:20-22.

183. The '126 provisional calls out position 450 (“(e.g., substitution of the threonine at position 450 in AAV2)”), and then calls out “one particular chimeric virus” that “comprises an asparagine substituted at this position into the capsid subunits.” EX1059 ('126 provisional), 21:30-31, 22:3-5; EX1063 (Samulski Dep. Tr.), 169:8-170:17, 171:7-13, 23-25, 172:1-10.

184. Here again, the '126 provisional touts a single amino acid substitution mutant of an AAV2 capsid involving the exchange of a phosphorylatable amino acid (threonine) for non-phosphorylatable *asparagine*, from AAV1, to confer some of the properties of AAV1 on AAV2.

185. Dr. Samulski testified that asparagine is singled out in the '126 provisional to replace threonine at position 450 of AAV2 because asparagine is the amino acid at the corresponding position of AAV1, and the named inventors sought to impart some of the properties of AAV1 onto AAV2. EX1063 (Samulski Dep. Tr.), 183:16-23, 25, 184:1-24.

186. In addition, the '126 provisional expressly teaches that these single amino acid changes are intended to produce rAAV variants with “enhanced transduction and/or an altered immunological profile as compared with the parent rAAV vector”:

The invention further provides chimeric virus vectors comprising a chimeric AAV capsid comprising a selective amino acid insertion and/or substitution selected from the group consisting of:

\*\*\*\*\*

(d) a selective amino acid insertion and/or substitution that results in an amino acid substitution *at amino acid position 716 in the AAV2 capsid subunits* or a corresponding change in other AAV capsids;

(e) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 447 to 453 (*e.g., position 450*) in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

\*\*\*\*\*

*wherein the chimeric virus vector has enhanced transduction and/or an altered immunological profile as compared with the parent rAAV vector.*

EX1059 ('126 provisional), 18:19-19:10 (emphases added).

187. The '126 provisional includes a claim, dependent claim 61, to an rAAV2 vector with a single amino acid substitution of an asparagine for a serine at position 450 (T450N), “wherein said chimeric virus vector has enhanced transduction as compared with the parent AAV vector.” EX1059 ('126 provisional), 59:28-29 (claim 61).

188. I have reproduced dependent claim 61, along with claims 57-60, from which it depends, below:

57. A chimeric virus vector comprising:

- (a) a recombinant adeno-associated virus (rAAV) genome; and
- (b) a chimeric AAV capsid comprising *a selective amino acid*

***insertion and/or substitution that results in an amino acid substitution at amino acid position 450 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;***

***wherein said chimeric virus vector has enhanced transduction as compared with the parent rAAV vector.***

58. The chimeric virus vector of claim 57, wherein the chimeric virus vector comprises a chimeric AAV2 capsid that has been modified to comprise non-AAV2 sequences and further comprises an amino acid substitution at the amino acid that corresponds to position 450 in the AAV2 capsid subunits.

59. The chimeric virus vector of claim 57, wherein the chimeric virus vector comprises a chimeric AAV2 capsid comprising an amino acid substitution at amino acid position 450 in the AAV2 capsid subunits.

60. The chimeric virus vector of claim 58 or claim 59, wherein the substitution is a substitution of serine, tyrosine, glycine, alanine, proline, valine, leucine, isoleucine, asparagine or glutamine for threonine.

***61. The chimeric virus vector of claim 60, wherein the substitution is a substitution of asparagine for threonine.***

EX1059 ('126 provisional), 59:6-26 (claims 57-60) (emphases added).

189. Notably, dependent claim 61, which depends from and therefore includes all the limitations of claim 57, recites the specific purpose of making the single amino acid substitution to provide “enhanced transduction as compared with the parent rAAV vector.” EX1059 ('126 provisional), 59:6-26 (claims 57-60); EX1063 (Samulski Dep. Tr.) 176:19-177:12, 14-25 (testifying that independent claim 57 recites a mutant AAV2 with a single amino acid insertion or substitution at

position 450, where the mutant has enhanced transduction as compared with the original AAV2); 179:19-180:10, 13-16, 19-21 (testifying that dependent claim 59 adds the limitation that it must be a substitution, not an insertion); 181:5-22, 24 (testifying that dependent claim 61 further limits the claim to a single amino acid substitution at position 450 in AAV2, where the threonine at position 450 in AAV2 is replaced with asparagine).

190. Dr. Samulski testified that he disclosed and claimed “everything” to cover his bases, and include everything that might turn out to be beneficial. EX1063 (Samulski Dep. Tr.), 197:5-21. He further testified that one of the “bases” he covered in the ’126 provisional was that the threonine to asparagine mutation in position 450 might enhance transduction. EX1063 (Samulski Dep. Tr.), 197:22-24, 198:2.

191. The ’126 provisional also calls out these substitutions (T716N and T450N) in combination with other mutations. *See, e.g.*, EX1059 (’126 provisional), 23:24-25 (“According to this embodiment, the chimeric virus vector can comprise a chimeric AAV capsid (*e.g.*, AAV2 capsid) comprising . . . an asparagine for threonine substitution at amino acid position 716 in the AAV2 capsid subunits . . . .”); 24:13-14 (“(b) an asparagine for threonine substitution at amino acid position 450 in the AAV2 capsid subunits”); 24:29-34 (“(i) an asparagine for threonine substitution at amino acid position 450 in the AAV2 capsid subunits”).

192. The '126 provisional discloses multiple additional embodiments with a single amino acid substitution in AAV1, AAV2, and chimeric AAV capsids, including substitution of asparagine to replace a different amino acid. *See, e.g.*, EX1059 ('126 provisional), 17:34-18:2, 19:14-29; 20:3-15, 21-33; 22:6-14, 24:14-25:34.

193. The '126 provisional also includes claims to each of the T450N and T716N substitutions in an AAV2 capsid that contains the threonine insertion after position 264. *See, e.g.*, EX1059 ('126 provisional) 53:31-32 (claim 36), 54:17-18 (claim 41).

194. The '126 provisional also includes claims to the T450N and T716N substitutions in combination with other mutations. *See, e.g.*, EX1059 ('126 provisional), 56:21-33 (claim 49), 57:1-12 (claim 50), 58:20-28 (claim 55), 58:30-59:4 (claim 56), 60:24-30 (claim 67), 60:32-61:4 (claim 68).

195. In my Declaration, I argued that a POSA would have used the teachings of Snowdy, combined with those of the '772 Publication, to substitute in asparagine, a non-phosphorylatable amino acid present at the corresponding position of AAV8, for serine, a phosphorylatable amino acid, in the capsid of AAVrh.10, to improve liver tropism. *See, e.g.*, EX1005 (Schaffer Dec.), ¶¶ 271-289.

196. The '126 provisional touts at least two “particular” AAV variants, each containing essentially the same single amino acid substitution I described in my

Declaration – namely, substitution of non-phosphorylatable asparagine in from AAV1, in place of phosphorylatable threonine at the corresponding position of AAV2.

197. As I have discussed above, the '126 provisional also teaches expressly that mutations such as this single amino acid substitution (asparagine from AAV1 to replace threonine in AAV2) should be understood as an intentional effort to alter phosphorylation of the capsid to confer some of the tropism properties of AAV1 onto AAV2. *See, e.g.*, EX1059 ('126 provisional), 4:28-30 (“It would be desirable to engineer a variant of AAV2 that exhibits the enhanced muscle tropism of AAV1, 6 and 7 . . .”); 4:33-2:2 (“The inventors have identified the key amino acid(s) of AAV1 responsible for enhanced in vivo transduction and selectively engineered these amino acids into the backbone<sup>9</sup> of AAV2 as well as other AAV. In particular embodiments, the chimeric viruses of the invention have enhanced transduction capability (*e.g.*, transduction of skeletal muscle, cardiac muscle, liver, retina and/or lung, *etc.*) and/or enhanced levels of transgene expression with earlier onset of expression”).

---

<sup>9</sup> Dr. Samulski testified that the term “backbone” as used in the '126 provisional means the original capsid sequence that has been identified for a serotype. EX1063 (Samulski Dep. Tr.), 104:2-15.

198. Dr. Samulski also testified that some of the embodiments of the '126 provisional have enhanced transduction capability across different tissues. EX1063 (Samulski Dep. Tr.), 104:24-105:2.

199. And the '126 provisional teaches that the disclosed AAV2 variants confer improved muscle tropism on AAV2 from AAV1 without destroying the desirable properties of AAV1. *See, e.g.*, EX1059 ('126 provisional), 5:2-6 (“these chimeras [based on AAV2] retain the desirable properties of AAV2 (*e.g.*, ease of purification and known safety) while exhibiting some of the advantageous properties of AAV1 (including enhanced transduction of skeletal muscle).” Dr. Samulski testified that they were trying to improve “muscle tropic transduction,” of AAV2 while retaining desirable attributes of AAV2 such as ease of purification and known safety. EX1063 (Samulski Dep. Tr.), 105:15-106:11, 106:14-22.

**D. Dr. Samulski's '809 Patent Contradicts His Argument**

200. Like the '126 provisional, Dr. Samulski's '809 patent contradicts his argument. I will not repeat all the disclosures in the '809 patent that are essentially the same as those in the '126 provisional, and instead focus on a few points below.

*See, e.g.*, EX1061 ('809 patent), Fig. 1B, cols. 1:59-2:15, 5:31-42, 17:6-32, 45-49 (SEQ ID NO: 3), 75:13-24 (claims 22, 23).<sup>10</sup>

201. In general, like the '126 provisional, the '809 patent discloses single amino acid mutants “designed to exhibit one or more properties of interest (e.g., *enhanced tissue tropism*).” EX1061 ('809 patent), col. 1:59-62; 5:31-42 (emphasis added). Like the '126 provisional, the '809 patent teaches that these substitutions are designed to confer new properties without destroying the favorable properties of the parent serotype. EX1061 ('809 patent), col. 2:8-15.

#### **1. Single Amino Acid Changes to Alter Tropism by Altering Phosphorylation**

202. Dr. Samulski's issued '809 patent includes claims to the T450N and T716N substitutions in AAV2, which substitute in nonphosphorylatable asparagine from the corresponding position in AAV1 to replace phosphorylatable threonine.

---

<sup>10</sup> Both the '809 patent and the '224 patent include claims that recite an AAV2 variant with a single amino acid insertion after position 264. *See, e.g.*, EX1061 ('809 patent), col. 73:14-25, 39-40 (claims, 1, 6); EX1065 ('224 patent), col. 73:15-27 (claim 2). The '809 patent contains a dependent claim specifying that the inserted amino acid is threonine. *See, e.g.*, EX1061 ('809 patent), col. 73:14-25, 39-40 (claims, 1, 6).

203. Specifically, dependent claim 23 recites an rAAV2 capsid with the single amino acid substitution T450N. EX1061 ('809 patent), col. 75:23-24 (claim 23). Dependent claim 23 depends from independent claim 22. EX1061 ('809 patent), col. 75:13-24 (claims 22, 23). I have reproduced both claims below:

22. A chimeric virus vector comprising:

(a) a chimeric adeno-associated virus (AAV) capsid comprising a selective amino acid substitution at amino acid position 450 in an AAV2 capsid subunit having the amino acid sequence of (SEQ ID NO:3) or a corresponding change in another AAV capsid subunit;

(b) a nucleic acid comprising an AAV terminal repeat sequence and a heterologous nucleic acid sequence;  
wherein the nucleic acid is packaged within the chimeric AAV capsid.

23. The chimeric virus vector of claim 22, wherein the substitution is a substitution of asparagine for threonine.

EX1061 ('809 patent) col. 75:13-24 (claims 22, 23).

204. Dr. Samulski confirmed that claim 23 recites a single amino acid substitution at position 450 of AAV2, where the threonine that is normally there has been replaced by asparagine. EX1063 (Samulski Dep. Tr.), 240:2-241:24, 242:1-2.

205. The '809 patent also includes a claim (dependent claim 17) to the T450N substitution in rAAV2 vectors that have an insertion, such as the threonine insertion, after position 264. EX1061 ('809 patent), col. 74:39-40 (claim 17).

206. The '809 patent also includes a claim (claim 28) to the T716N substitution in rAAV2 vectors in combination with other mutations. EX1061 ('809 patent), col. 76:5-22 (claim 28).

207. Therefore, the '809 patent, like the '126 provisional, contradicts Dr. Samulski's broad generalizations in his declaration about the state of the art as of 2005.

## **2. Single Amino Acid Changes That Expand the Tropism of an rAAV Vector**

208. Dr. Samulski's '809 patent is inconsistent with his argument that a POSA as of 2005 would not have sought to design a vector with tropism across multiple tissues, but would have sought to target only a single tissue, using a vector such as AAV1, AAV7, or AAV8. In the '809 patent, Dr. Samulski disclosed and claimed a variant of AAV2 that turned out to have tropism in a new tissue, astrocytes, in addition to the initial target, muscle. His '809 patent therefore is not consistent with his argument that people in the art at the time would not have understood that an AAV vector with tropism across multiple tissues could be useful as a basis for a gene therapy vector.

209. Dr. Samulski's '809 patent discloses and claims single amino acid substitutions from one serotype (AAV1) into another (AAV2) that can create tropism in a wholly new tissue (astrocytes), which both parent serotypes (AAV1 and

AAV2) are unable to transduce. Therefore, Dr. Samulski's '809 patent is not consistent with his arguments in this proceeding that a POSA would have always sought to restrict the tropism of an AAV vector to a narrower set of "target" tissues and minimize "off-target" tropism.

210. In the PCT application that claims priority to the '126 provisional, some additional experiments are described. EX1067 ('552 PCT), 63:1-31, Example 4. Specifically, Example 4 describes in vivo experiments to evaluate the efficiency of transduction of the 2.5 vector in brain and liver. EX1067 ('552 PCT), 63:1-31, Example 4.

211. The inventors found that the substitutions changed not only muscle tropism but also liver and brain tropism. EX1067 ('552 PCT), 63:1-31, Example 4. They found that "the 2.5 vector has a reduced ability to transduce liver as compared with the parent AAV2 virus." EX1067 ('552 PCT), 63:13-15, Example 4.

212. They also found that the 2.5 vector was able to transduce an additional type of cell in the brain, astrocytes, that the parent vector could not transduce. EX1067 ('552 PCT), 63:18-24, Example 4. The parent vectors, AAV1 and AAV2, are specific for neuronal transduction and were not able to transduce astrocytes, which are non-neuronal. EX1067 ('552 PCT), 63:18-24, Example 4.

213. The '552 PCT sums up these results as follows: "The sum of these experiments when testing the 2.5 vector for tissue-specific transduction in vivo

demonstrates that in addition to gaining tissue-specific tropism (e.g., muscle, skeletal or cardiac derived from the AAV serotype 1 parent), and losing cell type specific transduction (e.g., liver-hepatocyte-specific transduction of recipient AAV2), these vectors have gained a new tropism (non-neuronal/astrocytes) that is not present in either the donor parent (AAV1) or recipient parent capsid (AAV2) and is totally unique to the chimeric 2.5 vector.” EX1067 (’552 PCT), 63:25-31, Example 4.

214. Therefore, Dr. Samulski’s own patent application (and his issued ’809 patent) discloses single amino acid substitutions from one serotype (AAV1) into another (AAV2) that can change tropism, including increasing tropism for a particular tissue (muscle), decreasing tropism for another tissue (liver), and creating tropism in a wholly new tissue (astrocytes), which both parent serotypes (AAV1 and AAV2) are unable to transduce.

215. I note that the ’809 patent includes claims reciting the single amino acid insertion following position 264 in an AAV2 capsid. *See, e.g.*, EX1061 (’809 patent), col. 73:14-25, claim 1 (“A chimeric virus vector comprising: (a) a chimeric adeno-associated virus (AAV) capsid comprising a selective amino acid insertion immediately following amino acid position 264 in an AAV2 capsid subunit having the amino acid sequence of (SEQ ID NO:3) or a corresponding change in another AAV a capsid subunit from other AAV; and (b) a nucleic acid comprising an AAV

terminal repeat sequence and a heterologous nucleic acid sequence; wherein the nucleic acid is packaged within the chimeric AAV capsid”).

216. In fact, various dependent claims specifically recite enhancement of transduction and tropism as additional limitations for the chimeric AAV capsid containing the insertion after position 264. For example, dependent claim 2 recites: “The chimeric virus vector of claim 1, wherein said chimeric virus vector has enhanced transduction of a target cell as compared with the recipient parent AAV vector.” EX1061 (’809 patent), col. 73:26-28, claim 2.

217. Dependent claim 3 recites: “The chimeric virus of claim 2, wherein said chimeric virus vector has enhanced transduction of skeletal muscle.” EX1061 (’809 patent), col. 73:30-32, claim 3.

218. Dependent claim 4 recites: “The chimeric virus of claim 2, wherein said chimeric virus vector has enhanced transduction of cardiac muscle.” EX1061 (’809 patent), col. 73:33-35, claim 4.

219. Dependent claim 5 recites: “The chimeric virus of claim 2, wherein said chimeric virus vector has enhanced transduction of astrocytes and/or glial cells.” EX1061 (’809 patent), col. 73:36-38, claim 5.

220. To the extent that Dr. Samulski believed that expanding the tropism of an rAAV vector would be detrimental to its usefulness, as he has argued in this proceeding, he would not have patented variants that expand tropism beyond the

tropism of either parent serotype. His patent contradicts his arguments here.

