

SAREPTA THERAPEUTICS, INC.
v.
THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA
AND REGENXBIO INC.

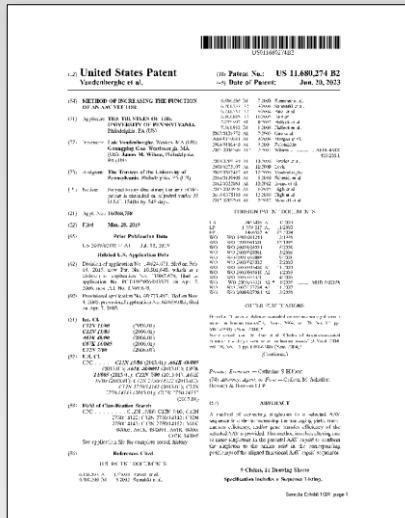
IPR2024-00580
Oral Hearing May 27, 2025
Patent Owner's Presentation

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE 1

Only Petitioner's Grounds 4-6 Remain

Ground	Claims	Description
1	1, 3-6	Obvious in view of the '772 Publication
2	1, 3-6	Obvious in view of the '772 Publication and Xie
3	1, 3-6	Obvious in view of the '772 Publication and Snowdy
4	8	Obvious in view of the '772 Publication and Fabb
5	8	Obvious in view of the '772 Publication, Xie, and Fabb
6	8	Obvious in view of the '772 Publication, Snowdy, and Fabb

The '274 Patent Claims an AAV with a Novel Capsid

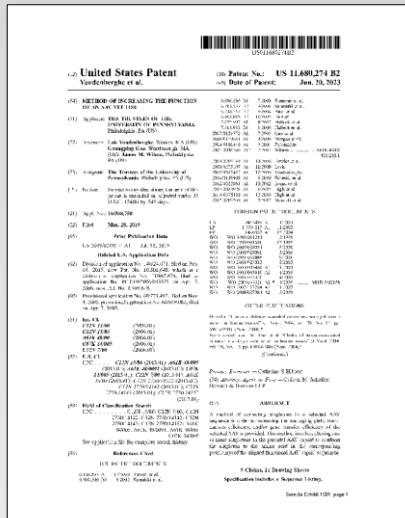


EX1001, cl. 1;

Paper 18 (POR) at 1-6, 30-31

1. A recombinant adeno-associated virus (AAV) comprising an AAV capsid and a minigene having AAV inverted terminal repeats and a **heterologous gene** operably linked to regulatory sequences which direct expression of the heterologous gene in a host cell, wherein the AAV capsid comprises AAV vp1 proteins, AAV vp2 proteins, and AAV vp3 proteins, wherein the AAV vp1 proteins have i) the sequence of amino acids 1 to 738 of **SEQ ID NO: 4 (AAVrh46)**, or ii) an amino acid sequence at least 95% identical to the full length of amino acids 1 to 738 of SEQ ID NO:4, wherein the **amino acid residue corresponding to position 665 in SEQ ID NO: 4 is N** when aligned along the full length of amino acids 1 to 738 of SEQ ID NO: 4.

Claim 6 is Directed to Various Heterologous Genes

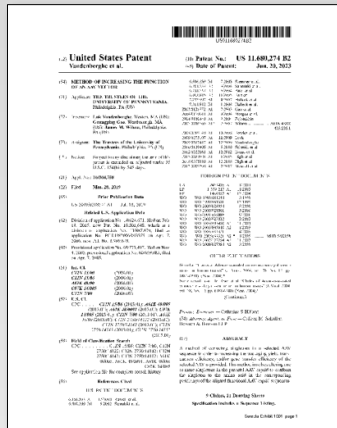


EX1001, cl. 6;

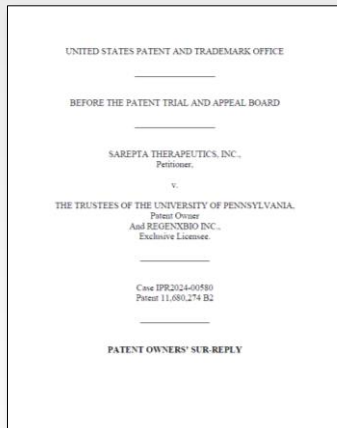
Paper 18 (POR) at 1-6, 30-31

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

Claim 8 Recites Dystrophin, Which Is Predominantly Expressed in Muscle



EX1001, cl. 8;
Paper 18 (POR) at 1-6, 30-31;
Paper 24 (Sur-Reply) at 1-4



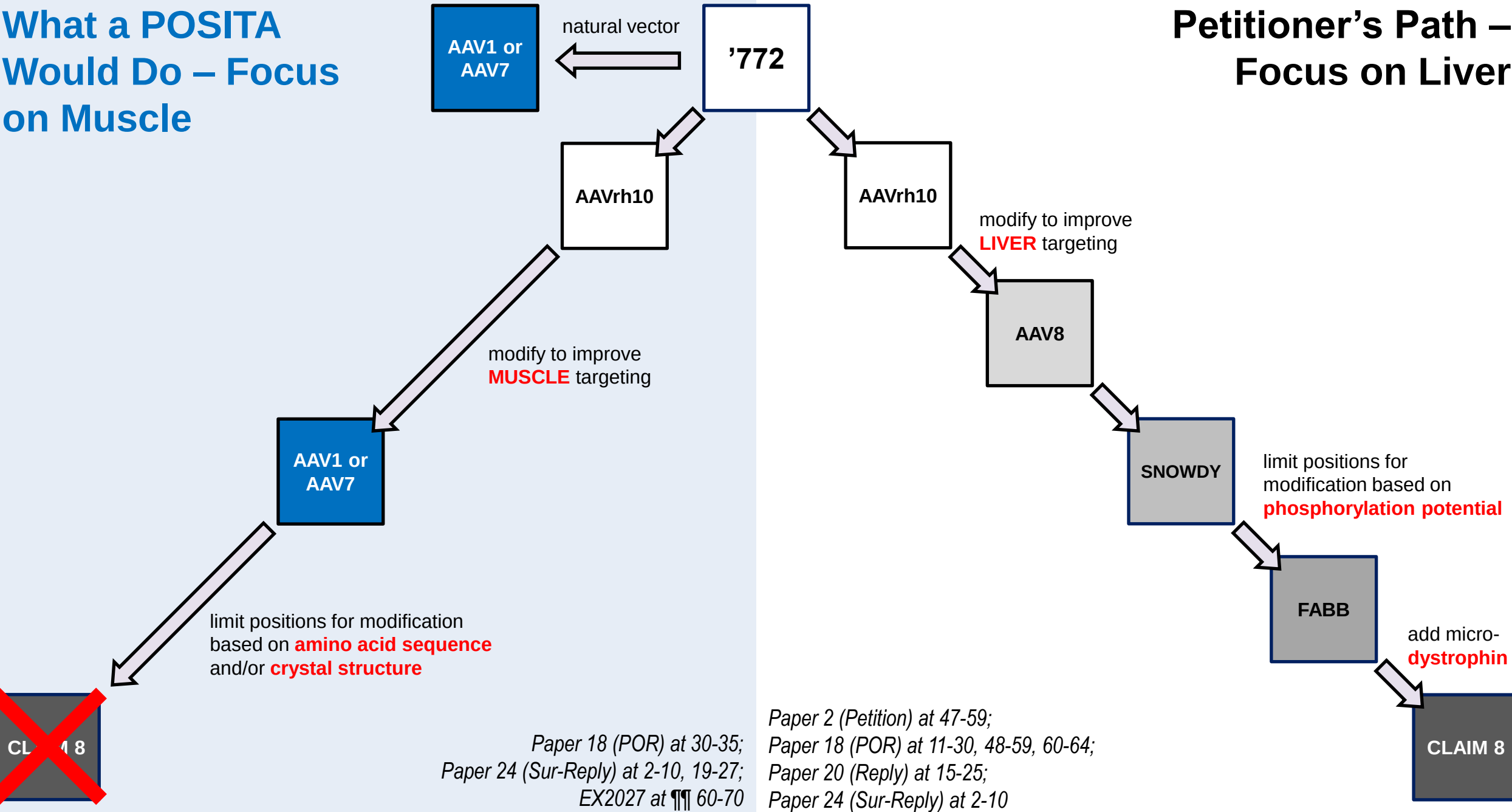
Paper 24 (Sur-Reply) at 2;
see also EX2027 at ¶ 60; EX1010 at
Abstract, 3, 5; EX1063 at 270:9-15

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

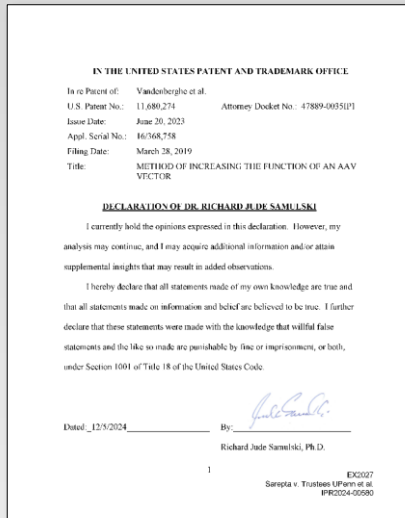
As Patent Owners' expert explained, "[d]ystrophin is a protein that is predominantly expressed in muscle, and a skilled artisan creating a gene therapy to deliver dystrophin would seek to target the muscles." EX2027, ¶60; *see also* EX1063, 270:9-15. Petitioner offers no meaningful dispute, and contends that its Petition addressed muscle targeting (though it did not). *See* Reply, 17-20 ("Petitioner and Dr. Schaffer Addressed Motivation to Target Muscle"). Thus,

What a POSITA
Would Do – Focus
on Muscle

Petitioner’s Path –
Focus on Liver



Dystrophin Is “Predominantly Expressed in Muscle”



EX2027 at ¶ 60;

*Paper 18 (POR) at 30-31;
Paper 24 (Sur-Reply) at 2*

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

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

Ground Reference Fabb Shows Dystrophin Delivery to Muscle

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Human Molecular Genetics, 2002, Vol. 11, No. 7 733-741

Adeno-associated virus vector gene transfer and sarcolemmal expression of a 144 kDa micro-dystrophin effectively restores the dystrophin-associated protein complex and inhibits myofibre degeneration in nude/*mdx* mice

Stewart A. Fabb, Dominic J. Wells¹, Patricia Serpente and George Dickson²

Centre for Biomedical Sciences, School of Biological Sciences, Royal Holloway, University of London, Surrey TW20 0EX, UK and ¹Gene Targeting Unit, Department of Neuroimmunology, Division of Neuroscience and Psychological Medicine, Imperial College Faculty of Medicine, Charing Cross Hospital, London WC8B 9PP, UK

Received October 28, 2001; Revised and Accepted February 4, 2002

Duchenne muscular dystrophy is a severe life-threatening X-linked recessive disorder, caused by mutations in the dystrophin gene, for which currently there is no effective treatment. Because of the large size of the dystrophin cDNA (14 kb) this precluded it from being used in early adenovirus- or retrovirus-based gene therapy vectors. However, some therapeutic success has been achieved in nude mice using adenovirus- and retrovirus-mediated transfer of a 6.3 kb recombinant mini-dystrophin cDNA. Despite this, problems with immunogenicity and inefficient transduction of mature myofibres make these vectors less than ideal for gene transfer to skeletal muscle. Adeno-associated viral (AAV) vectors overcome many of the problems associated with other vector systems. However, AAV vectors can only accommodate <5 kb of foreign DNA. For this reason we have produced a micro-dystrophin cDNA gene construct that is <3.8 kb. This construct, driven by a CMV promoter, was introduced into the skeletal muscle of 12-day-old nude/*mdx* mice using an AAV vector, resulting in specific sarcolemmal expression of micro-dystrophin in >50% of myofibres up to 20 weeks of age, and effective restoration of the dystrophin-associated protein (DAP) complex components. Additionally, evaluation of central nucleation indicated a significant inhibition of degenerative dystrophic muscle pathology. We have therefore shown that the current micro-dystrophin gene delivered *in vivo* using an AAV vector is not only capable of restoring sarcolemmal DAP complexes, but can also ameliorate dystrophic pathology at the cellular level.

INTRODUCTION

Duchenne muscular dystrophy (DMD) is a severe life-threatening X-linked disorder which affects 1/3500 males (prevalent at which are sporadic cases with no family history (1)) and is characterized by a progressive loss of muscle mass and replacement of myofibres with connective and adipose tissue (2). The genetic defect is clinical symptoms of weakness, posture and breathing difficulties. The symptoms of the disease usually appear before 5 years of age, the patients are wheelchair bound by their early teens and death is normally at 20 years of age.

The disease is caused by an absence of dystrophin, a 427 kDa cytoplasmic protein of the dystrophin gene (3). The DMD gene has one of the highest mutation rates reported in the world, with two thirds of disease-causing mutations being large deletions in the 5' and central portions of the gene. Interestingly, deletion size

has no influence over whether the dystrophin is DMD or the much less severe form, Becker muscular dystrophy (BMD). Very mild forms of the disease, when only a portion of the gene has been deleted, have been described (3-5). Conversely, other individuals with only small deletions display a DMD phenotype. This apparent anomaly depends on whether the dystrophin gene contains fewer or associated (DMD) or duplicated (BMD) DMDs. At the molecular level, the DMD phenotype also appears to be due either to the complete absence of the mutated allele or protein, or to a functional instability of the gene (6).

The dystrophin protein has two main structural domains, an N-terminal region, which binds to the F-actin of cytoskeletal myofibres, and a C-terminal region, which is associated with the dystrophin-associated protein (DAP) complex. The DAP complex is a group of proteins that are associated with the dystrophin protein. These DAPs provide a molecular link between the

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George Dickson, Patricia Serpente, Molecular Development Division, The National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

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EX1010 at Abstract;

Paper 18 (POR) at 31;

Paper 24 (Sur-Reply) at 2-3

Ground Reference Fabb Shows Dystrophin Delivery to Muscle

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Blood Molecular Genetics, 2002, Vol. 1, No. 7 733-741

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INTRODUCTION

Duchenne muscular dystrophy (DMD) is a severe life-threatening X-linked disorder which affects 1/3500 males (prevalent at which age depends on the family history (1)) and is characterized by a progressive loss of muscle mass and replacement of myofibres with connective and adipose tissue (2). This process leads to clinical symptoms of weakness, posture and breathing difficulties. The symptoms of the disease usually appear before 5 years of age, the patients are wheelchair bound by their early teens and death is normally at 20 years of age.

The disease is caused by an absence of dystrophin, a 427 kDa cytoplasmic protein of the dystrophin family (3,4). The DMD gene has one of the highest mutation rates reported in the world, with two thirds of disease-causing mutations being large deletions in the 5' and central portions of the gene. Interestingly, deletion size

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The dystrophin protein has two main structural domains, an N-terminal region, which binds to the F-actin of cytoskeletal myofibres, and a C-terminal region and repeat domain (12) which bind to the dystrophin-associated protein (DAP). These DAPs provide a molecular link between the

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Expression of micro-dystrophin $\Delta 3788$ in the skeletal muscle of nude/*mdx* mice transduced with rAAV

To evaluate the expression of micro-dystrophin $\Delta 3788$ *in vivo*, we injected rAAV $\Delta 3788$ particles into the tibialis anterior (TA) muscle of 12-day-old nude/*mdx* mice and performed

EX1010 at 3

DISCUSSION

In order to utilize an AAV vector system to provide gene transfer based on complementation of dystrophin deficiency in muscle tissues *in vivo*, we have reduced the cDNA sequence of this gene down from 14 to ~3.8 kb to produce a micro-dystrophin cDNA. The rationale behind the rod-domain coding

EX1010 at 5

EX1010 at 3, 5;
Paper 18 (POR) at 31

Dystrophin Is Primarily Expressed in Muscle



Dr. Samulski

EX1063 at 270:9-15;

Paper 24 (Sur-Reply) at 2

Q. Dr. Samulski, I just have a few questions for you.

Where is the dystrophin protein primarily expressed in the body?

A. Muscle.

Q. And if you were going to make a gene therapy that included a dystrophin gene, what tissues would you target with that gene therapy?

A. Muscle.

No Dispute that Dystrophin Is Expressed in Muscle

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Vanderschuerge et al.
U.S. Patent No.: 11,680,274 Attorney Docket No.: 47889-0031P1
Issue Date: June 20, 2023
Appl. Serial No.: 16/968,758
Filing Date: March 28, 2019
Title: METHOD OF INCREASING THE FUNCTION OF AN AAV VECTOR

DECLARATION OF DR. RICHARD JUDE SAMULSKI

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

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

Dated: 12/5/2024 By: 
Richard Jude Samulski, Ph.D.

