

IPR2024-00580

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

Demonstratives of Petitioner Sarepta Therapeutics

Oral Argument

May 27, 2025

Grounds for Inter Partes Review

Ground	Claims	Section	References
1	1, 3, 4, 5, 6	pre-AIA 103(a)	'772 publication
2	1, 3, 4, 5, 6	pre-AIA 103(a)	'772 publication and Xie
3	1, 3, 4, 5, 6	pre-AIA 103(a)	'772 publication and Snowdy
4	8	pre-AIA 103(a)	'772 publication and Fabb
5	8	pre-AIA 103(a)	'772 publication, Xie, and Fabb
6	8	pre-AIA 103(a)	'772 publication, Snowdy, and Fabb

Petition at 7-8.

Patent Owner Disclaimed Challenged Claims 1, 3, 4, 5, and 6

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.
Petitioner.

v.

THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA
Patent Owner
And REGENXBIO INC.,
Exclusive Licensee.

Case IPR2024-00580
Patent 11,680,274 B2

PATENT OWNERS' SUR-REPLY

¹ To narrow the issues for the Board, Patent Owners have statutorily disclaimed claims 1 and 3-6 of the '274 Patent, leaving only claim 8 in this proceeding. Thus, Petitioner's Grounds 1-3 are no longer at issue apart from where Grounds 4-6 rely on them.

Patent Owner's Sur-Reply at 1, n.1.

Claim 8

(12) **United States Patent**
Vandenberghe et al.

(10) **Patent No.:** US 11,680,274 B2
(45) **Date of Patent:** Jun. 20, 2023

(54) **METHOD OF INCREASING THE FUNCTION OF AN AAV VECTOR**
(71) **Applicant:** THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, Philadelphia, PA (US)
(72) **Inventors:** Luk Vandenberghe, Weston, MA (US); Guozhong Gao, Westborough, MA (US); James M. Wilson, Philadelphia, PA (US)
(73) **Assignee:** The Trustees of the University of Pennsylvania, Philadelphia, PA (US)
(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 545 days

(21) **Appl. No.:** 16/368,758
(22) **Filed:** Mar. 28, 2019
(65) **Prior Publication Data**
US 2019/0218574 A1 Jul. 18, 2019
Related U.S. Application Data
(62) Division of application No. 14/624,671, filed on Feb. 18, 2015, now Pat. No. 10,301,648, which is a division of application No. 11/987,679, filed as application No. PCT/US2006/013375 on Apr. 7, 2006, now Pat. No. 8,999,678.
(60) Provisional application No. 60/733,497, filed on Nov. 4, 2005; provisional application No. 60/669,083, filed on Apr. 7, 2005.

(51) **Int. Cl.**
C12N 15/00 (2006.01)
C12N 15/86 (2006.01)
A61K 48/00 (2006.01)
C07K 14/005 (2006.01)
C12N 7/00 (2006.01)
(52) **U.S. Cl.**
C12N 15/86 (2013.01); A61K 48/005 (2013.01); A61K 48/0091 (2013.01); C07K 14/005 (2013.01); C12N 7/00 (2013.01); A61K 48/00 (2013.01); C12N 2750/14122 (2013.01); C12N 2750/14142 (2013.01); C12N 2750/14143 (2013.01); C12N 2750/14152 (2013.01)
(58) **Field of Classification Search**
CPC C12N 15/86; C12N 7/00; C12N 2750/14122; C12N 2750/14142; C12N 2750/14143; C12N 2750/14152; A61K 48/005; A61K 48/0091; A61K 48/00; C07K 14/005
See application file for complete search history.
(56) **References Cited**
U.S. PATENT DOCUMENTS
6,156,303 A 12/2000 Russell et al.
6,410,300 B1 6/2002 Samilko et al.

9 Claims, 21 Drawing Sheets
Specification includes a Sequence Listing.

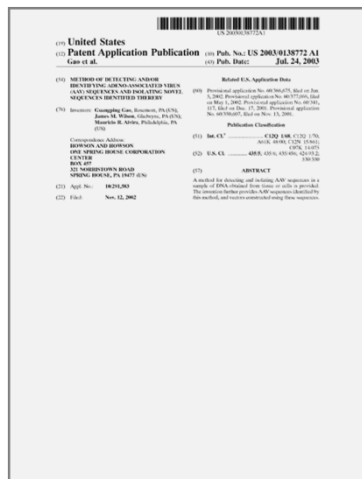
1. A recombinant adeno-associated virus (AAV) comprising an AAV capsid and a minigene having AAV inverted terminal repeats and a heterologous gene operably linked to regulatory sequences which direct expression of the heterologous gene in a host cell, wherein the AAV capsid comprises AAV vp1 proteins, 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.

6. The recombinant AAV according to claim 1, wherein the heterologous gene encodes an ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, arginase, fumarylacetylacetate 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), betaglucoisidase, pyruvate carboxylate, hepatic phosphorylase, phosphorylase kinase, beta-glucuronidase (GUSB), glycine decarboxylase, a low density lipoprotein (LDL) receptor, high density lipoprotein (HDL) receptor, very low density lipoprotein (VLDL) receptor, scavenger receptor, glucocorticoid receptor, estrogen receptor, Vitamin D receptor, nuclear receptor, cystic fibrosis transmembrane regulator (CFTR) sequence, Factor IX or variants thereof, Factor VIII or variants thereof, a dystrophin gene product, or an immunoglobulin.

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

EX1001 ('274 patent) at 193:2-15 (claim 1), 194:1-19 (claim 6), 194:24-26 (claim 8); Petition at xiv-xv, 9.

Ground 6: '772 Publication, Snowdy, and Fabb



'772 Publication

- AAV rh.10
 - “Intriguing biological significance”
 - Superb tropism in lung
 - Outstanding in liver and muscle
 - Close to best liver transducer, AAV8, and best muscle transducer, AAV1
- Liver tropism
 - Tropism in liver has been “relatively disappointing”
- Motivation
 - A POSA would have been motivated to modify rh.10 to confer some of the enhanced liver tropism of AAV8, while preserving excellent tropism in muscle and lung



Snowdy

- A single amino acid substitution enhanced transduction
 - Replacing a phosphorylatable amino acid with a non-phosphorylatable amino acid improved transduction efficiency



Fabb

- Micro-dystrophin cDNA gene construct in AAV that restored function

Petition at 12-42, 47-53, 58-60.

The Panel Credited the Unrebutted Testimony of Petitioner's Expert on Motivation in the Institution Decision

Trials@uspto.gov
571-272-7822

Paper 9
Entered: August 22, 2024

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

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IPR2024-00580
Patent 11,680,274 B2

Before ULRIKE W. JENKS, SUSAN L. C. MITCHELL, and
ROBERT A. POLLOCK, *Administrative Patent Judges*.

MITCHELL, *Administrative Patent Judge*.

DECISION
Granting Institution of *Inter Partes* Review
35 U.S.C. § 314

Based on the disclosure of the '772 Publication and the unrebutted testimony of Dr. Shaffer, Petitioner has sufficiently shown on the record before us that a POSA would have been motivated to select the promising AAVrh.10 as a starting point.

Based on the record before us, we credit Dr. Shaffer's testimony here and determine that Petitioner has sufficiently shown that a POSA would be motivated to modify AAVrh.10 based on a comparison with AAV8.

At this point in the proceeding, Dr. Shaffer's testimony is unrebutted. See Prelim. Resp. 51–57. We credit Dr. Shaffer's unrebutted testimony here and determine that Petitioner has shown sufficiently for purposes of institution that a POSA would have been motivated to make the substitution at the 665 position with a reasonable expectation of success

8/22/24 Decision Granting Institution of *Inter Partes* Review at 24, 25, 27.

The '772 Publication

**A POSA Would Have Been Motivated to Modify rh.10 to Enhance Liver Tropism,
While Preserving Tropism in Muscle and Lung**

Petition at 21-15, 22-42.

The '772 Publication Provides the Motivation to Select rh.10

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2003/0138772 A1
(43) Pub. Date: Jul. 24, 2003

(54) METHOD OF DETECTING AND/OR
IDENTIFYING ADENO-ASSOCIATED VIRUS
(AAV) SEQUENCES AND ISOLATING NOVEL
SEQUENCES IDENTIFIED THEREBY

(79) Inventors: Guoqiang Gao, Rosemont, PA (US);
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(21) Appl. No.: 10/291,583

(22) Filed: Nov. 12, 2002

Related U.S. Application Data

(60) Provisional application No. 60/366,675, filed on Jun.
5, 2002. Provisional application No. 60/377,066, filed
on May 1, 2002. Provisional application No. 60/341,
117, filed on Dec. 17, 2001. Provisional application
No. 60/350,607, filed on Nov. 13, 2001.

Publication Classification

(51) Int. Cl.⁷ C12Q 1/68; C12Q 1/70;
A61K 48/00; C12N 15/861;
C07K 14/075
(52) U.S. Cl. 435/5; 435/6; 435/456; 424/93.2;
530/350

(57) ABSTRACT

A method for detecting and isolating AAV sequences in a
sample of DNA obtained from tissue or cells is provided.
The invention further provides AAV sequences identified by
this method, and vectors constructed using these sequences.

[0256] The data from both experiments confirmed the superb tropism of clone 44.2 in lung-directed gene transfer.

[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.

EX1007 ('772 publication) at [0256]-[0257]; Petition at 13-14.

A POSA Would Have Been Motivated to Improve Liver Tropism of rh.10, While Maintaining Tropism in Lung and Muscle

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2003/0138772 A1
(43) Pub. Date: Jul. 24, 2003

(54) METHOD OF DETECTING AND/OR
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(79) Inventors: Guoqiang Gao, Rosemont, PA (US);
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A61K 48/00; C12N 15/861;
C07K 14/075
(52) U.S. Cl. 435/5; 435/6; 435/456; 424/93.2;
530/350

ABSTRACT

A method for detecting and isolating AAV sequences in a
sample of DNA obtained from tissue or cells is provided.
The invention further provides AAV sequences identified by
this method, and vectors constructed using these sequences.

Importantly, AAV2/8 provides a substantial advantage over the other serotypes in terms of efficiency of gene transfer to liver that until now has been relatively disappointing in terms of the numbers of hepatocytes stably transduced.

EX1007 ('772 publication) at [0250]; Petition at 26-27.