**E. Dr. Samulski's Scientific Publication (Wu 2006) Contradicts His Argument**

221. Dr. Samulski also published a paper in 2006 titled “Single Amino Acid Changes Can Influence Titer, Heparin Binding, and Tissue Tropism in Different Adeno-Associated Virus Serotypes.” EX1060 (Wu 2006). I note that the title itself contradicts Dr. Samulski's statements in his declaration that single amino acid changes had not been shown to increase tropism around the relevant date.

222. I note further that this manuscript was submitted to the journal on June 19, 2006. EX1060 (Wu 2006), p. 1. Therefore, I would expect that the work was carried out over some number of months preceding June 2006 – likely sometime in 2005. Dr. Samulski testified that he “assumed” the work for Wu 2006 began in 2005. EX1063 (Samulski Dep.), 202:7-9. He testified that, on average, the work on his published papers involved a four to five year effort, suggesting that some part of the work for Wu 2006 began even earlier, in the early 2000s. EX1063 (Samulski Dep.), 282:14-283:5.

223. In this paper, Samulski and his colleagues disclosed experiments in which they examined the six amino acids that differ between AAV1 and AAV6. EX1060 (Wu 2006), Abstract. Wu 2006 disclosed that, despite these two variants (AAV1 and AAV6) differing by only six amino acids, “these viruses have different

liver transduction profiles when tested as vectors.” EX1060 (Wu 2006), Abstract. Specifically, AAV6 vectors are better at transducing liver tissue than AAV1 vectors, *in vitro* and *in vivo*. EX1060 (Wu 2006), pp. 1, 11395-6.

224. Wu 2006 created single amino acid mutants in which they swapped an amino acid from AAV1 into AAV6, and then vice versa, swapping an amino acid from AAV6 into AAV1. EX1060 (Wu 2006), p. 2, Table 1; EX1063 (Samulski Dep. Tr.), 203:22-204:2. One purpose of making these single amino acid mutants was to investigate the molecular determinants of the different liver tropism of AAV1 and AAV6. EX1063 (Samulski Dep. Tr.), 204:20-205:1. The premise was that one or more of the amino acid differences between AAV1 and AAV6 could be responsible for the difference in liver tropism between the two variants. EX1063 (Samulski Dep. Tr.), 209:18-210:1, 3.

225. Wu 2006 identified a specific residue, K531 in AAV6, that seemed to be responsible for the ability of AAV6 to bind heparin. EX1060 (Wu 2006), pp. 1-3. AAV1 has a glutamic acid instead of a lysine at this position. EX1060 (Wu 2006), p. 2.

226. Wu 2006 tested the effects of swapping the glutamic acid and the lysine at position 531 in AAV1 and AAV6. They created two different reporter constructs (luciferase and human coagulation factor IX), each with a reporter transgene driven by a liver-specific promoter to test transduction of the different mutants. EX1060

(Wu 2006), p. 4. They packaged these reporter constructs into AAV1, AAV1-E531K, AAV6, and AAV6-K531E capsids. EX1060 (Wu 2006), pp. 3-4.

227. They first tested the ability of the different AAVs carrying the luciferase reporter construct to transduce hepatocytes *in vitro*. They found that the K531E mutation in AAV6 reduced the luciferase expression levels to that of AAV1, while the converse E531K change in AAV1 appeared to enhance the transduction efficiency substantially of AAV1. EX1060 (Wu 2006), p. 4, Figure 3(a); EX1063 (Samulski Dep. Tr.), 213:4-16.

228. To evaluate *in vivo* transduction, Wu 2006 infused the vectors packaging the hFIX expression cassette into the portal vein of 6-week-old male C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME) at a dose of  $5 \times 10^{10}$  particles per mouse. EX1060 (Wu 2006), p. 4, Fig. 3(b). Serum hFIX levels at 1 and 6 weeks following administration of AAV6 vectors were approximately an order of magnitude higher than those achieved with AAV1 vectors. EX1060 (Wu 2006), p. 4, Fig. 3(b).

229. In the case of AAV6-K531E vectors, hFIX expression was reduced to levels obtained with AAV1, identical to *in vitro* results and suggesting that K531 is critical for enhanced liver transduction by AAV6. EX1060 (Wu 2006), p. 4. Wu 2006 also found increased hFIX levels obtained with AAV1-E531K vectors, although at lower levels than those achieved with AAV6. EX1060 (Wu 2006), p. 4.

230. To characterize AAV capsid interactions with hepatocytes further, mouse liver parenchymal (PC) and nonparenchymal cells (NPC) were isolated at 6 weeks postadministration of different vectors. EX1060 (Wu 2006), p. 4. Total DNA from PC and NPC was extracted and dot blot DNA hybridization was performed using a radiolabeled hFIX cDNA-derived probe. EX1060 (Wu 2006), p. 4.

231. Wu 2006 found that the levels of vector DNA correlated well with the serum hFIX levels. EX1060 (Wu 2006), p. 4, Fig. 3c. Significantly larger amounts of vector DNA from AAV6 were detected in PC compared to those of AAV1. EX1060 (Wu 2006), p. 4, Fig. 3c.

232. The K-to-E change at residue 531 in the AAV6 capsids reduced the amount of vector DNA in PC to a level similar to that of AAV1, while the converse E-to-K change in AAV1 capsids markedly increased the amount of vector DNA accumulated in PC. EX1060 (Wu 2006), p. 4, Fig. 3c.

233. Wu 2006 concluded that they had identified “a single amino acid (K531) that is essential for conferring the heparin binding characteristics of AAV6. Introduction of an E531K change in AAV1 imparts a heparin binding ability similar to that of AAV6. These observations suggest that a minimum basic footprint is required to facilitate heparin binding through electrostatic interactions. Introduction of K531 in AAV type 8 capsid resulted in recombinant vector particles with heparin binding activity similar to that of AAV6 (Fig. 1d). . . . We also found that lysine 531

is important for higher transduction of hepatocytes by AAV6 than by AAV1.” EX1060 (Wu 2006), pp. 4-5.

234. Wu 2006 emphasized that their data showed that a single amino acid change in an AAV capsid could confer a property from a distantly related serotype: *“These data not only speak to the importance of the K531 position but also illustrate the ability of a single amino acid change to confer a serotype-specific trait in another distantly related serotype.”* EX1060 (Wu 2006), p. 4.

235. Wu 2006 noted that this phenomenon extended beyond AAV to other parvoviruses: “similar studies with autonomous parvoviruses suggest that the molecular determinants controlling host range and tropism in autonomous parvoviruses can often be narrowed down to a few critical amino acid residues in the capsid.” EX1060 (Wu 2006), p. 5.

236. Wu 2006 set out their expectation that “further studies mapping the potential role of single amino acid changes in closely related AAV serotypes and variants . . . are likely to shed light on molecular detrmnants of capsid-receptor interactions, tissue tropism, immune profile, and other aspects of the AAV life cycle in general.” EX1060 (Wu 2006), p. 5; EX1063 (Samulski Dep. Tr.), 228:11-15.

237. Such studies, they concluded, would “lay the foundation for rational engineering of cell type-specific AAV vectors in targeted gene delivery applications.” EX1060 (Wu 2006), p. 5.

**F. A 2003 Publication from Another Research Group Contradicts Dr. Samulski's Argument**

238. Dr. Samulski testified that, when he argued in his declaration that no researchers at the relevant time were investigating single amino acid substitutions in AAV to alter tropism, what he actually meant was that his group was the only group of researchers carrying out such experiments. EX1063 (Samulski Dep. Tr.), 143:13-18. I do not agree.

239. Other researchers were investigating single amino acid changes at the time. I discussed the disclosures of the '772 Publication and Snowdy above, both of which investigated single amino acid substitutions to change transduction and tropism. *See* Sections V.A. and V.B., *supra*. In the next section below, I discuss one of my own patent publications from 2005 that discloses single amino acid changes that we made in an effort to alter various properties of AAV including tropism. EX1066 ('922 publication).

240. In addition, at least one other group published an AAV mutagenesis study at the relevant time investigating single and double amino acid substitutions into AAV2, and their effects on various properties of AAV2, including heparin binding, HeLa cell binding, and tissue tropism *in vivo*. EX1068 (Kern).

241. Specifically, in 2003, a research group from Germany published a paper titled, "Identification of a Heparin Binding Motif on Adeno-Associated Virus Type

2 Capsids.” EX1068 (Kern). In this paper, Kern disclosed a series of point mutations in the AAV2 capsid that were tested for their effects on properties of the virus, including tissue tropism. EX1068 (Kern), pp. 4-6.

242. Kern stated that their purpose in making these mutations was to determine the region of AAV2 responsible for heparin and HeLa cell binding. EX1068 (Kern), Abstract.

243. Previous studies had also shown that AAV1, AAV4, AAV5, and AAV6 do not bind heparin. EX1068 (Kern), p. 3. Kern aligned the sequences of these variants with AAV2, and identified differences in the sequences between amino acids 443 and 602. EX1068 (Kern), p. 3. These amino acids are all located the loop IV region of AAV2. EX1068 (Kern), p. 3; *see also* EX1032 (Wu), p. 3, Figure 1.

244. Based on data from previous studies, Kern assumed that AAV2 and AAV3 bind to different primary receptors, although both serotypes were known to bind to heparan sulfate glucosaminoglycans (HSGAGs). EX1068 (Kern), pp. 1, 3.

245. Kern then modified the loop IV region of AAV2. EX1068 (Kern), p. 3. They tested the mutants for capsid, genomic, and infectivity titers, and for cell and heparin binding. EX1068 (Kern), pp. 3-6, Table 1, Figure 2. From these mutagenesis studies they identified certain residues that could form part of a heparin binding motif in AAV2. EX1068 (Kern), p. 6.

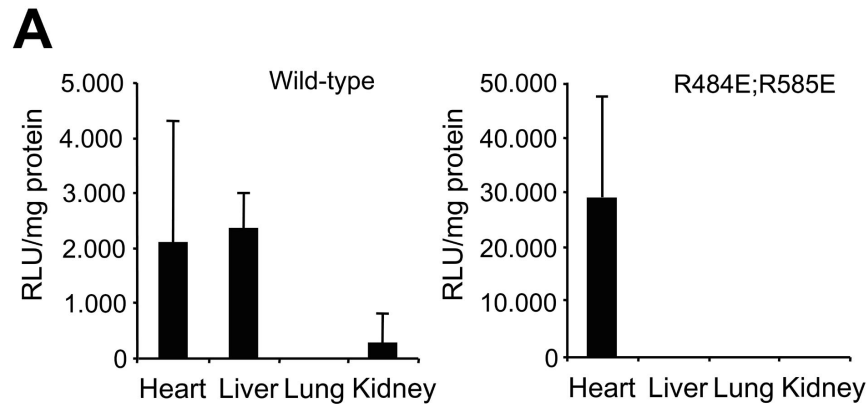
246. They also tested the tissue tropism of certain of the heparin-binding mutants *in vivo*, to see whether mutations in the heparin-binding motif affected tropism. EX1068 (Kern), p. 6.

247. Kern tested the tropism across various tissues of an AAV2 double mutant (mutant 17, with R484E and R585E substitutions) as compared to the tropism of wild-type AAV2. EX1068 (Kern), pp. 6-8. This double mutant had displayed wild-type packaging, reduced infectivity in a dot blot replication assay, and reduced cell and heparin binding. EX1068 (Kern), p. 4, Table 1.

248. Kern injected rAAV with either wild type or the double mutant capsids into the tail vein of mice and analyzed vector genome distribution and reporter gene expression in different tissues. EX1068 (Kern), pp. 6-7, Fig. 3.

249. They found that the double mutant lost the liver tropism of the wild type but had surprisingly good tropism in the heart, higher than the wild type in two different assays. EX1068 (Kern), pp. 6-7, Fig. 3. Both an *in vivo* luciferase reporter assay and detection by PCR of the luciferase gene showed the double mutant to be superior to the wild type in heart tissue. EX1068 (Kern), pp. 6-7, Fig. 3.

250. I have reproduced Figure 3A below, which shows the luciferase reporter gene expression in heart, liver, lung, and kidney of wild-type AAV2 and the double mutant:



EX1068 (Kern), p. 6, Figure 3A.

251. These results are interesting in a couple of respects. First, the tropism of the double mutant in heart tissue increased from around 2.000 RLU to nearly 30.000 RLU, an increase of about 15 fold. EX1068 (Kern), p. 6, Figure 3A. Therefore, the double mutant gained substantially improved tropism to the heart.

252. Second, Kern concluded that this increase could not be attributed merely to the possibility of increased mutant vector reaching the heart, as a result of the loss of liver tropism. Kern stated, “Although in this case the heart would have received a higher amount of virus (due to reduced retention by the liver) than in the wild-type experiment, *there must also be a significant permissivity of heart tissue*

*for the mutant virus to explain the observed expression levels.”* EX1068 (Kern), p. 8 (emphasis added).

253. The data for the kidney support this conclusion. The wild-type vector showed some amount of tropism in the kidney. EX1068 (Kern), p. 6, Figure 3A. However, the double mutant lost that small amount of kidney tropism. EX1068 (Kern), p. 6, Figure 3A. Thus, even though there might have been more mutant virus reaching the kidney as a result of the loss of liver tropism, the mutant did not show a corresponding increase in kidney tropism. EX1068 (Kern), p. 6, Figure 3A.

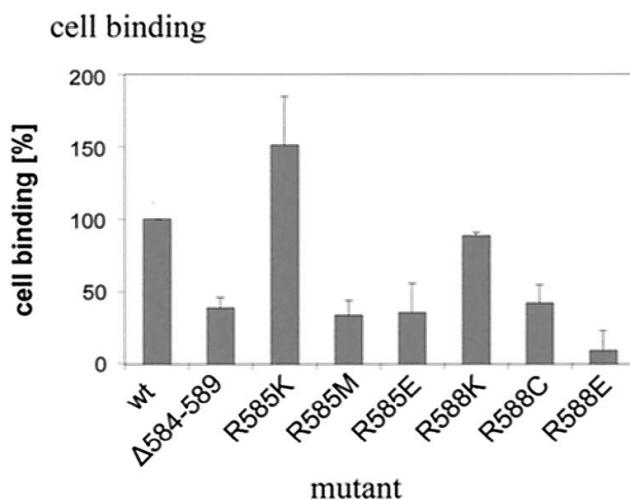
254. The data for the lung also support this conclusion. The wild-type vector showed no tropism in the lung. EX1068 (Kern), p. 6, Figure 3A. The loss of liver tropism in the mutant, and the possible increase in the amount of mutant virus reaching the lung as a result, did not confer any lung tropism on the mutant.

255. These experiments support my opinion, explained in more detail below (Section VII.C), that there is an important difference between biodistribution and infectivity, which Dr. Samulski does not acknowledge. *See, e.g.*, EX2027 (Samulski Dec.), ¶¶ 33, 35. In Kern, the double mutant that lost liver tropism showed increased infectivity of heart, but not lung or kidney, suggesting that this increase involved a “significant permissivity” of heart tissue in particular to the double mutant, relative to wild type. EX1068 (Kern), p. 8. The double mutant’s improved tropism in heart

tissue therefore seems to have been driven by improved infectivity, rather than strictly by a change in biodistribution.

256. Kern also observed enhanced function for one of the single amino acid mutants they tested (R585K), which involved one of the same amino acids from the double mutant, R585. The R585K mutation showed increased cell binding (to HeLa cells) as compared to wild type. EX1068 (Kern), pp. 2-4 (Table 1, Fig. 2a).

257. I have reproduced the relevant graph from Figure 2(a) below:



EX1068 (Kern), pp. 5-6, Figure 2(a).

258. This graph shows that the R585K single amino acid change enhanced cell binding by about 50%. EX1068 (Kern), pp. 5-6, Figure 2(a).

259. From these data, a POSA would have understood Kern to teach that a single amino acid change could enhance tropism. The R585K single amino acid mutant showed enhanced cell binding. And the mutant with a substitution at R585

in combination with only one other substitution showed substantially improved tropism to the heart.

**G. My 2005 Published Patent Application Contradicts Dr. Samulski's Argument**

260. U.S. Patent Application Publication No. 2005/0053922 (“the ’922 publication”) published on March 10, 2005 – before the earliest claimed priority date for the ’274 patent on April 7, 2005. EX1066 (’922 publication), p. 1. I am one of the named inventors on the ’922 publication, along with Brian Kaspar and Narendra Maheshri. EX1066 (’922 publication), p. 1.

261. The ’922 publication discloses our results that a single amino acid substitution in an AAV2 capsid (T716A) could enhance the ability of the rAAV to evade the immune system. EX1066 (’922 publication), [0246], Table 1.

262. We identified this mutant by creating a library of mutant rAAVs designed to produce mutant capsids with a low level of mutations (one or more) in a capsid gene, and selecting for various desired properties. EX1066 (’922 publication), [0009], [0188] – [207]. Those properties included altered tropism, altered heparin binding, and ability to evade the immune system. EX1066 (’922 publication), [0009].

263. I note that the ’922 publication claims priority to Provisional Application No. 60/484,111 (the ’111 provisional), filed on June 30, 2003. EX1066

('922 publication), p. 1. In the '111 provisional, we disclosed that the library comprised “one or more mutations” in a capsid gene of AAV2. EX1069 ('111 provisional”), [0039].

264. Therefore, in the time period from around 2003-2005, my lab was exploring single amino acid substitutions in capsid proteins to develop rAAVs with altered properties, including tropism and ability to evade the immune system.