1 EX2027
Serepta v. Trustees of Proton et al.
IPR2024-00580

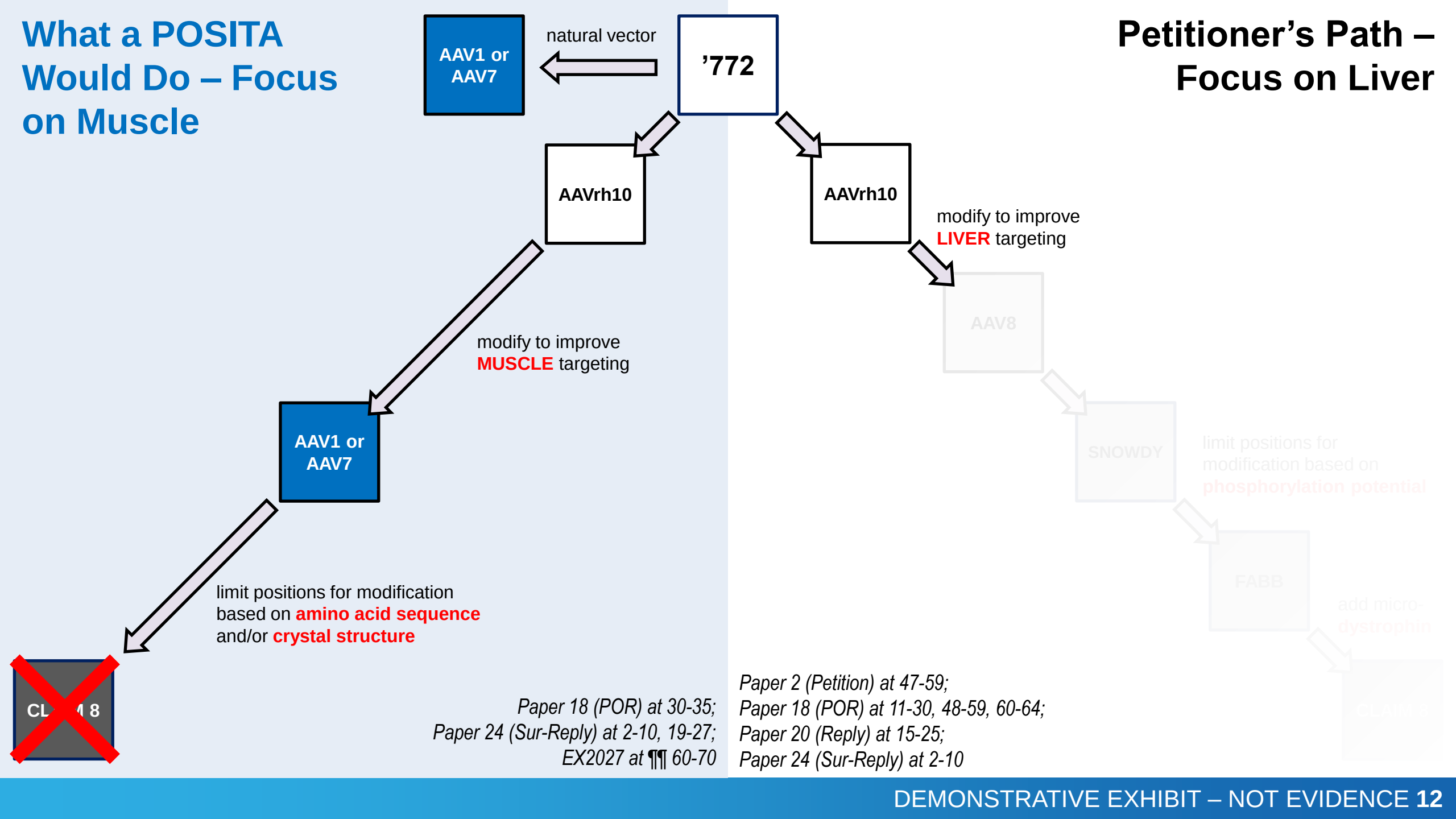
EX2027 at ¶ 61;

Paper 18 (POR) at 30-31

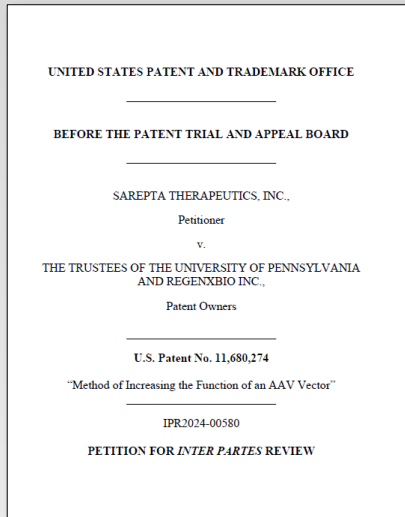
61. I have read Dr. Schaffer's declaration and the Petition, and I did not see any discussion of delivery to muscle specifically. I also saw that at his deposition, Dr. Schaffer would not agree that dystrophin is largely expressed in muscle. EX2030 (Schaffer Tr.), 359:8-360:10. I do not agree. Dystrophin is a protein well-known to be expressed primarily in muscle, as even the Fabb reference that Dr. Schaffer relies upon explains. EX1010, 1 ("Duchenne muscular dystrophy is a severe life-threatening X-linked recessive disorder, caused by mutations in the dystrophin gene"; describing how previous vector options were "less than ideal for gene transfer to skeletal muscle"); at 6 ("AAV vectors remain one of the most attractive systems for muscle gene transfer, and the studies described here further confirm the feasibility of using highly engineered micro-dystrophin AAV vectors as an approach towards gene therapy of DMD"); see also EX2040, 4-5 ("The cellular regulation of dystrophin expression appears to be a key aspect of the plasticity of muscle ... The dystrophin protein is also large (427 kDa) and requires a specialized intracellular structural niche within myofibers [i.e., muscle fibers] throughout the body (recalling that myofibers account for more cell volume in the body than any other cell type)."); EX2041 ("The dystrophin complex stabilizes the plasma membrane of striated muscle cells."). Therefore, it is my opinion that a skilled artisan seeking to deliver a mini- or micro-dystrophin, as required by claim 8, would seek to target muscle tissue.

What a POSITA
Would Do – Focus
on Muscle

Petitioner's Path –
Focus on Liver



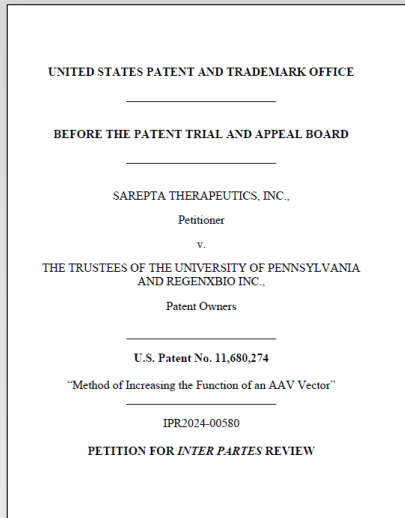
Petitioner's Motivation to Combine — Liver Delivery



Paper 2 (Petition) at 27

The '772 Publication thus teaches the superiority of AAVrh.10 overall and in lung in particular, the superiority of AAV8 in liver, and the importance of efficient gene transfer to the liver for a variety of gene therapy applications. Given these teachings and the express comparison between AAVrh.10 and AAV8 in the '772 Publication, a POSA would have considered substitutions between AAVrh.10 (best overall and best in lung) and AAV8 (best in liver) as a promising strategy for obtaining an artificial variant of AAVrh.10 with even more efficient gene transfer in liver. EX1005, ¶210.

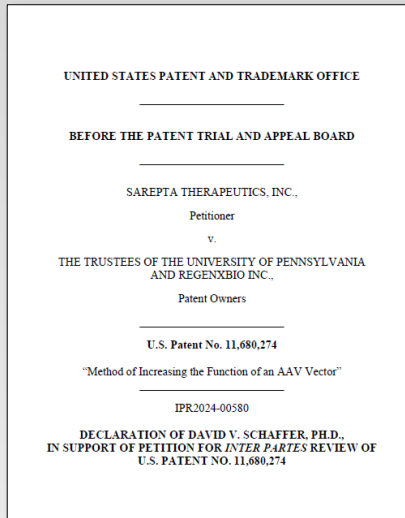
Petitioner's Motivation to Combine — Liver Delivery



Paper 2 (Petition) at 49

A POSA, seeking to improve the transduction of AAVrh.10 in liver to approach that of AAV8, in view of Snowdy, would have looked to see which positions in AAVrh.10 had phosphorylatable amino acids, where the corresponding position in AAV8 had a non-phosphorylatable amino acid. EX1005, ¶277.

Petitioner's Motivation to Combine — Liver Delivery



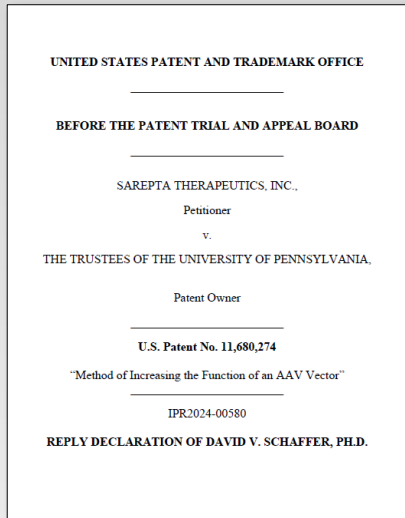
EX1005 at ¶ 210;

Paper 18 (POR) at 11-13;

Paper 2 (Petition) at 27

210. The '772 Publication thus teaches the superiority of AAVrh.10 overall and in lung in particular, the superiority of AAV8 in liver, and the importance of efficient gene transfer to the liver for a variety of gene therapy applications. Given these teachings and the express comparison between AAVrh.10 and AAV8 in the '772 Publication, a POSA would have considered substitutions between AAVrh.10 (best overall and best in lung) and AAV8 (best in liver) as a promising strategy for obtaining an artificial variant of AAVrh.10 with even more efficient gene transfer in liver.

Petitioner's Motivation to Combine — Liver Delivery



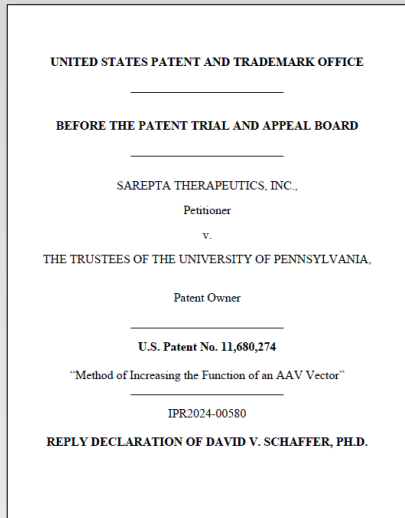
EX1072 at ¶¶ 9, 11;

*Paper 18 (POR) at 11-13;
Paper 24 (Sur-Reply) at 4*

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 the phosphorylatable residue from AAV8 to replace the phosphorylatable residue in AAVrh.10.

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.

Petitioner's Motivation to Combine — Liver Delivery

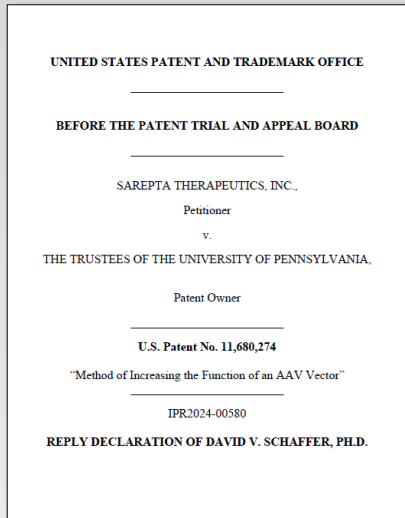


EX1072 at ¶ 57;

*Paper 18 (POR) at 11-13;
Paper 24 (Sur-Reply) at 4*

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.

Petitioner's Motivation to Combine — Liver Delivery



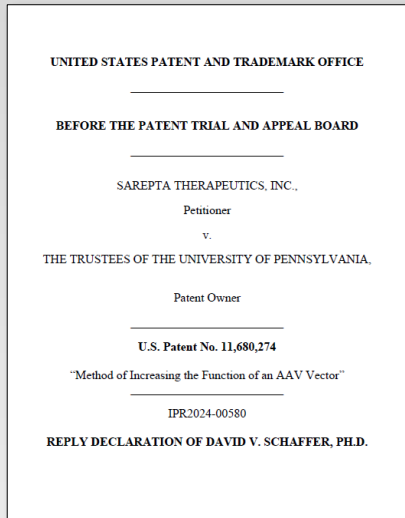
EX1072 at ¶ 195;

Paper 18 (POR) at 11-13;

Paper 24 (Sur-Reply) at 4

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.

Petitioner's Motivation to Combine — Liver Delivery



EX1072 at ¶¶ 325-26;

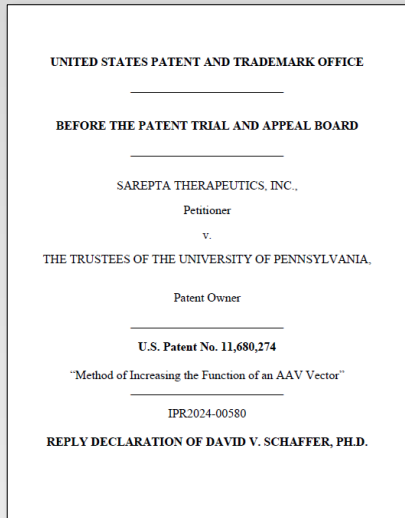
Paper 18 (POR) at 11-13;

Paper 24 (Sur-Reply) at 4

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.

Petitioner's Motivation to Combine — Liver Delivery



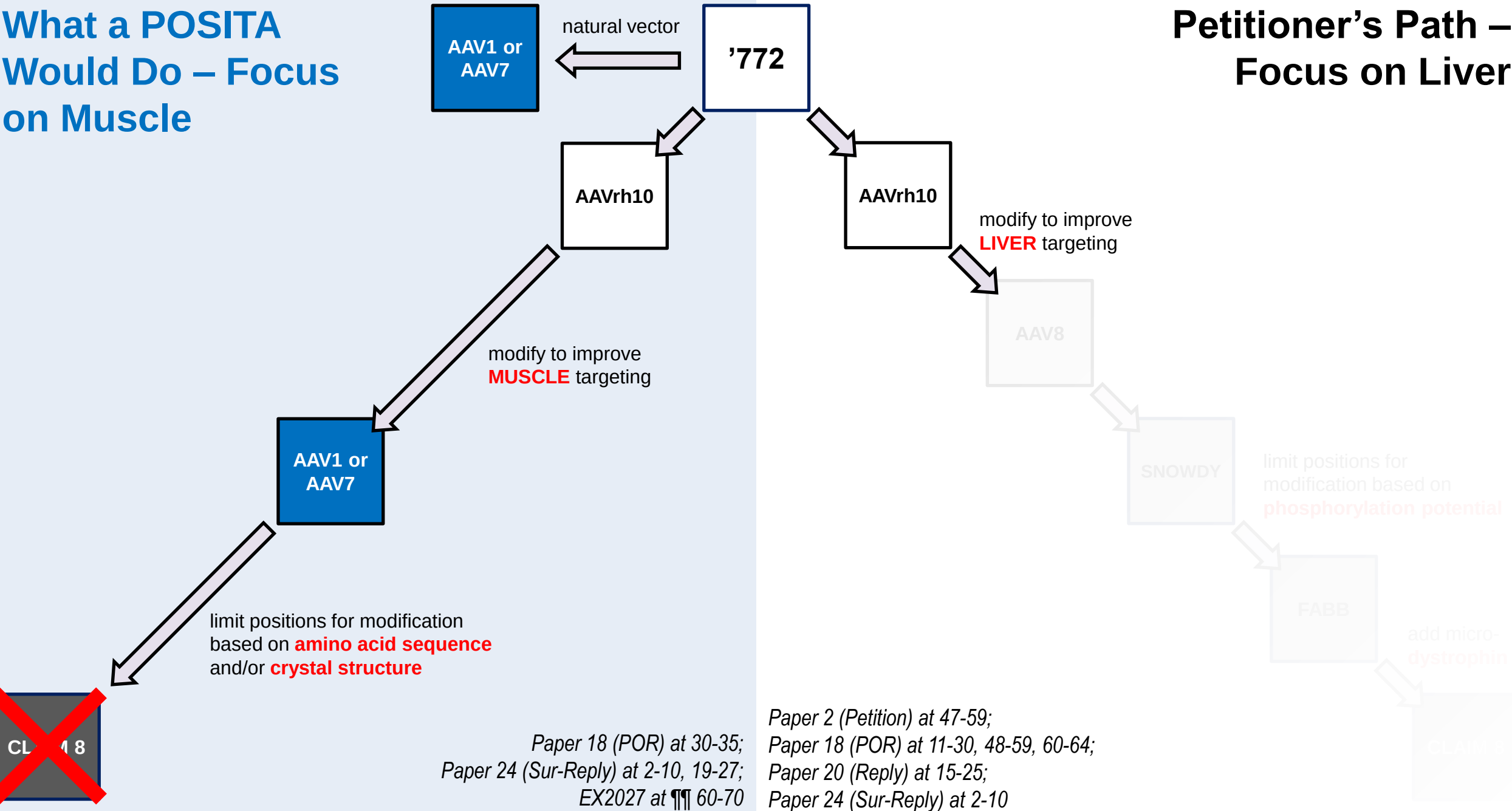
EX1072 at ¶ 368;

Paper 18 (POR) at 11-13;
Paper 24 (Sur-Reply) at 4

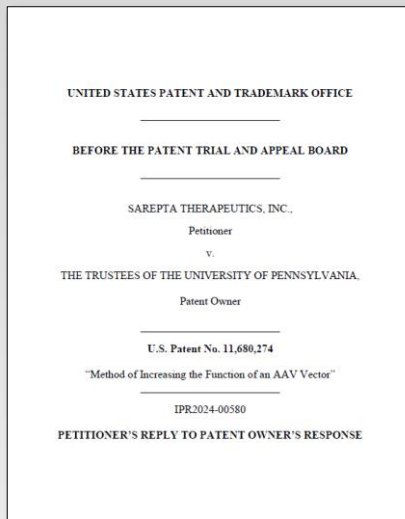
368. In my opinion, the superiority of AAV8 in liver would have motivated a POSA to try to improve the tropism of AAVrh.10 in liver, based on the guidance in the '772 Publication that the performance of AAVrh.10 "*in liver* and muscle *directed* gene transfer was also outstanding, *close to that of the 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.