A POSA Would Have Been Motivated to Improve Liver Tropism of rh.10, While Maintaining Tropism in Lung and Muscle

(19) **United States**
 (12) **Patent Application Publication** (10) Pub. No.: **US 2003/0138772 A1**
 Gao et al. (43) Pub. Date: **Jul. 24, 2003**

(54) **METHOD OF DETECTING AND/OR IDENTIFYING ADENO-ASSOCIATED VIRUS (AAV) SEQUENCES AND ISOLATING NOVEL SEQUENCES IDENTIFIED THEREBY**

(75) Inventors: **Guangping Gao, Rosemont, PA (US); James M. Wilson, Glacetyne, PA (US); Maurizio R. Alvira, Philadelphia, PA (US)**

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(21) Appl. No.: **10/291,583**
 (22) Filed: **Nov. 12, 2002**

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(60) Provisional application No. 60/366,675, filed on Jun. 5, 2002; Provisional application No. 60/377,066, filed on May 1, 2002; Provisional application No. 60/341,117, filed on Dec. 17, 2001; Provisional application No. 60/350,607, filed on Nov. 13, 2001.

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 (52) U.S. Cl. **435/5; 435/6; 435/456; 424/93.2; 530/350**

(57) **ABSTRACT**
 A method for detecting and isolating AAV sequences in a sample of DNA obtained from tissue or cells is provided. The invention further provides AAV sequences identified by this method, and vectors constructed using these sequences.

TABLE 8

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

EX1007 ('772 publication) at Table 8; Petition at 27.

The Panel Acknowledged the Disclosure in the '772 Publication That rh.10 Is Already “Outstanding” in Muscle

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Paper 9
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Before ULRIKE W. JENKS, SUSAN L. C. MITCHELL, and
ROBERT A. POLLOCK, *Administrative Patent Judges*.

MITCHELL, *Administrative Patent Judge*.

DECISION
Granting Institution of *Inter Partes* Review
35 U.S.C. § 314

We note that the '772 Publication states that “performance of clone 44.2 in liver and muscle directed gene transfer was also outstanding” indicating that AAVrh.10 (at least before modification) performed well with muscle tissue, but invite the parties to address this issue further at trial. *See* Ex. 1007 ¶ 257; *see id.* ¶ 253.

8/22/24 Decision Granting Institution of *Inter Partes* Review at 28.

A POSA Would Have Modified rh.10 to Improve Liver Tropism, While Preserving Tropism in Lung and Muscle

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Patent Owners

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

“Method of Increasing the Function of an AAV Vector”

IPR2024-00580

PETITION FOR *INTER PARTES* REVIEW

Moreover, given the already superior performance of AAVrh.10 in lung, a POSA would not have sought to make sweeping substitutions throughout the AAVrh.10 sequence, such as changing multiple amino acids at once, for fear of damaging or destroying the desirable properties of AAVrh.10. Instead, a POSA would have taken a finer, more directed approach, modifying a single amino acid residue at a time. EX1032, 5-6, Table 1; EX1005, ¶211.

Petition at 27; see also EX1005 (Declaration of David V. Schaffer, Ph.D.) at ¶211.

The Motivation in the '772 Publication Is to Improve Liver Tropism of rh.10, While Preserving Tropism in Lung And Muscle



Dr. David Schaffer

416. Importantly, I also stated that “a POSA would not have sought to make sweeping substitutions throughout the AAVrh.10 sequence, such as changing multiple amino acids at once, for fear of damaging or destroying the desirable properties of AAVrh.10.” EX1005 (Schaffer Dec.), ¶ 211 (emphasis added).

417. By the “desirable properties of AAVrh.10,” I meant all of the desirable properties of this serotype, including its excellent tropism in muscle and lung.

EX1072 (Reply Declaration of David V. Schaffer, Ph.D.) at ¶¶416-417; Reply at 17.

The Motivation in the '772 Publication Is to Improve Liver Tropism of rh.10, While Preserving Tropism in Lung And Muscle



Dr. David Schaffer

Q. Okay. But this – you’d agree the word “muscle” isn’t in 205; right?

A. 205 doesn’t discuss muscle. It does refer to, you know, for example, rh.10 and to the '772 publication which discusses the performance of rh.10 within muscle.

Q. Right but in 205 you’re just talking about a sequence alignment you did; right?

A. 205 discusses rh.10 and discusses sequence alignment.

Q. Okay.

A. However, you know, as I mentioned earlier it’s inherent earlier throughout the entire declaration that rh.10 is a superior vector within muscle.

EX1064 (Deposition of David V. Schaffer, Ph.D., with errata) (objection omitted) at 352:9-353:1; Reply at 19.

The '772 Publication Teaches That the Use of AAV Vectors Is Not Limited to the “Best” Natural Serotype in a Particular Tissue


US 2003/013872A1

(19) United States
(12) Patent Application Publication (10) Pub. No.: US 2003/013872 A1
Gao et al. (43) Pub. Date: Jul. 24, 2003

(54) METHOD OF DETECTING AND/OR IDENTIFYING ADENO-ASSOCIATED VIRUS (AAV) SEQUENCES AND ISOLATING NOVEL SEQUENCES IDENTIFIED THEREBY

(75) Inventors: Guoqiang Gao, Rosemont, PA (US); James M. Wilson, Glacetyne, PA (US); Maurizio R. Alvira, Philadelphia, PA (US)

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(21) Appl. No.: 10/291,583
(22) Filed: Nov. 12, 2002

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(60) Provisional application No. 60/366,675, filed on Jun. 5, 2002; Provisional application No. 60/377,066, filed on May 1, 2002; Provisional application No. 60/341,117, filed on Dec. 17, 2001; Provisional application No. 60/350,607, filed on Nov. 13, 2001.

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(51) Int. Cl.⁷ C12Q 1/68; C12Q 1/70; A61K 48/00; C12N 15/861; C07K 14/075
(52) U.S. Cl. 435/5; 435/6; 435/456; 424/93.2; 530/350

(57) ABSTRACT
A method for detecting and isolating AAV sequences in a sample of DNA obtained from tissue or cells is provided. The invention further provides AAV sequences identified by this method, and vectors constructed using these sequences.

[0142] It will be readily apparent to one of skill in the art one embodiment, that certain serotypes of the invention will be particularly well suited for certain uses. For example, vectors based on AAV7 capsids of the invention are particularly well suited for use in muscle; whereas vectors based on rh.10 (44-2) capsids of the invention are particularly well suited for use in lung. Uses of such vectors are not so limited and one of skill in the art may utilize these vectors for delivery to other cell types, tissues or organs. Further, vectors based upon other capsids of the invention may be used for delivery to these or other cells, tissues or organs.

EX1007 ('772 publication) at [0142]; Petition at 35; Reply 15-16.

The Panel Recognized That Other AAVs in the '772 Publication Do Not Diminish or Deflect From the Teachings on rh.10

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ROBERT A. POLLOCK, *Administrative Patent Judges*.

MITCHELL, *Administrative Patent Judge*.

DECISION
Granting Institution of *Inter Partes* Review
35 U.S.C. § 314

Based on the disclosure of the '772 Publication and the unrebutted testimony of Dr. Shaffer, Petitioner has sufficiently shown on the record before us that a POSA would have been motivated to select the promising AAVrh.10 as a starting point. The fact that other novel AAVs may also have promise for targeting particular types of tissues does not diminish or deflect from the teachings of the '772 Publication regarding AAVrh.10.

8/22/24 Decision Granting Institution of *Inter Partes* Review at 24.

Dr. Samulski's '126 Provisional Shows That People in the Art Did Not Limit Themselves to the Best Natural Serotype for a Given Tissue

Patent Docket No. 5470-427PR PATENT

COVER SHEET FOR FILING PROVISIONAL
PATENT APPLICATION (37 CFR §1.51(c)(1))

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Date: December 15, 2004

This is a request for filing a PROVISIONAL PATENT APPLICATION under 37 C.F.R. §1.53(c)

Docket No.	5470-427PR
Type a plus sign (+) inside this box	+

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TITLE OF THE INVENTION (280 characters maximum)
CHIMERIC VECTORS

Deposition Exhibit 1059

Select Exhibit 1059 page 1

SUMMARY OF THE INVENTION

The inventors have identified the key amino acid(s) of AAV1 responsible for enhanced *in vivo* transduction and selectively engineered these amino acids into the backbone of AAV2 as well as other AAV. In particular embodiments, the chimeric viruses of the invention have enhanced transduction capability (e.g., transduction of skeletal muscle, cardiac muscle, liver, retina and/or lung, etc.) and/or enhanced levels of transgene expression with earlier onset of expression. With respect to chimeric viruses based on AAV2, these chimeras retain the desirable properties of AAV2 (e.g., ease of purification and known safety) while exhibiting some of the advantageous properties of AAV1 (including enhanced transduction of skeletal muscle).

EX1059 ('126 provisional application) at 4:32-5:6; Reply at 21.

Dr. Samulski Chose to Modify AAV2 to Target Muscle



Dr. Jude Samulski

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.

EX1063 (Deposition of Richard Jude Samulski, Ph.D.) (objections omitted) at 106:23-107:23; Reply at 21.

Patent Owner Admits That the '126 Provisional Is Directed Towards Conferring Muscle Tropism from AAV1 onto AAV2

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PATENT OWNERS' SUR-REPLY

The premise of the work in the '126 provisional was to take the best known and characterized AAV to date—AAV2—and confer muscle targeting onto it. EX1059, 4. At that time, AAV1, AAV6, and AAV7 were “the most promising for delivery to skeletal muscle.” *Id.*; EX1072, ¶129. Therefore, it was “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.” EX1059, 4. In addition to AAV2’s ease of purification, Dr. Samulski testified that regulatory concerns were central—AAV2 was the only vector FDA would allow for use in clinical trials in 2005. See EX1063, 106:23-107:5, 257:21-258:17.

Patent Owner’s Sur-Reply at 19-20.