#### **H. My Publications Do Not Support Dr. Samulski’s Argument Regarding Single Amino Acid Substitutions**

265. The POR argues that two of my publications, Jang and Bartel, “confirm that single amino acid changes would not be expected to alter tropism.” POR, pp. 20-21. This argument is incorrect and is based on misreadings of my publications.

##### **1. Jang**

266. Jang is a 2007 review that I wrote, titled “Library Selection and Directed Evolution Approaches to Engineering Viral Vectors.” EX2024 (“Jang”). The POR argues that I discussed “strategies to modify AAV tropism,” but did not discuss single amino acids as a strategy to modify tropism. POR, pp. 20-21.

267. The POR quotes selectively from this part of my review. The full quote reads as follows:

There have been significant efforts to engineer AAV to overcome its challenges. For example, numerous studies have attempted to enhance selective AAV vector gene delivery to specific cell types via altering binding affinity to receptors expressed on the target cells. Several such

strategies to modify AAV tropism *include*: (i) association with “adaptor” bispecific antibodies, with one arm directed against AAV and the other against a target receptor (Bartlett et al., 1999), (ii) chemical or metabolic conjugation of biotin to the virus, followed by the addition of avidin and a biotinylated-targeting molecule (Arnold et al., 2006; Ponnazhagan et al., 2002), and (iii) genetic modification of the viral capsid structures through the insertion of defined peptide sequences (Girod et al., 1999; Grifman et al., 2001; Nicklin et al., 2001; Work et al., 2004).

EX2024 (Jang), p. 6 (emphasis added).

268. As I explained at my deposition, the use of the word “include” in the passage above indicates that the review here was not attempting to provide an exhaustive list of every possible strategy to modify tropism. EX1064 (Schaffer Dep. Tr.), 187:10-13.

## **2. Bartel**

269. In 2011, I published a review titled “Enhancing the clinical potential of AAV vectors by capsid engineering to evade pre-existing immunity.” EX2023 (Bartel).

270. The POR points specifically to a portion of Bartel where we discuss an earlier study by Maersch.<sup>11</sup> EX2023 (Bartel), p. 8; EX1070 (Maersch). The POR incorrectly characterizes both Bartel and Maersch.

---

<sup>11</sup> I note that the POR appears to contain some typographical errors in this section, repeatedly referring to “EX1032,” rather than EX2023. POR, p. 21.

271. The POR again eliminates important language from the portion of my review that is quoted, as shown below:

Two of these mutants were significantly less neutralized by human sera from single donors and by IVIG compared to AAV2 and AAV2 R459K/N551D. In agreement with the initial hypothesis that the level of immune evasion can be improved if an optimal amino acid substitution is introduced to disrupt an immunogenic epitope, both mutants contained novel substitutions at positions 459, 493, and 551. In addition, the relatively minor sequence changes - ***compared for example to the 120 amino acid differences between AAV2 and AAV1*** - likely did not change the mutant's tropism relative to AAV2.

EX2023 (Bartel), p. 8 (emphasis added).

272. The statement “compared for example to the 120 amino acid differences between AAV2 and AAV1,” omitted in the POR, distinguishes my discussion in this review from my opinions regarding AAV8 and AAVrh.10, which differ by only 48 amino acids. EX1005 (Schaffer Dec.), ¶ 212.

273. The Maersch study compared the ability of particular mutants of AAV2 (containing randomized mutations at five positions previously shown to be involved in immunogenicity) to evade anti-AAV2 antibodies with the ability of AAV1 and AAV2 to evade those antibodies. EX1070 (Maersch), p. 2. The Maersch study comments that AAV1 and AAV2 differ by 120 amino acids, which likely accounts for the ability of AAV1 to evade anti-AAV2 antibodies. EX1070 (Maersch), p. 2.

274. Our point in the passage quoted above was simply that an AAV2 mutant with enhanced ability to evade anti-AAV2 antibodies, with a relatively small number

of mutations as compared to AAV2, might nonetheless not exhibit the substantially different tropism of AAV1 as compared to AAV2.

275. Maersch makes essentially the same point in the passage I have reproduced below discussing two mutants, H6 and A11, that were found to be the best evaders of anti-AAV2 antibodies:

***Compared to AAV-2, H6 yielded higher transduction rates in all tested cell types (from 1.15- to 1.71-fold higher).*** Conversely, A11 yielded lower relative transduction rates than AAV-2 (0.09- to 0.23-fold lower). AAV-1 yielded lower transduction rates on most cell lines (between 0.1- and 0.27-fold), but was comparable to AAV-2 on HEK293 cells (0.89-fold). Therefore, while large structural differences distinguishing different natural AAV serotypes result in a radically different tropism, ***immune-escaping mutants carrying a limited number of appropriate mutations, depending on the identity of the introduced amino acids can retain a tropism that resembles (or even outperforms) the original.***

EX1070 (Maersch), p. 6 (emphases added).

276. Notably, as described in this passage, Maersch found that mutant H6 (the best performing mutant as far as ability to evade anti-AAV2 antibodies) actually exhibited ***enhanced*** ability to transduce human melanoma cells (BLM and MV3), human breast cancer cells (MDA), human embryonic kidney cells (293), human keratinocytes (HaCat), and murine embryonic fibroblasts (NIH3T3) as compared to the parent AAV2. EX1070 (Maersch), p. 6. Maersch concluded from these studies that such an immune-escaping mutant could demonstrate tropism that “outperforms” the original parent AAV. EX1070 (Maersch), p. 6.

277. Maersch emphasizes in this passage that immune escaping mutants “carrying a limited number of appropriate mutations, depending on the identity of the introduced amino acids” can retain or even enhance the tropism of the parent serotype. Maersch further comments: “Depending on the nature of the introduced amino acid, substitutions at the five positions could . . . dramatically alter viral tropism.” EX1070 (Maersch), p. 8.

278. Therefore, neither Bartel nor Maersch is in any way inconsistent with the opinions I set out in my Declaration. Bartel’s statements are in reference to Maersch, which found that small numbers of changes, depending on the identity of the changes, could improve the ability to evade the immune system without dramatically changing the tropism of the parent serotype. Notably, Maersch found that the best immune evader had enhanced tropism as compared to the parent AAV2.

279. These references therefore actually support my opinions that a single amino acid change, where the substitution is appropriately selected, can enhance tropism without radically changing the overall tropism of the parent serotype. EX1005 (Schaffer Dec.), ¶ 211.

## **VI. DR. SAMULSKI INCORRECTLY DESCRIBES AND LIMITS SNOWDY’S TEACHINGS**

280. Dr. Samulski argues that “[a]t a high level, Snowdy does not teach or discuss making mutations to change the tissue to which a particular AAV serotype

is targeted.” EX2027 (Samulski Dec.), ¶ 71. He further argues, “this study is unrelated to improving or changing tissue tropism, which is the sole motivation Sarepta identifies for combining Snowdy with the ’772 Publication.” EX2027 (Samulski Dec.), ¶ 72.

281. In my opinion, Dr. Samulski incorrectly describes and limits the teachings of Snowdy in several different respects, in an effort to support an argument that a POSA would not have been motivated to combine the ’772 Publication with Snowdy.

**A. Snowdy’s Teachings Are Not Limited to Nuclear Localization of AAV**

282. In his declaration, Dr. Samulski incorrectly attempts to limit Snowdy’s teachings to nuclear localization of AAV. He states, “Snowdy uses alanine and aspartic acid substitutions on or near putative nuclear localization signals to determine whether phosphorylation impacts nuclear localization of AAVs.” EX2027 (Samulski Dec.), ¶ 71. He also argues that “nuclear localization relates to infectivity, and is unrelated to the tissue-specific tropism driving Sarepta’s motivation to combine.” EX2027 (Samulski Dec.), ¶ 71.

283. Even if Dr. Samulski were correct that “nuclear localization” relates to “infectivity” rather than “tropism,” which, as I have discussed, differs from the

definition of tropism of numerous others (*see* Section IV above), Snowdy's teachings in any event are not limited to nuclear localization.

284. Importantly, in his declaration, Dr. Samulski incorrectly interprets the unexpected nature of Snowdy's findings, and Snowdy's own conclusions that the effects he saw of the S181A substitution on transduction might not have been related to nuclear localization. *See, e.g.*, EX2027 (Samulski Dec.), ¶ 75; EX1009 (Snowdy), p. 129; EX1005 (Schaffer Dec.), ¶¶ 186-89, 275-76.

285. Dr. Samulski quotes key language in Snowdy that states: "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.*" EX2027 (Samulski Dec.), ¶ 75; EX1009 (Snowdy), p. 129; EX1005 (Schaffer Dec.), ¶¶ 186-89, 275-76.

286. Yet Dr. Samulski, in his declaration, does not meaningfully address this portion of Snowdy, which teaches that the effects of the S181A and S181D mutations on AAV2 transduction efficiency might have been unrelated to nuclear localization, other than to say, "I agree with Snowdy on this point." EX2027 (Samulski Dec.), ¶ 75.

287. As I discuss in my Declaration, the fact that Snowdy's results were unexpected – in fact, they were the *opposite* of what would have been predicted based on other studies of nuclear localization signals – broadens his results out

beyond effects relating to nuclear localization, and makes his teachings highly relevant to a POSA seeking to alter tropism by modulating phosphorylation. EX1005 (Schaffer Dec.), ¶¶ 186-89, 275-76.

288. Specifically, Snowdy expected the S181A substitution to ***decrease*** transduction efficiency, and did not expect the S181D substitution to result in a total loss of transduction efficiency: “Based on Jans’ data for T-ag discussed above, we expected the serine to alanine mutations to result in lowered transduction efficiency ***if the residue is important for regulating the putative NLS***. Likewise, we expected the serine to aspartic acid mutation to suffer, at most, a slight decrease in transduction efficiency versus the total loss of transduction ability that is shown in figure 23.” EX1009 (Snowdy), p. 128 (emphasis added).

289. Dr. Samulski testified at his deposition that Snowdy’s result was the “opposite observation of what was expected from nuclear localization signals.” EX1063 (Samulski Dep. Tr.), 97:23-98:4.

290. Dr. Samulski testified that a POSA would have understood that Snowdy’s results suggested that another function other than nuclear localization was affected by the S181A mutation and that “another hypothesis” was required. EX1063 (Samulski Dep. Tr.), 98:23-99:2, 4-13, 15. He further testified that a POSA would have understood that Snowdy’s data implied that this other function had been affected by removal of a single putative phosphorylation site by substituting in a

nonphosphorylatable residue in a way that enhanced AAV transduction. EX1063 (Samulski Dep. Tr.), 98:23-99:2, 4-13, 15.

291. A POSA would have understood that Snowdy's expression data could have resulted from effects at any point in the transduction process caused by the S181A substitution. EX1009 (Snowdy), pp. 126-27, Figure 23. There is nothing in Snowdy that would have led a POSA to conclude that the effects of the S181A mutation on transduction were caused by interference with nuclear localization. In fact, as I have explained, a POSA would have understood the opposite from Snowdy's disclosures – that the improved transduction was the result of altering a function other than nuclear localization. EX1005 (Schaffer Dec.), ¶ 280.

292. Given the unexpected results, Snowdy could not conclude that S181 was “important for regulating the putative NLS.” Instead, Snowdy hypothesized that S181 “may play some role in AAV2's *ability to infect its target cell*.” EX1009 (Snowdy), p. 128 (emphasis added).

293. The phrase “ability to infect its target cell” broadens Snowdy's results beyond the function of nuclear localization, and beyond the *in vitro* transduction assays he conducted with HeLa cells. A POSA would have understood the phrase “infect its target cell” to mean that S181 may be an important determinant of the ability of AAV to infect target cells *in vivo*.

294. It is my opinion that a POSA at the time would have understood

Snowdy to teach that removing a putative phosphorylation site in the AAV capsid could “play some role” in the virus’ “ability to infect its target cell,” which, in turn, would result in altered tropism.

**B. Snowdy’s Teachings Are Not Limited to Substitution of Alanine for Serine**

295. Dr. Samulski argues that Snowdy’s results are limited to substituting either an alanine or an aspartic acid for a serine, and have no application to the substitution of an asparagine for a serine. EX2027 (Samulski Dec.), ¶ 76. I disagree.

296. First, Dr. Samulski argues inaccurately that Snowdy’s approach is “consistent with” a “wreck and check alanine scanning approach.” EX2027 (Samulski Dec.), ¶ 76. Importantly, Snowdy mutated serines and threonines to aspartic acid, in addition to mutating them to alanine. *See, e.g.*, EX1009 (Snowdy), p. 125. These mutations were not intended to abrogate nuclear localization. *See, e.g.*, EX1009 (Snowdy), p. 128. In fact, their purpose was just the opposite, to show that mimicking constitutive phosphorylation would not destroy function. *See, e.g.*, EX1009 (Snowdy), p. 128.

297. Dr. Samulski points to Wu as an example of “alanine scanning” or a “wreck and check” approach. EX2027 (Samulski Dec.), ¶ 49. A comparison of Wu with Snowdy shows that the two publications used completely different mutagenesis strategies.

298. Wu expressly describes some of the mutations they made in the AAV2 capsid as “alanine scanning” mutations. EX1032 (Wu), p. 2 (“Initially, 48 alanine scanning mutations were made in which two to five charged amino acids in the AAV capsid ORF were mutated to alanine residues by site-directed mutagenesis. We reasoned that since the mutations were an average of 15 to 20 amino acids (aa) apart and spanned the whole capsid gene, some of them would inevitably fall in or near the functional domains of AAV capsid”).

299. In fact, Wu created an alanine scanning mutant, *mut17*, with substitutions in residues surrounding S181. EX1032 (Wu), p. 5, Table 1. This mutant involved the following substitutions at residues 180-184: DSVPD (aspartic acid – serine – valine – proline – aspartic acid) to ASVPA (alanine – serine – valine – proline – alanine).

300. Notably, in this alanine scanning mutant (*mut17*), S181 was not changed to alanine (or to anything else). Only the two surrounding aspartic acid residues were changed to alanine (D180A and D184A). Interestingly, the phenotype Wu observed for *mut17* was wild type. EX1032 (Wu), pp. 3, 5, Fig. 1, Table 1.

301. Notably, neither substitution mutant that Snowdy created at position 181 had a wild-type phenotype – S181D abrogated transduction and S181A substantially enhanced transduction. EX1005 (Schaffer Dec.), ¶¶ 183-87; EX1009 (Snowdy), pp. 5, 126-30.

302. In this way, Wu supports my opinion and differs from Dr. Samulski's opinion, by demonstrating Snowdy's approach was materially different from Wu's alanine scanning and produced completely different results.

303. A POSA would have understood that Snowdy was exploring a wholly different theory from Wu in creating the S181A and S181D mutants. Snowdy theorized that phosphorylation at S181 could affect nuclear localization, and made two specific substitutions at that position to test this theory. EX1005 (Schaffer Dec.), ¶¶ 183-87; EX1009 (Snowdy), pp. 5, 126-30.

304. Importantly, Snowdy found that substitution of alanine at position 181 *enhanced* a particular function – transduction efficiency – rather than revealing the relevance of the amino acid that was removed by destroying a function. EX1005 (Schaffer Dec.), ¶¶ 183-87; EX1009 (Snowdy), pp. 5, 126-30.

305. A POSA would further have understood that this result indicated that removing a putative phosphorylation site at this position was important for transduction, but would not have had any reason to believe that only alanine could serve the function of removing the phosphorylatable serine. Snowdy does not include any teaching to suggest that alanine in particular was important to this result. On the contrary, Snowdy repeatedly refers generally to the significance of “the phosphorylation status of S181,” rather than attributing the enhancement of function to the substitution of alanine in particular. *See, e.g.*, EX1009 (Snowdy), pp. 128-30.

306. As I discussed in my Declaration, a POSA at the time would have been well aware of which amino acids were phosphorylatable, and which were not. EX1005 (Schaffer Dec.), ¶¶ 45-48. As I also discussed in my Declaration, a POSA would have been guided by the comparison between AAV8 and AAVrh.10, to identify phosphorylatable residues in AAVrh.10 where the corresponding residue in AAV8 was nonphosphorylatable. EX1005 (Schaffer Dec.), ¶¶ 272-280.

307. In any event, Dr. Samulski again appears to disregard the principal bases for my opinions in asserting that “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.” EX2027 (Samulski Dec.), ¶ 77.

308. As I have set out in detail in my Declaration, a POSA at the time would have chosen to substitute in a nonphosphorylatable asparagine from AAV8 to replace phosphorylatable serine at the corresponding position (665) of AAVrh.10, to confer the superior liver tropism of AAV8 onto AAVrh.10. EX1005 (Schaffer Dec.), ¶¶ 271-280. The choice of asparagine specifically as the nonphosphorylatable amino acid to substitute in would have been guided by the presence of asparagine at the corresponding position of AAV8. EX1005 (Schaffer Dec.), ¶¶ 271-280.