What a POSITA
Would Do – Focus
on Muscle

Petitioner’s Path –
Focus on Liver



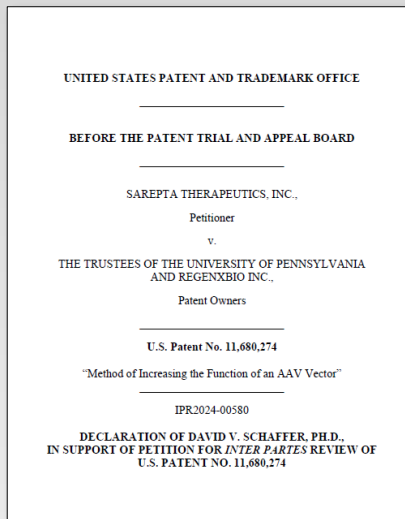
The Petition Lacks Evidence on Muscle Delivery



Paper 20 (Reply) at 18

Both the Petition and Dr. Schaffer explained that there was a need in the art to improve transduction of AAV vectors in various tissues, including muscle. EX1005, ¶¶85, 89-90; Petition, 8. Importantly, both the Petition and Dr. Schaffer

The Petition Lacks Evidence on Muscle Delivery



EX1005 at ¶ 85;

*Paper 24 (Sur-Reply) at 2;
Paper 20 (Reply) at 18*

V. TECHNICAL BACKGROUND AND STATE OF THE PRIOR ART

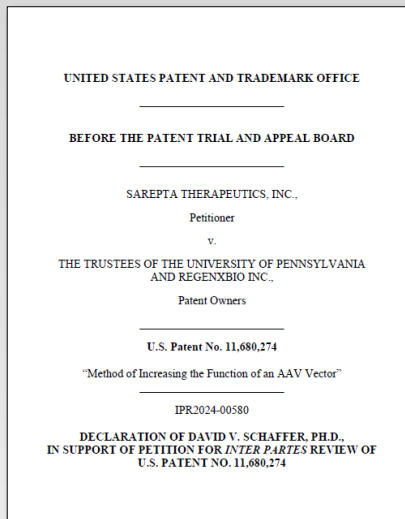
* * *

E. Recombinant AAV (rAAV) Vectors for Gene Therapy

* * *

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

The Petition Lacks Evidence on Muscle Delivery



EX1005 at ¶¶ 89, 90;

Paper 24 (Sur-Reply) at 2;

Paper 20 (Reply) at 18

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

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

EX1031 (Grimm), Abstract

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

Petitioner's Expert's Testimony



Dr. Schaffer

EX2030 at 350:1-359:7;

Paper 24 (Sur-Reply) at 2-3

Q. And I think I asked you, and you said "I'd have to go through every paragraph," where you talked about that in your declaration.

Do you remember that conversation?

A. I do.

Q. Okay. So I want to just look through your analysis in ground 1 and talk about where you talk about muscle.

And so in -- so ground 1 starts at paragraph 194.

Do you see that?

A. I'm at paragraph 194.

Q. Okay. And paragraph 194 is just a summary paragraph is that fair summary of your overall opinion?

A. That's correct.

Q. Okay. And paragraph 195 is just talking about the disclosure of a recombinant adeno-associated virus in the '772 publication; right?

A. That's correct.

Q. And you don't talk about targeting muscle in paragraph 195; right?

A. Within that particular paragraph, I don't talk

about targeting muscle.

Q. And the same is true for paragraph 196; right?

A. In paragraph 196, I don't discuss muscle.

Q. And the same is true for paragraph 197? You don't discuss muscle?

A. In paragraph 197, muscle is not mentioned; correct.

Q. And the same is true in paragraph 198 muscle is not mentioned; right?

A. That is correct.

Q. And same is true in paragraph 199 muscle is not mentioned?

A. That's correct. Muscle is not mentioned in 9.

Q. And in 200 the same is true muscle is not mentioned?

A. 200 doesn't mention muscle.

Q. Nor does 201?

A. 201 doesn't mention muscle.

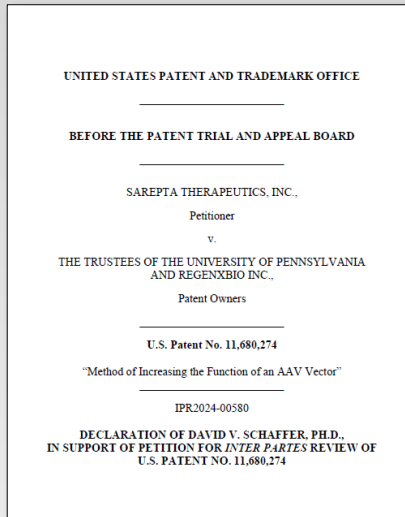
Q. Nor does 202 mention muscle; correct?

A. 202 does not mention muscle.

Q. And 203 does not mention muscle?

A. 203 does not discuss muscle.

Only Two Paragraphs in Petitioner's Expert's Grounds Discuss Muscle



*EX1005 at ¶¶ 208, 294;
Paper 24 (Sur-Reply) at 2-3*

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

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

POSITA Would Not Target Liver To Deliver Gene To Muscle

PROGRESS

Engineering adeno-associated viruses for clinical gene therapy

Melissa A. Kottemann and David V. Schaffer

Advances in clinical gene therapy have been increasingly successful using both an enhanced molecular understanding of human disease and the progressive improvement of gene delivery technologies. Among these technologies, adeno-associated viruses (AAV) have emerged as safe and effective and, in some recent cases, have had the regulatory approval. Although challenging in scale, vector properties will enable treatment of both common and many other human diseases challenging, new approaches to engineer and improve AAV vectors and their genetic cargo are increasingly helping to overcome these barriers.

The vast majority of the ~7,000 monogenic diseases – which collectively affect millions of people worldwide with often debilitating permanent and/or fatal consequences – have no treatment options, leaving patients and their families with little hope for the future.

Gene therapy is a promising approach to treat these diseases, but the complexity of gene delivery is often the major barrier to its clinical application. The complexity of gene delivery is often the major barrier to its clinical application. The complexity of gene delivery is often the major barrier to its clinical application.

In this review, we discuss the challenges of gene delivery and the progress made in the field of gene delivery.

Gene delivery is a promising approach to treat these diseases, but the complexity of gene delivery is often the major barrier to its clinical application.

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Petitioner's Expert, Dr. Schaffer:

Transport to and tropism for target cells

For systemically administered viruses, the liver is often the default destination, which can

represent a barrier when other organs are the intended targets. In addition, endothelial cell layers,

especially those within the blood–brain barrier, pose a physical barrier for entry into a tissue.

A vector that gains access to an organ, or that is directly administered to that organ, can then

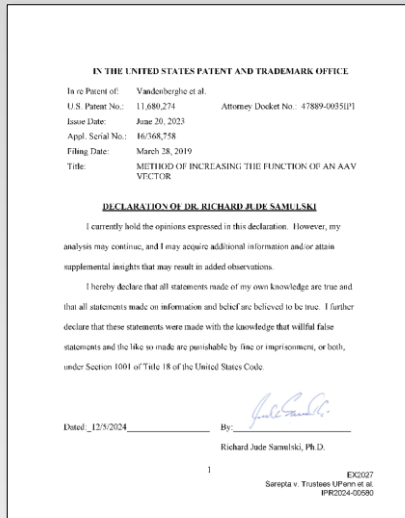
encounter numerous transport barriers to efficient transduction of the often large tissue volumes

involved in disease, including cell bodies and intervening extracellular matrix to which many AAV

variants bind⁵³ (for example, heparan sulphate⁵⁴).

EX1030 at 3;
Paper 18 (POR) at 24-25

Off-Target Delivery to the Liver Is Potentially Toxic



EX2027 at ¶¶ 33-34;

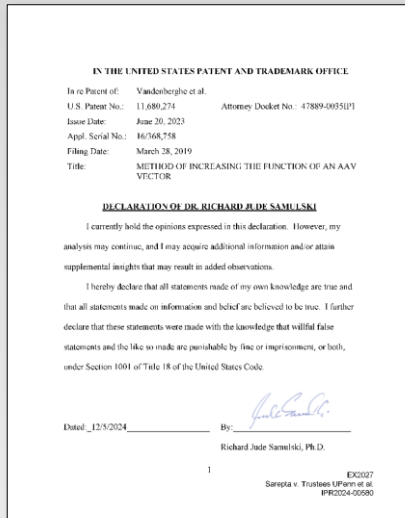
Paper 18 (POR) at 23-24;

Paper 24 (Sur-Reply) at 5-6

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

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

Off-Target Delivery to the Liver Is Potentially Toxic

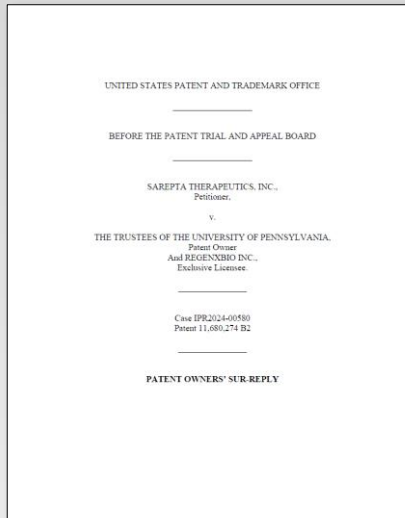


EX2027 at ¶ 35;

*Paper 18 (POR) at 23-24;
Paper 24 (Sur-Reply) at 5-6*

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

Off-Target Delivery to the Liver Is Potentially Toxic



Paper 24 (Sur-Reply) at 5-6

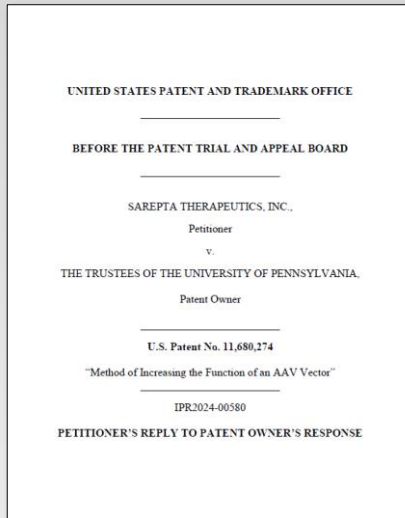
Nor does Petitioner meaningfully rebut Patent Owners' evidence that off-target delivery would increase manufacturing costs and risk life-threatening toxicity. *See* EX2027, ¶¶33-35. Tissue-specific promoters, (Reply, 22), do not mitigate immune responses to the AAV capsid itself, as the virus would still be everywhere, (EX2027, ¶35; EX1063, 214:15-19). Nor does local administration solve the problem, as multiple high-dose injections across the body are required to reach widespread muscle, increasing exposure and triggering immune responses. *See* EX1062, 1, 10 (discussing "the very large mass of target tissue...widely distributed throughout the body with layers of biological barriers").

Petitioner Cannot Change Its Motivation to Combine

“[T]he Board did not err in refusing the reply brief as improper under 37 C.F.R. § 42.23(b) because [Petitioner] relied on an entirely new rationale to explain why [a POSITA] would have been motivated to combine Tsien or Ju with a modification of Zavgorodny.”

–*Intelligent Bio-Sys., Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359, 1370 (Fed. Cir. 2016)

Petitioner Cannot Change Its Motivation to Combine



Paper 20 (Reply) at 5-6;

Paper 18 (POR) at 22-23;

Paper 24 (Sur-Reply) at 7

Additionally, the '772 Publication touts the superior *gene transfer* capability and *transduction* of AAVrh.10 across three tissues of AAVrh.10. EX1007, [0257]; EX1072, ¶¶26, 59-63. And Snowy 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; EX1072, ¶¶26, 59-63. Therefore, a POSA would be motivated to combine these references to improve *transduction* of AAVrh.10. Patent Owners' arguments based on Dr. Samulski's unusual definitions of tropism fail for this additional reason.²

“Preserving Tropism to the Muscle and Lung” Results in Overwhelming Number of Options

Number of Modified Sites	Possible Combinations (Rounded to nearest whole number)
1	764
2	291,732
3	7.399×10^7
4	1.4038×10^{10}
5	2.125×10^{12}

$$\sum_{n=1}^x \left\{ \frac{x!}{(n! * (x - n)!)} * 1.984^n \right\}$$

“Preserving Tropism to the Muscle and Lung” Results in Overwhelming Number of Options



Dr. Samulski

EX1063 at 137:10-138:5;

Paper 24 (Sur-Reply) at 25

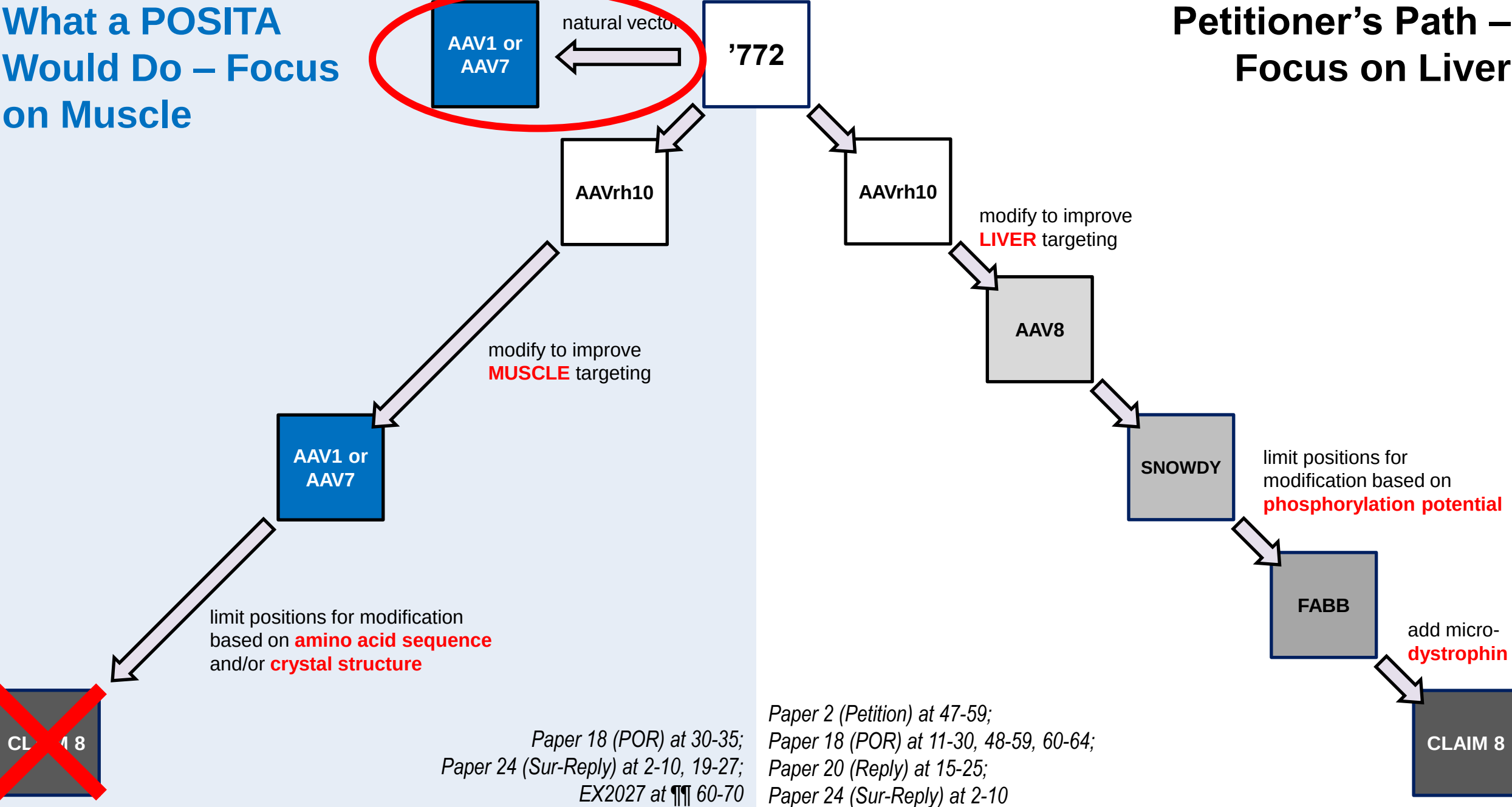
Q. Okay. But to get back to then sort of the state of the art, but you do agree that people at the time were making single amino acid changes in AAV capsid to affect function of the capsid, you and others, right?

A. What we were doing was a procedure we referred to as **wreck and check**. Mutate something and see what the phenotype was, systematically making changes across the AAV genome.

A. Yeah, absolutely, because in the end we narrowed it down to one amino acid. We couldn't have done that without a wreck, check, wreck, check, wreck, check. You had to do five, then you did four, then you did four plus the fifth one missing, then you did three, then you did three with the second and the third one missing. **So by the time you do that, that wreck and check is an exponential how many variations are there.**

What a POSITA
Would Do – Focus
on Muscle

Petitioner’s Path –
Focus on Liver



The Field Used Natural Serotypes



Dr. Samulski

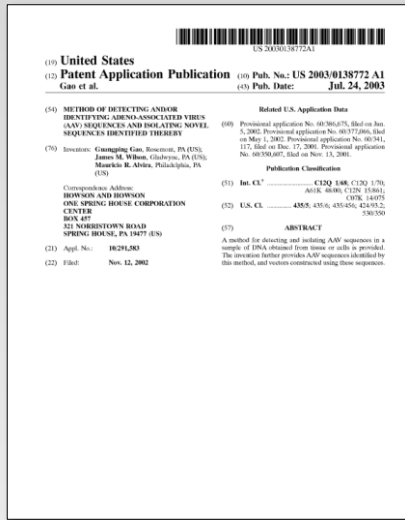
*EX1063 at 130:12-131:21;
Paper 24 (Sur-Reply) at 19-
20*

A. So let me put it in historical perspective. AAV2 is the only serotype that we had sequence information on. In the beginning, it was the first serotype, the one I cloned as a graduate student, and that was the only candidate that was being utilized for gene therapy. It was later determined that in animals, there may be other serotypes that were preferred over to. So anyone stilled in the art quickly switched to other serotypes, like 1 for muscle 8 for liver, so forth and so on.

As a consequence of that, basic science was trying to understand why is 1 different than 2 and that's where we were coming in with basic science approaches to genetically decipher what is responsible. Until then, large domains were being swapped, and large domains basically said something in this region of interstate blah, blah, blah, causes your radios to go out.

What we end up finding out was that systematically taking that approach down to traditional mutagenesis and genetics and crystal structures, that -- it's this tower that's responsible for it. And that was just a foundation that was constantly being laid down. For clinical applications, everybody went from AAV2 serotypes to natural serotypes that had predilection to go into certain cell types, muscle, liver, lung, whatever they were.