The '772 Publication Discloses Tissue-Specific Promoters as a Strategy for Adapting Different Serotypes to Desired Targets

 US 2003/013872A1	
(19) United States (12) Patent Application Publication (10) Pub. No.: US 2003/013872 A1 Gao et al. (43) Pub. Date: Jul. 24, 2003	
(54) METHOD OF DETECTING AND/OR IDENTIFYING ADENO-ASSOCIATED VIRUS (AAV) SEQUENCES AND ISOLATING NOVEL SEQUENCES IDENTIFIED THEREBY (75) Inventors: Guoqiang Gao, Rosemont, PA (US); James M. Wilson, Glacetyne, PA (US); Maurizio R. Alvira, Philadelphia, PA (US) Correspondence Address: HOWSON AND HOWSON ONE SPRING HOUSE CORPORATION CENTER BOX 457 321 NORRISTOWN ROAD SPRING HOUSE, PA 19477 (US) (21) Appl. No.: 10/291,583 (22) Filed: Nov. 12, 2002	Related U.S. Application Data (60) Provisional application No. 60/386,675, filed on Jun. 5, 2002; Provisional application No. 60/377,066, filed on May 1, 2002; Provisional application No. 60/341,117, filed on Dec. 17, 2001; Provisional application No. 60/350,607, filed on Nov. 13, 2001. Publication Classification (51) Int. Cl. C12Q 1/68; C12Q 1/70; A61K 48/00; C12N 15/861; C07K 14/075 (52) U.S. Cl. 435/5; 435/6; 435/456; 424/93.2; 530/350 (57) ABSTRACT A method for detecting and isolating AAV sequences in a sample of DNA obtained from tissue or cells is provided. The invention further provides AAV sequences identified by this method, and vectors constructed using these sequences.

[0111] Another embodiment of the transgene includes a transgene operably linked to a tissue-specific promoter. For instance, if expression in skeletal muscle is desired, a promoter active in muscle should be used. These include the promoters from genes encoding skeletal β -actin, myosin light chain 2A, dystrophin, muscle creatine kinase, as well as synthetic muscle promoters with activities higher than naturally-occurring promoters (see Li et al., *Nat. Biotech.*, 17:241-245 (1999)). Examples of promoters that are tissue-

EX1007 ('772 publication) at [0111]; see also *id.* at [0107]; Reply at 22.

The '772 Publication Discloses Route of Administration as a Strategy for Adapting Different Serotypes to Desired Targets

US 2003/08772A1

(19) United States
(12) Patent Application Publication (10) Pub. No.: US 2003/0138772 A1
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(54) METHOD OF DETECTING AND/OR IDENTIFYING ADENO-ASSOCIATED VIRUS (AAV) SEQUENCES AND ISOLATING NOVEL SEQUENCES IDENTIFIED THEREBY

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(21) Appl. No.: 10/291,583
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(60) Provisional application No. 60/366,675, filed on Jun. 5, 2002; Provisional application No. 60/377,066, filed on May 1, 2002; Provisional application No. 60/341,117, filed on Dec. 17, 2001; Provisional application No. 60/350,607, filed on Nov. 13, 2001.

Publication Classification
(51) Int. Cl.⁷ C12Q 1/68; C12Q 1/70; A61K 48/00; C12N 15/861; C07K 14/075
(52) U.S. Cl. 435/3; 435/6; 435/456; 424/93.2; 530/350

(57) ABSTRACT
A method for detecting and isolating AAV sequences in a sample of DNA obtained from tissue or cells is provided. The invention further provides AAV sequences identified by this method, and vectors constructed using these sequences.

[0150] The viral vectors are administered in sufficient amounts to transfect the cells and to provide sufficient levels of gene transfer and expression to provide a therapeutic benefit without undue adverse effects, or with medically acceptable physiological effects, which can be determined by those skilled in the medical arts. Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, direct delivery to the selected organ (e.g., intraportal delivery to the liver), oral, inhalation (including intranasal and intratracheal delivery), intraocular, intravenous, intramuscular, subcutaneous, intradermal, and other parental routes of administration. Routes of administration may be combined, if desired.

[0242] The performance of AAV vectors in skeletal muscle was analyzed following direct injection into the tibialis anterior muscles.

EX1007 ('772 publication) at [0150], [0242] (muscle); *see also id.* at [0244] (liver), [0254] (lung); Reply at 22.

Dr. Samulski Confirmed That the Route of Administration Is a Viable Strategy for AAV Targeting



Dr. Jude Samulski

Q. You can have a vector that can have multiple different tissue tropisms, but the way you use it, can still -- it can still be useful to target one of the multiple tropisms of the vector.

A. So I think what you're proposing is if you had multiple tropisms attached to a capsid, the route of administration may restrict access to those tropisms. I would put forth that if it's intravenous delivery, it has access to essentially everything. If it's intramuscular, it's restricted. If it's intravitreal, it's restricted, intracranial, so forth and so on. So that the virus is not restricted. You're not using the virus based on its promiscuity. You're restricting it based on the route of administration.

EX1063 (Deposition of Richard Jude Samulski, Ph.D.) (objection omitted) at 248:13-249:4; Reply at 22.

Liver Tropism Can Be Enhanced By Increasing the Efficiency of Expression, Without Increasing the Vector Dosage

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.,

Petitioner

v.

THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA,

Patent Owner

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

"Method of Increasing the Function of an AAV Vector"

IPR2024-00580

REPLY DECLARATION OF DAVID V. SCHAFER, PH.D.

C. Dr. Samulski Conflates Biodistribution and Infectivity

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

EX1072 (Reply Declaration of David V. Schaffer, Ph.D.) at ¶¶325-326; Reply 22-23.

Dr. Samulski's PCT Application Highlights a New Tropism in the Chimeric AAV2.5 Vector

5470.427.WO
PCT/US05/45552 1/5
PCT REQUEST
Original (for SUBMISSION)

0	For receiving Office use only	
0-1	International Application No.	PCT/US05/45552
0-2	International Filing Date	15 DEC 2005 (15.12.05)
0-3	Name of receiving Office and "PCT International Application"	PCT INTERNATIONAL APPLICATION RO/US
0-4	Form PCT/RO/101 PCT Request Prepared Using	PCT-SAFE [EASY mode] Version 3.50 (Build 0002.175)
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	United States Patent and Trademark Office (USPTO) (RO/US)
0-7	Applicant's or agent's file reference	5470.427.WO
I	Title of Invention	CHIMERIC VECTORS
II	Applicant	applicant only
II-1	This person is	all designated States except US
II-2	Applicant for	
II-4	Name	UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
II-5	Address	308 Bynum Hall Campus Box 4105 Chapel Hill, North Carolina 27599-4105 United States of America
II-6	State of nationality	US
II-7	State of residence	US
II-8	Telephone No.	919-966-3929
II-9	Facsimile No.	919-962-0646

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

EX1072 at ¶213 (quoting EX1067 (PCT/US2005/045552, issued as U.S. Patent No. 7,892,809) at 63:25-31);
Reply at 23.

A POSA Would Have Understood That There Are Potential Benefits to an AAV Vector With Tropism to Multiple Tissues

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.,
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v.

THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA,
Patent Owner

U.S. Patent No. 11,680,274
"Method of Increasing the Function of an AAV Vector"

IPR2024-00580

REPLY DECLARATION OF DAVID V. SCHAFER, PH.D.

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

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

EX1072 (Reply Declaration of David V. Schaffer, Ph.D.) at ¶¶ 396-397; Reply at 16.

Dystrophin Is Expressed in Many Different Tissues Throughout the Body, Besides Muscle

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.,

Petitioner

v.

THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA,

Patent Owner

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

"Method of Increasing the Function of an AAV Vector"

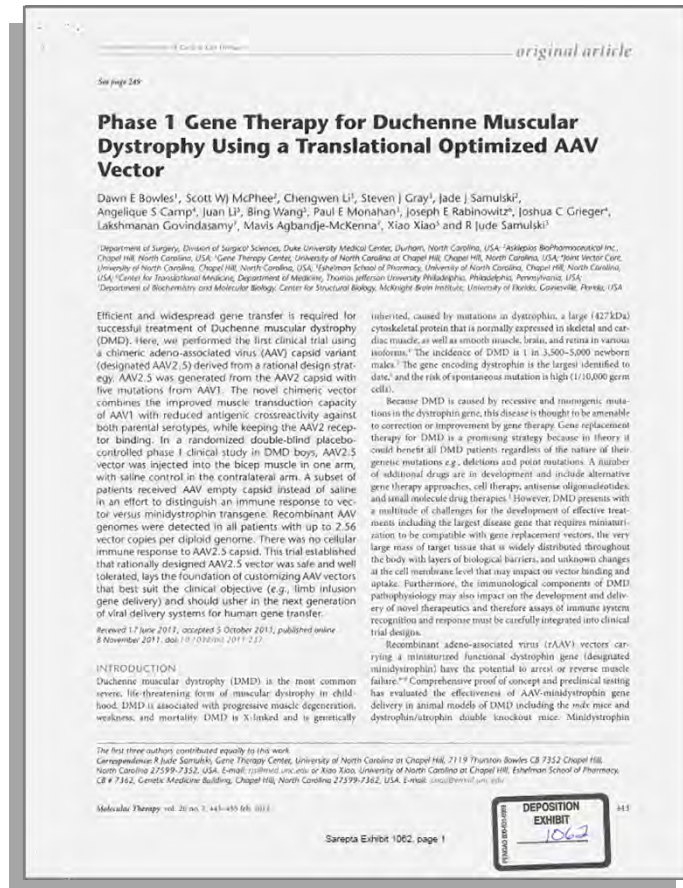
IPR2024-00580

REPLY DECLARATION OF DAVID V. SCHAFER, PH.D.

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

EX1072 (Reply Declaration of David V. Schaffer, Ph.D.) at ¶¶ 398; Reply at 20.

Dr. Samulski Has Published That Dystrophin Is Expressed in Tissues Other Than Muscle



INTRODUCTION

Duchenne muscular dystrophy (DMD) is the most common severe, life-threatening form of muscular dystrophy in childhood. DMD is associated with progressive muscle degeneration, weakness, and mortality. DMD is X-linked and is genetically inherited, caused by mutations in dystrophin, a large (427 kDa) cytoskeletal protein that is normally expressed in skeletal and cardiac muscle, as well as smooth muscle, brain, and retina in various isoforms.¹ The incidence of DMD is 1 in 3,500–5,000 newborn males.² The gene encoding dystrophin is the largest identified to date,¹ and the risk of spontaneous mutation is high (1/10,000 germ cells).

EX1062 (Bowles) at 1; Reply at 20.

Snowdy

Snowdy Teaches a POSA How to Improve Transduction Efficiency By Replacing a Phosphorylatable Amino Acid with a Non-Phosphorylatable Amino Acid

Petition at 16-20, 47-53.

Snowdy Teaches Replacement of a Phosphorylatable Amino Acid with a Non-Phosphorylatable Amino Acid to Improve Transduction Efficiency

Nuclear targeting by adeno-associated virus capsid proteins and virions

Stephen Snowdy

A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the curriculum of Neurobiology

Chapel Hill
2003

Approved by:

Advisor: Richard Jude Samulski

Reader: Mohanish Doshmukh

Reader: William Marzluff

Reader: Thomas McCown

Reader: John Olsen

Sanepa Exhibit 1009, page 1

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).