309. Dr. Samulski’s criticisms of my opinions are particularly surprising given that, as I discussed in detail above (Sections V.C and V.D), the ’126

provisional and the '809 patent both disclose substituting in a nonphosphorylatable asparagine from AAV1 to replace a phosphorylatable threonine at the corresponding position in AAV2, for the same reasons that I set out in my Declaration – namely, to confer some of the desirable tropism properties of AAV1 onto AAV2.<sup>12</sup>

---

<sup>12</sup> Dr. Samulski does not accurately interpret my statement in my Declaration that “Snowdy teaches the substitution of asparagine (N) as a nonphosphorylatable amino acid to remove a phosphorylation site.” EX1005 (Schaffer Dec.), ¶ 281. This statement was in reference to my discussion of one typographical error on pp. 28-29 of Snowdy, where the word “asparagine” should read “aspartic acid.” EX1005 (Schaffer Dec.), ¶¶ 178-181; EX1009 (Snowdy), pp. 28-29. I note that Dr. Samulski agrees with my opinion that the word “asparagine” is a typographical error that should instead read “aspartic acid.” EX2027 (Samulski Dec.), ¶ 77. I explained that a POSA would have understood that asparagine does not mimic a constitutively phosphorylated state (which is the literal meaning of the sentence with the typographical error), but rather provides an unphosphorylated state. EX1005 (Schaffer Dec.), ¶¶ 178-181. To support this conclusion, I referred to Jans & Jans, because Snowdy cites Jans & Jans at the end of the sentence containing the typographical error. EX1009 (Snowdy), p. 15. Jans & Jans expressly states that aspartic acid mimics a constitutively phosphorylated state, and also uses asparagine

**C. The Fact That Some Mutations Disclosed in Snowdy Had No Effect Does Not Diminish Snowdy's Teachings**

310. Dr. Samulski argues in his declaration that because substitutions of serines and threonines at two other positions in the AAV capsid had little or no effect on the ability of the virus to infect HeLa cells, “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.” EX2027 (Samulski Dec.), ¶ 78. I disagree.

---

to replace aspartic acid to destroy the function of a CKII recognition sequence. EX1005 (Schaffer Dec.), ¶¶ 180-181. I explained that, given these disclosures in Jans & Jans, a POSA would have understood that Snowdy taught that aspartic acid, not asparagine, mimics constitutive phosphorylation, particularly given that asparagine cannot provide the function of the charged amino acid aspartic acid, when substituted in for aspartic acid in the context of the mutants used in Jans & Jans. It would have been clear to a POSA that the single reference to asparagine in Snowdy was a typographical error, and that Snowdy, like any other POSA, understood that asparagine was nonphosphorylatable, and could be substituted in to remove a phosphorylation site. I discuss Jans & Jans in more detail below.

311. A POSA at the time would have understood that not all experiments work, but that does not diminish the value or significance of the experiments that do work. At his deposition, Dr. Samulski testified that a researcher would be “lucky” to get one positive result out of three experiments, let alone an unexpected enhancement of function, as Snowdy did. EX1063 (Samulski Dep. Tr.), 86:24-25, 87:2-9, 11.

312. The effects that Snowdy found at position 181 were substantial and surprising, and included enhancing transduction by removing a phosphorylation site. A POSA would have been guided by this result to create and test similar mutations in AAVrh.10, based on a comparison with AAV8.

313. The POR argues that my testimony that I “would have used the NetPhos program to identify the phosphorylation sites in AAVrh.10” is “misguided” because a POSA would not have known in advance which (if any) of the four positions where AAV8 has a nonphosphorylatable amino acid and AAVrh.10 has a phosphorylatable amino acid are actually phosphorylated in AAVrh.10. POR, pp. 53-54.

314. This argument seems to be based on a fundamental misconception about the teachings of Snowdy. As Snowdy teaches, and as a POSA would have understood at the time, the purpose of a program such as NetPhos is to predict potential phosphorylation sites, based on known kinase recognition sequences. *See, e.g.,* EX1005 (Schaffer Dec.) ¶ 184, n.3; EX1009 (Snowdy), p. 125.

315. A POSA would have understood that the predictions of a program such as NetPhos would need to be tested experimentally, just as Snowdy tested them, after identifying three potential phosphorylation sites of interest in the vicinity of the putative NLS. EX1009 (Snowdy), p. 125.

316. Snowdy found that mutating one of these sites (S181) to remove the phosphorylatable residue (and replace it with alanine) greatly increased the efficiency of transduction of HeLa cells. EX1009 (Snowdy), pp. 125-26. Snowdy's experiments therefore produced an unexpectedly beneficial and encouraging result, teaching that this experimental approach should be followed as a method of improving the tropism of an AAV vector, and motivating a POSA to try similar experiments with AAVrh.10.

317. I note in addition that the '126 provisional and the '809 patent disclose substitutions that produced no effect. *See, e.g.*, EX1059 ('126 provisional) 5:30-31 ("Figure 2A. Changing the amino acid of AAV2 at the 324, 328 positions did not enhance muscle transduction"); EX1061 ('809 patent), col. 3:52-53 ("FIG. 2A. Changing the amino acid of AAV2 at the 324, 328 positions did not enhance muscle transduction").

318. Nonetheless, Dr. Samulski disclosed and claimed interesting variants that did enhance tropism, despite these negative results. Neither the '126 provisional nor the '809 patent includes any suggestion that these negative results in any way

diminished the importance or usefulness of the substitutions that enhanced tropism.

## **VII. DOSAGE AND OFF-TARGET LOCALIZATION**

319. Dr. Samulski argues that it would have been very important to a POSA to limit “off-target expression” of the gene of interest. EX2027, ¶ 33. Dr. Samulski argues that off-target expression could require that a higher dosage of the AAV vector be administered to achieve an effective level of expression in the target tissue. EX2027, ¶ 33. He further argues that such a higher dosage could produce undesirable immune responses. EX2027, ¶ 35.

320. I disagree for several reasons, set out below.

### **A. Tissue-Specific Promoters**

321. In my opinion, it was well known to a POSA as of 2005 that tissue-specific promoters could be used to control transgene expression, and limit expression to the desired cell target or targets. I note that both the ’772 Publication and Dr. Samulski at his deposition recognized the use of tissue specific promoters in this way. *See, e.g.*, EX1007 (’772 Publication), [0111]; EX1063 (Samulski Dep. Tr.), 214:8-215:13, 15-25.

### **B. Route of Administration**

322. A POSA as of 2005 would also have understood that localization of AAV vectors could be controlled through the route of administration. Administration directly into muscle (intramuscular), lung (intratracheal), or liver

(interportal), could reduce the chance that the vector would travel widely throughout the body, and thus minimize concerns about tropism to non-target tissues.

323. The '772 Publication supports my opinion, in that it discloses intramuscular injection for muscle, intratracheal injection for lung, and intraportal injection for liver. *See, e.g.*, EX1007 ('772 Publication), [0242] (“The performance of AAV vectors in skeletal muscle was analyzed following direct injection into the tibialis anterior muscles”); [0244] (“Similar murine models were used to evaluate liver-directed gene transfer. Identical doses of vector based on genome copies were infused into the portal veins of mice that were analyzed subsequently for expression of the transgene”); [0254] (“First, AAV vector carried [*sic*] CC10hA1AT minigene for lung specific expression were pseudotyped with capsids of novel AAVs were given to Immune deficient animals (NDR nude) in equal volume (50  $\mu$ l each of the original preps without dilution) via intratracheal injections”); *see also* [0150] (“Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, direct delivery to the selected organ (e.g., intraportal delivery to the liver), oral, inhalation (including intranasal and intratracheal delivery), intraocular, intravenous, intramuscular, subcutaneous, intradermal, and other parental routes of administration”).

324. Dr. Samulski acknowledged at his deposition that local administration could obviate concerns about off-target expression, testifying that “if you had

multiple tropisms attached to a capsid, the root [*sic*] of administration may restrict access to those tropisms. . . . If it's intramuscular, it's restricted. . . . You're not using the virus based on its promiscuity. You're restricting it based on the root [*sic*] of administration." EX1063, 248:19-249:3.

**C. Dr. Samulski Conflates Biodistribution and Infectivity**

325. I also disagree that improving liver tropism in AAVrh.10 would require administering a higher dosage of the vector. In fact, as I explain below, I believe that improving liver tropism by increasing the efficiency of expression of the transgene in liver tissue could actually reduce the necessary dosage and therefore improve safety.

326. Dr. Samulski appears to conflate two different concepts – biodistribution and infectivity. I argue in my Declaration that a POSA would be motivated to improve the tropism of AAVrh.10 in the liver by making some single amino acid substitutions from AAV8 into AAVrh.10. The fact that liver tropism in AAVrh.10 might be improved by a single amino acid substitution would not necessarily mean that more of the AAVrh.10 vector would be directed to the liver, or that there would be any difference in localization of the vector in different tissues – rather, it could mean that the amount of the vector that reaches the liver would be more effective at expressing the transgene in liver tissues.

327. For example, Dr. Wilson published a paper in 2015 titled, “Comparative Study of Liver Gene Transfer with AAV Vectors Based on Natural and Engineered AAV Capsids.” EX1071 (Wang).

328. Wang investigated the liver transduction of several different natural and engineered AAV capsids, including AAV2, AAV8, and AAVrh.10. EX1071 (Wang), Abstract, p. 3, Table 2.

329. Their results demonstrate that the distribution of an AAV vector in different tissues does not always correlate with the expression of that vector in any particular tissue. EX1071 (Wang), p. 3, Table 2. In other words, a vector may localize well to a particular tissue, but not express its transgene in that tissue in the transduced cells, whereas another vector might localize poorly to that tissue, but express the transgene at high levels in that tissue in the transduced cells. In short, localization does not always correlate with efficiency of transgene expression.

330. As a result, a POSA at the relevant time would have understood that improving tropism of an rAAV vector in a particular tissue does not necessarily mean altering localization of that vector to different tissues, but instead could mean improving efficiency of transgene expression in a tissue to which the vector already localizes.

331. I have reproduced Table 2 from Wang below (I have highlighted the data in the table I am focusing on):

Table 2 Summary of gene transfer in macaques after systemic vector administration

| Vector serotype | Animal no. | Pre AAV Nab (1:dilution) | Capsid in spleen <sup>a</sup> | GFP transduction in liver                   |                                        |                                                | GFP DNA copy/diploid genome <sup>e</sup> |        | Spleen/liver GFP DNA ratio |
|-----------------|------------|--------------------------|-------------------------------|---------------------------------------------|----------------------------------------|------------------------------------------------|------------------------------------------|--------|----------------------------|
|                 |            |                          |                               | Efficiency (%) <sup>b</sup> (mean $\pm$ SD) | Intensity <sup>c</sup> (mean $\pm$ SD) | GFP protein <sup>d</sup> (ng/ $\mu$ g protein) | Liver                                    | Spleen |                            |
| AAV3B           | RQ9759     | <1:5                     | ++                            | 25.6 $\pm$ 5.6                              | 1.02 $\pm$ 0.27                        | 0.73                                           | 17.1                                     | 9.6    | 0.56                       |
|                 | RQ9831     | <1:5                     | ++                            | 24.4 $\pm$ 17.2                             | 1.16 $\pm$ 0.19                        | 0.65                                           | 13.1                                     | 19.6   | 1.50                       |
| AAVLK03. L125I  | RQ8982     | <1:5                     | +++                           | 17.8 $\pm$ 9.2                              | 0.66 $\pm$ 0.14                        | 0.49                                           | 7.0                                      | 30.9   | 4.4                        |
|                 | RQ9284     | <1:5                     | +++                           | 3.8 $\pm$ 1.8                               | 0.51 $\pm$ 0.09                        | 0.12                                           | 1.2                                      | 31.0   | 25.8                       |
| AAVLK03         | RQ9828     | <1:5                     | ++                            | 15.2 $\pm$ 9.0                              | 0.43 $\pm$ 0.07                        | 0.18                                           | 0.5                                      | 34.0   | 68.0                       |
|                 | RQ9837     | 1:20                     | ++                            | 0.1 $\pm$ 0.1                               | 0.31 $\pm$ 0.04                        | 0                                              | 0.003                                    | 25.3   | 8433.3                     |
| AAVrh10         | 090-0266   | <1:5                     | +                             | 14.5 $\pm$ 10.1                             | 0.47 $\pm$ 0.11                        | 0.18                                           | 9.6                                      | 7.1    | 0.74                       |
|                 | 090-0283   | <1:5                     | +/-                           | 15.8 $\pm$ 7.0                              | 0.58 $\pm$ 0.21                        | 0.45                                           | 20.1                                     | 9.4    | 0.47                       |
| AAV8            | RQ8082     | <1:5                     | +                             | 23.0 $\pm$ 17.6                             | 0.79 $\pm$ 0.26                        | 0.45                                           | 26.2                                     | 2.8    | 0.11                       |
|                 | RQ8083     | <1:5                     | -                             | 20.5 $\pm$ 12.7                             | 0.92 $\pm$ 0.24                        | 0.47                                           | 29.4                                     | 3.0    | 0.10                       |
| AAV2            | 03D313     | <1:5                     | ++                            | 1.6 $\pm$ 1.8                               | 0.35 $\pm$ 0.04                        | 0.12                                           | 28.8                                     | 28.5   | 0.99                       |

AAV, adeno-associated virus; GFP, green fluorescent protein; Nab, neutralizing antibody.

<sup>a</sup>Detection of capsid protein in spleen by immunofluorescence. <sup>b</sup>Measured as the percentage of GFP-positive area per liver section regardless of brightness. Ten sections per animal were analyzed. <sup>c</sup>Determined as the total intensity of each image with background level subtracted (see Materials and Methods). Ten sections per animal were analyzed. <sup>d</sup>Determined by an enzyme-linked immunosorbent assay on liver lysate. <sup>e</sup>Determined by quantitative polymerase chain reaction of transgene GFP, according to a standard curve generated with linearized plasmid DNA, pAAV.CMV.GFP. The limit of detection is  $7 \times 10^{-3}$  copies/diploid genome.

EX1071 (Wang), p. 3, Table 2 (highlights added).

332. For example, as can be seen in Table 2, Wang found that AAV2 showed a large number of vector transgenes per diploid genome (meaning per cell) localized to liver (28.8 GFP DNA copies / diploid genome), but showed relatively poor expression of the GFP transgene in liver tissue overall ( $1.6 \pm 1.8$ ). EX1071 (Wang), p. 3, Table 2. In other words, Table 2 shows that AAV2 localized well to liver tissue, but showed poor transgene expression in the liver cells to which it localized.

333. In contrast, AAV8 showed equally good localization to liver tissue (26.2 and 29.4 GFP DNA copies / diploid genome), yet also showed good expression of the GFP transgene in the liver cells to which it localized ( $23.0 \pm 17.6$  and  $20.5 \pm$

12.7). EX1071 (Wang), p. 3, Table 2. In other words, AAV8 both localized well to liver cells and expressed the transgene well in liver cells. For AAVrh.10, Wang found that it did not localize to liver cells as well as AAV2 or AAV8 (20.1 and 9.6 GFP DNA copies / diploid genome). EX1071 (Wang), p. 3, Table 2. However, AAVrh.10 expressed the GFP transgene considerably better in liver cells ( $14.5 \pm 10.1$  and  $15.8 \pm 7.0$ ) than AAV2, although not as well as AAV8. EX1071 (Wang), p. 3, Table 2.

334. Wang therefore shows that AAV vector localization does not necessarily correlate with efficiency of transgene expression – an AAV vector can show good localization to a particular tissue but poor transgene expression, or vice versa. Wang therefore supports my opinion, and does not support Dr. Samulski's argument. A POSA at the relevant time would have understood that a single amino acid substitution into AAVrh.10 from AAV8 could improve the liver tropism of AAVrh.10 by increasing the levels of transgene expression in liver tissue without having any effect on localization of AAVrh.10 to liver, lung, or muscle. In that case, there would be no concern about off-target localization or about needing a higher dose because more of the vector would not localize to the liver.

335. In another example, Dr. Wilson published an abstract in May, 2005 titled, "Transductional and Genome Biodistribution of Novel AAV Serotype Vectors after Systemic Delivery and Their Liver Specific Dose Response Profiles in

Mice.” EX1073 (Lu). In this study, they compared transduction of mouse tissues after intravenous administration of AAVs 2, 5, 7, 8 and 9. They found differences in transduction, where for example AAV8 performed well in liver, and AAV9 performed well in cardiac myocytes. However, they report that “In contrast to distinguished tissue transductional profiles of AAV serotype vectors, there was no serotype specific genome targeting observed. In most of tissues analyzed, genome copies of all rAAVs in a particular tissue are quite similar, suggesting that the capsid directed genome processing pathway represent the determining factor for the serotype specific vector biology.” EX1073 (Lu), p. 1.

336. Lu therefore supports my opinion, and does not support Dr. Samulski’s argument. A POSA at the relevant time would have understood from Lu that localization, as measured by the number of genome copies in a particular tissue, was distinct from gene expression – given that Lu found the genome copies of the various serotypes to be similar, despite different transduction profiles. EX1073 (Lu), p. 1. Therefore, a POSA at the time would have further understood that a single amino acid substitution into AAVrh.10 from AAV8 could improve the liver tropism of AAVrh.10 by increasing the levels of transgene expression in liver tissue without having any effect on localization of AAVrh.10 to liver, lung, or muscle. In that case, there would be no concern about off-target localization or about needing a higher dose because more of the vector would not localize to the liver.

**D. My Publications Are Wholly Consistent with My Opinions in This Proceeding and Do Not Support Dr. Samulski's Arguments**

337. The POR incorrectly characterizes two of my publications, Kotterman and Schaffer 2014, in arguing that a POSA at the time would not have been motivated to improve liver tropism while preserving muscle and lung tropism in AAVrh.10. *See, e.g.*, POR, pp. 23-25.