'772 Publication Teaches AAV8 for Targeting Liver

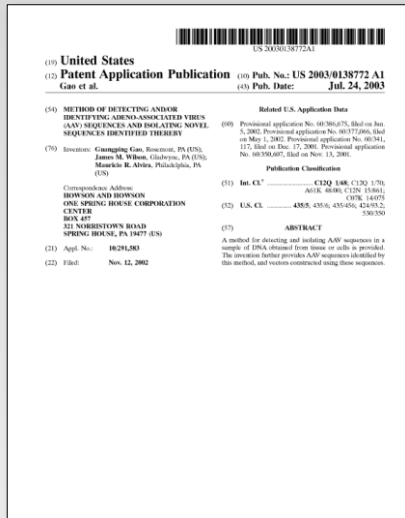


EX1007 at [0250];

Paper 18 (POR) at 14-16

[0250] The tropism of each new vector is favorable for in vivo applications. AAV2/7 vectors appear to transduce skeletal muscle as efficiently as AAV2/1, which is the serotype that confers the highest level of transduction in skeletal muscle of the primate AAVs tested to date [Xiao, W., cited above; Chou (2001), cited above, and Chou (2000), cited above]. Importantly, AAV2/8 provides a substantial advantage over the other serotypes in terms of efficiency of gene transfer to liver that until now has been relatively disappointing in terms of the numbers of hepatocytes stably transduced. AAV2/8 consistently achieved a 10 to 100-fold improvement in gene transfer efficiency as compared to the other vectors. The basis for the improved efficiency of AAV2/8 is unclear, although it presumably is due to uptake via a different receptor that is more active on the basolateral surface of hepatocytes. This improved efficiency will be quite useful in the development of liver-directed gene transfer where the number of transduced cells is critical, such as in urea cycle disorders and familial hypercholesterolemia.

'772 Publication Teaches AAV8 for Targeting Liver



EX1007 at [0246];

Paper 18 (POR) at 14-16

[0246] The amount of vector DNA found in liver 56 days after injection correlated with the levels of transgene expression (See Table 7). For this experiment, a set of probe and primers targeting the SV40 polyA region of the vector genome was used for TaqMan PCR. Values shown are means of three individual animals with standard deviations. The animals were sacrificed at day 56 to harvest liver tissues for DNA extraction. These studies indicate that AAV8 is the most efficient vector for liver-directed gene transfer due to increased numbers of transduced hepatocytes.

TABLE 7

Real Time PCR Analysis for Abundance of
AAV Vectors in nu/nu Mouse
Liver Following Injection of 1×10^{11} Genome Copies of Vector.

AAV vectors/Dose	Genome Copies per Cell
AAV2/1AlbA1AT	0.6 ± 0.36
AAV2AlbA1AT	0.003 ± 0.001
AAV2/5AlbA1AT	0.83 ± 0.64
AAV2/7AlbA1AT	2.2 ± 1.7
AAV2/8AlbA1AT	18 ± 11

'772 Publication Teaches AAV8 for Targeting Liver

TABLE 6

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

*Not determined in this experiment.

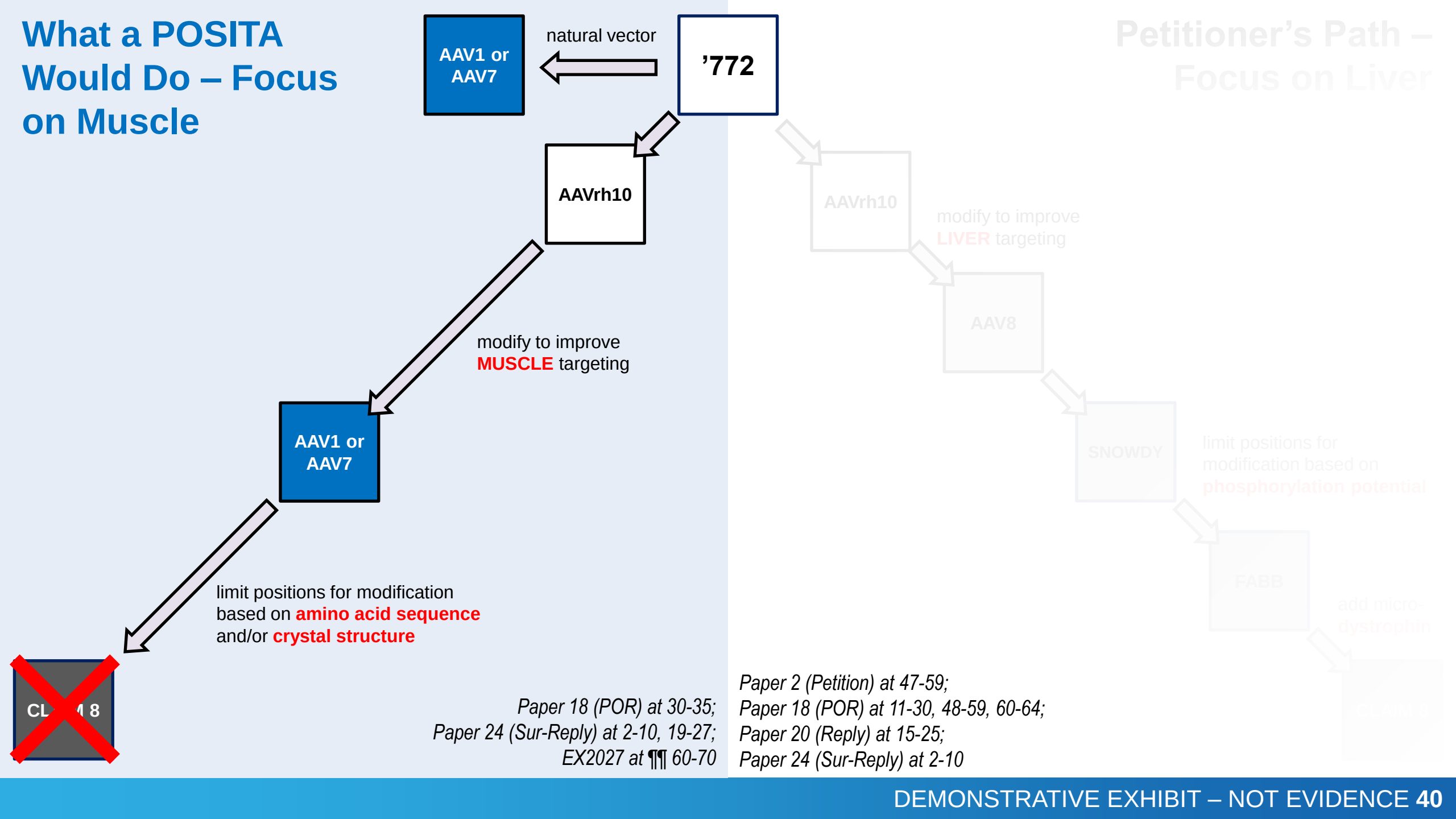
TABLE 8

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

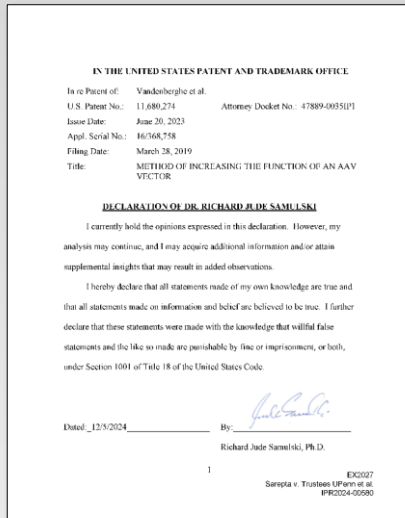
EX1007 at [0244], [0253]; EX2027 at ¶¶ 40-43; Paper 18 (POR) at 14-16

What a POSITA
Would Do – Focus
on Muscle

Petitioner's Path –
Focus on Liver



POSITA Would Start With Muscle-Targeting Vector

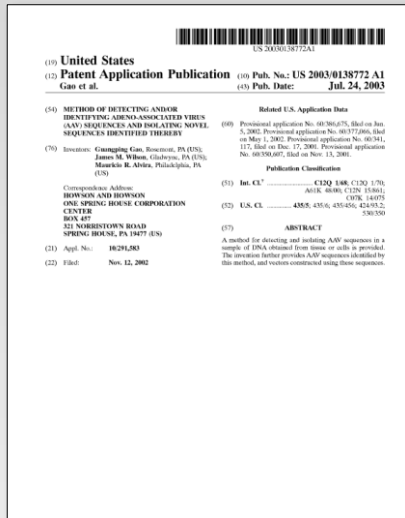


EX2027 at ¶ 62;

*Paper 18 (POR) at 32-34;
Paper 24 (Sur-Reply) at 8-10*

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

To Target Muscle, a POSITA Would Look to AAV7 and AAV1

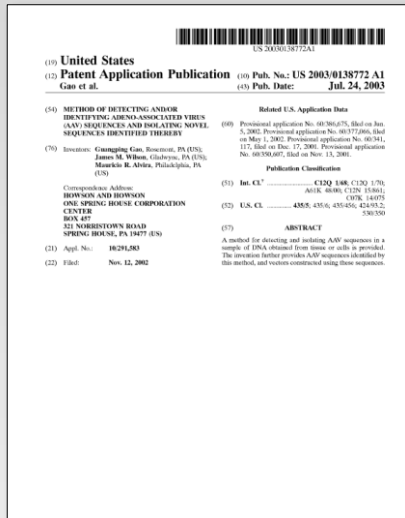


*EX1007 at [0142];
see also id. at [0243], [0251];*

*Paper 18 (POR) at 32-34;
Paper 24 (Sur-Reply) at 9*

[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.

POSITA Would Start With Muscle-Targeting Vector

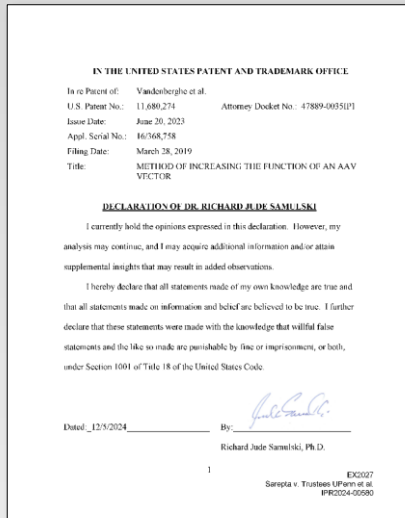


*EX1007 at [0251], [0257];
Paper 18 (POR) at 32-34;
Paper 24 (Sur-Reply) at 9;
see also id. at 8-10, 20*

[0251] Thus, the present invention provides a novel approach for isolating new AAVs based on PCR retrieval of genomic sequences. The amplified sequences were easily incorporated into vectors and tested in animals. The lack of pre-existing immunity to AAV7 and the favorable tropism of the vectors for muscle indicates that AAV7 is suitable for use as a vector in human gene therapy and other in vivo applications. Similarly, the lack of pre-existing immunity to the AAV serotypes of the invention, and their tropisms, renders them useful in delivery of therapeutic molecules and other useful molecules.

[0257] 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.

POSITA Would Start With Muscle-Targeting Vector



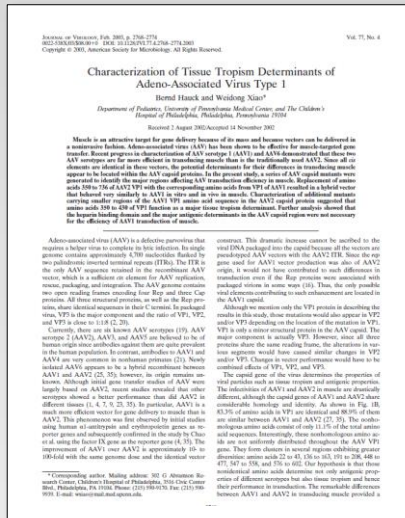
EX2027 at ¶ 63;

*Paper 18 (POR) at 32-34;
Paper 24 (Sur-Reply) at 8-10*

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

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

FOSTA Would Start With Muscle-Targeting Vector



EX1052 at 1;

Paper 18 (POR) at 32-34;

Paper 24 (Sur-Reply) at 8-10

different tissues (1, 4, 7, 9, 23, 35). In particular, AAV1 is a much more efficient vector for gene delivery to muscle than is AAV2. This phenomenon was first observed by initial studies using human α 1-antitrypsin and erythropoietin genes as reporter genes and subsequently confirmed in the study by Chao et al. using the factor IX gene as the reporter gene (4, 35). The improvement of AAV1 over AAV2 is approximately 10- to 100-fold with the same genome dose and the identical vector construct. This dramatic increase cannot be ascribed to the viral DNA packaged into the capsid because all the vectors are pseudotyped AAV vectors with the AAV2 ITR. Since the *rep* gene used for AAV1 vector production was also of AAV2 origin, it would not have contributed to such differences in transduction even if the Rep proteins were associated with packaged virions in some ways (16). Thus, the only possible viral elements contributing to such enhancement are located in the AAV1 capsid.

A POSITA Seeking to Target Muscle Would Use AAV7 or AAV1

TABLE 6

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

*Not determined in this experiment.

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

POSITA Selecting AAVrh10 Would Modify Based On AAV7 or AAV1

```
#=====
#
# Aligned_sequences: 2
# 1: rh10
# 2: AAV7
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 739
# Identity:   655/739 (88.6%)
# Similarity: 692/739 (93.6%)
# Gaps:                   3/739 ( 0.4%)
# Score: 3578.0
#
#=====
```

```
600 SQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQI 649
    :||||||||||||||||||||||||||||||||||||||||||||||
599 NQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQI 648

650 LIKNTVPVADPPTTSIAKLASFITQYSTGQVSVEIEWELQKENS KRWNP 699
    |||||:|.:.:|.||||||||||||||||||||||||||
649 LIKNTVPV PANPPEVTIAKFASFITQYSTGQVSVEIEWELQKENS KRWNP 698

700 EIQYTSNYYKSTNVDFAVNTDGT YSEPRPIGTRYLTRNL 738
    |||||:|.|.|.|||:|.||||||||||||||
699 EIQYTSNFEKQTGVDFAVDSQGVYSEPRPIGTRYLTRNL 737
```

POSITA Selecting AAVrh10 Would Modify Based On AAV7 or AAV1

```
#=====
#
# Aligned_sequences: 2
# 1: rh10
# 2: AAV1
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 738
# Identity: 627/738 (85.0%)
# Similarity: 673/738 (91.2%)
# Gaps: 2/738 ( 0.3%)
# Score: 3449.0
#
#=====
```

```
601 QGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPMLGGFGLKHPPPQIL 650
    .|||||||:|||||||:|||||||:|||||||
599 MGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPMLGGFGLKNPPPQIL 648

651 IKNTPVPADPPTTS AKLASFITQYSTGQVSVEIEWELQKENS KRWNPE 700
    |||||:|..|.|.|||||||
649 IKNTPVPANPPAEIS TKFASFITQYSTGQVSVEIEWELQKENS KRWNPE 698

701 IQYTSNYYKSTNVDFAVNTDGTYSERPIGTRYLTRNL 738
    :|||||.|||.|||.||:|.||:|||||||.
699 VQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL 736
```

POSITA Selecting AAVrh10 Would Modify Based On AAV7 or AAV1

AAV7	PPEVITPAKFASFITQYSTGQVSVEIEWELQKENS	SKRWNPEIQYTSNFEKQTGVDFAVDS	718
rh10	PPTTISPAKLASFITQYSTGQVSVEIEWELQKENS	SKRWNPEIQYTSNYYKSTNVDFAVNT	719
AAV8	PPTTINQSKLNSFITQYSTGQVSVEIEWELQKENS	SKRWNPEIQYTSNYYKSTSVDFAVNT	719
AAV1	PPAETSATKFASFITQYSTGQVSVEIEWELQKENS	SKRWNPEVQYTSNYAKSANVDFTVDN	717
AAV2	PSTTISPAKFASFITQYSTGQVSVEIEWELQKENS	SKRWNPEIQYTSNYNKSVNVDFTVDT	716
AAV3	PPTTISPAKFASFITQYSTGQVSVEIEWELQKENS	SKRWNPEIQYTSNYNKSVNVDFTVDT	717
	* * . : * : ***** : ***** : * . . . * * * : * : .		

Manufacturing, Safety, and Regulatory Issues Motivate a POSITA Against AAVrh10



Dr. Samulski

EX1063 at 106:23-107:23;

Paper 24 (Sur-Reply) at 9-10

Q. Now, you didn't decide here to work with AAV1, which is better in muscle than AAV2, right?

A. At that time, AAV2 was the only virus that the FDA would approve for going into patients. So it was thought and decided that if we did it in 2, we would have a better chance of the FDA accepting the modified 2 versus a completely new AAV serotype.

Q. Right. And just because AAV1 had these superior muscle transduction properties over AAV2, you still chose, for other reasons, to modify AAV2 rather than to work with AAV1, correct?

A. That is correct.

Manufacturing, Safety, and Regulatory Issues Motivate a POSITA Against AAVrh10



Dr. Samulski

EX1063 at 258:9-259:2;

see also id. at 257:21-259:2;

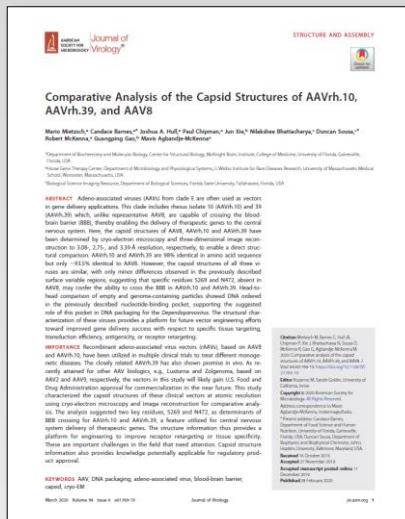
Paper 24 (Sur-Reply) at 9-10

A. So when we say safety, I think I'm going back to historical perspective. AAV2 was the only one in humans. It was the only one that the FDA had to rely on. So there was what we called a safety profile attached to that. Nobody died. And nobody -- so that was like if you're going to choose something to go in, try to choose 2. And if your 2 won't do what you want it to do, then you're stuck trying to use rational design to make 2 potentially successful.