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.

EX1009 (Snowdy) at 126, 128; Petition at 19.

Patent Owner Says the Issue Is Not Whether “Snowdy’s Data Are Valid”

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.
Petitioner.

v.

THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA,
Patent Owner
And REGENXBIO INC.,
Exclusive Licensee.

Case IPR2024-00580
Patent 11,680,274 B2

PATENT OWNERS' SUR-REPLY

The issue is not whether Snowdy’s data are valid, but whether they are
relevant.

Patent Owner’s Sur-Reply at 10.

Dr. Samulski Testified That Serine and Asparagine Are “Different” Because Serine Is Phosphorylatable While Asparagine Is Not



Dr. Jude Samulski

Q. And when you were talking about amino acids that are similar versus different, I want to focus on serine and asparagine. Are those similar, or are they different?

A. So, again, we would look for those that are different, whether they're charged and be phosphorylated and things of that nature over ones that are not likely to be what's called post-translated modified, PTMs.

EX1063 (Deposition of Richard Jude Samulski, Ph.D.) (objection omitted) at 277:6-15; Reply at 24.

Patent Owner Relied on Dr. Samulski's Opinion That the '772 Publication Is "Silent" as to Single Amino Acid Substitutions

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Vandenberghe et al.
U.S. Patent No.: 11,680,274 Attorney Docket No.: 47889-0035IP1
Issue Date: June 20, 2023
Appl. Serial No.: 16/368,758
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
Sarepta v. Trustees UPenn et al.
IPR2024-00580

At the outset, I note that the '772 Publication is silent as to using single amino acid substitutions to alter tissue tropism.

EX2027 (Declaration of Dr. Richard Jude Samulski) at ¶¶53; Patent Owner Response at 17.

The '772 Publication Teaches Single Amino Acid Substitutions to Change Functional Characteristics, Including Tropism

US 2003/08772A1

(19) United States
(12) Patent Application Publication (10) Pub. No.: US 2003/0138772 A1
Gao et al. (43) Pub. Date: Jul. 24, 2003

(54) METHOD OF DETECTING AND/OR IDENTIFYING ADENO-ASSOCIATED VIRUS (AAV) SEQUENCES AND ISOLATING NOVEL SEQUENCES IDENTIFIED THEREBY

(75) Inventors: Guoqiang Gao, Rosemont, PA (US); James M. Wilson, Glacetyne, PA (US); Maurizio R. Alvira, Philadelphia, PA (US)

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SPRING HOUSE, PA 19477 (US)

(21) Appl. No.: 10/291,583
(22) Filed: Nov. 12, 2002

Related U.S. Application Data
(60) Provisional application No. 60/386,675, filed on Jun. 5, 2002; Provisional application No. 60/377,066, filed on May 1, 2002; Provisional application No. 60/341,117, filed on Dec. 17, 2001; Provisional application No. 60/350,607, filed on Nov. 13, 2001.

Publication Classification
(51) Int. Cl.⁷ C12Q 1/68; C12Q 1/70; A61K 48/00; C12N 15/861; C07K 14/075
(52) U.S. Cl. 435/5; 435/6; 435/456; 424/93.2; 530/350
(57) ABSTRACT
A method for detecting and isolating AAV sequences in a sample of DNA obtained from tissue or cells is provided. The invention further provides AAV sequences identified by this method, and vectors constructed using these sequences.

Thus, the inventors anticipate this it is desirable to alter the start codon of the vp3 of other AAV serotypes to contain this rare GTG start codon, in order to improve packaging efficiency, to alter the virion structure and/or to alter location of epitopes (e.g., neutralizing antibody epitopes) of other AAV serotypes.

In another example, it may be desirable to alter the start codon of the vp3 protein to GTG. These modifications may be to increase expression, yield, and/or to improve purification in the selected expression systems, or for another desired purpose (e.g., to change tropism or alter neutralizing antibody epitopes).

EX1007 ('772 publication) at [0072], [0091]; Reply 8-9.

Dr. Samulski Admitted That the '772 Publication Teaches Single Amino Acid Substitutions to Change Tropism



Dr. Jude Samulski

Q. So the inventors of the '772 publication here are recommending that you might want to alter the normal start code on a VP3, which is ATG to GTG, right?

A. That's what they are suggesting.

Q. And a person of ordinary skill in the art would also understand that this mutation to GTG would be present in VP1 and VP2 proteins in that mutant capsid, right?

A. That's correct.

Q. Of course. So it doesn't really matter, for our purposes here, whether or not it's on the surface or not, the point is the '772 publication is suggesting that you might want to make this mutation, because it might alter the surface, and particularly alter the location of epitopes on the surface, right?

A. That's what they're proposing.

Q. And even under your definition of tropism, which stops at opening the door, altering an epitope on the surface could affect whether or not the virus can open the door, right?

A. And based on the way you're describing it, that's correct.

EX1063 (Deposition of Richard Jude Samulski, Ph.D.) (objections omitted) at 60:23-61:6, 68:12-69:1; Reply 8-9.

Dr. Samulski Admitted That He Did Not Consider the Teachings in the '772 Publication on Single Amino Acid Substitutions



Dr. Jude Samulski

Q. Right. But it's what we call a disclosure. This is what they're suggesting. This is a teaching. You may not agree with it, but this is what the '772 publication is suggesting?

A. It's suggesting.

Q. Okay. But it is suggested here, and you didn't consider this as part of your opinion, right?

A. I had no reason to consider it as part of my --

Q. Okay. So --

A. It's a hypothesis I don't believe in.

EX1063 (Deposition of Richard Jude Samulski, Ph.D.) (objection omitted) at 63:13-17, 64:4-10; Reply at 9.

Dr. Samulski Acknowledged the Significance of Snowdy's Results



Dr. Jude Samulski

Q. And you're thrilled when one of them works, one of out three?

A. If you're lucky.

Q. If you're lucky, you get one out of three to work right? Yes?

A. Correct.

Q. And in fact, what Snowdy did was he didn't just get his experiment to work, he got an unexpected result that he enhanced a function that he didn't expect to enhance, right?

A. That's the data suggests that, correct.

Q. Right. And so what he found when he mutated a single amino acid, right, in an AAV2, he found that he enhanced transduction efficiency with that single amino acid change, correct?

A. That is an outcome from his study.

EX1063 (Deposition of Richard Jude Samulski, Ph.D.) (objections omitted) at 86:24-87:16; Reply at 7-8; EX1072, ¶¶111, 311.

Dr. Samulski Agreed With Dr. Schaffer's Opinions About How a POSA Would Have Understood Snowdy's Results



Dr. Jude Samulski

Q. And so a person of ordinary skill in the art reading this disclosure would understand that Snowdy's results suggest that another function, other than nuclear localization, was affected by the S181A mutation, right?

A. He was coming up with other hypothesis.

. . . .

Q. And so a person of ordinary skill in the art reading this disclosure would further understand that this hypothetical other function had been affected by removal of a single putative phosphorylation site by substituting a nonphosphorylatable residue in somehow that enhanced AAV transduction, right?

A. The data implied that.

EX1063 (Deposition of Richard Jude Samulski, Ph.D.) (objections omitted) at 98:23-99:15; Reply at 1.

Fabb

Fabb Teaches Creation of a Micro-Dystrophin cDNA Gene Construct That Could Restore Dystrophin Function in Muscle Tissue

Petition at 20-21, 58-60.

Fabb Teaches a Micro-Dystrophin cDNA Gene Construct That Can Be Packaged in an AAV Capsid

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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 Neuromuscular Diseases, Division of Neuroscience and Psychological Medicine, Imperial College Faculty of Medicine, Charing Cross Hospital, London W6 8RP, UK

(Received October 29, 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 *mdx* 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 >60% 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 (one-third of 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]. This process leads to clinical symptoms of weakness, paralysis and breathing difficulties. The symptoms of the disease usually occur before 3 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 cytoskeletal protein of the spectrin family [3,4]. The DMD gene has one of the highest mutation rates reported to date, with two-thirds of disease-causing mutations being large deletions in the 5' and central portions of the gene. Interestingly, deletion size

has an influence over whether the phenotype is DMD or the much less severe form, Becker muscular dystrophy (BMD). Very mild forms of the disease, where ~50% or more of the gene has been deleted, have been described [5-8]. Conversely, other individuals with only small deletions display a DMD phenotype. This apparent anomaly depends on whether the dystrophin open reading frame is maintained (BMD) or disrupted (DMD). At the molecular level, the DMD phenotype thus appears to be due either to the metabolic instability of the truncated mRNAs or proteins, or to a functional inactivity of the latter [9].

The dystrophin protein has four main structural domains: an N-terminal region, which binds to the F-actin of cytoskeletal structures; 24 right-handed coiled coils, interspersed with four hinge regions; and two C-terminal regions (CR and CT) which bind a complex of dystrophin-associated proteins (DAPs). These DAPs provide a molecular link between the

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Present address: Patricia Serpente, Muscular Dystrophy Division, The National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.

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, 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.

EX1010 (Fabb) at Abstract; Petition at 20-21, 55.

Fabb Demonstrated That the Disclosed Micro-Dystrophin cDNA Gene Construct Restored Function in Muscle

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

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

Centre for Biomedical Sciences, School of Biological Sciences, Royal Holloway, University of London, Surrey TW20 0EX, UK and ¹Gene Targeting Unit, Department of Neuromuscular Diseases, Division of Neuroscience and Psychological Medicine, Imperial College Faculty of Medicine, Charing Cross Hospital, London W6 8RP, UK

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The disease is caused by an absence of dystrophin, a 427 kDa cytoskeletal protein of the spectrin family [3,4]. The DMD gene has one of the highest mutation rates reported so far, 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 phenotype is DMD or the much less severe form, Becker muscular dystrophy (BMD). Very mild forms of the disease, where <50% of the gene has been deleted, have been described [5-8]. Conversely, other individuals with only small deletions display a DMD phenotype. This apparent anomaly depends on whether the dystrophin open reading frame is maintained (BMD) or disrupted (DMD). At the molecular level, the DMD phenotype thus appears to be due either to the metabolic instability of the truncated mRNAs or proteins, or to a functional inactivity of the latter [9].