**1. Kotterman**

338. The POR argues incorrectly that my “previous work shows” that “the liver represents a ‘barrier’ for targeting non-liver tissues,” citing Kotterman, and that Kotterman therefore supports the arguments of Dr. Samulski and the POR. POR, pp. 24-25; EX1030 (Kotterman).

339. In the passage the POR cites, Kotterman states that “For systemically administered viruses, the liver is often the default destination, which can represent a barrier when other organs are the intended targets.” EX1030 (Kotterman), p. 3.

340. This statement does not support Dr. Samulski's arguments, or the arguments in the POR. As a preliminary matter, the statement refers only to “systemically administered viruses.” EX1030 (Kotterman), p. 3. As I discussed above (Section VII.B), there are gene therapies that are directly injected into a particular organ, rather than systemically administered, and pose different challenges. *See also* EX1030 (Kotterman), p. 4.

341. I have set out my opinions that a POSA at the relevant time would have been motivated by the teachings of the '772 Publication to modify AAVrh.10, a vector that showed superior performance across three tissues, muscle, lung, and liver, to obtain “an artificial variant of AAVrh.10 with even more efficient gene transfer in liver,” while preserving its already desirable properties. EX1005 (Schaffer Dec.), ¶¶ 210-211.

342. As I explained above, improving AAVrh.10 liver tropism through a single amino acid substitution would not necessarily mean that more of the AAVrh.10 vector would be directed to the liver, or that there would be any difference in localization of the vector in different tissues – rather, it could mean that the amount of the vector that reaches the liver would be more effective at expressing the transgene in liver tissues.

343. Therefore, the statement in Kotterman that liver can sometimes be a barrier to an AAV vector’s reaching other tissues is completely consistent with my opinions in this proceeding, and does not support Dr. Samulski’s arguments or the those of the POR.

## **2. Schaffer 2014**

344. The POR also incorrectly characterizes a similar statement in Schaffer 2014. EX2025 (Schaffer 2014). Schaffer 2014 states that “upon systemic administration, natural serotypes typically exhibit tropism for liver, or alternatively

for multiple organs, which renders targeted delivery to other tissues difficult.” EX2025 (Schaffer 2014), p. 1.

345. This statement, like the statement in Kotterman cited in the POR, refers to systemic administration. As I discussed above (and in Section VII.B), there are gene therapies that are directly injected into a particular organ, rather than systemically administered, and pose different challenges. *See, e.g.*, EX1030 (Kotterman), p. 4.

346. In addition, the statement from Schaffer 2014 refers to a situation in which an AAV vector localizes to the liver and to tissues other than the target tissue of interest. EX2025 (Schaffer 2014), p. 1 (discussing targeted delivery to “other tissues,” meaning to tissues other than the liver or the target tissue of interest).

347. As I have explained, however, the specific guidance of the ’772 Publication about AAVrh.10 is that it has superior tropism across three tissues (muscle, lung, and liver), and therefore “has some intriguing biological significance.” EX1007 (’772 Publication), [0257]; EX1005 (Schaffer Dec.), ¶¶ 123, 208. It is my opinion that a POSA at the time would have sought to improve the liver tropism of AAVrh.10, without damaging its tropism in the other two tissues, to use it as a vector in all of these tissues. EX1005 (Schaffer Dec.), ¶¶ 210-11.

348. And I have also explained that improving liver tropism would not necessarily involve altering the tissue distribution of AAVrh.10. *See* Section VII.C, *supra*.

349. Improving the liver tropism of AAVrh.10, while preserving its tropism in lung and muscle, would therefore not make the vector any less useful for targeting muscle and lung.

350. Therefore, the statement from Schaffer 2014 is completely consistent with my opinions in this proceeding and does not support Dr. Samulski's arguments or those of the POR.

### **VIII. MOTIVATION TO MODIFY AAVrh.10 BASED ON AAV8**

351. Dr. Samulski also does not correctly characterize other aspects of the invalidity argument I set out in my original Declaration.

352. In my Declaration, I specifically called out the need for improved rAAV vectors in all three of these tissues: "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." EX1005 (Schaffer Dec.), ¶¶ 85 (emphases added), 89-90.

353. I argued that a POSA, based on the express teachings of the '772 Publication, would have focused on AAVrh.10 as a serotype of “intriguing biological significance” based on its excellent tropism *across these three separate tissues* – liver, lung, and muscle. EX1007 ('772 publication), [0257]; EX1005 (Schaffer Dec.), ¶¶ 123, 139-41, 207-211.

354. I further argued that a POSA, given these interesting properties of AAVrh.10, would also have been guided by some additional express teachings of the '772 Publication, namely, that there was a particular need in the art for rAAV vectors with improved liver transduction, and that AAV8 addressed this need: “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.” EX1007 ('772 publication), [250]; EX1005 (Schaffer Dec.), ¶¶ 137-38.

355. Therefore, it is my opinion that a POSA, aware of the superiority of AAVrh.10 across the three important tissues – liver, lung, and muscle – and aware that there had been particular difficulty in the art achieving satisfactory transduction of liver tissue before the discovery of AAV8, would have been motivated to modify AAVrh.10 to achieve even better transduction of liver – specifically the level of liver transduction observed with AAV8. EX1005 (Schaffer Dec.), ¶¶ 207-211. To do so, a POSA would have been motivated, as I have set out in my original Declaration, to

make certain mutations in AAVrh.10 based on the AAV8 sequence, to try to confer some of the superior liver transduction of AAV8 onto AAVrh.10. EX1005 (Schaffer Dec.), ¶¶ 207-211.

356. But to be clear, I am not arguing that a POSA would have been motivated to modify AAVrh.10 *merely to obtain a superior liver transducer*. For this reason, it would not make sense for the POSA to work with AAV8 instead of modifying AAVrh.10.

357. Instead, it is my opinion that a POSA would have been motivated to develop AAVrh.10 into an even better transducer of liver tissue, *while preserving its excellent properties in lung and muscle* – given that its superiority across tissues was touted by the '772 Publication as the reason for its “intriguing biological significance.” EX1005 (Schaffer Dec.), ¶¶ 207-211. The point would be to create a vector that maintained this interesting and unusual superiority across tissues while improving its transduction of one tissue, liver, even further, based on the guidance in the '772 Publication that liver transduction had proved particularly challenging previously in the art. EX1005 (Schaffer Dec.), ¶¶ 137-38, 207-211.

358. And improving liver transduction at the expense of lung and muscle would negate the entire purpose of modifying AAVrh.10 in the first place, and would ignore the teachings of the '772 Publication about the importance of the superiority of AAVrh.10 across multiple tissues. EX1007 ('772 publication), [0257].

359. Nor have I changed my opinion in this regard. As I stated in my original Declaration: “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.” EX1005 (Schaffer Dec.), ¶ 211 (emphasis added). By the phrase “the desirable properties of AAVrh.10,” I was referring to its excellent tropism across all three tissues. Two paragraphs earlier, I quoted the passage from [0257] discussing the importance of the superior performance of AAVrh.10 in lung, liver, and muscle. EX1005, ¶ 209.

360. And, as I discussed above, earlier in my Declaration I discussed the importance to the field of achieving improved transduction of liver, lung, and muscle cells. EX1005 (Schaffer Dec.), ¶ 85. I quoted Grimm (EX1031), describing the concerns in the field that AAV2 vectors were inefficient transducers of liver and muscle cells, and noted this problem. EX1005 (Schaffer Dec.), ¶¶ 89, 90.

361. To the extent I focused on liver transduction in the section of my Declaration addressing the teachings of the '772 Publication regarding the comparison of AAVrh.10 with AAV8, it was because I was explaining the motivation of a POSA to make changes to AAVrh.10 based on AAV8 to improve

liver transduction. EX1005 (Schaffer Dec.), ¶¶ 207-11. I did not argue or suggest at any point that preserving the functions of AAVrh.10 in lung and muscle was unimportant – in fact, I expressly stated the opposite. EX1005 (Schaffer Dec.), ¶ 211.

**A. A POSA Would Not Have Chosen AAV8 Instead of Modifying AAVrh.10**

362. Dr. Samulski argues that a POSA following the teachings of the '772 Publication and interested in “finding or creating a vector that targeted liver,” would have begun the analysis with AAV8. EX2027 (Samulski Dec.), ¶ 40.

363. However, Dr. Samulski appears to miss a key point of my argument (as I discussed above), that a POSA, following the teachings of the '772 Publication, would be interested in developing an rAAV vector with superiority across the three tissues most important to the field at the time – liver, lung, and muscle – and would not be solely focused on the liver.

364. Dr. Samulski argues that “the '772 Publication showed that AAV8 had relatively low expression in muscle, which is desirable for reducing off-target expression if the goal was targeting liver.” EX2027 (Samulski Dec.), ¶ 41. But the fact that AAV8 exhibited low expression in muscle supports my argument that a POSA interested in developing an rAAV vector that had superior tropism across the three important tissues would *not* have started with AAV8.

365. Moreover, Dr. Samulski relies on Table 6 for his discussion of the expression of AAV8 in muscle – but I note that Table 6 does not include any data for AAVrh.10, and therefore cannot reliably be used to compare the tropism of AAV8 with that of AAVrh.10. EX2027 (Samulski Dec.), ¶ 41; EX1007 (’772 Publication), [244].

366. Dr. Samulski further argues that the “relatively low expression in muscle” of AAV8 would be “desirable for reducing off-target expression if the goal was targeting the liver.” EX2027 (Samulski Dec.), ¶ 41. As I discussed above (in VII.A), I do not believe that “off-target expression” would have dissuaded a POSA from trying to improve the tropism of AAVrh.10 in liver. A POSA would have understood that tissue-specific promoters and different routes of administration were available to control expression. Moreover, a POSA would have understood, as I explained above (Section VII.C), that improving liver tropism does not require redistributing the vector across multiple tissues, but rather, improving the efficiency in liver. And given that a POSA would have sought, based on the guidance in the ’772 Publication, to develop a vector that was superior across all three tissues, the POSA would not have chosen to develop AAV8.

367. Dr. Samulski argues that a POSA would have understood that AAV8 was superior to AAVrh.10 in liver. EX2027 (Samulski Dec.), ¶ 42. Specifically, Dr. Samulski argues that “AAV8 was shown to have *over fifty percent (50%)*

*greater expression* in liver than AAVrh.10.” EX2027 (Samulski Dec.), ¶ 42 (emphasis in original).

368. In my opinion, the superiority of AAV8 in liver would have motivated a POSA to try to improve the tropism of AAVrh.10 in liver, based on the guidance in the '772 Publication that the performance of 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 . . . .” EX1007 ('772 Publication), [0257] (emphases added). Whatever the actual percentage difference between AAVrh.10 and AAV8, the '772 Publication describes the tropism rAAVrh.10 as “oustanding,” and as “close” to that of AAV8.

369. Dr. Samulski also argues that a POSA would not have tried to modify a vector to try to improve tropism to a particular tissue, but “would have merely selected the best available vector for the tissue she wanted to target.” EX2027 (Samulski Dec.), ¶ 43. I have discussed above at length (in Section V) that researchers in the field, including Dr. Samulski himself, sought to modify AAV vectors to improve tropism to particular tissues, including through single amino acid modifications, and did not simply “select[] the best available vector” for the tissue to be targeted.

370. As I have discussed above, Dr. Samulski’s own patents and scientific research publications, in addition to the work of others in the field including myself,

directly contradict his arguments that “a skilled artisan would not have had a reasonable expectation that changes could be made to improve or alter tropism.” EX2027 (Samulski Dec.), ¶ 43. In fact, as I discussed above, Snowdy, in his work as Dr. Samulski’s graduate student, disclosed a single amino acid change in AAV2 that improved transduction efficiency. *See* Section V.A, *supra*.

**B. A POSA Would Have Arrived at the S665N Substitution**

**1. A POSA Would Have Used a Rational Design Approach Rather Than Making Large Numbers of Mutations**

371. Dr. Samulski also argues that, even if a POSA selected AAVrh.10 and sought to modify it based on AAV8 to improve liver tropism, the POSA “would have made a large number of potential insertions, deletions, and other modifications to try to test which sites might be important to different properties of the capsid protein.” EX2027 (Samulski Dec.), ¶ 44. Dr. Samulski goes so far as to argue, “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.” EX2027 (Samulski Dec.), ¶ 44.

372. Again, this argument is contradicted by Snowdy, and by Dr. Samulski’s own patents and research publications, and by the work of others in the field, including myself. *See* Section V, *supra*.

373. Dr. Samulski appears to disregard the fact that researchers in the field, including his own student, Snowdy, were using rational design to engineer rAAV vectors with improved tropism. *See* Section V, *supra*. These rational design approaches did not involve creating “large numbers” of random mutations, but rather, small numbers of selected mutations, chosen, for example, by comparison of one AAV serotype with another. *See* Section V, *supra*.

374. Dr. Samulski again disregards the use of rational design methods by researchers in the field, such as those disclosed in the '772 Publication, Snowdy, his own research papers and patents, and work from other groups, including my own, to argue that “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.” EX2027 (Samulski Dec.), ¶ 45. Whether or not the field was still mapping out “functional domains” of AAV vectors, the field was also, as I have discussed at length above, using rational design, including single amino acid substitutions, to alter properties of rAAV vectors, including tropism. *See* Section V, *supra*.

375. Contrary to Dr. Samulski’s arguments, researchers in the field were not restricted to “alanine scanning” (what he also calls “wreck and check”) to explore AAV domains, but used rational design techniques, including single amino acid substitutions from one AAV serotype into another. EX2027 (Samulski Dec.), ¶¶ 45-

46; *see* Section V, *supra*. And again, he disregards rational design in arguing that a POSA “would not have been able to predict which amino acids might impact tropism.” EX2027 (Samulski Dec.), ¶ 46. As his own work demonstrates, studying the differences between two closely related variants of AAV that have different tropism would guide a POSA to be able to make predictions about which residues could affect tropism specifically. *See* Section V, *supra*.

376. Instead, Dr. Samulski continues the argument that in comparing AAVrh.10 and AAV8, and observing that they differed in 48 amino acids, a POSA would ignore the amino acids present in AAV8 but not in AAVrh.10 and instead carry out alanine scanning. EX2027 (Samulski Dec.), ¶ 47.

377. In a statement that elicits some surprise in light of his own work at the time, Dr. Samulski asserts that “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.” EX2027 (Samulski Dec.), ¶ 48.

378. As Dr. Samulski is aware, I have relied extensively on Snowdy for my opinions. Snowdy made a single amino acid change (mutating a serine to an alanine, S181A) that *increased* transduction efficiency. EX1005 (Schaffer Dec.), ¶ 95. A POSA at the time would therefore have understood Snowdy to teach that a particular

type of single amino acid change – the removal of a phosphorylation site – substantially increased AAV transduction of target cells, and therefore that such a change could alter tropism.

379. Dr. Samulski’s statement that he is “unaware of any studies before 2005 where a single amino acid was shown to alter AAV tropism” is even more surprising, given his own patent filing in December 2004, and his paper published in 2006, both of which I discussed at length above (Sections V.C and V.E). EX2027 (Samulski Dec.), ¶ 48; EX1059 (’126 provisional); EX1060 (Wu 2006).

380. I also disagree with Dr. Samulski’s discussion of Wu, which I cite in my Declaration. EX2027 (Samulski Dec.), ¶ 49; EX1032 (Wu); EX1005 (Schaffer Dec.), ¶¶ 93, 211. Specifically, I disagree that Wu “stands for the opposite proposition” that a POSA at the relevant time “would not have expected a single amino acid change to alter tropism in any meaningful way.” EX2027 (Samulski Dec.), ¶¶ 48-49.

381. Nothing in Wu contradicts my argument that the ’772 Publication in combination with Snowdy would have motivated a POSA to create single amino acid mutations in AAVrh.10 based on the sequence of AAV8 to try to improve tropism to the liver.

382. Dr. Samulski does not correctly describe the reasons that I cited Wu in my Declaration, suggesting that I argued in my Declaration that Wu made single

amino acid substitutions that altered tropism. I did not. Instead, I cited Wu to support my opinion that people of ordinary skill in the art were commonly making single amino acid mutations in AAV capsids to test the effects of those mutations. *See, e.g.*, EX1005 (Schaffer Dec.), ¶¶ 93, 211. Dr. Samulski does not appear to dispute this point in his declaration, but characterizes the single amino acid mutations that Wu described as “single alanine wreck and check substitutions (mutant numbers 29, 31, 45, and 48).” EX2027 (Samulski Dec.), ¶ 49.

**2. The Fact That Asparagine and Serine Both Have Uncharged Polar Side Chains Would Not Have Dissuaded a POSA From Making the Substitution**

383. Dr. Samulski argued in his declaration that he would expect the substitution of asparagine in place of a serine in the AAV capsid “to have little to no effect on tropism, or of any other function, because it is a conservative substitution.” EX2027 (Samulski Dec.), ¶ 50. Dr. Samulski defined a “conservative substitution” as one that “does not alter the electrical charge or polarity of the side chain of the amino acid.” EX2027 (Samulski Dec.), ¶ 50.

384. Yet at his deposition, Dr. Samulski walked back this opinion, admitting that serine and asparagine are “different” because serine is capable of being phosphorylated while asparagine is not, and therefore that there would be a motivation to substitute asparagine in to replace serine. EX1063 (Samulski Dep. Tr.), 277:6-9, 11-15.