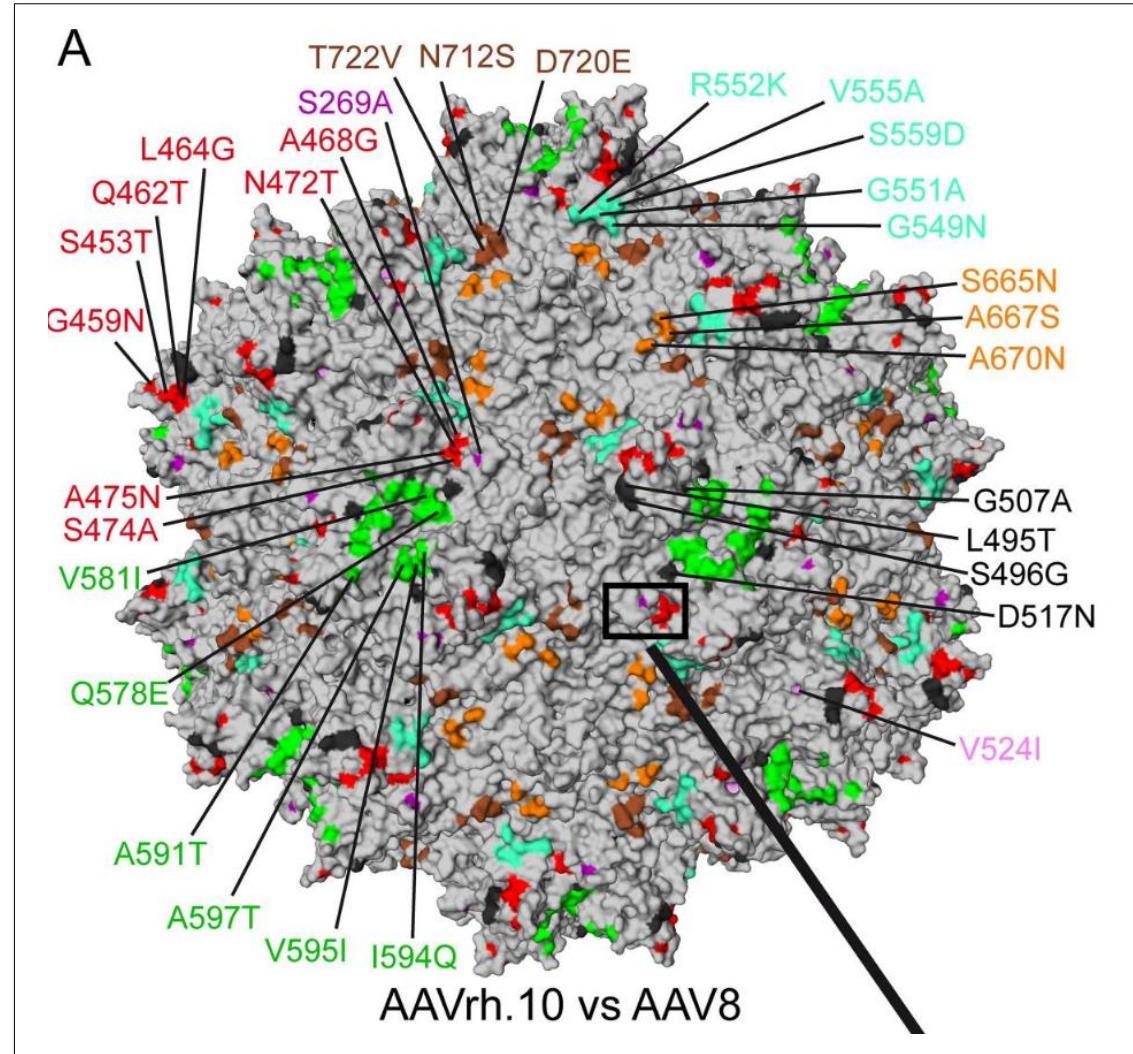
After that, there was an onslaught of forget rational design, just take AAV1, that was Glybera the first drug approved, SMA, which was AAV9, which was another. So people stopped worrying about safety and just starting use you different serotypes.

But we were hamstrung by what the FDA would say they would let go into a human. And I think in order to be fair, the amount of resources it took to do a clinical trial was too expensive to take each one in. So we were trying to attach it to AAV2.

48 Amino Acids Are Different Between AAVrh10 and AAV8

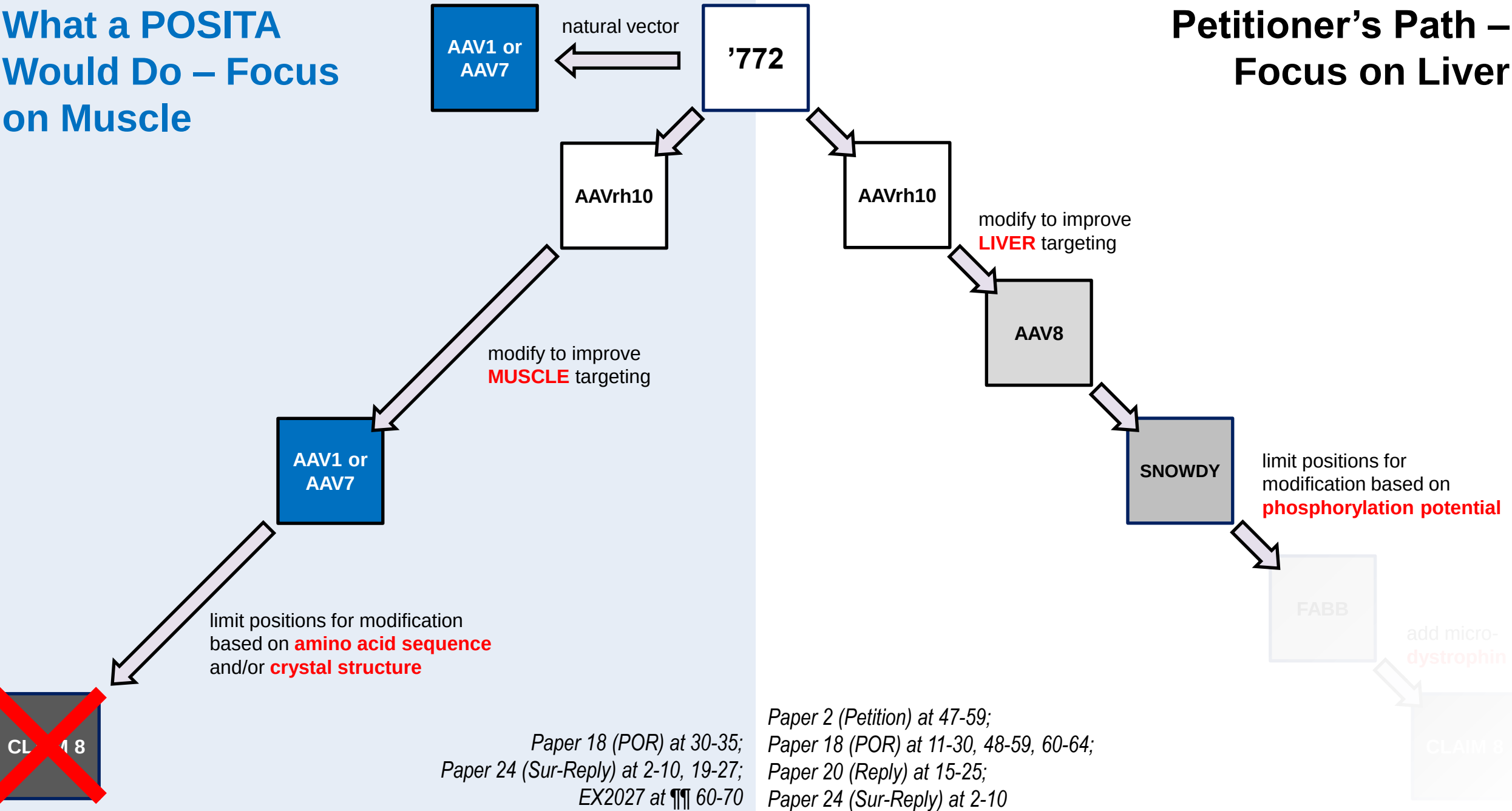


EX2019 at 11;
Paper 18 (POR) at 35-39



What a POSITA
Would Do – Focus
on Muscle

Petitioner’s Path –
Focus on Liver



Snowdy's Work Was Supervised by Dr. Samulski



Dr. Samulski

EX2027 at ¶ 71;

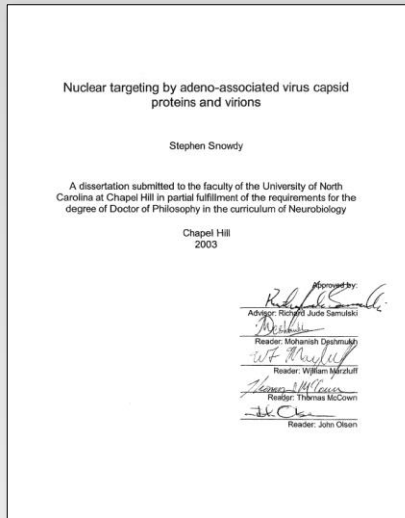
*Paper 18 (POR) at 49-50;
Paper 24 (Sur-Reply) at 10-
12*

71. In Grounds 4 and 6, I understand that Sarepta relies on a section of a dissertation written by one of my former Ph.D. students, Stephen Snowdy, entitled “Nuclear targeting by adeno-associated virus capsid proteins and virions”

(EX1009). Dr. Snowdy was a student in my lab at University of North Carolina Chapel Hill from ~1998-2003, and this work was performed under my supervision.

I therefore have first-hand knowledge of the work described in Dr. Snowdy's dissertation, and can put this work in proper context. At a high level, Snowdy does

Snowdy Demonstrates Unpredictability in the Art



EX1009 at 126;

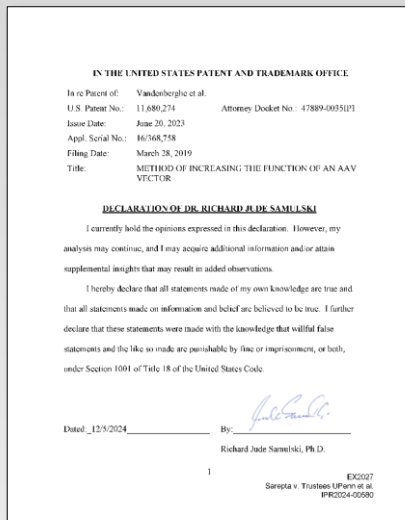
Paper 18 (POR) at 8-9, 55-56;

see also id. at 49-60;

Paper 24 (Sur-Reply) at 10-12

All of the mutants were capable of forming virus at normal titers. We infected cultured HeLa cells with 500 particles/cell of each of the 6 mutants to establish whether the potential phosphorylation sites are important in the virus' ability to deliver its genome to the target cell during an infection. Infected cells were assayed for green fluorescence by fluorescent microscopy and the results are shown in figure 23. AAV2-eGFP made from wild-type capsids was used as a positive control. The S148A, S148D, T159A, and T159D mutations had no effect, or little effect, on the virus' ability to infect the HeLa cells and to deliver the eGFP gene carried as their cargo. However, the S148 and S148D had large effects on the infectivity of the virus. 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 (Figure 23).

Snowdy Demonstrates Unpredictability in the Art



EX2027 at ¶ 78;

Paper 18 (POR) at 8-9, 55-56;

see also id. at 49-60;

Paper 24 (Sur-Reply) at 10-12

78. Additionally, Sarepta and Dr. Schaffer ignore that, in addition to the serine at position 181 that they focus on, Snowdy tested two other amino acids at putative phosphorylation sites—a threonine at position 159 and a different serine at position 148. EX1009, 125-126. Notably, mutating these two amino acids “had no effect, or little effect on the virus’ ability to infect the HeLa cells and to deliver the eGFP gene carried as their cargo.”¹ EX1009, 126. Therefore, even within Snowdy, mutating a serine residue had significantly different results. This experiment demonstrates that removing a phosphorylatable amino acid would not have been expected to have any impact on the function of the AAV, and certainly would not have been expected to impact tissue tropism.

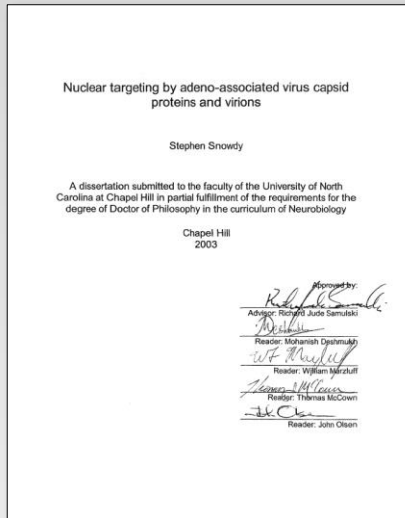
Snowdy's Results Were Inconsistent with Jans & Jans

All of the mutants were capable of forming virus at normal titers. We infected cultured HeLa cells with 500 particles/cell of each of the 6 mutants to establish whether the potential phosphorylation sites are important in the virus' ability to deliver its genome to the target cell during an infection. Infected cells were assayed for green fluorescence by fluorescent microscopy and the results are shown in figure 23. AAV2-eGFP made from wild-type capsids was used as a positive control. The S148A, S148D, T159A, and T159D mutations had no effect, or little effect, on the virus' ability to infect the HeLa cells and to deliver the eGFP gene carried as their cargo. However, the S148 and S148D had large effects on the infectivity of the virus. 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 (Figure 23).

Nuclear import of SV40 large T-antigen (T-ag) is completely dependent on the T-ag nuclear localization sequence (amino acids 126–132), but the rate of nuclear import is greatly increased by the additional presence of the N-terminal flanking sequence (amino acids 111–125), which includes a site for casein kinase II (CKII) (Ser^{111/112}). The role of this site was investigated by site-directed mutagenesis and analysis of the effects on phosphorylation and on nuclear import at the single cell level using microinjection and quantitative fluorescent techniques. Removal of the CKII site either by substitution of S^{111/112} by nonphosphorylatable amino acid residues, or mutation of the Asp-Asp-Glu^{113/115} CKII recognition sequence to Asn-Asn-Gln, resulted in nuclear import rates less than 4% wild type, demonstrating that the CKII site was responsible for the enhancement of nuclear import conferred by T-ag amino acids 111–125. The substitution of Asp for Ser¹¹², the serine preferentially phosphorylated by purified CKII, enhanced nuclear import to about 45% maximal wild type rates. It is concluded that negative charge at the CKII site, normally provided by phosphorylation, is mechanistically important for nuclear transport enhancement. There was no evidence of a direct role for phosphatases or dephosphorylation in the transport process.

Compare EX1009 at 126 with EX1017 at 2; Paper 18 (POR) at 55-57; Paper 24 (Sur-Reply) at 24-25

Snowdy's Results Were Inconsistent with Jans & Jans

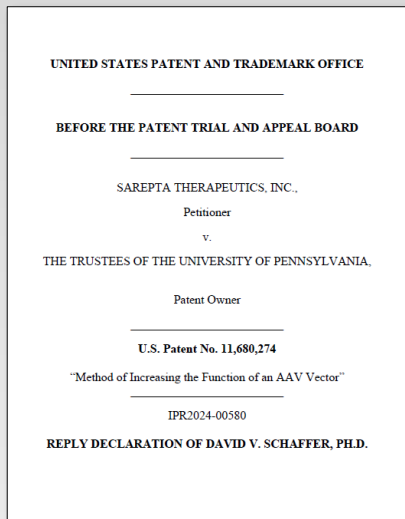


EX1009 at 128-129;

Paper 18 (POR) at 53, 59

We present preliminary evidence that one of the possible phosphorylation sites, serine 181, may play some role in AAV2's ability to infect its target cell. When S181 was mutated to a nonphosphorylatable alanine, the result was a virus that transduces target cells with greater efficiency than did the wild-type virus. When S181 was mutated to a residue that simulates phosphorylation, the result was a virus that transduced the target cells very poorly. 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. It may be premature in the field's development to assume that a given phosphorylation status will have the same effect on all NLSs, as there is insufficient evidence in the literature to support this assumption. This issue will be better understood with future studies on other nuclear localization signals.

Petitioner's Expert Admits Snowdy's Inconsistent Results

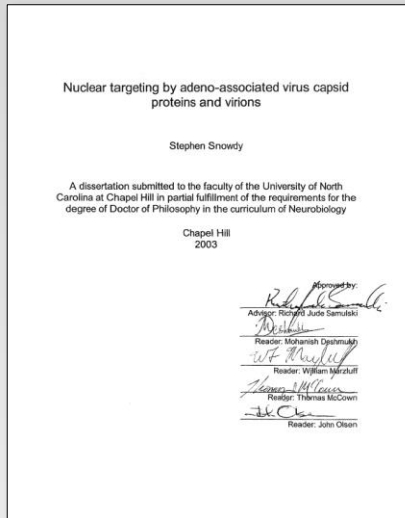


EX1072 at ¶ 461;

Paper 24 (Sur-Reply) at 24-25

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.

Snowdy Is Irrelevant to Tissue Targeting



EX1009 at 126;

Paper 18 (POR) at 8-9, 55-56;

see also id. at 49-60;

Paper 24 (Sur-Reply) at 10-12

All of the mutants were capable of forming virus at normal titers. We infected cultured HeLa cells with 500 particles/cell of each of the 6 mutants to establish whether the potential phosphorylation sites are important in the virus' ability to deliver its genome to the target cell during an infection. Infected cells were assayed for green fluorescence by fluorescent microscopy and the results are shown in figure 23. AAV2-eGFP made from wild-type capsids was used as a positive control. The S148A, S148D, T159A, and T159D mutations had no effect, or little effect, on the virus' ability to infect the HeLa cells and to deliver the eGFP gene carried as their cargo. However, the S148 and S148D had large effects on the infectivity of the virus. 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 (Figure 23).