The dystrophin protein has four main structural domains: an N-terminal region, which binds to the F-actin of cytoskeletal structures; 24 right-handed coiled coil repeats, interspersed with four hinge regions; and two C-terminal regions (CR and CT) which bind a complex of dystrophin-associated proteins (DAPs). These DAPs provide a molecular link between the

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Present address: Patricia Serpente, Muscular Dystrophy Division, The National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.

Serpente Exhibit 1010, page 1

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 (OAP) 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 OAP complexes, but can also ameliorate dystrophic pathology at the cellular level.

EX1010 (Fabb) at Abstract; Petition at 20-21, 55.

Patent Owner Argues That Claim 8 Requires Delivery to Muscle

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.
Petitioner.

v.

THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA,
Patent Owner
And REGENXBIO INC.,
Exclusive Licensee.

Case IPR2024-00580
Patent 11,680,274 B2

PATENT OWNERS' SUR-REPLY

Petitioner's Reply fails to address the critical issue underlying this IPR—that claim 8 of the '274 Patent requires an adeno-associated virus ("AAV") that delivers a dystrophin gene product to *muscle* tissue.¹ Instead, all of Petitioner's

Thus,
there is no real dispute that claim 8 requires an AAV that can deliver dystrophin to *muscle*, as even Petitioner's ground reference, Fabb, confirms. *See, e.g.,* EX1010, abstract.

But enhancing liver tropism has no bearing on,
and may even conflict with, claim 8's requirement to deliver dystrophin to *muscle*.

Patent Owner's Sur-Reply at 1, 2-3, 9.

Expert Testimony

Dr. Samulski's Opinions Are Unreliable

Reply at 1-14.

Patent Owner Argued That the Results in Snowdy Were Not Published or Peer-Reviewed

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Vandenberghe et al.
U.S. Patent No.: 11,680,274 Attorney Docket No.: 47889-0035IP1
Issue Date: June 20, 2023
Appl. Serial No.: 16/368,758
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
Sarepta v. Trustees UPenn et al.
IPR2024-00580

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

EX2027 (Declaration of Dr. Richard Jude Samulski) at ¶72; Reply at 1.

Dr. Samulski Confirmed That He Supervised, Approved, and Signed the Snowdy Thesis



Dr. Jude Samulski

Q. Dr. Snowdy carried out his thesis work in your laboratory at the University of North Carolina, right?

A. Correct.

Q. You were his thesis adviser?

A. That's correct.

Q. You supervised Dr. Snowdy's graduate work, correct?

A. I did.

Q. And he worked on a project that you helped him select, correct?

A. I did.

Q. You helped him design the project that he worked on?

A. I helped guide him on that project, yep.

EX1063 (Deposition of Richard Jude Samulski, Ph.D.) at 16:3-8, 16:23-17:6; Reply at 2.

Dr. Samulski Confirmed That He Supervised, Approved, and Signed the Snowdy Thesis



Dr. Jude Samulski

Q. But the fact that your signature is on the first page of his dissertation means that you thought that it was worthy of being granted a Ph.D, correct?

A. Yeah, me and the other four colleagues.

Q. So the fact that all of those signatures are on the first page of Dr. Snowdy's dissertation indicates that you and the whole rest of the committee --

A. Correct.

Q. Let me just finish my question.

A. Sorry.

Q. -- thought that the dissertation was worthy of the grant of a Ph.D, correct?

A. That is the correct assumption.

Q. It's not an assumption. It's a statement, right? It's correct to say that you and the rest of the committee believed that the thesis was worthy of being granted a Ph.D when you all signed off on it, correct?

A. I would say that's correct.

EX1063 (Deposition of Richard Jude Samulski, Ph.D.) at 19:20-20:14; Reply at 2.

Patent Owner Argued That a POSA Would Not Have Combined Snowdy and the '772 Publication Based on Dr. Samulski's Definition of Tropism

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Vandenberghe et al.
U.S. Patent No.: 11,680,274 Attorney Docket No.: 47889-0035IP1
Issue Date: June 20, 2023
Appl. Serial No.: 16/368,758
Filing Date: March 28, 2019
Title: METHOD OF INCREASING THE FUNCTION OF AN AAV VECTOR

DECLARATION OF DR. RICHARD JUDE SAMULSKI

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

1 EX2027
Sarepta v. Trustees UPenn et al.
IPR2024-00580

In my view, nuclear localization relates to infectivity, and is unrelated to the tissue-specific tropism driving Sarepta's motivation to combine. As I discuss in detail above in paragraph 30, tropism relates to the steps that occur that allow an AAV vector to reach and be taken up by the cell or tissue of interest—steps 1 and 2 in the Figure I describe above; the intracellular steps that follow affect infectivity. Snowdy's hypothesis regarding the effect of phosphorylation was focused on step 4 in the Figure—nuclear pore entry. EX2029, 4.

EX2027 (Declaration of Dr. Richard Jude Samulski) at ¶¶73; *see also id.* at ¶¶ 30, 38; Patent Owner Response at 52.

Dr. Samulski Admitted That His Definition of “Tropism” Is Different From How the Term Is Used in the '772 Publication



Dr. Jude Samulski

Q. And you didn't formulate your definition based on however the term is used in the '772 publication?

A. I'm sure I did not use it as my sole definition of tropism.

Q. Well, you exclude gene expression from the definition of a tropism, right?

A. Yes.

Q. And these are gene expression studies, right?

A. So they are using tropism in another manner.

Q. Yes, they are using tropism in a different way from the way you use it in your declaration?

A. Correct.

EX1063 (Deposition of Richard Jude Samulski, Ph.D.) at 43:8-12, 47:22-48:6; Reply at 4.

Dr. Samulski Admitted That His Definition of “Tropism” Is Different From How the Term Is Used in His Own Patents and Publications



Dr. Jude Samulski

Q. So you have a different definition here in the '126 than you just defined in your declaration?

A. I think it says “the term tropism used herein.” So it’s already making a clear distinction that it’s different. So you’re right in saying it’s different than the one I’m declaring.

EX1063 (Deposition of Richard Jude Samulski, Ph.D.) at 162:11-16; Reply at 5.

Patent Owner Says That Addressing Dr. Samulski's Use of the Term Tropism Is a "Red Herring"

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.
Petitioner.

v.

THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA,
Patent Owner
And REGENXBIO INC.,
Exclusive Licensee.

Case IPR2024-00580
Patent 11,680,274 B2

PATENT OWNERS' SUR-REPLY

Rather than engage with these concerns, Petitioner attacks Dr. Samulski's
use of the term "tropism"—a red herring. Reply, 3-6. The word "tropism" is not
in the claims.

Patent Owner's Sur-Reply at 6.

Patent Owner Argued That a POSA Would Not Have Expected Single Amino Acid Substitutions to Affect Tissue Tropism

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Vandenberghe et al.
U.S. Patent No.: 11,680,274 Attorney Docket No.: 47889-0055IP1
Issue Date: June 20, 2023
Appl. Serial No.: 16/368,758
Filing Date: March 28, 2019
Title: METHOD OF INCREASING THE FUNCTION OF AN AAV VECTOR

DECLARATION OF DR. RICHARD JUDE SAMULSKI

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Dated: 12/5/2024

By: 

Richard Jude Samulski, Ph.D.

1

EX2027
Sarepta v. Trustees UPenn et al.
IPR2024-00580

28. The kind of modifications that I understand are being proposed by Petitioner here—targeting a single amino acid in the AAV capsid to allegedly improve or change its functionality, such as its tissue targeting ability—had not been reported in the literature by 2005.

Notably, as I will discuss more below, while some techniques had been shown by 2005 to improve or change tropism, none involved a single amino acid substitution. Nor am I aware today of any example where tropism is significantly increased or changed to a materially different tissue type because of a single amino acid substitution.

48. In my opinion, however, a skilled artisan would not have expected a single amino acid change to alter tropism in any meaningful way. I am unaware of any studies before 2005 where a single amino acid change was shown to alter AAV tropism.

EX2027 (Declaration of Dr. Richard Jude Samulski) at ¶¶28, 32, 48; Reply at 6.

The '126 Provisional Discloses Single Amino Acid Substitutions That Confer the Favorable Muscle Tropism of AAV1 onto AAV2

Attorney Docket No. 5470-427PR PATENT

COVER SHEET FOR FILING PROVISIONAL
PATENT APPLICATION (37 CFR §1.51(c)(1))

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Date: December 15, 2004

This is a request for filing a PROVISIONAL PATENT APPLICATION under 37 C.F.R. §1.53(c).

Docket No.	5470-427PR
Type in plus sign (+) inside this box	+

INVENTOR(S)/APPLICANT(S)

Name: Dawn E. Bowles
Address: Durham, NC

Name: Josh Greiger
Address: Chapel Hill, NC

Name: Chengwen Li
Address: Chapel Hill, NC

Name: Mavis McKenna
Address: Gainesville, FL

Name: Joseph E. Rabinowitz
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Name: Richard Jude Samulski
Address: Chapel Hill, NC

TITLE OF THE INVENTION (280 characters maximum)
CHIMERIC VECTORS

Deposition Exhibit 1059

Sample Exhibit 1059, page 1

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

EX 1072 at ¶128 (quoting EX1059 ('126 provisional application) at 8:23-27 (emphasis added); Reply at 10-11.

Dr. Samulski's '126 Provisional Discloses Single Amino Acid Substitutions with Asparagine to Remove a Phosphorylation Site

Attorney Docket No. 5470-427PR PATENT

COVER SHEET FOR FILING PROVISIONAL PATENT APPLICATION (37 CFR §1.51(c)(1))

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Date: December 15, 2004

This is a request for filing a PROVISIONAL PATENT APPLICATION under 37 C.F.R. §1.53(c).

Docket No.	5470-427PR
Type if plus sign (+) inside this box	+

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Address:

Name: Richard Jude Samulski
Address: Chapel Hill, NC

TITLE OF THE INVENTION (280 characters maximum)
CHIMERIC VECTORS

Deposition Exhibit 1059

Sample Exhibit 1059, page 1

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

EX1059 ('126 provisional application) at 21:20-27; *see also id.* at 21:28-22:5, 56:6-29 (claims 57-61); Reply at 25.

The '809 Patent Claims the AAV2 Vectors in the '126 Provisional With Single Amino Acid Substitutions to Enhance Muscle Tropism



(12) **United States Patent**
Bowles et al.