385. A POSA would have understood this important difference between serine (in AAVrh.10) and asparagine (at the corresponding position in AAV8). EX1005 (Schaffer Dec.), ¶¶ 272-73. A POSA would further have understood that there were only four positions at which AAV8 had a non-phosphorylatable amino acid, while AAVrh.10 had a phosphorylatable amino acid. EX1005 (Schaffer Dec.) ¶¶ 277-86. A POSA would have been motivated to create four variants of AAVrh.10, each having a single amino acid substitution at one of these four positions, substituting in the non-phosphorylatable amino acid from AAV8 for the phosphorylatable amino acid at that position in AAVrh.10. EX1005 (Schaffer Dec.) ¶¶ 277-81. And a POSA would have had a reasonable expectation of success in creating these single amino acid variants. EX1005 (Schaffer Dec.) ¶¶ 282-86.

386. Moreover, the fact that both asparagine and serine have uncharged, polar side chains supports my arguments and contradicts Dr. Samulski's. As Dr. Samulski notes, I stated expressly in my Declaration that "[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." *See, e.g.*, EX1005 (Schaffer Dec.), ¶ 182; EX1040 (Alberts I), p. 5; EX2027 (Samulski Dec.), ¶ 51.

387. The fact that an asparagine-serine substitution is more conservative than the alanine-serine substitution that Snowdy made would increase the POSA's reasonable chance of success in making the claimed combination. As I stated in my Declaration, Snowdy found that the six mutants he constructed (S148A, S148D, T159A, T159D, S181A, and S181D) were all capable of forming virus at normal titers. EX1009 (Snowdy), pp. 125-26. The fact that an S to N substitution is more conservative than an S to A substitution further supports my argument that a POSA would have had a reasonable expectation of success that the four AAVrh.10 capsid variants would similarly be able to form virus at normal titers. EX1005 (Schaffer Dec.), ¶ 285.

**C. A POSA Would Not Have Used Methods of Modifying Tropism Other Than Single Amino Acid Substitutions**

388. Dr. Samulski argues that a POSA would have used approaches other than single amino acid substitutions. EX2027 (Samulski Dec.), ¶ 52. Dr. Samulski points to serpin insertion mutants disclosed in Wu, arguing that they would have encouraged a POSA to try similar insertions, rather than single amino acid substitutions. EX2027 (Samulski Dec.), ¶ 52.

389. Dr. Samulski argues incorrectly that this approach would be “entirely consistent with the disclosures in the '772 Publication,” because “the '772 Publication is silent as to using single amino acid substitutions to alter tissue

tropism.” EX2027 (Samulski Dec.), ¶ 53. But, as I discussed above (Section IV.A), and as Dr. Samulski acknowledged at his deposition, the ’772 Publication discloses a single amino acid substitution in AAV that could alter properties of the virus including tropism. EX1007 (’772 Publication), [0072], [0091]; EX1005 (Schaffer Dec.), ¶ 229; EX1063 (Samulski Dep. Tr.), 50:9-16, 60:23-61:6, 61:14-19, 61:21-62:11, 68:20-23, 68:25-69:1.

390. Therefore, the ’772 Publication is entirely consistent with my argument that a POSA would have been motivated to use single amino acid substitutions, following the guidance of the ’772 Publication and Snowdy. EX1005 (Schaffer Dec.), ¶¶ 211, 229.

**D. A POSA Would Have Been Motivated to Maintain the Superior Tropism of AAVrh.10 Across All Three Tissues**

391. Dr. Samulski argues that a POSA would not have been motivated to maintain the superior tropism of AAVrh.10 across all three tissues, while improving tropism to liver, for several different reasons. EX2027 (Samulski Dec.), ¶¶ 54-59.

392. First, he argues that a POSA would have sought to minimize the tropism of AAVrh.10 to “off-target” tissues, muscle and lung, “that would not benefit from receiving the gene of interest.” EX2027 (Samulski Dec.), ¶ 54. As I discussed above (Section VII), a POSA would not have been dissuaded by “off-target expression” of AAVrh.10.

393. Dr. Samulski also argues that a POSA “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.” EX2027 (Samulski Dec.), ¶ 55. As I discussed above (Section VII.C), improving the tropism of AAVrh.10 to liver would not necessarily involve reducing or in any way affecting its tropism to other tissues.

394. Moreover, as I also discussed above, Dr. Samulski disregards the guidance of the '772 Publication that AAVrh.10 had “intriguing biological significance” precisely because it exhibited excellent tropism across all three tissues. As I explained at my deposition, a POSA at the time would have understood that, in some cases, it would be desirable to have an AAV gene therapy vector with broad tropism, if multiple tissues were affected by the condition to be treated. EX1064 (Schaffer Dep. Tr.) 50:13-15.

395. Dr. Samulski also expresses skepticism that “a gene of interest exists that would be desirable to target to liver, muscle, and lung at the same time.” EX2027 (Samulski Dec.), ¶ 55. As a preliminary matter, Dr. Samulski misunderstands my opinions. A POSA’s decision to work with AAVrh.10 would not be limited to a situation in which the POSA would seek to target all three tissues in which AAVrh.10 displays superior tropism (lung, liver, and muscle) with a single gene at the same time.

396. As I explained in my deposition, the usefulness of such an AAVrh.10-based vector would be that it could be the basis for multiple different gene therapies, delivering different genes of interest to different tissues – not merely, or even primarily, useful for delivering a single gene of interest to all three tissues. EX1064 (Schaffer Dep. Tr.), 59:8-13; 131:15-132:3; 134:18-21.

397. A POSA would understand that developing a single vector with tropism across multiple tissues for multiple gene therapies, such as AAVrh.10, rather than multiple vectors with different tropisms for different diseases, could also have other potential benefits. These include ease and consistency of manufacturing, an established safety profile, and expedited review for regulatory approval.

398. Moreover, as I explained at my deposition, a POSA at the relevant time would have been well aware that the dystrophin gene is an example of a gene expressed in many different tissues throughout the body, including brain, cerebellum, retina, muscle, and liver. EX1064 (Schaffer Dep. Tr.) 343:17-18, 359:16-360:1.

399. Dr. Samulski also argues that I am actually “identifying two separate motivations, or goals” – namely, “improving tropism to liver versus maintaining tropism in muscle and lung.” EX2027 (Samulski Dec.), ¶ 56. He argues that these two separate goals “would be addressed by a skilled artisan in completely different

ways.” EX2027 (Samulski Dec.), ¶ 56. I am not sure that I fully understand his argument here, but I will do my best to respond, nonetheless.

400. First, Dr. Samulski refers back to his prior discussion about improving tropism to liver, which I have already addressed (Section VII, *supra*). EX2027 (Samulski Dec.), ¶ 56.

401. Then, he argues that a POSA would have had two options “to *maintain* existing tropism in AAVrh.10, such as to muscle and/or lung.” EX2027 (Samulski Dec.), ¶ 57 (emphasis in original). As one option, he says that “a skilled artisan could simply have left the vector as is and unmodified, therefore ensuring tropism was maintained.” EX2027 (Samulski Dec.), ¶ 57.

402. I do not believe that this approach is consistent with the guidance of the ’772 Publication, or with the way that a POSA at the time would have approached the need in the field for improved gene therapy vectors in tissues such as muscle, lung, and liver. *See, e.g.*, EX1005 (Schaffer Dec.), ¶¶ 85, 89-90, 123, 208.

403. He then argues that, as a second option, “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.” EX2027 (Samulski Dec.), ¶ 57.

404. I do not understand Dr. Samulski’s argument that a POSA’s options would be limited to making no mutations at all in AAVrh.10 or to considering “an

exceedingly large number of potential changes,” and would not include the approach I have set out, namely, a limited set of substitutions following a rational design approach. As I discussed above (Sections V.C, V.D, V.E), the approach I have set out was precisely the approach that Dr. Samulski followed in his own patent application, patent, and publication at the relevant time.

405. Dr. Samulski, as part of this argument, does not correctly characterize part of my Declaration. EX2027 (Samulski Dec.), ¶ 58. Dr. Samulski refers to “the framework that Dr. Schaffer sets forth in paragraphs 214-226 of his declaration . . . .” EX2027 (Samulski Dec.), ¶ 58. Dr. Samulski, however, fails to mention that the “framework” I discuss in that section of my declaration is not my own framework, but rather is a framework set out by *Patent Owners’ expert*, Dr. Paola Leone, in an earlier litigation against Sarepta. EX1005 (Schaffer Dec.), ¶¶ 214-226.

406. Dr. Leone argued that a POSA would have understood the ’772 Publication to teach the creation of a large number of artificial sequences from the sequences disclosed in Figure 2. EX1005 (Schaffer Dec.), ¶¶ 214-226; EX1012 (Leone Rebuttal Report), ¶¶ 313, 316-19. Dr. Leone set out a framework that she argued would guide a POSA in constructing these artificial sequences. EX1005 (Schaffer Dec.), ¶¶ 214-226; EX1012 (Leone Rebuttal Report), ¶¶ 313, 316-19.

407. My reason for discussing Dr. Leone’s opinion in this regard was to explain that, according to the framework Dr. Leone set out, one of the artificial

sequences a POSA would have constructed from Figure 2 would have been AAVrh.10 with an asparagine substituted in at position 665 (AAVrh.10 with an S665N substitution). EX1005 (Schaffer Dec.), ¶¶ 223-26.

408. Dr. Samulski again disregards my argument that a POSA would have been guided by the teachings of the '772 Publication, in combination with Snowdy, to adopt a rational design approach to making single amino acid substitutions at a limited number of positions in AAVrh.10, substituting in the corresponding amino acid from AAV8. Dr. Samulski argues that a POSA would have contemplated hundreds, thousands, or even millions of substitutions in AAVrh.10. EX2027 (Samulski Dec.), ¶¶ 58-59.

409. For unclear reasons, he argues that “a skilled artisan would have been extremely unlikely to pluck a single serine to asparagine substitution at position 665 out of thin air.” EX2027 (Samulski Dec.), ¶ 59. I have never argued that a POSA would make only the S665N substitution, let alone that such a substitution would have been “plucked out of thin air.” Instead, I have argued that a POSA would have been motivated to combine the teachings of the '772 Publication with Snowdy to make four specific single amino acid substitutions in AAVrh.10, one of which would be the S665N substitution. EX1005 (Schaffer Dec.), ¶¶ 271-85. I set out in detail in my Declaration the sequence of steps a POSA would have followed that would

have led to the selection and creation of these four variants. EX1005 (Schaffer Dec.), ¶¶ 271-85.

**E. I Did Not Express a “New” Opinion at My Deposition Regarding Preserving Tropism of AAVrh.10 in Muscle**

410. Dr. Samulski states incorrectly that at my deposition, I expressed opinions not found in my expert declaration regarding preserving tropism of AAVrh.10 in muscle. EX2027 (Samulski Dec.), ¶ 54. Specifically, Dr. Samulski says I expressed the new opinion at my deposition 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.” EX2027 (Samulski Dec.), ¶ 54. This opinion was not a “new” opinion – I stated this opinion expressly in my Declaration.

411. As I discussed above, I explained in my Declaration that there was a need in the art to improve transduction by AAV vectors of tissues including muscle: “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.” EX1005 (Schaffer Dec.), ¶¶ 85 (emphasis added), 89-90.

412. I discussed the experiments in the '772 Publication, including the transduction experiments in Example 9 in three different tissues, lung, liver, and muscle, showing that AAVrh.10 was “found to be a very potent gene transfer vehicle in all three tissues.” EX1005 (Schaffer Dec.), ¶¶ 139-41. I discussed Table 8 from the '772 Publication, which showed that AAVrh.10 performed “well in muscle, producing about the same amount of reporter expression as the other best construct in muscle, AAV2/1.” EX1005 (Schaffer Dec.), ¶¶ 140-41; EX1007 ('772 Publication), Table 8, p. 133. I have reproduced Table 8 below:

| 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 ('772 Publication), Table 8, p. 133.

413. In my Declaration, I also repeatedly quoted the key discussion in the '772 Publication comparing AAVrh.10 to AAV8 and AAV1, and describing AAVrh.10 as an excellent muscle transducer: “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.” EX1007 (’772 Publication), [0257]; EX1005 (Schaffer Dec.), ¶¶ 123, 208.

414. As I discussed above, and at length in my Declaration, I believe this guidance from the ’772 Publication would have motivated a POSA at the relevant time to have selected AAVrh.10 as a particularly “intriguing” serotype, given its excellent transduction across all three tissues, lung, liver, and muscle. EX1005 (Schaffer Dec.), ¶¶ 207-208. I stated that the ’772 Publication taught “the superiority of AAVrh.10 overall,” by which I meant its excellent transduction across all three tissues, lung, liver, and muscle. EX1005 (Schaffer Dec.), ¶ 210.

415. I also set out my opinion that a POSA, given the additional guidance in the ’772 Publication regarding a particular need in the field for AAV vectors with improved transduction of liver tissue, would have sought to modify AAVrh.10 to improve its liver transduction. EX1005 (Schaffer Dec.), ¶¶ 209-210.

416. Importantly, I also stated that “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.*” EX1005 (Schaffer Dec.), ¶ 211 (emphasis added).

417. By the “desirable properties of AAVrh.10,” I meant *all* of the desirable properties of this serotype, including its excellent tropism in muscle and lung. As I have discussed, it is my opinion that a POSA, guided by the teachings of the ’772

Publication, would have chosen to work with AAVrh.10 because it showed “intriguing” and excellent transduction across *three* key tissues for gene therapy applications, lung, liver, and muscle.

418. As I discussed above (Sections V.C and V.D), both the ’126 provisional and the ’809 patent include statements that are, in essence, the same as the discussions in my Declaration regarding a POSA’s motivation to preserve the desirable properties of the parent serotype, while making limited substitutions to confer additional advantageous properties. *See, e.g.*, EX1059 (’126 provisional), 5:2-6; EX1061 (’809 patent), col. 2:8-15.

419. I also reiterated these points regarding the importance of delivery to muscle throughout my deposition. *See, e.g.*, EX1064 (Schaffer Dep. Tr.), 128:6-21, 131:15-132:3, 132:7-13, 134:1-7, 134:11-15, 134:18-21, 136:9-15, 136:20-137:1, 138:1-10, 139:12-15, 141:3-11, 141:14-15, 142:10-15, 143:6-11, 215:19-216:12, 241:2-9, 242:1-12, 259:14-18.

**F. I Discussed Muscle Tropism in the Section of My Declaration Addressing Ground 1 of the Petition, and I Did Not “Admit” Otherwise at My Deposition**

420. The POR also incorrectly characterizes my Declaration and my deposition testimony relating to delivery to muscle. The POR states that “Dr. Schaffer admitted at deposition that his declaration does not discuss targeting to muscle in Ground 1.” POR, p. 31 (citing EX1064 (Schaffer Dep. Tr.), 349:9-359:7).

421. My Declaration discusses targeting to muscle in the section addressing Ground 1 of the Petition, and I did not “admit” otherwise at my deposition.

422. For example, I state in ¶ 208 of my Declaration (which is part of Ground 1): “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.” EX1005 (Schaffer Dec.), ¶ 208; EX1007 (’772 Publication), [0257].

423. My reference here to my discussion “above,” referred back to an earlier section of my Declaration, in which I discussed the ’772 Publication in detail, including this particular quote, and also including Example 9, which disclosed experiments demonstrating the excellent tropism of AAVrh.10 in muscle. EX1005 (Schaffer Dec.), ¶¶ 121-23, 139-41.

424. Also in the portion of my Declaration addressing Ground 1, I stated that the ’772 Publication “teaches the superiority of AAVrh.10 *overall* . . . .” EX1005 (Schaffer Dec.), ¶ 210 (emphasis added). By the word “overall,” I meant in all three tissues, muscle, lung, and liver.

425. I further stated that a POSA seeking to improve the tropism of AAVrh.10 in liver “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.” EX1005 (Schaffer Dec.), ¶ 211 (emphasis added).

426. By the phrase “the desirable properties of AAVrh.10,” I was referring to its excellent tropism across all three tissues.

427. As I also explained at my deposition, whether or not the word “muscle” appears in every paragraph of my Declaration, my references to, for example, overall superiority of AAVrh.10 and its desirable properties inherently address muscle tropism also. *See, e.g.*, EX1064 (Schaffer Dep. Tr.), 352:4-8, 11-13; 352:21-353:1; 353:10-14; 355:1-4, 8-11; 356:2-9, 13-16.

## **IX. DR. SAMULSKI INCORRECTLY DESCRIBES MY ARGUMENTS ABOUT CLAIM 8**

428. In discussing Claim 8 of the '274 patent, Dr. Samulski repeats the incorrect argument that I did not discuss delivery to muscle specifically in my Declaration. EX2027 (Samulski Dec.), ¶ 61.

### **A. I Discussed Targeted Delivery to Muscle in My Declaration in the Sections Addressing Grounds 4-6**

429. I stated expressly in my discussion of Ground 4 (relating to claim 8) in my Declaration that Fabb teaches a “micro-dystrophin” that, when delivered as part of an AAV gene therapy vector restored dystrophin associated protein complexes

and inhibited degenerative dystrophic muscle pathology in an animal model.<sup>13</sup>  
EX1005 (Schaffer Dec.), ¶ 294.