Snowdy Is Irrelevant to Tissue Targeting



Dr. Samulski

*EX1063 at 55:19-24;
id. at 93:21-25;*

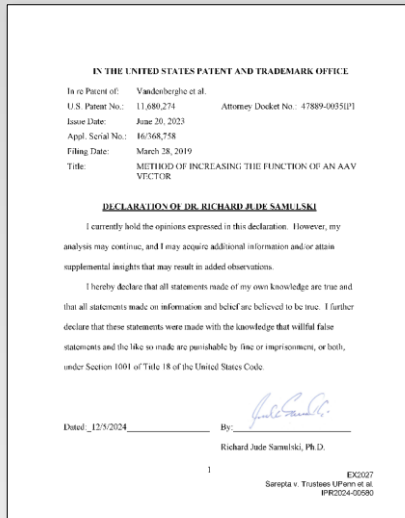
*Paper 18 (POR) at 8-9, 55-56; see also id. at 49-60;
Paper 24 (Sur-Reply) at 11;
see also id. at 10-12*

Q. You're not suggesting that cell culture studies can't provide information that could be related to tropism, right?

A. I am saying definitively that cell culture cannot define things that are related to tropism.

culture. We've been burnt enough where if we made this assumption, it did the same thing, and it didn't work, it would be simple like, okay, we study something unique to this cell type and that's not explorable to everything. It's restricted to this cell type.

Snowdy Never Proved Actual Phosphorylation Site

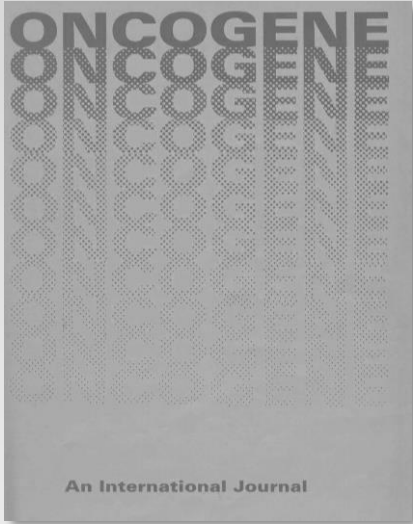


EX2027 at ¶ 74;

Paper 18 (POR) at 53

74. The study in Chapter IV of Snowdy was designed to determine whether putative (potential) phosphorylation sites in the vicinity of a putative nuclear localization signal in AAV2 affected intracellular infectivity. Critically, Snowdy never showed that the putative nuclear localization signal was in fact a nuclear localization signal, or that the putative phosphorylation sites were in fact phosphorylated on AAV2. Just because an amino acid could potentially be phosphorylated does not in and of itself make it a phosphorylation site. Indeed, Snowdy found that mutating two of three potential phosphorylatable residues had no impact, and never provided proof that any of the three were or were not phosphorylation sites. Therefore, any conclusions drawn are, in my opinion, too theoretical to be relied upon as determinative of the structure-function relationship of this particular site in AAV2. In any event, Snowdy's results and conclusion are completely irrelevant to tropism. There is no evidence that I am aware of, in Snowdy that suggests that removing a phosphorylation site at a nuclear localization signal would in any way change or improve tissue tropism.

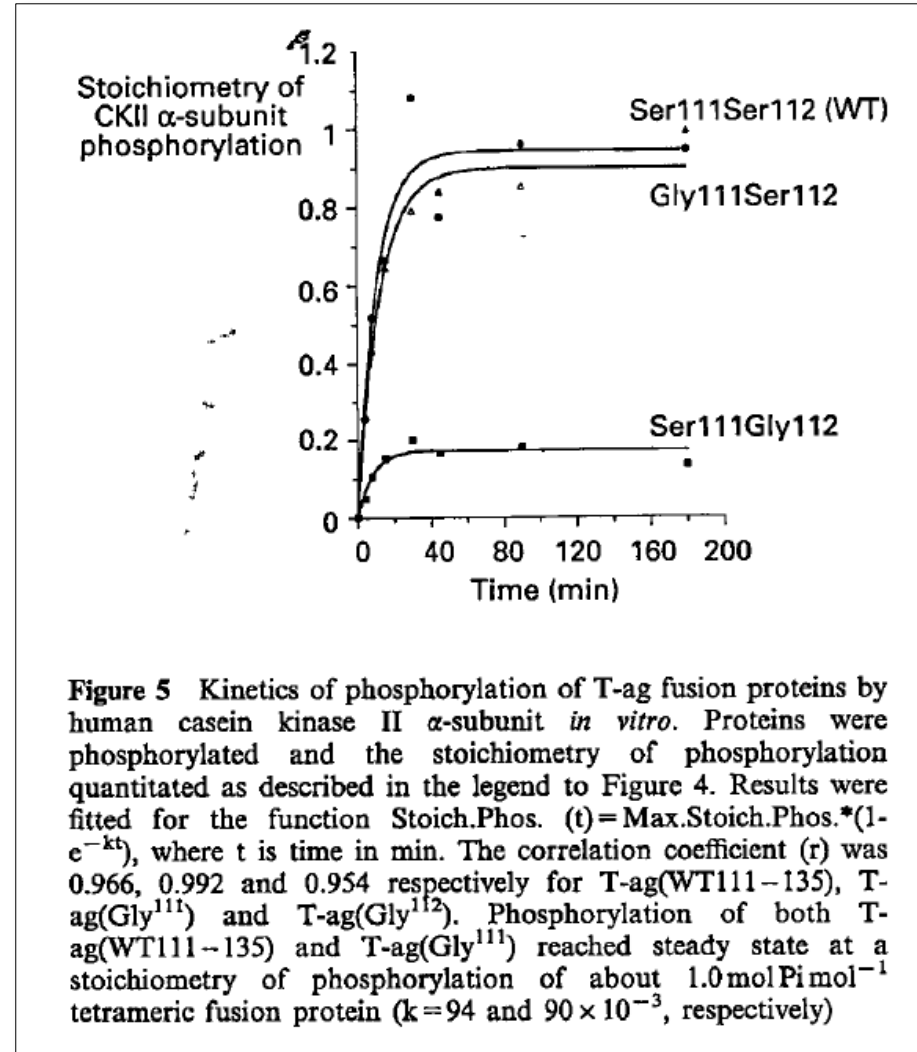
Jans & Jans Showed Unpredictable Results



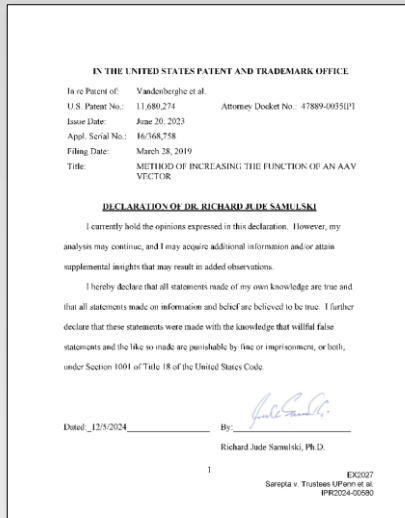
EX1017 at 6 (Fig. 5);

Paper 18 (POR) at 57-58;

Paper 24 (Sur-Reply) at 24-25



Jans & Jans Showed Unpredictable Results



EX2027 at ¶ 84;

Paper 24 (Sur-Reply) at 24-25

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

Extraordinarily Skilled Artisans Collaborated for '126 Provisional

“A [POSITA] is also presumed to be one who thinks along the line of conventional wisdom in the art and is not one who undertakes to innovate, whether by patient, and often expensive, systematic research or by extraordinary insights[.]”

– See *Standard Oil Co. v. Am. Cyanamid Co.*, 774 F.2d 448, 454 (Fed. Cir. 1985)

Extraordinarily Skilled Artisans Collaborated for '126 Provisional

US Patent and Trademark Office
Docket No. 5470-427PR PATENT
COVER SHEET FOR FILING PROVISIONAL
PATENT APPLICATION (37 CFR §1.510(i)(1))
Commissioner for Patents Date: December 15, 2004
P.O. Box 1450
Alexandria, VA 22315-1450
This is a request for filing a PROVISIONAL PATENT APPLICATION under 37 C.F.R.
§1.53(c).

Docket No.	5470-427PR
Type of patent application made	
File No.	

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Name: Richard Jude Samulski
Address: Chapel Hill, NC

TITLE OF THE INVENTION (250 characters maximum)
CHIMERIC VECTORS

EX1059 at 1;
Paper 24 (Sur-Reply) at 16-17

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Extraordinarily Skilled Artisans Ahead of the Field



Dr. Samulski

EX1063 at 283:18-21;

id. at 126:25-127:6;

Paper 24 (Sur-Reply) at 18-19

Q. Sure. Was any -- to your knowledge, was anyone else doing this sort of rational design that's described in this application at this time in 2005?

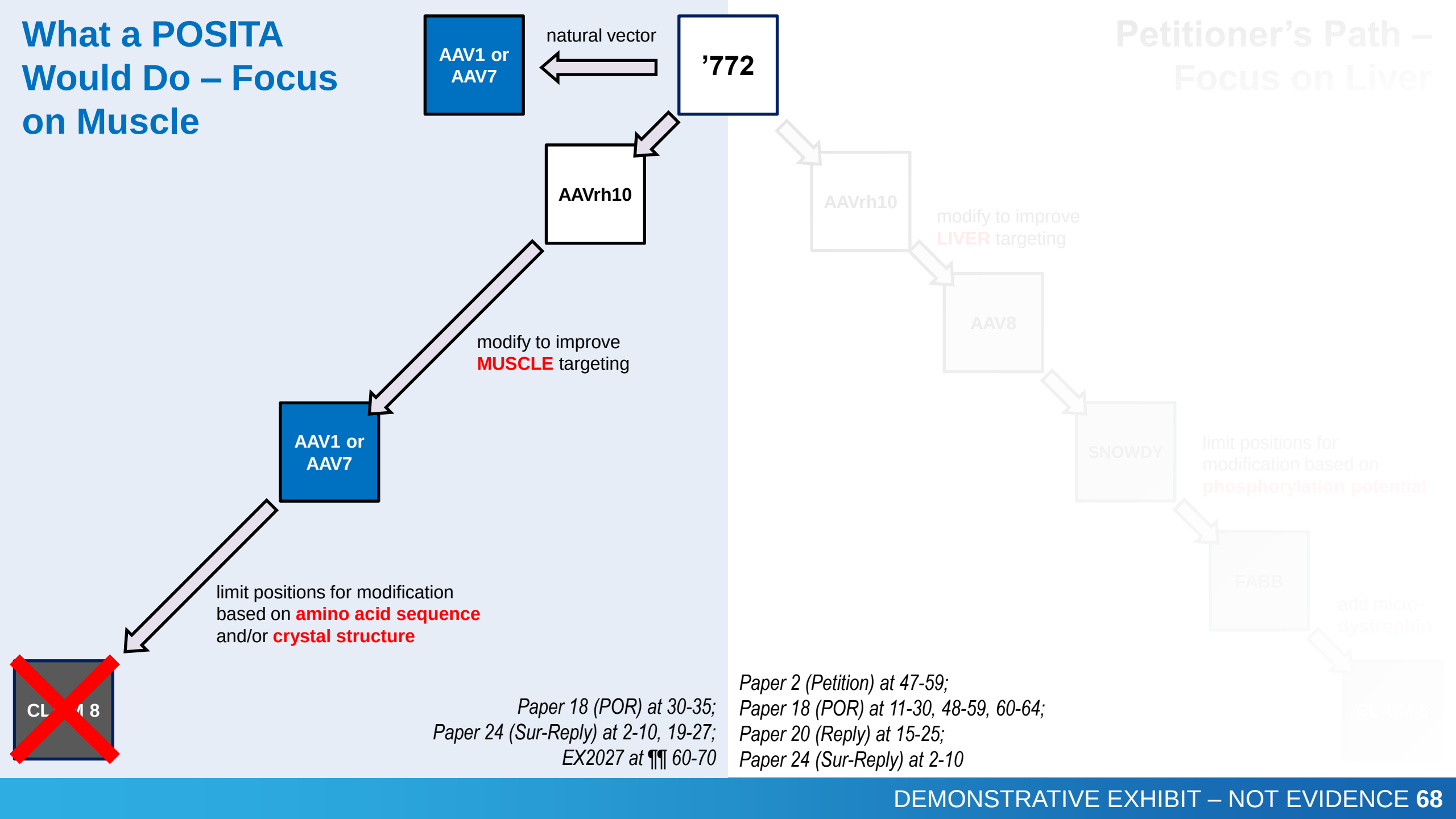
A. I think we were the lead in this and essentially all the other efforts that were going on, which meant we had like a three or four-year head start over everybody else. Once these were published, I think it was fairly straightforward that people can say oh, I see what he's doing. We should start doing that.

Q. Okay. Well, someone else might be able to use a different strategy to narrow down differences between two serotypes to decide which amino acids to test, right?

A. In 2004 and '5, I think we were the cutting edge, because everybody else was doing fragment swapping.

What a POSITA
Would Do – Focus
on Muscle

Petitioner's Path –
Focus on Liver



'126 Provisional Shows Desirable to Confer Muscle Targeting of AAV1, -6, -7 onto AAV2

USPTO Docket No. 6470-427PB PATENT
COVER SHEET FOR FILING PROVISIONAL
PATENT APPLICATION (37 CFR § 1.510(i)(1))
Date: December 15, 2004
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22315-1450
This is a request for filing a PROVISIONAL PATENT APPLICATION under 37 C.F.R. § 1.53(c).
Docket No. 6470-427PB
Type of filing (original or continuation)
Title of the Invention (250 characters maximum)
CHIMERIC VECTORS
INVENTOR/ASSIGNEE
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Name: Joseph E. Rabinowitz
Address: Chapel Hill, NC
Name: Richard Jude Samulski
Address: Chapel Hill, NC
TITLE OF THE INVENTION (250 characters maximum)
CHIMERIC VECTORS
EXHIBIT 1000, page 1

EX1059 at 4;

Paper 24 (Sur-Reply) at 19-20

AAV serotype 2 (AAV2) is the best studied of the AAV isolates. Over the past decade, inroads have been made in the evaluation of the tissue tropism of alternative AAV serotypes. These studies have shown that distinct AAV serotypes may be better suited for particular applications. In this regard, serotypes 1, 6 and 7 are the most promising for delivery to skeletal muscle. As compared with AAV2, AAV1 can be administered at lower dosages (*i.e.*, fewer particles) and can express the transgene at earlier time points and at higher levels of expression. Additional concerns relate to re-administration issues and immune responses.

The purification schemes of AAV2 are well defined. Less streamlined are the purification parameters for serotypes 1, 6, and 7. It would be desirable to engineer a variant of AAV2 that exhibits the enhanced muscle tropism of AAV1, 6 and 7 but still maintains its ease of purification.

AAV2 Was Only AAV Allowed in Clinical Trials



Dr. Samulski

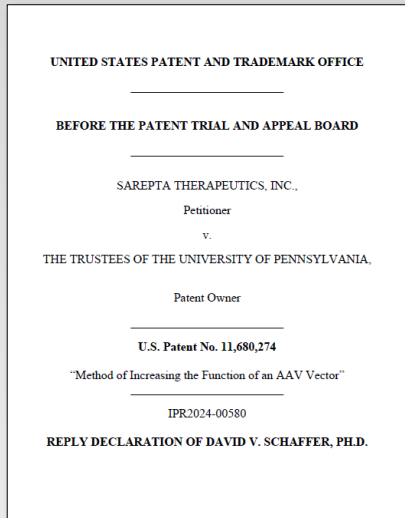
EX1063, 106:23-107:5;

Paper 24 (Sur-Reply) at 19-
20

Q. Now, you didn't decide here to work with AAV1, which is better in muscle than AAV2, right?

A. At that time, AAV2 was the only virus that the FDA would approve for going into patients. So it was thought and decided that if we did it in 2, we would have a better chance of the FDA accepting the modified 2 versus a completely new AAV serotype.

AAV1 and AAV7 Were Best In Muscle; AAV8 Was Inferior

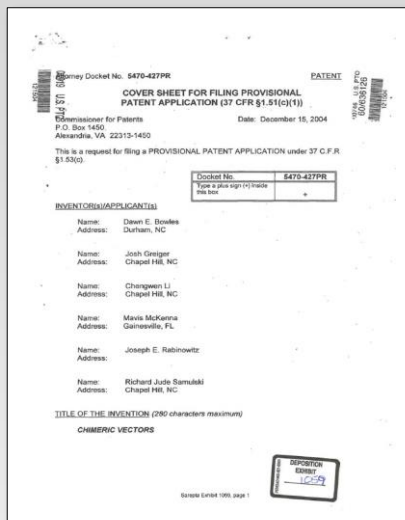


EX1072 at ¶ 129;

Paper 24 (Sur-Reply) at 19-20

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.

'126 Provisional Taught Using Amino Acids Distinct From AAV8



EX1059 at 5 (Fig. 1A);

Paper 24 (Sur-Reply) at 20-21

Figure 1A. Rationale of the triage approach. Four AAV serotypes (AAV1, AAV2, AAV7, and AAV8) were subjected to a multiple amino acid alignment using the Align program in the Vector NTI software suite. Amino acids similar in AAV1 and AAV7 and distinct from AAV2 and AAV8 or positions that contained amino acids that were different in the 4 serotypes were pinpointed as potential candidates. This led to the 36 potential candidates to engineer into AAV2. Elimination of amino acids that could not be modeled onto the crystal structure and molecular modeling of candidates onto the surface of the capsid led to the testing of the amino acids shown in **Figure 1B.**

'126 Provisional Identified 83 Amino Acids



Dr. Samulski

EX1063 at 113:6-17;

*Paper 24 (Sur-Reply) at 21-
22*

Q. Again, it's a little bit hard to read, so I'm not going to ask you to read all the stuff that's blacked out, I promise. But the heading says 36 candidates based on triage and then (versus 83 from AAV1 to 2 alignment). Do you see that?