(10) **Patent No.:** **US 7,892,809 B2**
(45) **Date of Patent:** **Feb. 22, 2011**

(54) **CHIMERIC VECTORS**

(57) **Inventors:** **Dawn E. Bowles**, Durham, NC (US);
Chengwen Li, Chapel Hill, NC (US);
Joseph E. Rabinowitz, Secaucus, NJ (US);
Josh Grigier, Chapel Hill, NC (US);
Mavis Aghajany-McKenna, Gainesville, FL (US);
Richard Jude Samulski, Chapel Hill, NC (US)

(73) **Assignees:** **The University of North Carolina at Chapel Hill**, Chapel Hill, NC (US);
The University of Florida Research Foundation, Gainesville, FL (US)

(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 211 days.

(21) **Appl. No.:** **11/793,430**

(22) **PCT Filed:** **Dec. 15, 2005**

(86) **PCT No.:** **PCT/US2005/045552**

8,371 (c)(1),
(2), (4) **Date:** **Aug. 22, 2007**

(87) **PCT Pub. No.:** **WO2006/06666**

PCT Pub. Date: **Jun. 22, 2006**

(65) **Prior Publication Data**
US 2008/0269149 A1 Oct. 30, 2008

Related U.S. Application Data
(60) Provisional application No. 60/636,126, filed on Dec. 15, 2004.

(51) **Int. Cl.** **C12N 7/01** (2006.01)

(52) **U.S. Cl.** **435/253.1; 435/320.1; 536/24.5**

(58) **Field of Classification Search** **None**
See application file for complete search history.

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Primary Examiner—James S. Ketter
(74) **Attorney, Agent, or Firm—Myers Bigel Sibley & Sejovick, P.A.**

(57) **ABSTRACT**

The present invention is based, in part, on the discovery that parvovirus (including AAV) capsids can be engineered to incorporate small, selective regions from other parvoviruses that confer desirable properties. The inventors have discovered that in some cases as little as a single amino acid insertion or substitution from a first parvovirus (e.g., an AAV) into the capsid structure of another parvovirus (e.g., an AAV) to create a chimeric parvovirus is sufficient to confer one or more of the desirable properties of the first parvovirus to the resulting chimeric parvovirus and to confer a property that is not exhibited by the first parvovirus or is present to a lesser extent.

66 Claims, 14 Drawing Sheets

22. A chimeric virus vector comprising:

- (a) a chimeric adeno-associated virus (AAV) capsid comprising a selective amino acid substitution at amino acid position 450 in an AAV2 capsid subunit having the amino acid sequence of (SEQ ID NO:3) or a corresponding change in another AAV capsid subunit;
- (b) a nucleic acid comprising an AAV terminal repeat sequence and a heterologous nucleic acid sequence; wherein the nucleic acid is packaged within the chimeric AAV capsid.

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

EX1061 ('809 patent) at 75:13-24 (claims 22, 23); see also *id.* at 17:6-32; Reply at 25.

Dr. Samulski Confirmed That the Purpose of the Substitutions in the '126 Provisional Was to Confer the Muscle Tropism of AAV1 to AAV2



Dr. Jude Samulski

Q. All right. So all of the disclosures we looked at, where it was singling out, substituting in asparagine, asparagine was chosen there and singled out because that's the corresponding amino acid in the AAV1, right?

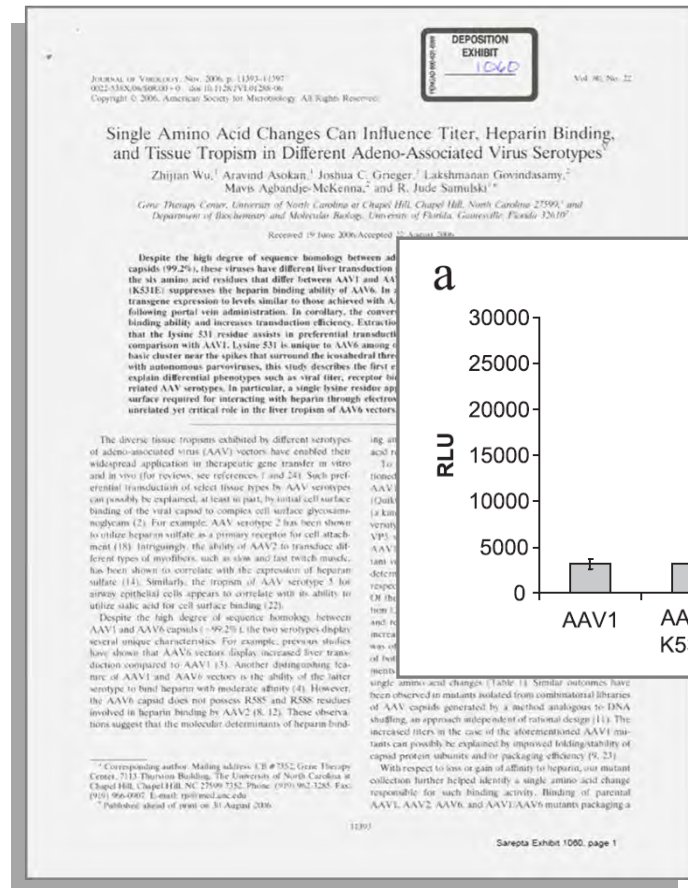
A. Correct.

Q. And this '126 provisional is looking to import some of the properties of AAV1 into AAV2?

A. Correct.

EX1063 (Deposition of Richard Jude Samulski, Ph.D.) at 184:16-24; Reply at 25.

Dr. Samulski Published in Wu (2006) a Single Amino Acid Substitution That Conferred on AAV1 Some of the Heparin Binding Characteristics of AAV6



In summary, of the six amino acids that differ between AAV types 1 and 6, residues at positions 129, 584, and 598 appear critical in determining viral genome titer. In addition, we have identified a single amino acid (K531) that is essential for conferring the heparin binding characteristics of AAV6. Introduction of an E531K change in AAV1 imparts a heparin binding ability similar to that of AAV6. These observations suggest that a minimum basic footprint is required to facilitate heparin binding through electrostatic interactions. Introduction of K531 in AAV type 8 capsid resulted in recombinant vector particles with heparin binding activity similar to that of AAV6 (Fig. 1d). These data not only speak to the importance of the K531 position but also illustrate the ability of a single amino acid change to confer a serotype-specific trait in another distantly related serotype.

EX1060 (Wu 2006) at 4-5, Figure 3a; Reply at 13.

Other Researchers Were Making Single Amino Acid Substitutions That Changed AAV Function, Including Cell Binding In Vitro

JOURNAL OF VIROLOGY, Oct. 2003, p. 11072-11081
0022-538X/03/0040-11072-10\$15.00/0
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Vol. 77, No. 20

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

A. Kern,¹ K. Schmidt,¹ C. Leder,¹ O. J. Müller,¹ C. E. Wobus,¹ K. Bettinger,²
C. W. Von der Lieth,² J. A. King,³ and J. A. Kleinschmidt^{1,4}

¹Forschungsschwerpunkt Angewandte Tumorstudiologie und Zentrale Spektroskopie,
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Received 8 May 2003/Accepted 15 July 2003

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 motif 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 R584 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.

Attachment of a virus to a host cell requires a specific interaction of the virus shell with cellular receptor molecules. These permit uptake and thus intracellular processing of the virus so that it can successfully infect the cell. For adeno-associated virus (AAV) type 2 (AAV-2), a member of the parvovirus family containing a nonenveloped icosahedral capsid, heparan sulfate proteoglycan has been shown to act as a primary receptor (62). However, the contribution of an additional receptor(s) has been postulated because AAV-2 binding to cells and recombinant AAV-2 transduction did not quantitatively correlate with the amount of heparan sulfate on the surface of different cell types tested (26, 51). After binding to the cell surface, AAV-2 is thought to engage a secondary receptor which mediates cell entry. To date, αVβ5 integrin and human fibroblast growth factor receptor 1 have been proposed (49, 61), but the involvement of other molecules has also been suggested (50, 52).

Heparan sulfate glycosaminoglycans (HSGAGs) are complex polysaccharides with high structural diversity. They consist of repeating disaccharide units each composed of glucosamine or iduronic acid linked to glucosamine (17, 48). The immense structural diversity of HSGAGs arises from the modification of individual disaccharide units within the oligosaccharide. This diversity enables these molecules to interact with a wide variety of proteins, such as growth factors, chemokines, morphogens, enzymes, matrix proteins, lipoproteins, and antimicrobial peptides, which are involved in diverse biological processes, such as morphogenesis, tissue repair, energy balance, host defense, cell adhesion, proliferation, and growth factor signaling (3, 40, 48). Numerous viruses, e.g., herpes simplex virus type 1 (HSV-1), pseudorabies virus (6), vaccinia virus (11), Sindbis virus (34, 58), cytomegalovirus (36, 62), and others, bind to assembled while attached to far, three major protein families: membrane-spanning syndecan, tol-linked glypicans, and the can perlecan and agrin (17), does not correlate with the p bound but rather correlates HSGAGs have been synthesized HSGAGs interact with protein interactions of basic amino acid sulfate and carboxyl groups or, ever, hydrogen bond formation hydrophobic interactions can tions (1). Heparin-binding domains of heparin-mimicking proteins

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¹ This article is dedicated to Harald zur Hausen on the occasion of his retirement as head of the German Cancer Research Center with gratitude and appreciation for 20 years of leadership.