430. Here, I cited portions of Fabb, including the Abstract, which states that the micro-dystrophin construct was “introduced into the skeletal muscle” of mice. EX1005 (Schaffer Dec.), ¶ 294 (citing EX1010 (Fabb), Abstract).

431. In the section addressing Ground 4, I also referred to the micro-dystrophin disclosed in Fabb as a “working example” of a micro-dystrophin that could fit in an AAV capsid. EX1005 (Schaffer Dec.), ¶ 296. By “working example,” I meant a micro-dystrophin gene that was packaged in an AAV capsid and delivered to muscle to restore function to muscle tissues.

432. In my discussion of Grounds 5 and 6, I referred back to Ground 4. EX1005 (Schaffer Dec.), ¶¶ 302, 308.

433. And, as I have discussed above (Section IX.A), I addressed targeted delivery to muscle by a modified AAVrh.10 vector throughout my declaration. As

---

<sup>13</sup> I note that in setting out my argument in my Declaration that the combination of the ’772 Publication, Snowdy, and Fabb renders claim 8 of the ’274 patent obvious, I referred back to my arguments regarding the obviousness of claims 1, and 3-6 over the ’772 Publication and Snowdy, in addition to my earlier discussion of Fabb. EX1005 (Schaffer Dec.), ¶¶ 271-89, 294, 305, 308.

I also discussed above, I reiterated these points regarding the importance of delivery to muscle throughout my deposition. *See, e.g.*, EX1064 (Schaffer Dep. Tr.), 128:6-21, 131:15-132:3, 132:7-13, 134:1-7, 134:11-15, 134:18-21, 136:9-15, 136:20-137:1, 138:1-10, 139:12-15, 141:3-11, 141:14-15, 142:10-15, 143:6-11, 215:19-216:12, 241:2-9, 242:1-12, 259:14-18.

**B. Dystrophin Is Expressed Throughout the Body**

434. At my deposition, I explained that dystrophin is expressed throughout the body, and that I was not aware whether anyone had done a quantitative study to determine how much of the protein is expressed in muscle, as compared with all the other tissues. EX1064 (Schaffer Dep. Tr.), 359:16-360:1, 360:7-10.

435. Dr. Samulski, while disagreeing with my statements here, does not cite any reference that actually supports his statement that “[d]ystrophin is a protein well-known to be expressed primarily in muscle, as even the Fabb reference that Dr. Schaffer relies on explains.” EX2027 (Samulski Dec.), ¶ 61. Dr. Samulski does not cite any part of Fabb, for example, that states that dystrophin is expressed “primarily” in muscle. EX2027 (Samulski Dec.), ¶ 61. Dr. Samulski also refers to a 2016 reference by Gao and McNally, “The Dystrophin Complex: structure, function and implications for therapy,” but again does not cite any part that states that dystrophin is expressed primarily in muscle. EX2027 (Samulski Dec.), ¶ 61; EX2041 (Gao and McNally).

436. Here, I note that Dr. Samulski's arguments are not consistent. On the one hand, Dr. Samulski contends that I nowhere address targeting AAVrh.10 to muscle. Yet, at the same time, Dr. Samulski contends that I rely on a reference that teaches that dystrophin is expressed in muscle to show that a POSA would insert a dystrophin micro-gene into an AAVrh.10 construct.

437. Moreover, Dr. Samulski testified at his deposition that dystrophin is expressed in a variety of tissues, including skeletal and cardiac muscle, in addition to smooth muscle, brain, and retina. EX1063 (Samulski Dep. Tr.), 253:8-254:4, 7-20.

438. The POR improperly accuses me of having "feigned ignorance" at my deposition of "where in the body dystrophin should be targeted." POR, p. 31 n. 3. The POR here completely mischaracterizes my deposition testimony.

439. At my deposition, I was asked whether I would agree that dystrophin is a protein found in muscle, that "you'd want to target dystrophin to muscle," and that "the Fabb paper says this throughout." EX1064 (Schaffer Dep. Tr.), 343:12-15. I responded that "Dystrophin is expressed in many tissues including muscle." EX1064 (Schaffer Dep. Tr.), 343:17-18.

440. My point here was that because dystrophin is expressed in many tissues including muscle, I was not prepared to say that dystrophin would only ever be targeted to muscle – and I stand by this testimony.

**C. A POSA Would Not Have Started with AAV7**

441. Dr. Samulski then argues again that a POSA would take an “entirely different path” from the one I have set out “to target delivery of a gene therapy to muscle in 2005.” EX2027 (Samulski Dec.), ¶ 62. Just as he argued in relation to liver tropism that a POSA would have started with AAV8 instead of AAVrh.10, he argues that in relation to muscle tropism, a POSA would have started with AAV7 instead of AAVrh.10. EX2027 (Samulski Dec.), ¶ 65. And just as before, Dr. Samulski disregards the guidance of the ’772 Publication about the “intriguing biological significance” of AAVrh.10. *See, e.g.*, EX1007 (’772 Publication), [0257].

442. Dr. Samulski argues that the data in Table 6 of the ’772 Publication “show that the performance of AAV7 in muscle was approximately three times better than the previously known best muscle transducer, AAV1.” EX2027 (Samulski Dec.), ¶ 63.

443. As a preliminary matter, given the reported standard errors in the measurements disclosed in Table 6 (AAV2/1  $4.5 \pm 2.1$ , and AAV2/7  $14.2 \pm 2.4$ ), the data could be interpreted to show that AAV7 was about 1.8 times as good as AAV1

in this assay (11.8 compared to 6.6). EX1007 ('772 Publication), [0244], Table 6. I have reproduced Table 7 below:

TABLE 6

---

| Expression of $\beta$ -unit of Rhesus Monkey<br>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 ('772 Publication), [0244], Table 6.

444. In addition, as Dr. Samulski notes, AAVrh.10 was not tested in this set of experiments. EX2027 (Samulski Dec.), ¶ 63; EX1063 (Samulski Dep. Tr.), 70:24-71:4. In fact, the '772 Publication does not disclose any set of experiments in which the muscle tropism of AAVrh.10 is directly compared to that of AAV7. Therefore, no conclusion can be drawn about the relative muscle tropism of the two serotypes. *See also* EX1063 (Samulski Dep. Tr.), 73:24-74:2 (“If I had a concern, I would have everything in the same experiment”).

445. Table 8 of the '772 Publication, however, does disclose experiments that directly compare the muscle tropism of AAV1 to that of AAVrh.10:

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 ('772 Publication), [0253], Table 8.

446. And in these experiments, the muscle tropism of AAV1 and AAVrh.10 was comparable (AAV2/1 45 ± 11, and AAV2/rh.10 46 ± 11). Moreover, the '772 Publication teaches that “performance of [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 . . .*” EX1007 ('772 Publication), [0257] (emphasis added). The '772 Publication therefore teaches that an appropriate comparison can be drawn between the muscle tropism of AAVrh.10 and that of AAV1, and that the two are comparable. In contrast, the '772 Publication does not discuss a comparison between the muscle tropism of AAVrh.10 and AAV7.

447. Dr. Samulski quotes a portion of the '772 Publication to try to support his argument. He states, “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.’” EX2027 (Samulski Dec.), ¶ 65.

448. The entire paragraph, however, from which Dr. Samulski has excerpted this quote, is as follows:

[0142] It will be readily apparent to one of skill in the art one embodiment, that certain serotypes of the invention will be particularly well suited for certain uses. For example, 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. ***Uses of such vectors are not so limited and one of skill in the art may utilize these vectors for delivery to other cell types, tissues or organs.*** Further, vectors based upon other capsids of the invention may be used for delivery to these or other cells, tissues or organs.

EX1007 (’772 Publication), [0142] (emphasis added).

449. This paragraph supports my argument that a POSA would have been guided by the teachings of the ’772 Publication to select AAVrh.10 as the basis for a vector because it showed excellent tropism across three important tissues. This paragraph expressly teaches that a POSA could use a vector based on AAVrh.10 for delivery to tissues in addition to lung. A POSA would have understood that considerations other than the capability of a particular serotype to transduce a particular tissue – such as usefulness for targeting multiple tissues, safety, and ease

of purification – could determine which serotype to choose as the basis for a vector to target a given tissue.

450. I note that Dr. Samulski testified at his deposition that he himself, in his '126 provisional, chose to work with AAV2 as a vector to target muscle, despite the fact that AAV1 had superior muscle tropism as compared to AAV2. EX1063 (Samulski Dep. Tr.), 106:23-24, 107:1-20, 23. He testified that his lab chose to work with AAV2 for reasons in addition to tropism – including the regulatory track record of AAV2 at the FDA and route of administration considerations. EX1063 (Samulski Dep. Tr.), 106:23-24, 107:1-20, 23.

451. Dr. Samulski argues that if a POSA chose to start with AAVrh.10, the POSA would have chosen to “modify AAVrh.10 with AAV7,” instead of AAV8. EX2027 (Samulski Dec.), ¶ 65. This argument appears to be a corollary of his argument that a POSA interested in targeting muscle would choose the best natural serotype to target muscle as the basis for an AAV vector.

452. This approach, however, is also not supported by the teachings of the '772 Publication, which does not include any data allowing a direct comparison of the muscle tropism of AAVrh.10 and AAV7. A POSA would have understood that it would not have been appropriate to try to draw comparisons across different sets of data between AAVrh.10 and AAV7.

453. Moreover, a POSA would have been guided by the express teachings of the '772 Publication, which appropriately compared the muscle tropism of AAVrh.10 with AAV8, based on experiments that directly compared the two. EX1007 ('772 Publication), [0253], Table 8, [0257].

454. Dr. Samulski testified that [0257] of the '772 Publication refers to the results of Table 8 in comparing AAVrh.10, AAV1, and AAV8. EX1063 (Samulski Dep. Tr.), 77:9-14, 16. Dr. Samulski further testified AAV7 is not mentioned in [0257] or tested in Table 8. EX1063 (Samulski Dep. Tr.), 77:17-22. Therefore, he testified, for a POSA to try to draw a comparison directly between AAVrh.10 and AAV7, that person would have to compare between experiments in the '772 Publication. EX1063 (Samulski Dep. Tr.), 77:23-78:1, 3-5.

455. Dr. Samulski also comments that Fabb may have used AAV2, and that Fabb discloses that vectors based on AAV1, AAV3, and AAV5 “can increase levels of protein production by 100-1000-fold.” EX1010 (Fabb), p. 6; EX2027 (Samulski Dec.), ¶ 69. Dr. Samulski seems to suggest here that these disclosures in Fabb would motivate a POSA to use one of these serotypes, rather than AAVrh.10.

456. Yet these disclosures in Fabb support my opinion because the '772 Publication touted the excellent tropism of AAVrh.10 in muscle, which was “close to that of . . . the best muscle transducer AAV1.” EX1007 ('772 Publication), [0257]; EX1005 (Schaffer Dec.), ¶¶ 123, 208. Given the teaching of Fabb that

AAV1 could be particularly efficacious in transducing muscle to deliver a micro-dystrophin construct, and given the teachings of the '772 Publication that the muscle tropism of AAVrh.10 was close to that of AAV1, a POSA interested in using AAVrh.10 as a vector to deliver a dystrophin gene in muscle tissue would have been motivated to create the micro-dystrophin construct disclosed in Fabb for use in an AAVrh.10 vector. EX1005 (Schaffer Dec.), ¶ 300.

457. The POR also argues that Fabb does not “suggest implementing individual point mutations to improve tropism in any tissue.” POR, p 10. This argument is beside the point. In my opinion, as I set out in my Declaration, the combination of the '772 Publication and Snowdy would have motivated a POSA to make certain substitutions in the AAVrh.10 capsid to improve tropism in liver, while preserving tropism in muscle and lung. EX1005 (Schaffer Dec.), ¶¶ 271-80. Fabb, in combination with the '772 Publication and Snowdy, would have motivated a POSA at the time to construct a micro-dystrophin for use with an AAVrh.10-based vector for targeting to muscle. EX1005 (Schaffer Dec.), ¶ 300.

#### **X. DR. SAMULSKI DOES NOT CORRECTLY CHARACTERIZE JANS & JANS**

458. Dr. Samulski does not correctly characterize either Jans & Jans or the reasons that I discussed Jans & Jans in my Declaration.

459. Jans & Jans discloses essentially two sets of experiments to test the

effect of altering the phosphorylation state of residues near the nuclear localization sequence (NLS) of SV40 large T-antigen on the ability of the protein to localize to the nucleus. EX1017 (Jans & Jans), Abstract.

460. The first series of experiments involved mutating the putative binding site of a particular kinase, casein kinase II (CKII), near the NLS, to observe the effect on nuclear localization. EX1017 (Jans & Jans), Abstract. In my Declaration, I discussed this set of experiments in relation to my opinion that pp. 28-29 of Snowdy contained a typographical error, reading “asparagine” where it should have read “aspartic acid.” See Footnote 12, *supra*; EX1005 (Schaffer Dec.), ¶¶ 179-81.

461. The second set of experiments in Jans & Jans involved investigating the effect of phosphorylation of neighboring serines by CKII on nuclear localization. EX1017 (Jans & Jans), Abstract. I discussed this set of experiments in my Declaration as part of my discussion of Snowdy’s teachings that his results for the S181A and S181D mutations were the opposite of what he would have expected, based on Jans & Jans. EX1005 (Schaffer Dec.), ¶¶ 188-89; EX1007 (Snowdy), pp. 128-29.

462. As I discuss below, Dr. Samulski does not correctly characterize my opinions and the teachings of Jans & Jans and those of Snowdy.

## A. CKII Binding

463. Jans & Jans investigated a region near the SV40 large T-antigen NLS, an N-terminal flanking sequence (amino acids 111-125) that includes a CKII recognition site (amino acids 111-115, SSDDE), where CKII would be expected to bind to the charged residues (DDE), and phosphorylate the neighboring serines (SS). EX1017 (Jans & Jans), p. 3, Table 1.

464. Jans & Jans tested three novel mutations in the CKII site, as shown in the table below (I have highlighted the wild type sequence in yellow and the three novel mutations in blue and green):

**Table 1** Nuclear import parameters and SV40 T-antigen sequence of casein kinase II site mutated derivatives of the T-ag/ $\beta$ -galactosidase fusion proteins in this study

| <i>Fusion protein</i>                        | CKII-site            | SV40 T-ag sequence*<br>NLS                    | Nuclear import<br>rate $\uparrow$<br><i>k</i> ( $10^{-3}/\text{min}$ ) |
|----------------------------------------------|----------------------|-----------------------------------------------|------------------------------------------------------------------------|
| T-ag(WT126-135)                              |                      | <sup>126</sup> PKKKRKVEDP <sup>135</sup>      | 1                                                                      |
| T-ag(CKII Del) (WT120-135)                   |                      | <sup>120</sup> SQHSTPPKKRKVEDP <sup>135</sup> | 2                                                                      |
| T-ag(WT111-135)                              | <sup>111</sup> SSDDE | ATADSQHSTPPKKRKVEDP <sup>135</sup>            | 88                                                                     |
| T-ag(Gly <sup>111</sup> )                    | gSDDE                | ATADSQHSTPPKKRKVEDP <sup>135</sup>            | 100                                                                    |
| T-ag(Asn-Asn-Gln <sup>113-115</sup> )        | SSnnq                | ATADSQHSTPPKKRKVEDP <sup>135</sup>            | 2                                                                      |
| T-ag(Ala-Gly <sup>111/112</sup> )            | agDDE                | ATADSQHSTPPKKRKVEDP <sup>135</sup>            | 3                                                                      |
| T-ag(Gly <sup>111</sup> Asp <sup>112</sup> ) | gaDDE                | ATADSQHSTPPKKRKVEDP <sup>135</sup>            | 40                                                                     |

EX1017 (Jans & Jans), p. 3, Table 1 (highlights added).

465. As I discuss below, these three mutants were designed to test whether CKII binds to the DDE sequence and then phosphorylates neighboring serines (111 and 112), and, if so, the relevance of phosphorylation of these serines by CKII to nuclear localization of large T-antigen. EX1017 (Jans & Jans), pp. 3-4, Table 1.

466. The first mutant, highlighted in blue, was designed to abrogate binding of CKII to the recognition site by replacing the negatively charged, acidic residues (DDE) with uncharged asparagine and glutamine, to confirm that CKII is the kinase that carries out the phosphorylation. *See, e.g.*, EX1040 (Alberts I), p. 5.

467. A POSA would have understood that replacement of a sequence of three charged residues with a sequence of three uncharged residues would be expected to abrogate the function that the charged residues carried out. *See, e.g.*, EX1032 (Wu), pp. 2, 5, Table 1 (replacing charged residues, such as aspartic acid, glutamic acid, arginine, and lysine, throughout the AAV capsid with uncharged alanine to determine the effect on various functions of the AAV capsid).

468. The second and third mutants, highlighted in green, were designed to test whether phosphorylation of serines 111 and 112 is required for nuclear localization. EX1017 (Jans & Jans), pp. 3-4, Table 1.

469. Jans & Jans hypothesized that if mutating the CKII binding site affected nuclear import of large T-antigen, that result would support the hypothesis that CKII is the kinase responsible for phosphorylating the neighboring serines (111 and 112), and that such phosphorylation is important for nuclear localization. EX1017 (Jans & Jans), p. 3 (“The result for the Asn-Asn-Gln<sup>113-115</sup> fusion protein strongly implies that CKII is indeed the kinase responsible for phosphorylation at the site in vivo

since mutation of the recognition sequence alone, leaving Ser<sup>111/112</sup> intact, eliminates nuclear import enhancement”).