A. I do see that.

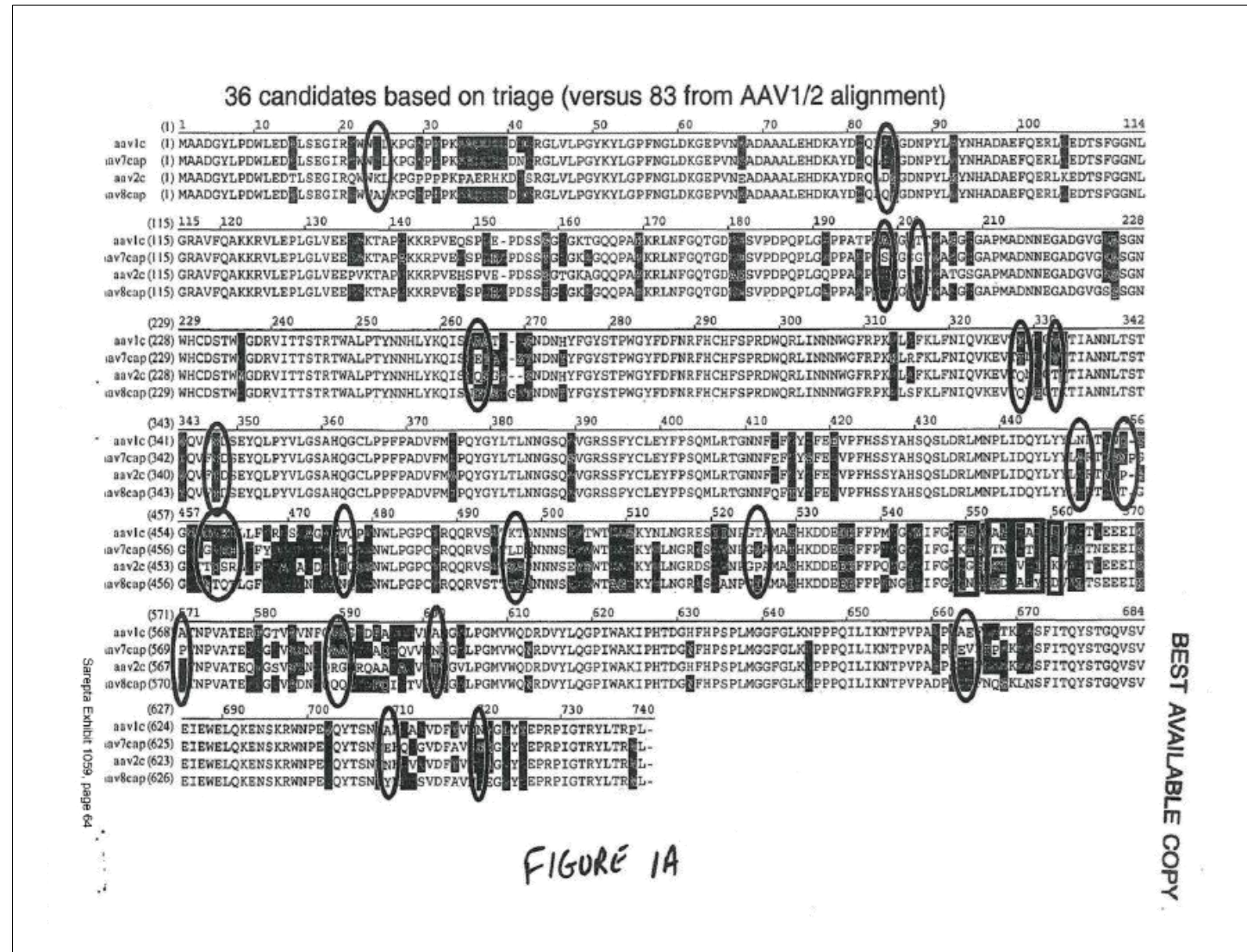
Q. So they're saying here that the alignment showed 83 positions at which the sequences deferred, right?

A. The alignment infers that 83 were different between AAV1 and 2 by the alignment.

'126 Provisional Identified 83 Amino Acids

Exhibit 1059 at 64 (Fig. 1A);
Paper 24 (Sur-Reply) at 21-22

Exhibit 1059 at 64 (Fig. 1A);
Paper 24 (Sur-Reply) at 21-22



'126 Provisional Narrowed 83 to 36



Dr. Samulski

EX1063 at 115:3-20;

*Paper 24 (Sur-Reply) at 21-
22*

Q. Right, okay. So you analyzed the sequence alignments, and, again, we're just talking about the first step. I agree we'll get to the crystal structure and other things. But just in terms of this step of sequence alignment, okay, you analyzed the sequence alignments, and out of the 83 places where AAV1 and AAV2 differ, you selected 36 -- based on considerations such as you mentioned of would it have been a conservative substitution, for example, if you were to make a change versus a nonconservative, correct?

A. Correct.

'126 Provisional Narrowed 36 to 12 using Crystal Structure



Dr. Samulski

EX1063 at 115:14-117:13;

*Paper 24 (Sur-Reply) at 22-
23*

Q. Now, in fact, since we're right there, let's just look at Figure 1B, which is right next door, and it says exactly what you were just beginning to tell us about, which is that it was then reduced to 12 candidates based on amino acid and crystal structure analysis, correct?

A. Correct.

Q. So you used the crystal structure to reduce further the number of positions that you decided you would mutate here, correct?

A. Yeah, so let me clarify. Mavis used the crystal structure. She was the only one qualified to do that analysis.

Q. This initial pool of candidate positions was narrowed considerably based on the 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 biologies of virus.

Do you see that?

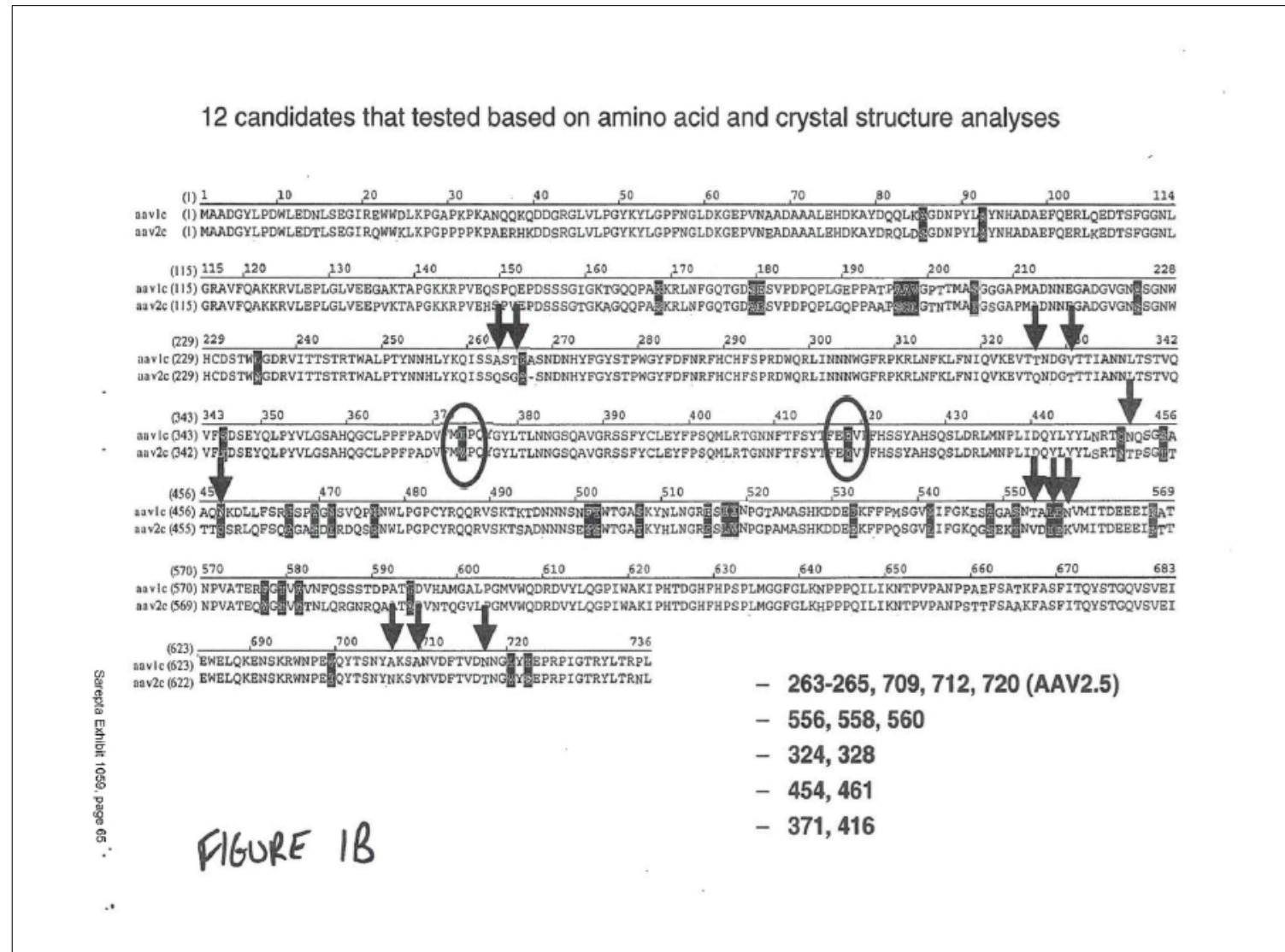
A. Correct.

'126 Provisional Narrowed 36 to 12 using Crystal Structure

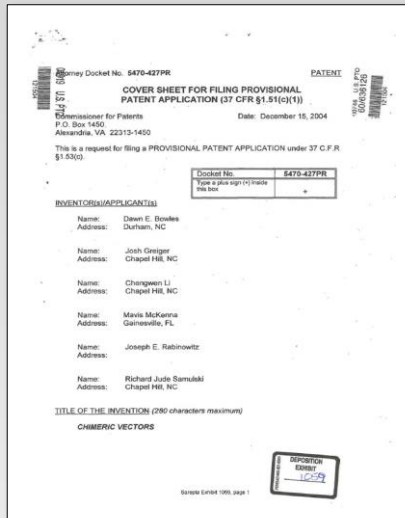
US Patent and Trademark Office
Cover Sheet for Filing Provisional Patent Application (37 CFR §1.510(i))
Date: December 15, 2004
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22315-1450
This is a request for filing a PROVISIONAL PATENT APPLICATION under 37 C.F.R. §1.53(c).
Docket No. 6470-427PB
Type of filing (original/continuation/other):
INVENTOR(S)/APPLICANT(S)
Name: Dawn E. Bowles
Address: Durham, NC
Name: Josh Greiger
Address: Chapel Hill, NC
Name: Changwen Li
Address: Chapel Hill, NC
Name: Maria McKenna
Address: Gainesville, FL
Name: Joseph E. Rabinowitz
Address: Chapel Hill, NC
Name: Richard Jude Samulski
Address: Chapel Hill, NC
TITLE OF THE INVENTION (250 characters maximum)
CHIMERIC VECTORS
Exhibit 1059, page 1

EX1059 at 65 (Fig. 1B);

Paper 24 (Sur-Reply) at 22-23



'126 Provisional Narrowed 36 to 12 using Crystal Structure



USPTO Cover Sheet for Filing Provisional Patent Application (37 CFR § 1.510(i)(1))

Patent Docket No. 5470-427PR PATENT

COVER SHEET FOR FILING PROVISIONAL
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TITLE OF THE INVENTION (250 characters maximum)

CHIMERIC VECTORS

EX-1059

EX1059 at 44:26-27;

Paper 24 (Sur-Reply) at 22-23

Alignments were generated of these very similar proteins from the different serotypes. Amino acids in common between AAV1 and AAV7 and distinct from AAV2 and AAV8 were identified. 36 amino acid candidates were identified via this triage approach (Figure 1A). Based on modeling of the crystal structure, we choose the 12 best candidates to test for enhanced muscle transduction (Figure 1B).

No Candidates Near Position 665



Dr. Samulski

EX1063 at 279:17-280:4;

*Paper 24 (Sur-Reply) at 22-
23*

Q. And in Figure 1B, I see there are -- it mentions 12 candidates. What are the arrows in this figure describing. There's arrows, do you see those?

A. Yes.

Q. Are those the 12 candidates?

A. Those are 12 candidates.

Q. Okay. And is there an arrow at position 665?

A. No.

Q. Is there an arrow anywhere within 50 amino acids of position 665?

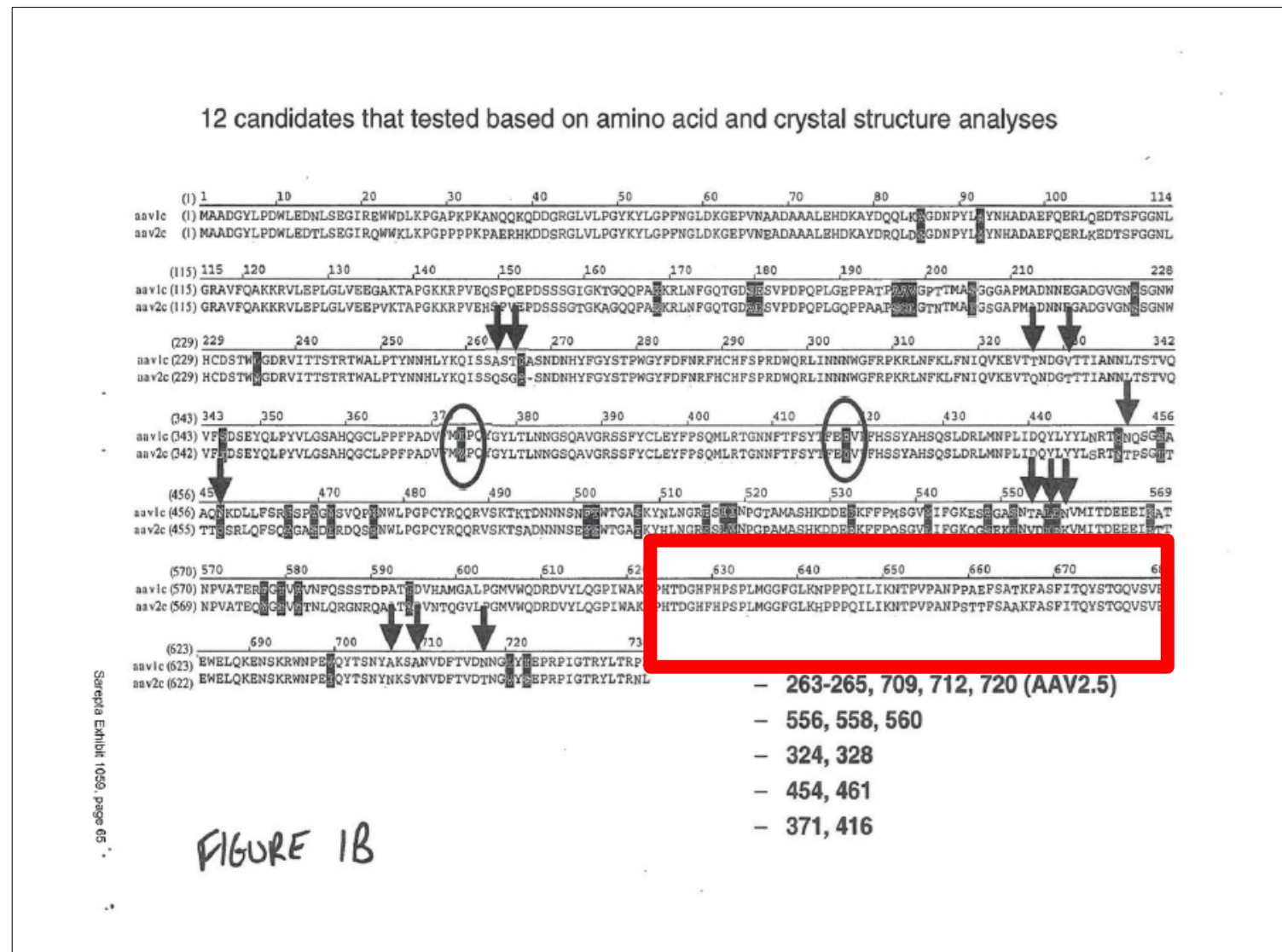
A. I'd have to count those, but I don't think there's anything within proximity.

No Candidates Near Position 665

US Patent and Trademark Office
Cover Sheet for Filing Provisional Patent Application (37 CFR § 1.516(i)(1))
Date: December 18, 2004
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Type of publication (circle one)
INVENTOR(S) (APPLICANT(S))
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TITLE OF THE INVENTION (250 characters maximum)
CHIMERIC VECTORS
Exhibit 1059, page 1

EX1059 at 65 (Fig. 1B);

Paper 24 (Sur-Reply) at 22-23



'126 Provisional Shows Unpredictability of the Field

US Patent and Trademark Office
Cover Sheet for Filing Provisional
Patent Application (37 CFR §1.510(i))
Date: December 15, 2004
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This is a request for filing a PROVISIONAL PATENT APPLICATION under 37 C.F.R. §1.53(c).
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TITLE OF THE INVENTION (250 characters maximum)
CHIMERIC VECTORS
EX-1059

EX1059 at 45:10-19;

Paper 24 (Sur-Reply) at 23

To investigate whether all 5 amino acids were necessary for this enhanced muscle tropism we made the 263, 265 variants and the 709, 712, 720 variants. These were tested in similar imaging experiments (**Figure 4**). The 709, 712, 720 variant exhibited a muscle transduction similar to AAV2, while the 263, 265 variant exhibited an enhanced muscle transduction similar to what was observed with the 2.5 variant containing all 5 amino acid changes. A 263 and a separate 265 variant were constructed and tested in similar experiments (**Figure 5**). These experiments revealed that the amino acid mostly responsible for enhanced AAV1 transduction is located at the 265 position. This is essentially an insertion of a threonine in the AAV2 capsid sequence.