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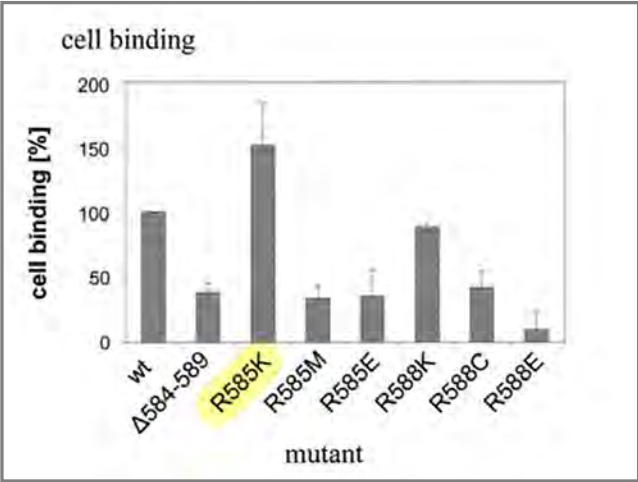
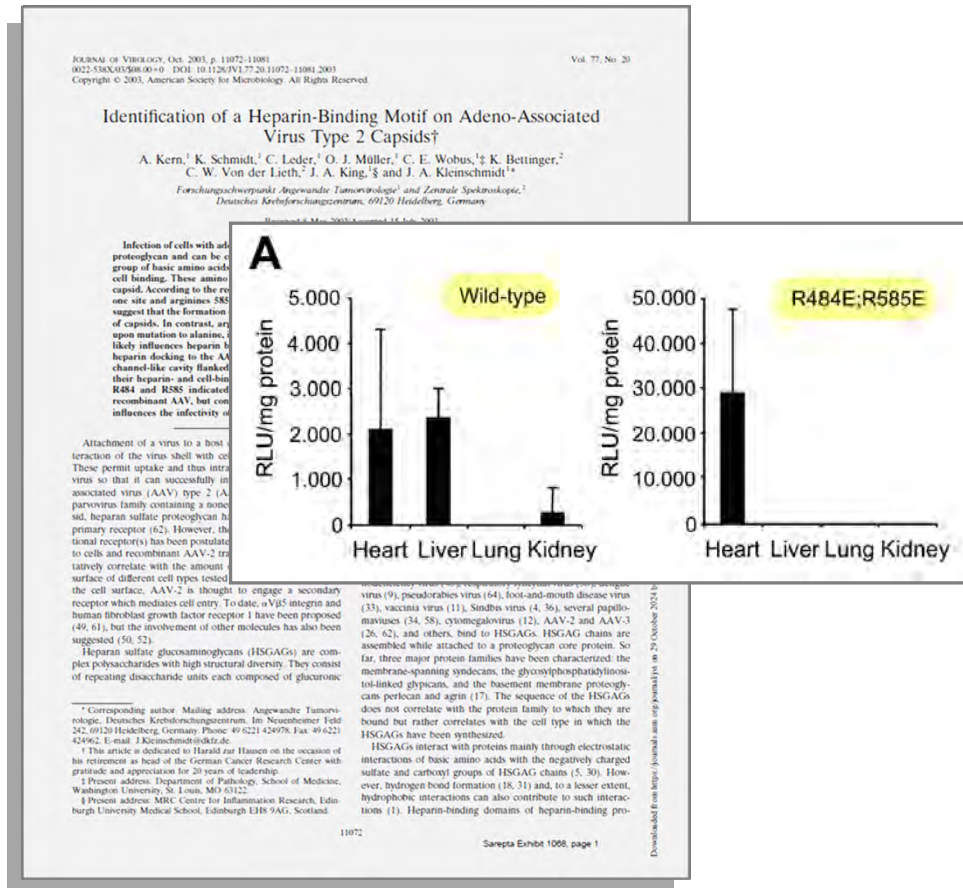


TABLE 1. Mutant analysis

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
28	R585K	2 × 10 ³	WT	2	WT	+	+

EX1068 (Kern) at Table 1, Figure 2(a); Reply at 9.

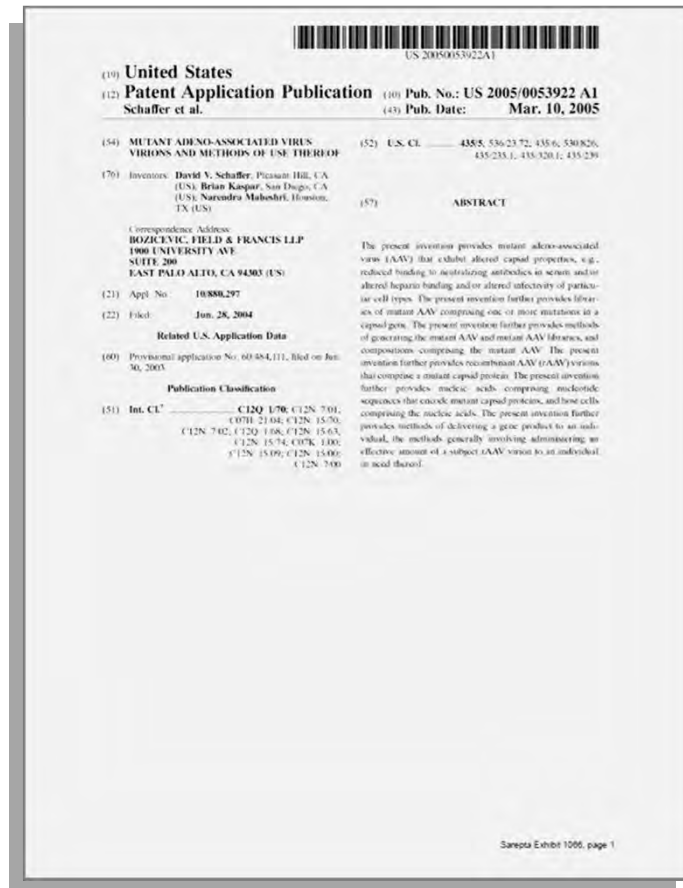
Other Researchers Were Making Amino Acid Substitutions That Changed AAV Function, Including Tropism



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 10¹¹ 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.

EX1068 (Kern) at 6-7, Figure 3; Reply at 9-10.

Other Researchers Were Making Single Amino Acid Substitutions That Changed AAV Function, Including Antibody Evasion



[0246] Table 1 provides the clone number and amino acid changes for various AAV neutralizing antibody escape mutants.

TABLE 1

Mutations in 5 rabbit antisera evader clones

Clone	Mutation	Region
rAbE1	T713R	2-fold dimple*
	T716A	2-fold dimple*
rAbE2	V418L	Antigenic peptide 53*
	T713R	2-fold dimple*
	T716A	2-fold dimple*
rAbE3	T716A	2-fold dimple*
rAbE4	D180N	A69 linear epitope†
	T716A	2-fold dimple*
rAbE5	A493G	C37-B conformational epitope†
	T716A	2-fold dimple*

EX1066 (U.S. Patent Application Publication No. US 2005/0053922) at [0246], Table 1; Reply at 10.

Dr. Samulski Agreed That One Approach Available to a POSA Was to Select and Test Amino Acids for Their Effect on AAV Function, Such as Tropism



Dr. Jude Samulski

Q. So Dr. Schaffer's opinion in this case that you're responding to, right, is that a person of ordinary skill in the art would have combined the teachings of the '772 publication with Dr. Snowdy's thesis, correct? That's his opinion that you're responding to? I'm not asking that you agree with it. I'm saying you are responding to that opinion?

A. Correct.

Q. And his opinion is that this person of ordinary skill, looking at the teachings of both, would have selected several amino acids to test to see if any them affected functions such as tropism, transduction. Do we agree with that?

A. That would have been one approach.

EX1063 (Deposition of Richard Jude Samulski, Ph.D.) at 83:10-23; Reply at 12.

Patent Owner Says the Issue Is Not Whether Single Amino Acid Substitutions Were Generally Known to Affect AAV Function

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.
Petitioner.

v.

THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA,
Patent Owner
And REGENXBIO INC.,
Exclusive Licensee.

Case IPR2024-00580
Patent 11,680,274 B2

PATENT OWNERS' SUR-REPLY

Petitioner devotes much of its Reply to arguing that single amino acid substitutions were generally known to affect AAV function. But that is not the issue here.

Patent Owner's Sur-Reply at 13.

Xie Teaches That the Amino Acid Residues in Figure 4 Are on the Surface of the AAV Capsid

The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy

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 Edited by Donald L. D. Casper, Florida State University, Tallahassee, FL, and approved June 5, 2002 (received for review April 26, 2002)

The structure of the adeno-associated virus (AAV-2) has been determined to 3.4 Å resolution by x-ray crystallography. AAV is being developed as a vector for gene therapy to treat diseases including hemophilia, cancer, and cystic fibrosis. As in the distantly related nonretroviral parvoviruses, the capsid protein has a β -barrel fold, but long loops between the β -strands share little structural homology with other parvoviruses, leading to unique surface features. Most prominent are groups of threefold-related peaks, each an intimate association of loops from two neighboring subunits. Mutations affecting cell entry and receptor binding are clustered near the positively charged side of each peak, implicating the region in attachment to the cellular receptor, heparan sulfate proteoglycan. Amino acids involved in antibody binding are in the same general vicinity. The structure will guide rational engineering of vector capsids to tailor cellular targeting and to avoid immediate neutralization by an immune system sensitized by prior exposure to AAV.

Gene therapy is being developed as a treatment for a wide range of diseases for which a genetic cause or an inherited predisposition exists. Gene therapy involves the introduction of new genetic material to correct an abnormality, and one of the biggest challenges remains the development of suitable vectors for the delivery of DNA into targeted cells (1). Along with retroviruses and adenoviruses, recombinantly engineered adeno-associated viruses (rAAV) have emerged as leading candidates (2), in which the therapeutic DNA replaces much of the viral genome and is contained within the protein capsid shell. Each viral vector has its own utility. The advantages of AAV include transduction of nondividing cells and a mild immune reaction (3). Clinical trials with rAAV vectors are underway for treatment of several diseases including cystic fibrosis (4) and hemophilia (5), whereas success in animal models is encouraging development of treatments for high blood pressure (6), Parkinson's disease (7, 8), muscular dystrophy (9, 10), and many other afflictions.

The natural hosts of the wild-type virus are human. Indeed ~80% of the population is seropositive because of natural exposure to AAV (11, 12). However, AAV has not been associated with disease (13). The replication of AAV depends on co-infection by a helper virus, and no pathogenic effects apparently exist beyond those caused by the helper virus. The helper is usually adenovirus (14). Hence the name "adeno-associated virus" and their classification as "dependoviruses," although they share no other relation to adenoviruses. The dependence on a helper virus makes AAV a satellite virus, leading to technical challenges (see below) in this first structure determination of an animal satellite virus. Before replication, the natural life cycle involves latent integration of AAV's DNA into the host's genome. Wild-type AAV is the only known eukaryotic virus that integrates at a specific site in the human genome (15). Vector constructs have generally not taken advantage of this specificity, because the gene for the replication protein that encodes the site specificity is usually replaced.