470. As Table 1 shows, they found that when they replaced both aspartic acid residues with asparagine, and the glutamic acid residue with glutamine, in the CKII sequence (the mutant highlighted in blue in the figure above), nuclear import of the protein was abrogated. EX1017 (Jans & Jans), p. 3, Table 1.<sup>14</sup>

---

<sup>14</sup> A POSA would have understood that aspartic acid and glutamic acid have an acidic group as a side chain, and are negatively charged. *See, e.g.*, EX1040 (Alberts I), p. 5. It is for this reason, in fact, that aspartic acid can simulate a constitutively phosphorylated state – phosphate groups are negatively charged. *See, e.g.*, EX1048 (Mandal), p. 4, Figure 1; EX1017 (Jans & Jans), pp. 5-6 (“Asp 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”). A POSA at the time would have understood that Jans & Jans chose to substitute in asparagine for aspartic acid (and glutamine for glutamic acid) to render the CKII binding site nonfunctional by removing the negative charges at the site, which are required for recognition by CKII. *See* EX1017 (Jans & Jans), p. 3 (explaining that the T-Ag (Asn-Asn-Gln<sup>113/115</sup>) mutant possesses a non-functional CKII-phosphorylation site). Therefore, a POSA would have understood that, as I

471. Jans & Jans concluded from this result that CKII was in fact the kinase that phosphorylated one or both of the neighboring serine residues (111 and 112) and that this phosphorylation was important for nuclear localization of large T-antigen. EX1017 (Jans & Jans), p. 3.

472. Dr. Samulski does not correctly describe this result and its relevance to Snowdy's teachings. He argues that "Jans & Jans teaches that substituting in asparagine could have a dramatic negative effect on functionality, the opposite of what Sarepta contends a skilled artisan would have gleaned from Snowdy." EX2027 (Samulski Dec.), ¶ 80.

473. On the contrary, a POSA would have understood from Jans & Jans that substituting in *uncharged* residues for *charged* residues can abrogate the function of the charged residues at that position. Wu's experiments contained similar teachings in the context of the AAV capsid, where Wu substituted uncharged alanine for charged residues (aspartic acid, glutamic acid, lysine, arginine) throughout the

---

said in my Declaration, Jans & Jans (and therefore Snowdy in citing Jans & Jans) teaches that asparagine can be substituted in to remove negative charge. By a different but analogous means, asparagine could be substituted in to remove a phosphorylation site, where addition of a phosphate group to a phosphorylatable residue produces a negative charge at the site. EX1005 (Schaffer Dec.), ¶ 181.

AAV capsid and observed the effect on function. *See, e.g.*, EX1032 (Wu), pp. 2, 5, Table 1 (“Initially, 48 alanine scanning mutations were made in which two to five charged amino acids in the AAV capsid ORF were mutated to alanine residues by site-directed mutagenesis”).

474. Snowdy made similar substitutions in the putative AAV NLS, replacing charged residues with uncharged residues, to investigate what functions would be affected by these substitutions. EX1009 (Snowdy), pp. 78-79. Specifically, Snowdy replaced two charged residues, arginine and lysine, with uncharged asparagines (mutating the wild type sequence <sup>166</sup>PARKRLN<sup>173</sup> to <sup>166</sup>PANNRLN<sup>173</sup>). EX1009 (Snowdy), pp. 78-79.

475. Snowdy found that these substitutions, unexpectedly, had no effect on capsid assembly, viral particle production, or nuclear localization. EX1009 (Snowdy), pp. 78-81. The mutant virus particles, however, could not transduce HeLa cells in culture. EX1009 (Snowdy), pp. 78-81.

476. Notably, Dr. Samulski does not describe this portion of Snowdy’s dissertation correctly, stating, “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’.” EX2027 (Samulski Dec.), ¶ 81.

477. However, Jans & Jans did not mutate the putative SV40 large T-antigen NLS. Instead, Jans & Jans mutated the putative CKII binding site. *See, e.g.*, EX1017

(Jans & Jans), p. 3, Table 1. And, notably, Snowdy does not cite Jans & Jans in this section. EX1009 (Snowdy), p. 78. Dr. Samulski therefore appears not to have correctly characterized either the experiments of Snowdy or of Jans & Jans.

478. In any event, none of this has any bearing on my opinions regarding Snowdy's substitutions of the serine at position 181, which do not involve substitution of an uncharged residue in place of basic or acidic residues (*e.g.*, arginine, lysine, glutamic acid, or aspartic acid), but rather involve substitution of an uncharged residue in place of an uncharged, polar residue.

479. Dr. Samulski disregards my opinions and states inaccurately that "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." EX2027 (Samulski Dec.), ¶ 81.

480. As I discussed extensively in my Declaration, a POSA would have been guided by the teachings of the '772 Publication in combination with Snowdy to substitute in asparagine *from AAV8* into the corresponding position in AAVrh.10. EX1005 (Schaffer Dec.), ¶¶ 271-280. A POSA would have understood that asparagine at this position in AAV8 did not "have a dramatic negative impact" on the functionality of interest, namely transduction of liver tissue, given that AAV8

was disclosed to be the best liver transducer tested. EX1005 (Schaffer Dec.), ¶¶ 271-280.

481. As I also discussed above (Sections V.C and V.D), Dr. Samulski is familiar with this approach, and with substitution in of asparagine from one serotype in to replace a phosphorylatable amino acid (serine or threonine) in another serotype to alter tropism, given the disclosures and claims of his '126 provisional and '809 patent.

### B. Phosphorylation of Serines Adjacent to the CKII Site

482. As I mentioned above, Jans & Jans also discloses a second set of experiments in which they mutated the two serines next to the DDE sequence in the CKII recognition site. *See, e.g.*, EX1017 (Jans & Jans), p. 3, Table 1. I have reproduced my highlighted version of Table 1 below:

**Table 1** Nuclear import parameters and SV40 T-antigen sequence of casein kinase II site mutated derivatives of the T-ag/ $\beta$ -galactosidase fusion proteins in this study

| <i>Fusion protein</i>                        | CKII-site                                              | SV40 T-ag sequence*<br>NLS                    | Nuclear import<br>rate†<br><i>k</i> ( $10^{-3}/\text{min}$ ) |
|----------------------------------------------|--------------------------------------------------------|-----------------------------------------------|--------------------------------------------------------------|
| T-ag(WT126-135)                              |                                                        | <sup>126</sup> PKKKRKVEDP <sup>135</sup>      | 1                                                            |
| T-ag(CKII Del) (WT120-135)                   |                                                        | <sup>120</sup> SQHSTPPKKRKVEDP <sup>135</sup> | 2                                                            |
| T-ag(WT111-135)                              | <sup>111</sup> SSDDEATADSQHSTPPKKRKVEDP <sup>135</sup> |                                               | 88                                                           |
| T-ag(Gly <sup>111</sup> )                    | gSDDEATADSQHSTPPKKRKVEDP <sup>135</sup>                |                                               | 100                                                          |
| T-ag(Asn-Asn-Gln <sup>113-115</sup> )        | SSnnqATADSQHSTPPKKRKVEDP <sup>135</sup>                |                                               | 2                                                            |
| T-ag(Ala-Gly <sup>111/112</sup> )            | agDDEATADSQHSTPPKKRKVEDP <sup>135</sup>                |                                               | 3                                                            |
| T-ag(Gly <sup>111</sup> Asp <sup>112</sup> ) | gaDDEATADSQHSTPPKKRKVEDP <sup>135</sup>                |                                               | 40                                                           |

EX1017 (Jans & Jans), p. 3, Table 1 (highlights added).

483. The two mutants shown in green involve replacing the two serines with either alanine and glycine (the T-ag(Ala-Gly<sup>111/112</sup>) mutant), or glycine and aspartic acid (the T-ag(Gly<sup>111</sup>Asp<sup>112</sup>) mutant). EX1017 (Jans & Jans), p. 3, Table 1.

484. As Table 1 shows, they found that the substitution of alanine and glycine for the two serines abrogated nuclear localization, while the substitution of glycine and aspartic acid produced a protein that could localize to the nucleus about 45% as well as wild-type (40 vs. 88). EX1017 (Jans & Jans), p. 3, Table 1.

485. Jans & Jans also teaches that aspartic acid can simulate a constitutively phosphorylated serine or threonine. EX1017 (Jans & Jans), pp. 5-6. Therefore, the fact that the T-ag(Gly<sup>111</sup>Asp<sup>112</sup>) mutant localized to the nucleus at a rate 45% that of wild type, while the T-ag(Ala-Gly<sup>111/112</sup>) mutant, containing two nonphosphorylatable residues, did not, supports the relevance of phosphorylation at that site to nuclear localization. *See, e.g.*, EX1017 (Jans & Jans), p. 6.

486. For reasons that are not clear, Dr. Samulski argues that “Jans & Jans also teaches that substituting serine (i.e., the native residue of AAVrh.10 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.” EX2027 (Samulski Dec.), ¶ 82.

487. Jans & Jans sought to test the importance of phosphorylation at the CKII site by mutating two native serine residues in the wild-type protein. EX1017 (Jans & Jans), p. 3, Table 1. In particular, they found that substituting in a nonphosphorylatable glycine in place of serine 112 abrogated nuclear localization, while substituting in an aspartic acid, which mimics constitutive phosphorylation, produced about 45% of wild-type nuclear localization. EX1017 (Jans & Jans), p. 3, Table 1.

488. Snowdy, following these teachings, made mutations in three positions in the AAV capsid, near a putative NLS, to test the effect of phosphorylation on nuclear localization. *See, e.g.*, EX1009 (Snowdy), pp. 125-26.

489. As I have discussed, the fact that Snowdy unexpectedly found the opposite results to those of Jans & Jans – namely, that the S181A greatly enhanced transduction, while the S181D abrogated transduction – suggested to Snowdy, and would have suggested to a POSA at the time, that these effects were related to a phenomenon other than nuclear localization and therefore had even broader applicability. EX1005 (Schaffer Dec.), ¶¶ 186-89, 275-76; EX1009 (Snowdy), p. 129.

490. Therefore, the unexpected nature of Snowdy's findings would have encouraged a POSA to make the substitution of nonphosphorylatable asparagine

from AAV8 in place of phosphorylatable serine in AAVrh.10. EX1005 (Schaffer Dec.), ¶¶ 186-89, 275-76.

491. Surprisingly, Dr. Samulski argues that “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.” EX2027 (Samulski Dec.), ¶ 83.

492. This statement is contradicted by the experimental approach and dissertation of Dr. Samulski’s own student, Snowdy, and by Dr. Samulski’s ’126 provisional and ’809 patent.

493. As I discussed above, Snowdy modeled these AAV capsid experiments on the work of Jans & Jans in SV40 large T-antigen, expressly relying on specific substitutions from one study, Jans & Jans, to draw inferences about what would happen if those substitutions were made in his own study, in different proteins, the AAV capsid proteins. EX1009 (Snowdy), p. 129.

494. Similarly, in his ’126 provisional and his ’809 patent, Dr. Samulski claimed not only specific substitutions in specific proteins, but also these substitutions in all different AAV serotypes. *See, e.g.*, EX1059 (’126 provisional), 59:6-29 (claims 57-61) (reciting an AAV capsid with a substitution at position 450 in the AAV2 capsid “or a corresponding change in other AAV capsids”); EX1061 (’809 patent), col. 75:13-24 (claims 22 and 23) reciting an AAV2 capsid subunit

with a substitution at position 450 “or a corresponding change in another AAV capsid subunit”).

495. Given that neither the '126 provisional or the '809 patent disclose the effects of these substitutions in all the serotypes encompassed by the claims, Dr. Samulski's patent claims draw inferences about the effect of these substitutions across all serotypes, differing from his opinions in this proceeding.

496. The '772 Publication also teaches the applicability of substitutions from one AAV serotype to another, for example in discussing the unusual GTG start codon in AAV7, and suggesting that it would be desirable to alter the start codons of other serotypes to GTG, based on the AAV7 start codon. *See, e.g.*, EX1007 ('772 Publication), [0072] (“the inventors anticipate this it is desirable to alter the start codon of the vp3 *of other AAV serotypes* to contain this rare GTG start codon, in order to improve packaging efficiency, to alter the virion structure and/or to alter location of epitopes (e.g., neutralizing antibody epitopes) *of other AAV serotypes*. The start codons may be altered using conventional techniques including, e.g., site directed mutagenesis. Thus, *the present invention encompasses altered AAV virions of any selected serotype*, composed of a vp3, and/or optionally, vp1 and/or vp2 having start codons altered to GTG”) (emphases added); see also EX1063 (Samulski Dep. Tr.), 61:21-24..

497. To provide support for his assertion, Dr. Samulski refers to the Jans & Jans findings that mutating serine 111 produced different results from mutating serine 112. EX2027 (Samulski Dec.), ¶ 83. Specifically, they found that serine 111 was not phosphorylated to the same extent as serine 112. EX2027 (Samulski Dec.), ¶ 83; EX1017 (Jans & Jans), pp. 4-6, Figure 5.

498. Dr. Samulski argues that “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.” EX2027 (Samulski Dec.), ¶ 84.

499. As a preliminary matter, Jans & Jans disclosed that, based on the known consensus CKII phosphorylation site, they predicted that both serine 111 and serine 112 would be phosphorylated by CKII. EX1017 (Jans & Jans), p. 4. They actually demonstrated that one of the two is phosphorylated by CKII. EX1017 (Jans & Jans), pp. 4-6, Figure 5. In my experience, this result supports the validity of the method they used to make the prediction and would teach a POSA at the time to pursue a similar approach to the extent the POSA sought to investigate phosphorylation of serines.

500. Snowdy strongly supports my opinion rather than Dr. Samulski’s opinion. Snowdy states expressly that he selected three sites (S148, T159, S181) in

the vicinity of the putative AAV2 NLS where there are phosphorylatable serines and threonines, and mutated them “in a manner consistent with” the mutations in Jans & Jans. EX1009 (Snowdy), p. 125 (“These sites were mutated in a manner consistent with the mutations of Jans *et al* (Jans, Ackermann et al. 1991; Jans and Jans 1994; Jans and Hubner 1996), i.e. we mutated each of them to either a nonphosphorylatable alanine, to simulate an unphosphorylated serine, or to an aspartic acid to simulate a constitutively phosphorylated serine, resulting in six mutants, S148A, S148D, T159A, T159D, S181A, and S181D”).

501. Clearly, in this way, Snowdy expressly taught the applicability of the Jans & Jans findings regarding serine 111 and 112 in the vicinity of the SV40 large T-antigen to phosphorylatable residues in the vicinity of the AAV NLS, contradicting Dr. Samulski’s opinion.

502. Dr. Samulski also does not correctly characterize the statement from Snowdy that “It is unknown what receptor mediates infection by AAV1, AAV3, AAV7, and AAV8.” EX1009 (Snowdy), p. 132. Dr. Samulski argues that this statement shows that “Snowdy itself recognized this additional limitation regarding the applicability from one AAV to another AAV.” EX2027 (Samulski Dec.), ¶ 85. But Snowdy nowhere teaches that his findings are not applicable to other AAV serotypes.

503. The fact that it was unknown what receptor mediates infection by certain serotypes of AAV would not have taught a POSA at the time that Snowdy's results regarding substitution of nonphosphorylatable residues for phosphorylatable residues were limited to AAV2. For example, a POSA would have understood that, even if a different receptor mediated infection by other serotypes, the phosphorylation status of the capsid proteins could still be highly relevant to transduction efficiency and tropism.

504. And potential differences in receptors that mediate infection of the various serotypes did not prevent Dr. Samulski from broadly claiming the applicability across all serotypes of his single amino acid substitutions of nonphosphorylatable residues (such as asparagine) from AAV1 to replace phosphorylatable residues in AAV2 to alter tropism. *See, e.g.*, EX1059 ('126 provisional), 59:6-29 (claims 57-61) (reciting an AAV capsid with a substitution at position 450 in the AAV2 capsid "or a corresponding change in other AAV capsids"); EX1061 ('809 patent), col. 75:13-24 (claims 22 and 23) reciting an AAV2 capsid subunit with a substitution at position 450 "or a corresponding change in another AAV capsid subunit").

505. In addition, Dr. Samulski specifically claimed substitutions from AAV1 into AAV2 (*see* V.C and V.D *supra*), though he now argues in his declaration that the fact AAV1 and AAV2 might bind to different receptors would make findings

relating to AAV2 inapplicable to AAV1. *See, e.g.*, EX1059 ('126 provisional), 59:6-29 (claims 57-61) (reciting an AAV capsid with a substitution of asparagine for threonine at position 450 in the AAV2 capsid “or a corresponding change in other AAV capsids”); EX1061 ('809 patent), col. 75:13-24 (claims 22 and 23) reciting an AAV2 capsid subunit with a substitution of asparagine for threonine at position 450 “or a corresponding change in another AAV capsid subunit”).

## **XI. SECONDARY CONSIDERATIONS**

506. I note that neither Dr. Samulski’s declaration nor the Patent Owners’ response addresses secondary considerations. As I mentioned in my original Declaration, I am unaware of any secondary considerations supporting the validity of the '274 patent. EX1005 (Schaffer Dec.), Sections X.F, XI.D., XII.D., XIII.C., XIV.C., XV.C.

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".

---

David V. Schaffer

Date: March 20, 2025