Wu 2000 Does Not Teach Single Amino Acid Substitution

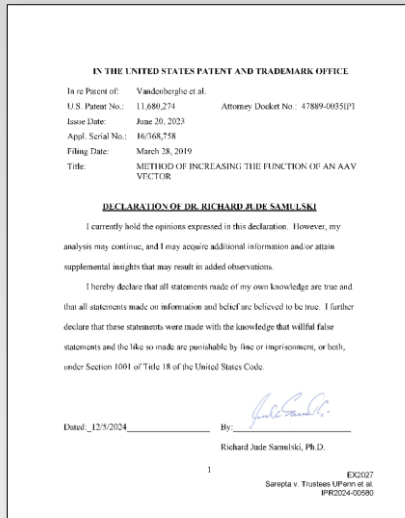


EX1032 at 4-5;
Paper 18 (POR) at 18-19

TABLE 1. Summary of all mutants

Mutant ^a	Type ^b	Amino acid positions ^b	Class	Phenotype ^c
<i>mut1</i> ¹	Ala sub	9-13 DWLED-AWLAA	1	wt
<i>mut29</i> ¹	Ala sub	389 R-A	1	wt
<i>mut30</i> ²	Ala sub	415-419 FEDVP-FAAVP	2a	pd, hep ⁺
<i>mut31</i> ⁴	Ala sub	432 R-A	4c	ni, empty particle
<i>mut32</i> ²	Ala sub	454-456 TTT-AAA	1	wt
<i>mut33</i> ²	Ala sub	469-472 DIRD-AIAA	3a	hs
<i>mut34</i> ²	Ala sub	490-494 KTSAD-ATSAA	2a	pd, hep ⁺
<i>mut35</i> ²	Ala ins	509 AAAA	3b	cs, hep ⁻ , surface
<i>mut36</i> ¹	Ala sub	513-517 RDSLVAASLV	2a	pd, hep ⁺
<i>mut37</i> ²	Ala sub	527-532 KDDEEK-AAAAA	4a	ni, full particle
<i>mut38</i> ²	Ala sub	547-551 SEKTN-SAATN	1	wt
<i>mut39</i> ²	Ala sub	553-557 DIEKV-AIAAV	2b	pd, unstable capsid
<i>mut40</i> ²	Ala sub	561-565 DEEEI-AAAAI	4d	ni, hep ⁻ , full particle, surface
<i>mut41</i> ²	Ala sub	585-588 RGNR-AGAA	2c	pd, hep ⁻ , surface
<i>mut42</i> ²	Ala sub	607-611 QDRDV-QAAAV	4b	ni, no capsid
<i>mut43</i> ²	Ala sub	624-628 TDGHF-TAGAF	1	wt
<i>mut44</i> ¹	Ala sub	637-641 FGLKH-FGLAA	1	wt
<i>mut45</i> ²	Ala sub	665 K-A	1	wt
<i>mut46</i> ²	Ala sub	681-683 EIE-AAA	4b	ni, no capsid
<i>mut47</i> ²	Ala sub	689-693 ENSKR-ASSAA	4b	ni, no capsid
<i>mut48</i> ¹	Ala sub	706 K-A	2a	pd, hep ⁺

Wu 2000 Does Not Teach Single Amino Acid Substitution



EX2027 at ¶ 49;

Paper 18 (POR) at 18-19

49. The Wu paper that Sarepta cites (Pet., 27), in my opinion, stands for the opposite proposition. For example, Wu makes a number of mutations, including several single alanine wreck and check substitutions (mutant numbers 29, 31, 45, and 48). EX1032, 5. Two of those mutants (mut29 and mut45) were shown to have no impact on infectivity. EX1032, 5 (showing mutants 29 and 45 are Class 1); EX1032, 4 (“Class 1 contained mutants that have an infectious titer similar to the wt titer.”). The other two mutants (mut31 and mut48) formed a capsid but were found to have reduced or abolished infectivity. EX1032, 5 (showing mut31 was class 4c, non-infectious with an “empty particle” and mut48 was class 2a); EX1032, 4 (“Class 2 contained partially defective mutants with infectious titers 2 to 3 logs lower than the wt titer”), (“This meant that mut31 formed intact virus particles that were empty.”). Nothing in Wu suggests that any of these four amino acid substitutions could potentially change the tropism of the AAV. Nor am I aware of any other paper suggesting that.

Wu 2000 Shows Unpredictability of the Field



Dr. Schaffer

*EX2030 at 158:5-19;
see also id. at 157:10-
161:11;*

Paper 18 (POR) at 18-19

If you look at -- if you look at Mutant 29,
there's -- only a single amino acid changed, an arginine
to an alanine?

A. Yes.

Q. And that substitution resulted in wild type
phenotype; right?

A. Yes. That's what the table says.

Q. And Mutant 31, again, an arginine was chained
to an alanine; right?

A. That's correct.

Q. But for that mutation, there was not an
infectious virus formed; right?

A. NI, I believe, stands for noninfectious.

Empty particle speaks for itself. It would a particle
without a genome inside.

Wu 2006 Tested All Mutations Due to Unpredictability of Art



EX1060 at 2 (Table 1);
per 24 (Sur-Reply) at 25-26

Virus	Titer (v.g./ml) ^a
AAV1	$6.44\text{E} \times 10^{11}$
AAV1-L129F.....	$1.91\text{E} \times 10^{12}$
AAV1-E418D.....	$7.04\text{E} \times 10^{11}$
AAV1-E531K.....	$4.51\text{E} \times 10^{11}$
AAV1-F584L.....	$1.18\text{E} \times 10^{12}$
AAV1-A598V.....	$1.03\text{E} \times 10^{12}$
AAV1-N642H	$6.02\text{E} \times 10^{11}$
AAV6	$4.05\text{E} \times 10^{11}$
AAV6-F129L.....	$1.95\text{E} \times 10^{11}$
AAV6-D418E.....	$4.78\text{E} \times 10^{11}$
AAV6-K531E.....	$5.13\text{E} \times 10^{11}$
AAV6-L584F.....	$2.20\text{E} \times 10^{11}$
AAV6-V598A.....	$2.78\text{E} \times 10^{11}$
AAV6-H642N	$4.44\text{E} \times 10^{11}$

^a v.g., virus genome.

Wu 2006 Had Unpredictable Results



EX1060 at 1;
Paper 24 (Sur-Reply) at 25-26

To identify a critical residue(s) involved in the aforementioned features unique to AAV6, we generated a series of AAV1 and AAV6 mutants by site-directed mutagenesis (QuikChange; Stratagene) on plasmids pXR1 (15) and pXR6 (a kind gift from Joseph Rabinowitz at Thomas Jefferson University). Positions of the six amino acids (five located in the VP3 subunit and one in VP1) that were swapped between AAV1 and AAV6 capsids, genome titers of AAV1/AAV6 mutant vectors packaging the firefly luciferase transgene cassette determined as described previously (19) (Table 1), and their respective heparin binding profiles (Fig. 1a and b) are shown. Of the six amino acids studied, swapping amino acids at position 129, 584, or 598 between AAV6 and AAV1 independently and reproducibly affected viral genome titers (Table 1). The increase in titer from AAV1 recombinants 129, 584, and 598 was of keen interest, since these vector yields exceeded those of both parent serotypes and suggested that further improvements in other serotype-specific titers may also be improved by single amino acid changes (Table 1). Similar outcomes have been observed in mutants isolated from combinatorial libraries of AAV capsids generated by a method analogous to DNA shuffling, an approach independent of rational design (11). The increased titers in the case of the aforementioned AAV1 mutants can possibly be explained by improved folding/stability of capsid protein subunits and/or packaging efficiency (9, 23).

Kern Focused on Heparin-Binding Motif, AAV2 Function



EX1068 at 1;
Paper 24 (Sur-Reply) at 26-27

Identification of a Heparin-Binding Motif on Adeno-Associated Virus Type 2 Capsids†

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Infection of cells with adeno-associated virus (AAV) type 2 (AAV-2) is mediated by binding to heparan sulfate proteoglycan and can be competed by heparin. Mutational analysis of AAV-2 capsid proteins showed that a group of basic amino acids (arginines 484, 487, 585, and 588 and lysine 532) contribute to heparin and HeLa cell binding. These amino acids are positioned in three clusters at the threefold spike region of the AAV-2 capsid. According to the recently resolved atomic structure for AAV-2, arginines 484 and 487 and lysine 532 on one site and arginines 585 and 588 on the other site belong to different capsid protein subunits. These data suggest that the formation of the heparin-binding motifs depends on the correct assembly of VP trimers or even of capsids. In contrast, arginine 475, which also strongly reduces heparin binding as well as viral infectivity upon mutation to alanine, is located inside the capsid structure at the border of adjacent VP subunits and most likely influences heparin binding indirectly by disturbing correct subunit assembly. Computer simulation of heparin docking to the AAV-2 capsid suggests that heparin associates with the three basic clusters along a channel-like cavity flanked by the basic amino acids. With few exceptions, mutant infectivities correlated with their heparin- and cell-binding properties. The tissue distribution in mice of recombinant AAV-2 mutated in R484 and R585 indicated markedly reduced infection of the liver, compared to infection with wild-type recombinant AAV, but continued infection of the heart. These results suggest that although heparin binding influences the infectivity of AAV-2, it seems not to be necessary.

Kern Tested 33 Mutations



EX1068 at 4;
Paper 24 (Sur-Reply) at 26-27

TABLE 1. Mutant analysis							
Mutant	Mutation(s) (amino acid positions)	Infectivity ^a		Packaging ^b		Binding	
		No. of capsids/no. of infectious particles	Phenotype	Capsid/genome ratio	Phenotype	Cell ^c	Heparin ^d
1	DIRD → SMSL (469–472)	10 ³	WT	21	WT	ND	ND
2	SEK → TTA (547–549)	10 ³	WT	1	WT	ND	ND
3	VDI → AEL (552–554)	10 ³	WT	26	WT	ND	ND
4	RQA → TAP (588–590)	2 × 10 ⁶	LI	42	WT	±	–
5	A; AD → T; GT (591; 593, 594)	7 × 10 ⁴	RI	5	WT	+	+
6	ΔQ589-V600, L601N, NR → AC (587, 588)	>10 ⁶	LI	5	WT	ND	ND
7	ΔN582-G586	8 × 10 ⁵	LI	2	WT	–	–
8	ΔQ584-Q589	10 ⁶	LI	1	WT	–	–
9	ΔN587-A590	3 × 10 ⁶	LI	1	WT	–	–
10	R447A	5 × 10 ³	WT	4	WT	+	+
11	R459A	2 × 10 ³	WT	4	WT	±	+
12	R475A	10 ⁷	LI	0,3	WT	–	±
13	R484A	10 ⁷	LI	5	WT	–	–
14	R484E	10 ⁶	LI	6	WT	–	–
15	R487A	4 × 10 ⁴	RI	7	WT	–	–
16	R487E	3 × 10 ⁷	LI	5	WT	–	–
17	R484E, R585E	6 × 10 ⁸	LI	7	WT	–	–
18	K507A	4 × 10 ³	WT	27	WT	±	+
19	K527A	3 × 10 ⁴	RI	3	WT	±	±
20	K532A	2 × 10 ⁴	RI	3	WT	–	–
21	K544S	1.3 × 10 ⁴	RI	1	WT	±	+
22	K544E	1.1 × 10 ⁴	RI	1	WT	±	+
23	K556A	7 × 10 ²	WT	4	WT	±	+
24	R566A	6 × 10 ³	WT	19	WT	±	+
25	R566E	3 × 10 ³	WT	5	WT	±	+
26	R585E	1.3 × 10 ⁷	LI	3	WT	–	–
27	R585M	9 × 10 ⁴	RI	3	WT	–	–
28	R585K	2 × 10 ³	WT	2	WT	+	+
29	NR → AC (587, 588)	2 × 10 ⁶	LI	5	WT	±	–
30	R588E	2 × 10 ⁶	LI	3	WT	–	–
31	R588C	10 ⁶	LI	13	WT	±	–
32	R588K	10 ³	WT	3	WT	+	+
33	K620A	10 ⁴	WT	9	WT	+	+

^a Infectious AAV-2 particles were quantified by a dot blot replication assay as described previously (24). The number of assembled capsids was measured by a capsid ELISA (25). Infectivity was expressed as the ratio of the number of capsids to the number of infectious particles. The wild-type (WT) phenotype corresponds to a ratio of ≤10⁴; reduced infectivity (RI) corresponds to ratios of >10⁴ to <10⁶, and low infectivity (LI) corresponds to ratios of ≥10⁶.

^b Packaging was measured as the ratio of capsids to viral genomes as described previously (23). The WT phenotype indicates a packaging ratio of <50; reduced packaging was indicated by a ratio of >50.

^c Cell binding was measured as the binding of capsids to HeLa cells by an A20-based ELISA as described in Materials and Methods. It is expressed as the percentage of binding of WT AAV-2: +, more than 80% binding; ±, 40 to 80% binding; –, less than 40% binding. ND, not determined.

^d Heparin binding was measured as the percentage of viral particles which were applied to a heparin-agarose column and which were recovered after high-salt elution as determined with the A20-based capsid ELISA: +, more than 80% binding; ±, 20 to 80% binding; –, less than 20% binding.

Kern Showed Specific Mutations De-Targeted Liver



EX1068 at 1;

Paper 24 (Sur-Reply) at 26-27

Infection of cells with adeno-associated virus (AAV) type 2 (AAV-2) is mediated by binding to heparan sulfate proteoglycan and can be competed by heparin. Mutational analysis of AAV-2 capsid proteins showed that a group of basic amino acids (arginines 484, 487, 585, and 588 and lysine 532) contribute to heparin and HeLa cell binding. These amino acids are positioned in three clusters at the threefold spike region of the AAV-2 capsid. According to the recently resolved atomic structure for AAV-2, arginines 484 and 487 and lysine 532 on one site and arginines 585 and 588 on the other site belong to different capsid protein subunits. These data suggest that the formation of the heparin-binding motifs depends on the correct assembly of VP trimers or even of capsids. In contrast, arginine 475, which also strongly reduces heparin binding as well as viral infectivity upon mutation to alanine, is located inside the capsid structure at the border of adjacent VP subunits and most likely influences heparin binding indirectly by disturbing correct subunit assembly. Computer simulation of heparin docking to the AAV-2 capsid suggests that heparin associates with the three basic clusters along a channel-like cavity flanked by the basic amino acids. With few exceptions, mutant infectivities correlated with their heparin- and cell-binding properties. The tissue distribution in mice of recombinant AAV-2 mutated in R484 and R585 indicated markedly reduced infection of the liver, compared to infection with wild-type recombinant AAV, but continued infection of the heart. These results suggest that although heparin binding influences the infectivity of AAV-2, it seems not to be necessary.

Kern Reflects Unpredictability of the Art



EX1068 at 6-7;

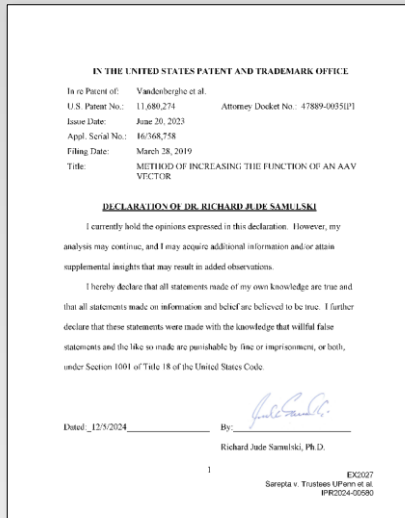
Paper 20 (Reply) at 9-10;

see also Paper 24 (Sur-Reply) at
26-27

Tissue tropism of AAV-2 heparin-binding mutants in vivo.

To test whether mutations in the heparin-binding motif also affect the in vivo tropism of the virus, we injected rAAV with either wild-type or heparin-binding mutant 17 (R484E and R585E) capsids into the tail vein of mice and analyzed vector genome distribution and reporter gene expression in different tissues. The reporter gene activity of rAAV packaged into the wild-type capsid could be detected in the liver and heart muscle after injection of 10^{11} vector genomes (Fig. 3A). Luciferase activity in the lungs and kidneys was close to background levels and could not be detected in the other tissues analyzed. The reporter gene activity of the double mutant was not detectable in the liver; however, it was surprisingly high in the heart sample, suggesting heparan sulfate-independent transduction of heart tissue in vivo. The difference in reporter gene expression between wild-type and double-mutant vectors was confirmed by PCR analysis of vector genomes in the heart and liver in several independently harvested samples (Fig. 3B). In contrast, vector genomes delivered by wild-type or mutant capsids could be detected by PCR in the lungs and kidneys in similar amounts (data not shown).

Kern Reflects Unpredictability of the Art

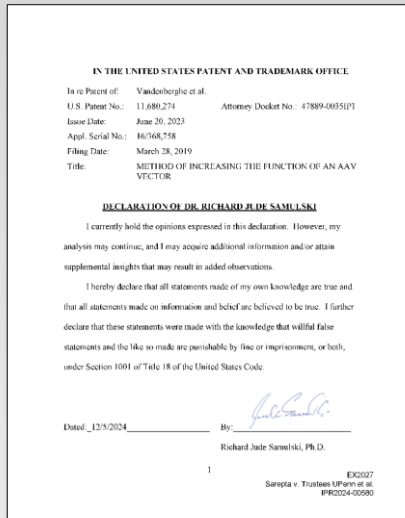


EX2027 at ¶ 27;

Paper 24 (Sur-Reply) at 26-27

27. At the time, AAV research for gene therapy was largely limited to basic science—we were figuring out how these vectors worked, and determining which portions of the vectors were important for specific AAV functions. By and large, mutagenesis and genetic engineering were not yet being used for the goal of enhancing a specific AAV function for clinical benefit. Instead, much of the research focused on understanding how variations in the AAV capsid structure influenced its functionality. For example, studies like the one by Hauck that Petitioner relies upon, (EX1052), explored how different regions of the AAV capsid affected its ability to infect muscle tissue. EX1052, 1-2 (“In the present study, a series of AAV capsid mutants were generated to identify the major regions affecting AAV transduction efficiency in muscle.”). But these studies were not aimed at engineering AAVs for specific therapeutic purposes; instead, they sought to better understand the relationship between capsid structure and tropism. *See, e.g.,* EX1052, 6 (“[W]e draw the conclusion that amino acids 350 to 423 are important in determining AAV1 tropism.”).

AAV Infection Pathway



EX2027 at ¶ 30;

Paper 18 (POR) at 50-52