Adeno-associated viruses are small single-stranded DNA viruses of the Dependovirus genus that constitute one of the four

Parvoviridae family genera (14). The other genera have no requirement for a helper virus, replicating autonomously. Atomic structures have been obtained for several from the Parvovirus genus. Canine parvovirus (CPV), feline panleukopenia virus, and minute virus of mouse share 50–97% capsid sequence identity, and similar three-dimensional structures (16–20). The genomic DNA is surrounded by a near-spherical protein shell that comprises 60 capsid protein subunits arranged with $T = 1$ icosahedral symmetry. The subunit has a β -barrel core that is common in viral capsid proteins (21). The structure of an insect parvovirus (*Iminovirus*) is also known: the subunit fold is the same, but the loops are a total of ~130 residues shorter and most of the surface features of the capsid are truncated (22). Hitherto, high-resolution structure has not been available for Dependoviruses like AAV. The genetic organization is similar to other parvoviruses, but the capsid sequence identities are low (17–22%), less than between the rhino- and erbo-subfamilies of picornaviruses (23), which indicates that homology between AAV and other parvoviruses might be limited. Indeed, recent electron microscopy images showed substantial differences in surface topology between AAV-2 and canine parvovirus (24). Although homology modeling has been attempted (see ref. 25, for example), an experimental atomic structure has been a high priority for development of viral gene therapy vectors.

AAV-2 is one of five distinct serotypes. Serotypes 1, 2, and 3 share ~85% capsid sequence identity, with 2 and 3 likely using the same cellular receptor, whereas serotypes 4 and 5 are more distantly related (~55% identity to each other and to AAV-2) and use different receptors (26, 27). The primary receptor for AAV-2 is heparan sulfate proteoglycan, but secondary receptors and alternative primary receptors may also be available (28–31). AAV-2 enters the cell through an endosomal route, is transported to the nucleus by motor proteins, and enters the nucleus, all within seconds (32, 33). Monoclonal antibodies have been used to study capsid assembly and to map sequence determinants of receptor attachment (see ref. 34, for example). Systematic mutagenesis has begun to yield the determinants of various phenotypes (see refs. 25, 35, and 36, for example), providing an emerging wealth of data to be integrated in mapping function to structure.

Materials and Methods

AAV-2 was propagated in cultured human cells that were infected with helper adenovirus. HeLa cells were transfected with the pAAV2 clone then infected with adenovirus-2 (37, 38). Production was scaled-up by optimization of the timing of transfection/infection and by switching to suspension culture. AAV was purified by three CsCl-gradient sedimentations by ultracentrifugation for ~24 h in an SW55 swinging-bucket rotor

This paper was submitted directly (Track II) to the PNAS office.
 Abbreviations: AAV, adeno-associated virus; CPV, canine parvovirus.
 Data deposition: The atomic coordinates have been deposited in the Protein Data Bank, www.rcsb.org (PDB code 1U01).
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 Sarapat Exhibit 1008, page 1

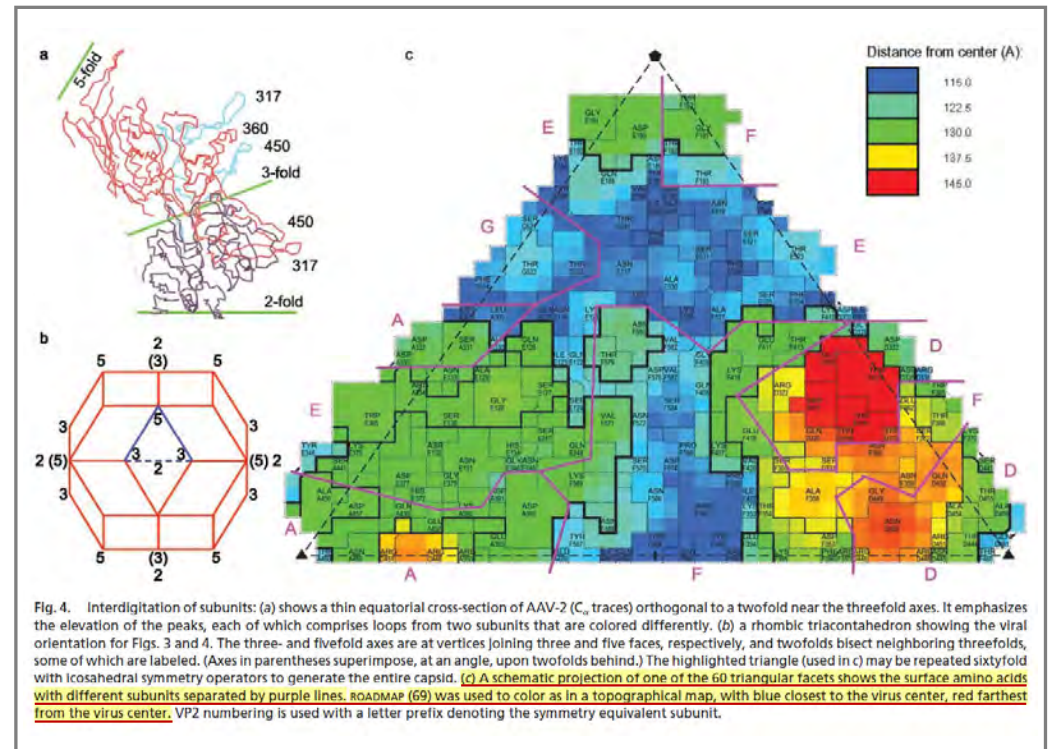
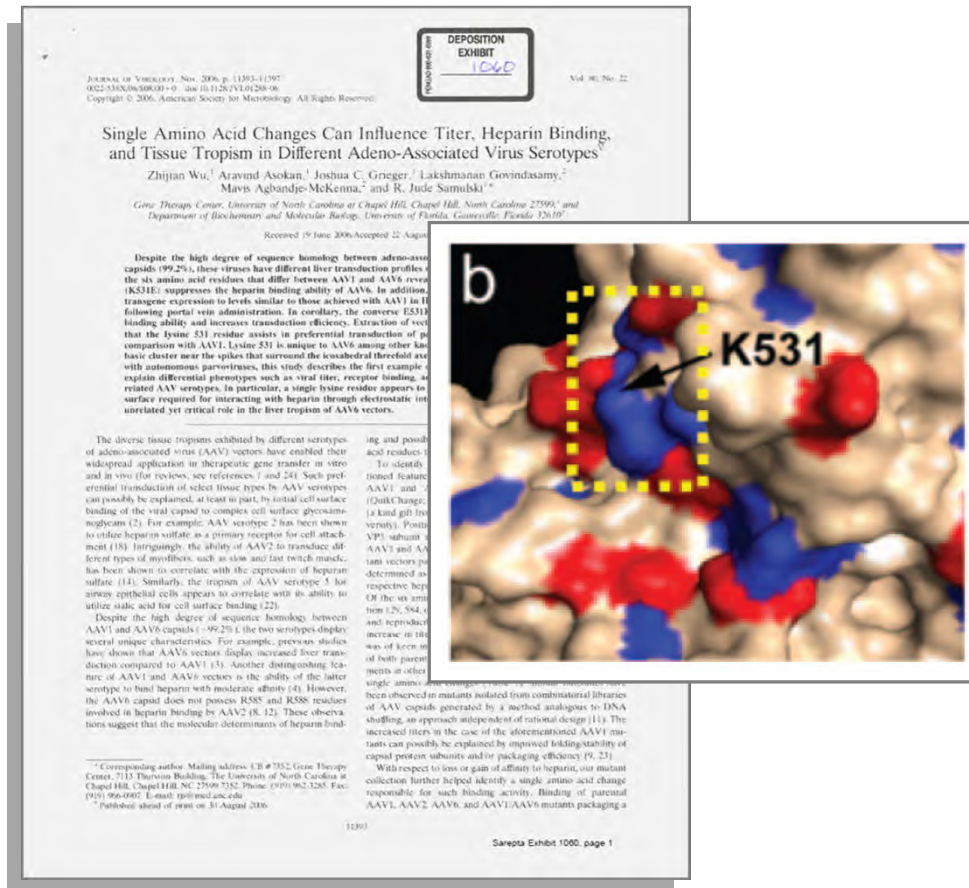


Fig. 4. Interdigitation of subunits: (a) shows a thin equatorial cross-section of AAV-2 (C_α traces) orthogonal to a twofold near the threefold axes. It emphasizes the elevation of the peaks, each of which comprises loops from two subunits that are colored differently. (b) a rhombic triacontahedron showing the viral orientation for Figs. 3 and 4. The three- and fivefold axes are at vertices joining three and five faces, respectively, and twofolds bisect neighboring threefolds, some of which are labeled. (Axes in parentheses superimpose, at an angle, upon twofolds behind.) The highlighted triangle (used in c) may be repeated sixtyfold with icosahedral symmetry operators to generate the entire capsid. (c) A schematic projection of one of the 60 triangular facets shows the surface amino acids with different subunits separated by purple lines. ROADMAP (69) was used to color as in a topographical map, with blue closest to the virus center, red farthest from the virus center. VP2 numbering is used with a letter prefix denoting the symmetry equivalent subunit.

EX1008 (Xie) at 10408, Figure 4; Petition 15-16.

Dr. Samulski Published in Wu (2006) That Less Accessible Amino Acids on the Surface of the Capsid May Affect Tropism



The potential contribution of lysine 531 to surface properties of the AAV6 capsid (and of E531 to that of AAV1) was analyzed by generating three-dimensional models of the surface charge distribution on the VP3 subunit (Fig. 2) of either serotype utilizing Swiss Model (17) with available coordinates of AAV2 (20) (Protein Databank accession no. 1LP3) supplied as a template (13). As shown in Fig. 2, insets a, b, and c, we have compared the capsid surface disposition of equivalent residues to those previously mapped for AAV2 heparin binding as well as neighboring basic residues located on icosahedral threefold symmetry-related VP3 subunits. The key positive residues and the 531 residue are proximal to the spikes that surround this symmetry axis. When the capsid is viewed perpendicular to the threefold axis, the side chain for K531 is surface exposed and forms a continuation for the basic patch between K533 and R488 as well as K528 and R567 at the base of each threefold spike of the AAV6 capsid surface model (Fig. 2, inset b, continuous blue patch, boxed with a dotted yellow line).

EX1060 (Wu 2006) at 3, Figure 2b; Reply at 13.

Dr. Samulski Agreed That Amino Acids at the Base of a Peak Can Affect Receptor Binding



Dr. Jude Samulski

Q. So just so I understand what you just said, things that are not on the peak or, you know, at the top of the peak, can still -- can influence the binding to the receptor based on conformational changes that occur upon binding?

A. Yep, correct.

Q. So that you can't, again, necessarily look at the surface and say well, you know, K531 is at the base of the peak so it won't affect binding?

A. Correct.

EX1063 (Deposition of Richard Jude Samulski, Ph.D.) at 232:25-233:9; Reply at 13.

